

## Selected Abstracts of the 8<sup>th</sup> International Workshop on Neonatology

### SYSTEMS MEDICINE IN PERINATOLOGY AND PEDIATRICS TAILORED BIOMARKERS, DRUGS AND TREATMENTS

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**ABS 1****URINARY METABOLOMICS AS A NEW STRATEGY TO DISCRIMINATE RESPONSE TO IBUPROFEN THERAPY IN PRETERM NEONATES WITH PATENT DUCTUS ARTERIOSUS**

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**INTRODUCTION**

Patent ductus arteriosus (PDA) is a frequent congenital cardiac malformation in the preterm infants. Intravenous ibuprofen is the standard treatment for pharmacological closure, but up to 30% of the patients do not respond and need to undergo surgery. Echocardiography is a very sensitive tool to diagnose PDA but does not predict effective response to ibuprofen. Our aim was to develop a model capable of predicting response to treatment applying a novel metabolomics-based strategy by means of analysing urinary metabolome.

**METHODS**

Prospective, observational, longitudinal study enrolling preterm infants < 32 weeks of gestation treated with ibuprofen for a hemodynamically significant PDA (> 1.5 mm). Ultrasound was performed before and after ibuprofen administration to determine response. Serial urinary samples were obtained by an internal validated non-invasive method (cotton-diaper technique) before and during treatment. Analysis of urine samples was carried out using LC-TOFMS. Multivariate analysis of the LCMS data was performed in MATLAB (Mathworks Inc. Natick, MA, USA) using in-house written scripts and the PLSToolbox (Eigenvector). Univariate statistical analysis was performed using SPSS v11 (SPSS Inc., Chicago, IL, USA).

**RESULTS**

Total of 34 patients were enrolled in the study with a median gestational age 27 weeks (CI: 24-31) and median birthweight 1,065 g (CI: 660-2,059 g). 19 patients did respond to ibuprofen (responder = R), 15 did not respond (nonresponder = NR). Median gestational age and birthweight were statistically higher in NR group ( $p < 0.001$ ). Ultrasound studies

did not show any differences between both groups. PLSDA multivariate analysis of LC-MS data showed a statistically significant difference in the metabolomic profiles between R and NR from urine samples collected after 72 h upon intervention. Based on a set of 33 selected potential biomarkers, a second PLSDA model was used for the prediction of an external sample subset. Results showed different dynamic metabolomic responses between R and NR during subsequent ibuprofen doses.

**CONCLUSIONS**

Urinary metabolomics appears to be a promising complementary strategy as it reflects PDA and its response to treatment. It might be a suitable approach for an early prediction of the response.

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**ABS 2****A METABOLOMIC APPROACH TO IDENTIFY PRETERM NEONATES BORN OF MOTHERS WITH CHORIOAMNIONITIS: PRELIMINARY DATA**

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**OBJECTIVE**

Histological chorioamnionitis (HCA) is a major risk factor for spontaneous preterm birth, especially at earlier gestational ages. Many studies have shown that antenatal exposure to inflammation makes neonatal outcome worse. This study was conducted to determine if metabolomic profiling of the urine can characterize preterm neonates born of mothers with HCA, also in order to identify biomarkers as early predictors of outcome in this population. To our knowledge, this is the first study of metabolomics performed on neonates exposed to intrauterine inflammation.

**METHODS**

We performed a matched case-control study to compare the metabolomic profile of urine collected from neonates born of mothers with HCA with a control group of neonates born of mothers without HCA, matched for gestational age (GA) at birth and birthweight (BW). A urine sample was taken non-invasively from each neonate on day 1, 7 and 14 of life. All samples were analyzed by <sup>1</sup>H nuclear magnetic resonance (NMR) spectroscopy. A multivariate statistical analysis (principal components analysis and partial least squares-discriminant analysis) was used to identify differences in the metabolic profile of the urine between the two groups.

**RESULTS**

Twelve neonates (4 born of mothers with HCA and 8 controls) were studied (mean GA = 31 + 3 weeks, SD 2 + 5; mean BW = 1,610 g, SD 606.5). Different metabolic patterns were found between the two groups. Individual metabolites discriminating were the following: mannitol, 4-hydroxy-phenylacetate, p-cresol, myo-inositol, trimethylamine-N-oxide and 1-methylnicotinamide. However, statistical significance was not achieved probably due to the small sample size.

**CONCLUSIONS**

Based on these preliminary data, metabolomic analysis seems to differentiate preterm infants born of mothers with HCA from those born of mothers without HCA, even if a larger sample is necessary to achieve statistical significance. The next step could be the identification of biomarkers as early predictors of outcome in preterm babies exposed to intrauterine inflammation, in order to plan a more accurate and targeted follow-up.

**REFERENCE**

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**ABS 3****URINARY METABOLOMICS IN TWINS AT BIRTH**

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**BACKGROUND**

Metabolomics, is helping to understand the field of metabolism. Through the rapid characterization of small molecule (metabolites), this new “omics”, has the opportunity to explore the interactions such as: genotype-phenotype and genotype-environment, which means it is possible to have a snapshot of the metabolic status, in normal or pathological conditions [1, 2]. Physiology of twins is quite different from that of singletons.

**OBJECTIVES**

The aim of this study is to evaluate metabolic trajectory of twins immediately after birth, and then after seven days through the metabolomics approach.

**METHODS**

Urine was collected (after ethical committee approval and parents consensus) from 15 twins after birth and then at seven days of life. A concentration of 1% of NaN<sub>3</sub> was added in order to avoid bacterial growth. Subsequently, <sup>1</sup>H-NMR-based metabolomics analysis was performed. Statistical analysis was carried out using Principal component analysis (PCA) and Partial Least Square-Data Analysis (PLS-DA).

**RESULTS**

Metabolomic approach showed characteristic profiles describing the difference between groups. In addition, the metabolites were used to build a predictive model for diagnosis. Differentiation between monozygotic and dizygotic twins was possible. Important metabolites were: galactitol, N-acetylcysteine, N-acetylglutamate, N-acetyltyrosine, methylguanidine, N-dimethylformamide, and 5-hydroxyindol-3-acetate.

**CONCLUSIONS**

We believe that this strategy will have a significant impact on the discovery of the new typical metabolic features at birth in twins, influencing immediate and long term epigenetic differences.

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<sup>1</sup>H NMR-based metabolomic analysis of urine from preterm and term neonates. *Frontiers Bioscience (Elite edition).* 2011;3:1005-12.

**ABS 4****FROM PRENATAL DIAGNOSIS TO NEONATOLOGY: RISK AND PROTECTIVE**

## FACTORS IN THE DEVELOPMENT OF MOTHER- PRETERM CHILD RELATIONSHIP

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### OBJECTIVES

Previous research has shown that premature birth can affect the quality of the parent-child relationship. Little is known about maternal and paternal fetal attachment during pregnancy in women at risk for preterm delivery. The aim of this study was to investigate the association between parents' prenatal attachment to the unborn child, gestational age and perinatal factors in a sample of inpatient pregnant women at risk for preterm birth and their partners.

### METHODS

A cross-sectional sample of 11 consecutive inpatient pregnant women at risk for preterm delivery between 24-32 weeks gestation were included in this pilot study, which is part of a larger longitudinal protocol. The quality and intensity of maternal and paternal attachment were assessed through the Maternal Antenatal Attachment Scale (MAAS) and the Paternal Antenatal Attachment Scale (PAAS), respectively. Sociodemographical, clinical and psychological data were also collected. Correlations were analyzed using Spearman's correlation coefficient. Results: The mean values ( $\pm$  SD) of the global scale of the MAAS and the MAAS quality and intensity subscales were 65.64 ( $\pm$  9.53), 37.36 ( $\pm$  5.07) and 28.27 ( $\pm$  4.98), respectively. All of the study participants scored below the cutoff value of 49 on the MAAS quality subscale. Five of the 11 women scored  $\geq$  27 on the MAAS intensity subscale, a finding which suggests the presence of a negatively preoccupied attachment style. The mean values ( $\pm$  SD) of the global scale of the PAAS and the PAAS quality and intensity subscales were 53.18 ( $\pm$  8.43), 30.18 ( $\pm$  7.78) and 23.00 ( $\pm$  2.97), respectively. No significant correlation was found between gestational age and MAAS and PAAS scores.

### CONCLUSIONS

These preliminary findings show that women hospitalized at risk for preterm delivery may

experience a negatively preoccupied attachment style. Further prosecution of the study will help to explore risk and protective factors affecting the mother-preterm child relationship and to develop targeted psychological intervention strategies.

### REFERENCE

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## ABS 5

### PROLONGED REFRIGERATED STORAGE OF HUMAN MILK: EFFECTS ON NUTRITIVE AND NON-NUTRITIVE CHARACTERISTICS

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### INTRODUCTION

Due to the limited knowledge on which common advice on human milk (HM) storage is based, current recommendations concerning safe human milk conservation are far from being univocal.

### OBJECTIVE

In an attempt to clarify some of the uncertainties existing on the storage of HM, we designed a comprehensive study to investigate the effects of prolonged refrigeration on the nutritive and non-nutritive characteristics of HM, and on its microbiological composition.

### METHODS

Human milk was collected and divided into 5 aliquots. One aliquot underwent analyses immediately, while the other aliquots were stored in the refrigerator for 24, 48, 72, and 96 hours before analysis. The temperature was constantly monitored. The samples were analysed for pH, free fatty acid (FFA) content, bile salt-dependent lipase (BSDL) activity, oxidative status, and bacterial profile. The experiment was replicated three times.

### RESULTS

The continuous temperature monitoring of the refrigerator showed a mean value of  $6.8 \pm 1.1^\circ\text{C}$ . Limited lipolysis was seen during prolonged refrigerated storage, consistently with a slight decrease in milk pH. Refrigeration preserved milk

overall oxidative status and the activity of BSDL. Moreover, the overall bacterial composition remained stable, with mean values for total bacteria, Enterobacteriaceae and coagulase positive Staphylococci counts under the recommended concentrations set for acceptability of milk in milk banks.

#### CONCLUSIONS

In conclusion, refrigeration of expressed human milk in controlled conditions for 96 hours allowed to maintain its bioactivity and nutritional quality, without compromising its microbiological safety.

#### ABS 6

##### USE OF DONOR HUMAN MILK IN NICU: IS DONOR MILK COMPETING WITH BREASTFEEDING OR SUPPORTING IT?

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#### INTRODUCTION

Recent research and systematic reviews have reinforced the conclusion that breastfeeding and human milk (HM) confer health benefits for infant and the mother. Human milk confers health benefits of vital importance for the sick and preterm infants in neonatal intensive care units (NICUs). Mother's own milk (MOM) is the first choice in preterm infant feeding and every effort should be made to promote lactation. When mother's milk is not available or insufficient, donor human milk (DHM) is recommended. Yet, occasionally the concern that the use of DHM might decrease breastfeeding is being raised. The present data collection planned by Italian Association of Human Milk Banks (AIBLUD) in collaboration with Italian Neonatal Network (INN) attempted to address this concern.

#### MATERIAL AND METHODS

A total of 4,277 VLBW infants from 83 Italian NICUs were evaluated for this comparative analysis. Eighty-three Italian NICUs were divided into two groups: centers with a human milk bank (HMB), centers without a HMB; the available parameters in the network – “any and exclusive breastfeeding

rates” and “formula only” at discharge – were compared.

#### RESULTS

The NICUs with and without HMB were comparable in terms of NICU characteristics and size. Exclusive breastfeeding rates at discharge were significantly higher in NICUs with a HMB when compared to NICUs without (29.6 vs. 16%, respectively,  $p=0.007$ ) (**Table 1**). Any breastfeeding rates at discharge were also slightly higher in the NICUs with HMB (60.4 vs. 52.8%), and formula only rates were slightly lower in the NICUs with HMB (26.5 vs. 31.3%), but these differences were not significant.

**Table 1.** Lactation rates at discharge for VLBW infants.

	Italian NICUs without a HMB N = 64	Range (%)	Italian NICUs with a HMB N = 19	Range (%)	P
Any breastfeeding at discharge (%)	52.8	4-85.1	60.4	29.7-84.1	0.087
Exclusive breastfeeding at discharge (%)	16	0-57.9	29.6	0-77.8	0.007
Formula only at discharge (%)	31.3	1.6-62.71	26.5	0-51.35	0.188

#### CONCLUSIONS

This report shows that the presence of a HMB and the use of DHM in NICU increase breastfeeding rates at discharge for VLBW infants.

#### ABS 7

##### PRENATAL DIAGNOSIS OF METHYLMALONIC ACIDURIA AND HOMOCYSTINURIA Cbl-C TYPE USING DNA ANALYSIS

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Methylmalonic aciduria (MMA) and homocystinuria, Cbl-C type is the most frequent inborn error of vitamin B12. Cbl-C patients present with a heterogeneous clinical picture [1]. Early-onset patients, presenting symptoms within the first year, show a multisystem disease with severe neurological, ocular, haematological, renal, gastrointestinal, cardiac, and pulmonary manifestations. Late-onset patients present a

milder clinical phenotype with acute or slowly progressive neurological symptoms and behavioral disturbance. The unfavorable outcome observed in most patients confirms that actual interventions, mostly focused on improving biochemical parameters, are not sufficient to prevent organ damage and that individual differences may also influence the response to therapies. The gene responsible for the Cbl-C defect, *MMACHC*, has been recently identified [2]. More than 40 mutations have been reported. A family of Sardinian origin with a 5-years old child affected by MMA and homocystinuria, Cbl-C type was referred to our Institution for genetic counseling. The patient was genetically analyzed for mutation in *MMACHC* gene and resulted homozygote, while both parents were heterozygote for c.271dupA mutation. Following not-directive genetic counseling during which the different options available were fully discussed, the parents opted for prenatal diagnosis. A transabdominal chorionic villi biopsy was performed at 12 weeks' gestation. DNA was extracted by standard methods. DNA was amplified for the exon 2 of *MMACHC* gene and sequencing analysis was performed that showed the normal sequence in the fetal DNA. To our knowledge this is the first case of prenatal diagnosis of MMA and homocystinuria, Cbl-C type using DNA analysis. The results suggest the importance of molecular analysis in prenatal diagnosis of MMA and homocystinuria.

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## ABS 8

### HUMAN BREAST MILK VS FORMULA MILK. IS <sup>1</sup>H-NMR METABOLOMICS ABLE TO HELP TO FIND THE RIGHT FORMULA?

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## RATIONALE

Currently the continuous progress of the neonatal intensive care unit allows the survival of almost 85% of low and extremely low premature infants. However several issues related to prematurity, including respiratory and intestinal immaturity makes difficult to adapt to the nutritional needs of these infants with a high risk of malnutrition and poor growth. The food choices are therefore a key role in the management of preterm infants. Although some premature babies grow and develop well with only breast milk, in the case of extremely premature infants and children, this food do not meet the adequate nutritional needs of the newborn. It therefore becomes necessary to use artificial products able to adapt well to the needs for growth and development of preterm. However, the effects of using artificial products to compensate the metabolic imbalances of the preterm infant is still a topic to debate.

## AIM

Aim of this experimental work is to analyze, through a multivariate approach, the profiles of the aqueous extract of human breast milk from mothers who had a preterm infants and to compare it with the infant formula milk for preterm infants.

## METHODS

Thirty complete 24 h expressions of milk were obtained at specific postpartum weeks (from 1 to 13), the total daily volume of milk was well mixed and an aliquot was removed and stored at -80°C for analysis. <sup>1</sup>H-NMR experiments on the aqueous extracts of milk were carried out on a Varian Unity 500 spectrometer. Multivariate analysis were performed using Simca P-13+.

## RESULTS

Multivariate analysis clearly indicate differences between the metabolic profile of the human breast and formula milk. In particular, the quantification of the following metabolites, choline, phosphocholine, creatine, creatinine are found to be predominant in the discrimination between the different types of milk.

## CONCLUSION

The results of this study, although still in a preliminary form, may represent a first step towards the development of a protocol for the optimization of the food in the formula.

## REFERENCE

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J Matern Fetal Neonat Med. 2012, in press.

## ABS 9

### A <sup>1</sup>H-NMR STUDY OF CRISPONI SYNDROME: CAN METABOLOMICS HELP TO DESCRIBE THE DISORDER?

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#### INTRODUCTION

Crisponi syndrome (MIM 601378) is a severe autosomal recessive disorder described for the first time in 1996 by Giangiorgio Crisponi [1] characterized by episodes of marked muscular contraction of the facial muscles like tetanus spasms associated with typical dysmorphic features, which include chubby cheeks, broad nose with anteverted nostrils, camptodactyly and long philtrum. This syndrome is often associated to hyperthermia, and sudden death in most cases. The syndrome is caused by mutations in the cytokine receptor-like factor 1 (CRLF1) gene, mutations of which have also been associated with cold-induced sweating syndrome, and it can be hypothesized that these two syndromes reflect different manifestations of the same entity [2]. Very little is known about the patho-physiology of the gene involved, so it's very important to develop new approach to investigate this disorder.

#### OBJECTIVES

Aim of this study is to identify metabolic differences between patients affected by Crisponi Syndrome and parents, carriers of the mutated gene CRLF1 to determine the possibility of <sup>1</sup>H-NMR metabolomic approaches in this kind of research.

#### MATERIAL AND METHODS

The study included 7 patients affected by Crisponi Syndrome and their parents, carriers of the mutated gene. A urine sample was collected from both, patients and parents, and an aliquot of 540 µl was mixed with 60 µl of 1.5 M phosphate buffer solution

(pH 7.4) 10% TSP (Trimethylsilyl propanoic acid) in D<sub>2</sub>O to stabilize the pH of the final solution. TSP was added to provide an internal reference for the chemical shifts (0 ppm), then urines were analyzed using <sup>1</sup>H-NMR Varian 500 MHz. NMR spectra were subjected to multivariate analysis in order to combine metabolic variables using SIMCA-P+ (version 13.0, Umetrics, Sweden).

#### RESULT

Data were analyzed using a PLS-DA (Partial least squares discriminant analysis) model which was able to discriminate between three groups, patients, mother and father. Using this mathematical approach we were able to find some metabolites that seem to be responsible of the groups separation. The metabolites are: hippurate, taurine, TMA-Oxide (decreased in Crisponi patients) and lactate (increased in Crisponi patients).

#### CONCLUSION

The use of metabolomics appears to be a new approach in Crisponi Syndrome. The multivariate analysis shows different metabolic profiles between three groups, patients, mothers and fathers. The investigation for the meaning of the discriminating metabolites may improve current knowledge on this rare disease and may describe the pathways involved.

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## ABS 10

### NESTIN IMMUNOREACTIVITY IN THE DEVELOPING HUMAN KIDNEY

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**BACKGROUND**

Nestin is a intermediate filament protein with a role in regulating cytoskeleton structure, also utilized as a stem cell marker in developing central nervous system. In adult kidneys, nestin expression is restricted to podocytes.

**DESIGN**

This study was aimed at analyzing nestin expression in the human kidney in different phases of development. To this end, twelve kidneys from seven fetuses (gestational age [GA] 9-19) and five newborns (GA at birth 26-36) were formalin-fixed, routinely processed and paraffin-embedded. Paraffin sections were immunostained for nestin.

**RESULTS**

In the fetal kidney, nestin expression was mainly detected in the undifferentiated metanephric mesenchymal. Moreover, nestin was expressed in developing glomeruli, in the area occupied by podocyte precursors. No significant reactivity for nestin was detected in the cap mesenchyme. Only rarely, we also observed nestin reactivity in cell of the Bowman's capsule. In newborn kidneys, nestin expression was detected in different cell types: in developing podocytes, in mesangial precursors and in cells committed toward the endothelial lineage. A strong interglomerular variability in nestin expression was also found.

**CONCLUSIONS**

Our data indicate that nestin is strongly expressed in the human developing kidney in different cell types, including podocyte precursors, endothelium of glomerular capillaries, and mesangial cells that populate the developing glomerulus. These preliminary findings imply that nestin may play a role in the modulation of human kidney development and function.

**ABS 11**

**A NON-INVASIVE APPROACH TO CHARACTERIZE EPILEPTIC CHILDREN BORN ELBW COMPARED TO NON EPILEPTIC: A METABOLOMICS POINT OF VIEW**

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**INTRODUCTION**

Extremely low birth weight (ELBW) are a class of preterm infants with a birth weight less than 1,000 g. They usually born at 27 weeks of gestational age or younger. These preterm infants represent a population at high risk for brain damage and neuroevolutionary disability. Epilepsy is a major neurological sequelae in ELBW infants [1] with a range of incidence between 4.1% and 18.7%. The occurrence of seizures in these children reflects the vulnerability of the immature brain for neuropathological processes that take place in that period of life. In particular, periventricular leukomalacia and severe intraventricular hemorrhage or post-hemorrhagic hydrocephalus, have been associated with a high risk of seizures in the first months of life as well as in the childhood [2].

**OBJECTIVES**

Aim of this study is to explore the metabolic differences between a group of children born ELBW who developed epilepsy and a group of children born ELBW without, as control.

**MATERIALS AND METHODS**

Urine samples were collected from 7 children with epilepsy and 9 controls. Children were matched for age, sex, birth weight, gestational age, growth curve, perinatal conditions and cerebral echography. The samples were prepared aliquoting 540 µl of urine and adding 60 µl of phosphate buffer (1.5M, pH 7.4 with 10% Trimethylsilyl propanoic acid in D20). Urines were analyzed using <sup>1</sup>H-NMR Varian 500 MHz. NMR spectra were subjected to multivariate analysis in order to combine metabolic variables using SIMCA-P+ (version 13.0, Umetrics, Sweden).

**RESULTS**

Spectra were aligned, binned, normalized and then analyzed using a PLS-DA (Partial least squares discriminant analysis) model. The mathematical model was able to discriminate between the group of epileptic children and the group of non-epileptic. The metabolites discriminating the separation between the two classes are: citric acid, alanine and taurine (increased in epileptics compared to controls), glycine, malonic acid, creatinine, sugars (decreased in epileptics compared to controls).

**CONCLUSION**

These preliminary results seems to be promising for the study of the postnatal metabolic maturation, as well as the identification of predictors biomarkers characterizing early events of the epilepsy which may help for the treatment of this type of diseases.

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## ABS 12

### EARLY CESSATION AND DERANGEMENT OF NEPHROGENESIS IN THE PRETERM HUMAN KIDNEY FOLLOWING IBUPROFEN TREATMENT

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## BACKGROUND

Preterms are commonly exposed to ibuprofen therapy in the neonatal period for the treatment of patent ductus arteriosus, exposing the developing kidney to a potentially nephrotoxic drug.

## DESIGNS

The aim of this study was to analyze at histology, the kidney of a 23 wks preterm affected by a patent ductus arteriosus who survived four weeks and underwent 2 cycles of ibuprofen therapy.

## RESULTS

At autopsy the histological study of kidneys evidenced the following pathological changes:

1. marked reduction of the subcapsular nephrogenic zone;
2. severe modifications of the sequence of events characterizing normal nephrogenesis (**Fig. 1**);
3. high percentage of abortive glomeruli;
4. apoptosis of podocyte precursor in developing glomeruli, leading to glomerular necrosis (**Fig. 2**);
5. apoptosis of proximal tubular cells.

## CONCLUSION

The effects of ibuprofen on the developing human kidney have been very scarcely studied. Recently a study in preterm baboons revealed the ability of ibuprofen to induce early cessation of nephrogenesis [1]. Our study clearly shows that ibuprofen therapy may cause not only a block but even a derangement of nephrogenesis, giving rise to abortive and mal

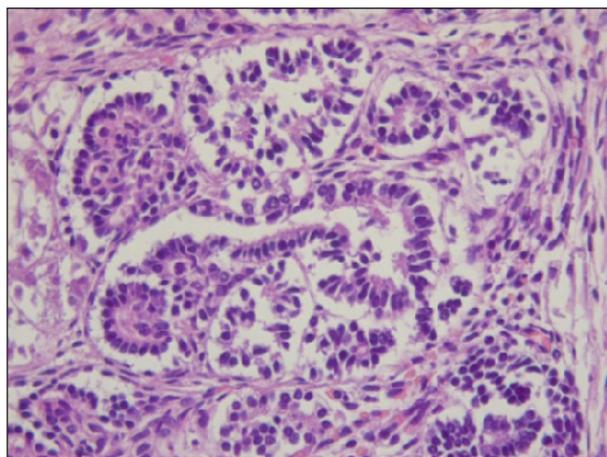


Figure 1.

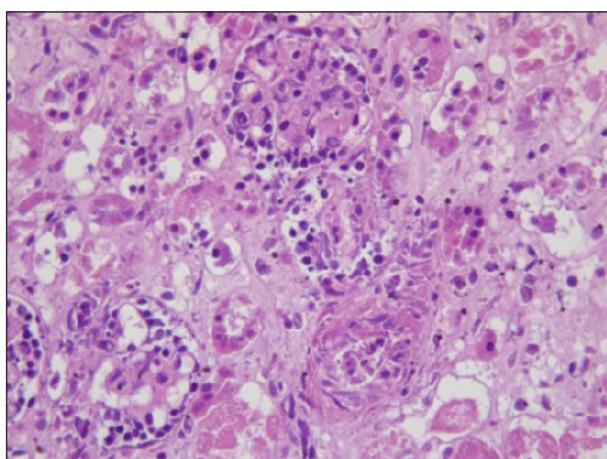


Figure 2.

developed glomeruli. The finding of massive podocyte apoptosis, suggests that ibuprofen may potentially play a role in podocytopathies. The alteration of number and function of nephrons likelihood affects not only the early stages of life but influence the renal susceptibility to disease in childhood and adulthood.

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## ABS 13

### THYMOSIN BETA-4 TRANSLOCATION FROM THE TRANS-GOLGI NETWORK TO THE NUCLEUS IN KIDNEY PROXIMAL TUBULE CELL LINE LLC-PK1 UNDER STARVATION

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## BACKGROUND

Thymosin beta-4 (T $\beta$ 4) is a multifunctional peptide involved in cytoskeletal organization, being considered the most important G-actin sequestering factor in mammalian cells. Recently, our group evidenced T $\beta$ 4 expression in the developing genitourinary tract and, in particular, in the developing newborn kidney [1].

## DESIGN

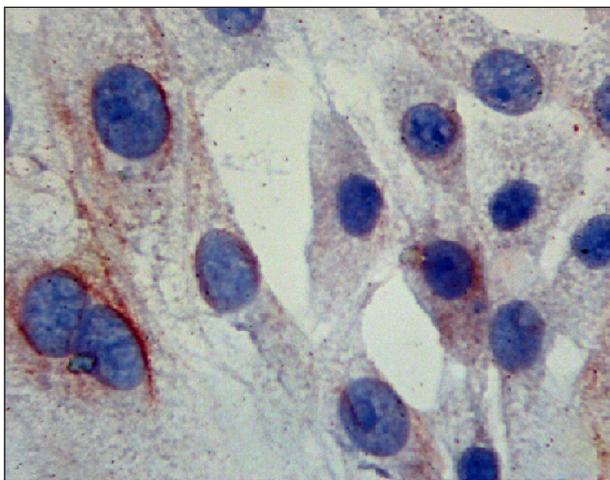
This study was aimed at verifying the T $\beta$ 4 expression pattern in a kidney proximal cell line (LLC-PK1), derived from a newborn piglet. T $\beta$ 4 immunoreactivity was analyzed, by a commercial antibody, in culture cells in normal conditions and under starvation, following 72 hours of serum deprivation.

## RESULTS

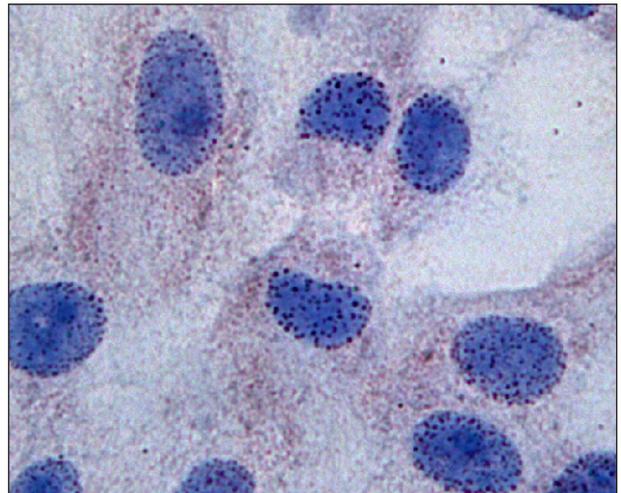
T $\beta$ 4 expression in normal conditions was restricted to the cytoplasm of culture cells, being mainly detected in perinuclear areas, a finding suggestive for its localization in the Golgi apparatus (**Fig 1**). After serum deprivation, we observed a redistribution of T $\beta$ 4 in the whole cytoplasm, paralleled by the translocation of the peptide into the nuclear envelope (**Fig 2**).

## CONCLUSIONS

Our preliminary data show the ability of T $\beta$ 4 to change its localization in proximal tubule cells, according with different cell conditions. The redistribution of the peptide in the cytoplasm and its migration into the nucleus clearly indicates that, under stress caused by serum deprivation, T $\beta$ 4 plays a role in a possible



**Figure 1.**



**Figure 2.**

cellular anti-stress response. Further studies at molecular level are needed, in order to better clarify the intimate nature of this finding.

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## ABS 14

### hCTR1 EXPRESSION IN THE DEVELOPING KIDNEY: HOW COPPER IS INVOLVED IN HUMAN NEPHROGENESIS

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## BACKGROUND

hCTR1 is a high affinity membrane copper permease that mediates the physiological uptake of copper ions. Given the role of copper in organogenesis, hCTR1 is considered an essential protein for early embryonic development.

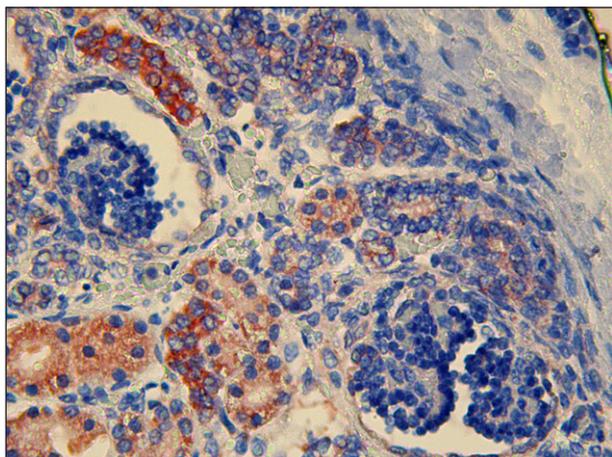
## DESIGN

Since few data are available regarding molecules involved in copper uptake in the developing human kidney, this study was aimed at verifying, by immunocytochemistry, hCTR1 expression in the kidney of fetuses and newborns, ranging from 15 up to 38 weeks of gestation.

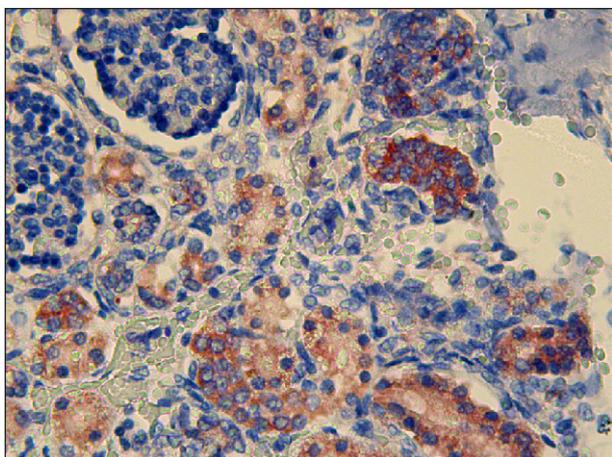
## RESULTS

Immunostaining for hCTR1 was detected in 20 out of 23 kidney examined. In the positive

cases, hCTR1 was mainly expressed in the cap mesenchymal cells localized in the nephrogenic zone under the kidney capsule (**Fig. 1**). Renal vesicles, comma- and S-shaped bodies also expressed the copper transport protein. Developing and mature glomeruli were constantly negative. hCTR1 was also found in proximal and distal tubules (**Fig. 2**).



**Figure 1.**



**Figure 2.**

#### CONCLUSIONS

Our preliminary data evidence a strong expression of hCTR1 in the developing human kidney. Immunoreactivity for this protein in cells undergoing mesenchymal-epithelial transition suggests a role for copper and for its transporter hCTR1 in the early phases of nephrogenesis. hCTR1 expression in proximal and distal tubules indicates the relevance of copper in the differentiation and function of renal tubular cells.

#### ABS 15

#### ULTRASTRUCTURAL ANALYSIS OF THE EARLY STAGES THAT CHARACTERIZE CAP MESENCHYMAL INDUCTION AND URETERIC BUD GROWING IN THE DEVELOPING NOD MOUSE KIDNEY

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#### BACKGROUND

The early events that in mouse and in several other animal species lead to the development of the adult kidney, are mainly induced by specific signals of the ureteric bud towards the surrounding metanephric mesenchyme. The aim of the present study was to investigate by means of electron microscopy the morphological events that characterize the early stages of the mesenchymal-to-epithelial transition of cap mesenchymal cells, analyzing the relationship between cap mesenchymal induction and ureteric bud growing.

#### DESIGN

Normal kidneys of newborn NOD mice were fixed in a mixture of 3% formaldehyde and 0.1% glutaraldehyde in 0.1 M cacodylate buffer and processed for standard electron microscopic techniques. The samples were dehydrated in a cold graded methanol series and embedded in LR gold resin. Ultrathin sections were cut with diamond knife, collected on formvar-coated 100-mesh grids, stained with uranyl acetate and bismuth subnitrate, observed and photographed in a JEOL 100S transmission electron microscope (TEM).

#### RESULTS

Evident nephrogenesis was observed in the outer portion of the renal cortex and resulted characterized by the interaction of two primordial derivatives: the metanephric mesenchyme and the ureteric buds. Mesenchymal cells were found to condense around the tips of the growing ureteric buds (UB), giving rise to specific cellular solid aggregates: the cap mesenchymal aggregates (CMA) (**Fig. 1**). At the ultrastructural level, the cellular constituents of the cap mesenchymal aggregates exhibited peculiar morphological features. In general they were characterized by a small cell

body with a scarce cytoplasm containing few cellular organelles, whereas the nucleus was usually large (Fig. 1-2).

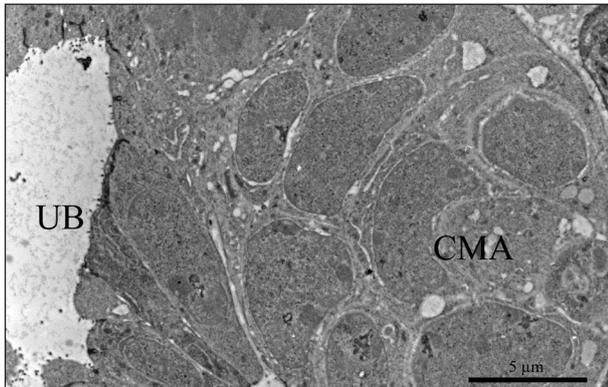


Figure 1.

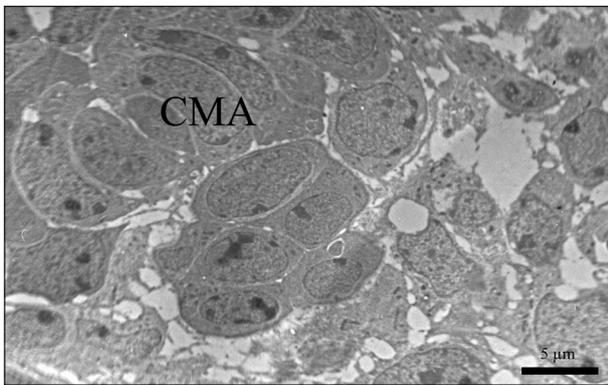


Figure 2.

## CONCLUSIONS

Cap mesenchymal induction is initiated by the growing ureteric buds, determining the differentiation and proliferation process of the mesenchymal cells that start to condense around the tips of the ureteric buds. In turn, cap mesenchymal cells seem to stimulate the growth and the branching of the ureteric bud. Although important questions concerning the factors that regulate the reciprocal induction process between ureteric buds and metanephric mesenchyme remain to be ascertained, the present study provides further evidences of the complex sequence of morphogenetic events that trigger the epithelialization of the metanephric mesenchyme.

## ABS 16

### THE SYNDROME OF THE EMILATUS

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## INTRODUCTION

We describe the “syndrome of the Emilatus”. This is probably a new syndrome characterized, since birth, by hypotrophy of the emisoma, left facial asymmetry, left micro enophthalmus, micrognathia, hypoplasia of the ipsilateral ear and of the left brain, dorsal-lumbar scoliosis right-convex, reduction in length of the lower left limb with curvature of the tibia and left foot launch supinated, reduction in length of the upper left, micropenis.

## CASE REPORT

Born at term (uneventful pregnancy), elective cesarean section, from non consanguineous parents and apparently healthy. Family history completely negative for malformation syndromes. Evident at birth marked hypotrophy of the left emisoma. The stages of psychomotor development are moderately slow.

The clinical picture, which was investigated by instrumental, laboratory and cytogenetic analysis, looking for microdeletions and microduplications, probably has a source post-zygotic. The question can regard a possible alteration of gene regulation mechanisms that are known to be able to explain the various forms of asymmetry. The genetic study using array CGH revealed a de novo deletion, not present in the parents, on the long arm of chromosome 12 (12p13.33 microdeletion). A further deletion is, however, also present in the father, the 4q13.3, but we don't believe that this has a pathological significance. Any way, we don't know specific genes in this area associated with the clinical picture of our little patient, but certainly the deletion is correlated with the framework malformation.

The tests performed showed a shortening of the left lower and in particular the measurement of the lower limbs, carried out by excluding the epiphyses, are: right femur 26.2 cm, left femur 25.5 cm, right tibia 21 cm, left tibia 18.4 cm. The radiogram showed a lower limb length discrepancy of 43 mm. We also evaluated the difference in length of the upper limbs and precisely: right humerus 19.2 cm, left humerus 18.6 cm, right radio 14.1 cm, left radio 13.6 cm, right ulna 14.9 cm, left ulna 14.5 cm. There is also a dorsal-lumbar scoliosis right-convex with hypoplasia of the left side of the corresponding vertebral bodies. MRI brain showed asymmetry of the volume of the splanchnocranium, hypoplasia of the left cerebral hemisphere and microphthalmia, small areas of altered signal probably gliosis or

malacic areas, in the context of the deep white matter of the semi-oval center and of the corona radiata, within the peritrigonal area.

The ophthalmologist found high myopia (11) to the right side and microphthalmos to the left eye. Audiometric assessment showed normoacusia.

#### DISCUSSION

This case had come to our attention as a possible example of atypical Goldenhar Syndrome. The grading in the facio-auriculo-vertebral spectrum appears unlikely because the more overt forms of expressiveness and involvement of the limbs determine primitively radial segment lesions, which are apparently absent in this baby. The question can involve a possible alteration of gene regulation mechanisms that are known to be able to explain various forms of asymmetry. One of the most significant aspect is the information to parents for a future pregnancy: all these forms of asymmetry, arising from clinically normal parents, are sparse.

Taking charge of this baby is essentially symptomatic: psychomotricity, periodic eye examinations and a follow-up for orthopedic treatment of the asymmetry.

#### ABS 17

### BIRTH IN ITALY: 2011 TRENDS AND 2012 FORECASTS

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#### OBJECT OF THE STUDY

To assess the state of birth in 2011 and to estimate the number of newborn babies in Italy in 2012.

#### MATERIAL AND METHODS

The study was based on provisional data of the 2011 ISTAT (National Statistical Institute).

#### RESULTS

In 2011, 546,606 children were born, 15,338 less than in 2010. A decreasing trend continues from 2008, when 576,659 were born.

September and October are classically the months with the highest number of births. But this trend has changed in 2011: in the North and in Sardinia the month of birth was highest in August, in the south and in Sicily in September, while in the center the record was recorded in November.

This probably means that in the North, where in the past the economy was strong, the crisis has been felt before: in winter, the population began to spend more time at home, and this has led to a slight advance of conceptions.

In the South, where the crisis was always present, the highest value of birth comes into play photoperiod and light effect that favors the conception in a certain period of the year, while the figure for the center is a bit more difficult to explain, and is primarily linked to the large number of babies born in Rome.

The month in which babies are born less for 2011 was April. This confirms that July and August are negative for conceptions, because with the summer solstice, the days are getting shorter, but mainly because such high temperatures damage sperm.

#### CONCLUSION

From September to December 2011 have been 'lost' almost 11,000 neonates in comparison with the previous year: the crisis has been felt heavily in the early months of 2011 and influenced the choice to conceive a child.

For 2012 a further loss of 25,000 neonates is expected (520,000).

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#### ABS 18

### NEONATAL TRANSPORT IN ITALIAN REGIONS

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#### OBJECT OF THE STUDY

To estimate the number of newborn babies who were assisted with different types of TNE (neonatal emergency transport); to evaluate the actual (2010) utility and availability of the service.

#### MATERIAL AND METHODS

On the basis of the regional TNE allocated and the annual number of newborn babies in each area (results provided by ISTAT 2008 – National Statistical Institute) we have estimated the number and percentage of newborn babies assisted with various types of TNE.

## RESULTS

Results obtained are presented in **Tab. 1-5**.

## CONCLUSION

Only 62.8% of newborn babies can be transported in a safety way (see **Tab. 1, 2**)

**Table 1.** Neonatal transport provided and arranged with doctors and nurses available in shifts for the transport.

Liguria	12,450	
Lazio	56,755	
Campania	60,742	
TOTAL	129,947	22.5%

**Table 2.** Neonatal emergency transport service provided just in case of need: doctors must arrive within 20 minutes.

Piedmont	39,551	
Aosta Valley	1,296	
Lombardy	98,672	
Trentino-Südtirol	10,885	
Veneto	48,615	
Friuli Venezia Giulia	10,501	
Umbria	8,271	
The Marche	14,637	
TOTAL	232,428	40.3%

**Table 3.** Regions where a neonatal transport system exists in part.

Tuscany	33,610	
Apulia	38,284	
Sicily	49,837	
TOTAL	121,731	21.16%

**Table 4.** Regions where a neonatal transport system does not exist.

Abruzzo	11,743	
Basilicata	4,923	
Calabria	17,995	
Emilia Romagna	41,915	
Molise	2,507	
Sardinia	13,470	
TOTAL	92,553	16.04%

**Table 5.** Those born in Italy in 2008.

TOTAL	576,659	100%
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## ABS 19

## GRANDPARENTS IN EIGHT PROVINCES IN SARDINIA

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## AIM

To evaluate the amount of money saved by each Sardinian family determined by grandparents' role.

## METHODS

Data processing using ISTAT (National Statistical Institute).

## RESULTS

In Sardinia, there are 315,268 grandparents (see **Tab. 1**). Actually, 22.3% (58,140 grandparents) look after their grandchildren while the parents are working and 30% (71,487 grandparents) while the parents have occasional engagements: grandparents; 13.2% (31,455 grandparents) when parents want some time to themselves; 10.2% (24,305 grandparents) when grandchildren are on holiday and 9.4% (22,400 grandparents) when their grandchildren are ill.

**Table 1.** Sardinia: estimated number of grandparents per province.

Province	Estimated no. of grandparents	Amount saved per family
Sassari	63,312	193,101,600
Nuoro	30,244	92,244,200
Cagliari*	105,285*	321,119,250
Oristano	34,462	105,109,100
Olbia-Tempio	28,191	85,982,550
Ogliastra	10,840	33,062,000
Medio Campidano	19,612	59,816,600
Carbonia-Iglesias	25,313	77,204,650
<b>Total in Sardinia</b>	<b>317,259</b>	<b>970,587,150</b>

\* Town of Cagliari: 32,471.

## CONCLUSION

A grandparent's role plays a significant part in household savings when the children are younger, especially up to three years of age.

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## ABS 20

## IBUPROFEN VERSUS INDOMETHACIN FOR PATENT DUCTUS ARTERIOSUS: PRACTICE

## ATTITUDE VARIATIONS AMONG EUROPE AND UNITED STATES

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### INTRODUCTION

Patent Ductus Arteriosus is very common in Very Low Birth Weight Infants affecting approximately 65% of the newborns < 28 weeks and up to 80% of the premature babies weighing < 800 g. Two main Non-Steroidal Drugs have been used in the treatment of PDA, indomethacin (INDO) and ibuprofen (IBU). The first clinical trials comparing IBU with INDO for PDA were small and only in the recent decades the studies have become more relevant.

### OBJECTIVE

The purpose of our study is to compare the efficacy and safety of IBU versus INDO in inducing closure of the PDA in preterm and to determine possible practice attitude variations among Europe and US in the use of NSAIDs for PDA.

### RESULTS

In the European group of trials (**Tab. 1**) we identified 8 main studies: 3 single centre randomized controlled, 2 multicenter randomized controlled, 1 single centre retrospective study, 1 prospective randomized controlled study and 1 single centre randomized controlled study with a total of 572 premature babies enrolled. The gestational age of the participants varied between 24-34 wk. Ductus closure was seen in 66 to 100% of the preterm babies belonging to the INDO group and varied between 62 to 82.6% in the newborn of the IBU group. The adverse effects (oliguria, increase in serum creatinine, reduction of cerebral and mesenteric blood flow) were more significant in the INDO group in all trials. None study reported the use of INDO prophylaxis. In case of failure of the first or reopening PDA most of the NICUs repeated a second course with the same drug or, only in one NICU, with INDO. In 5 of the 8 trials resistant PDA was legated.

Data from the US group were taken by 4 retrospective cohort studies (**Tab. 2**). Most of the participants were very low birth weight and had low gestational age (< 1,000 g, < 27 wk) with a total of 869 premature babies enrolled. Successful closure rate with INDO varied from 62 to 68% and was seen in the 58 to 71% of the participants of the IBU group. In one of the 4 trials INDO prophylaxis was

**Table 1.** IBU vs INDO Main studies published in Europe.

Authors and Study	Ibuprofen	Indomethacin	Results
Mosca et al., 1997, Italy Single centre randomized controlled study. 16 preterm, GA < 31 wk and MV. E	i.v 10 mg/kg and 5 mg/kg after 24-48 h	i.v 0.2 mg/kg every 24 hours	Ductus closure in 75% after the first dose of Indo and in 62.5% after the first dose of Ibu
Pezzati et al., 1999, Italy Single centre randomized controlled study. 17 preterm, GA < 33 wk with MV. E	i.v 10 mg/kg and 5 mg/kg after 24-48 h	i.v 0.2 mg/kg and 0.1 mg/kg after 24-48 h	Closure PDA Indo group 100% and in Ibu group 77%
Van Overmeire et al., 2000, Belgium Multicenter randomized controlled study. 148 infants, GA 24-32 wk with RDS. E	i.v 10 mg/kg and 5 mg/kg after 24-48 h	i.v 0.2 mg/kg every 12 hours	Closure PDA Indo group 66% and in Ibu group 70%
Patel et al., 2000, GB Prospective randomized controlled trial. 33 infants, GA 23-35 wk. E + C	i.v 10 mg/kg and 5 mg/kg after 24-48 h	i.v 0.2- 0.25 mg/kg every 12 hours	Closure PDA Indo group 93% and Ibu group 78%
Lago et al., 2002, Italy Multicenter randomized controlled study. 175 preterm, GA < 34 wk or postnatal age 48-72 hours, RDS treated with MV. E	i.v 10 mg/kg and 5 mg/kg after 24-48 h	i.v 0.2 mg/kg every 12 hours	Closure PDA 69% in Indo group and 73% in Ibu group
Fanos et al., 2004, Italy Single centre retrospective study. 90 preterm GA < 30 wk, < 1,300 g. E	Lysine i.v 10 mg/kg and 5 mg/kg after 24-48 h	i.v 0.2 mg/kg every 12 hours	Closure PDA Indo group 75% and 80% Ibu group
Gimeno-Navarro et al., 2005, Spain Single centre randomized controlled study. 47 preterm, GA < 34 wk with MV. E	i.v 10 mg/kg and 5 mg/kg after 24-48 h	i.v 0.2 mg/kg every 12 hours	Closure PDA 87.5% in the Indo group and 82.6% in the Ibu group
Zanardo et al., 2005, Italy Single centre randomized trial. 46 preterm GA < 34 wk, RDS and MV. E	Lysine 10 mg/kg i.v followed by 2 doses of 5 mg/kg each after 24 and 48 h	3 doses i.v of 0.2 mg/kg at 12 h intervals	Closure of the PDA Indo group 87.5% and Ibu 72%

E: diagnosis (echocardiography), C: diagnosis (clinical).

adopted in the 32% of INDO group and 42% of IBU group in another study indo prophylaxis was used in premature babies < 750 g or < 27 wk. In 1 of the 4 studies the greater increase of the creatinine and decrease in urine output happened in INDO group, death, medical and surgical necrotizing enterocolitis, or spontaneous intestinal perforation was 40% in the INDO group and 17% in the IBU group, without any differences in serum creatinine between the groups. In one of the remaining 2 studies

**Table 2.** IBU vs INDO Main studies published in USA.

Authors and Study	Ibuprofen	Indomethacin	Results
L Katakam et al., 2010, US Retrospective cohort study. 122 infants < 750 g or GA < 26 wk *Indo Prophylaxis in 32% of Indo group and 42% in Ibu group. C	3 doses of 10 mg/kg, 5 mg/kg, and 5 mg/kg at 24 hour intervals	3 doses every 12 h: < 48 h 0.2 mg/kg, 0.1 mg/kg and 0.1 mg/kg; 2-7 days 0.2 mg/kg; and > 7 days 0.2 mg/kg, 0.25 mg/kg, and 0.25 mg/kg	Closure of the PDA 62% in Indo group and 58% in Ibu group.
Kushnir, Pinheiro, 2011, Albany – US Retrospective cohort study. 350 inborn or outborn preterm neonates born at any gestation and birth weight. *No prophylaxis Indo. E + C	185 pts (2007-2008) 10 mg/kg then 5 mg/kg daily for 2 doses	165 pts (2005-2006) 0.2 mg/kg then 0.1 mg/kg IV daily for 2 doses < 750 g; 0.2 mg/kg daily for 3 doses 750-1,000 g; 0.2 mg/kg every 12 h > 1,000 g	Closure PDA 68% in Indo group and 71% in Ibu group
Ethington P. et al., US, 2011 Retrospective cohort study. 317 preterm, median GA 26 wk and median 850 g. *Prophylactic Indo (0.1 mg/kg/day x 3 doses) used < 750 g or GA < 27wk. E	(Dec 2006-Oct 2007) Lysine 10 mg/kg then 5 mg/kg daily for 2 doses	(Jan 2002-Nov 2006) < 48h 0.2 mg/kg then 0.1 mg/kg for 2 doses. 2-7days 0.2 mg/kg for 3 doses. > 7 days 0.25 mg/kg for 3 doses 12 h intervals	Successful medical closure similar regardless of direction of flow across the PDA (bidirectional 68% vs left to right 63%)
Adrouche-Amrani et al., 2012, US Retrospective cohort study. 80 infants < 1,000 g. *No prophylaxis Indo. E	i.v 10 mg/kg and 5 mg/kg after 24-48 h	0.2 mg/kg every 12 h for 3 doses	32.5% infants closed PDA after 1 <sup>st</sup> course, 20% after 2 <sup>nd</sup> course, 7% after 3 <sup>rd</sup> course

E: diagnosis (echocardio), C: diagnosis (clinical).

the incidence of medical and surgical NEC and SIP were similar in both study groups. Persistent PDA was treated with a second course of the same drug in 2 of the 4 trials followed by surgical ligation in case of resistant PDA. In one study, surgical ligation was performed after the first course failure and in the last study other 2 courses were performed when ligation could not be done expeditiously due to the scheduling issues.

#### CONCLUSIONS

We didn't observe significant difference in closure rates between INDO and IBU groups in European and American studies, respectively. Absolute closure rates are lower in USA, probably because of differences in GA, birth weight, timing of treatment and genetic differences (?). INDO is more nephrotoxic than IBU. In case of treatment failure there is wide spectrum of interventions. In USA is more frequent surgical ligation. It is not always reported in the studies, especially in Europe,

the use of prophylaxis with INDO. Further studies are needed to optimize PDA management.

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#### ABS 21

### FAST RECOVERY FOLLOWING SEVERE ACETAMINOPHEN-INDUCED LIVER DISEASE IN A NEWBORN: A CASE REPORT

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#### BACKGROUND

Acetaminophen is one of the most common causes of drug-induced hepatic toxicity in children. It has been reported to be the most common cause of acute liver failure in children, accounting for 15% of all drug-induced acute hepatitis.

Patients and methods. Here we report a case of acetaminophen-related acute hepatitis in a newborn, with some peculiar features, mainly related to the ability of the young patient to recover from a severe acute liver disease.

#### RESULTS

A 1 month old baby presented at our Paediatric Unit with a story of 3 days of fever treated for ten days with high doses of acetaminophen (125 mg/3 times a day) and antibiotic therapy. Laboratory tests showed important changes in coagulation in particular a growth of PT INR and PTT and a decrease of fibrinogen. Hepatic functionality was compromised with a rapid increase of cytotoxic indexes (ALT: 2,600 U/L; N.V. < 40 U/L). Viral markers, metabolic indexes and urinary organic acid were constantly negative. At 24 hours after the acetaminophen suspension, a suddenly decrease of serum levels of transaminases along with an increase of hepatic functionality

tests was observed. After 72 hours, the hepatic functionality and the parameters of coagulation were in the normal range.

#### CONCLUSIONS

The case here reported shows some peculiar features, that deserve some consideration. It is one of the rare cases of severe acute liver necrosis caused by acetaminophen reported in a newborn, being acetaminophen-related liver toxicity relatively rare in the perinatal period. In spite of this dramatic clinical picture, discontinuation of acetaminophen resulted in an extremely fast recovery, ALT levels decreasing toward normal values within 3 days from the diagnosis. These data confirm the hypothesis that acetaminophen metabolism is different in newborns as compared to adult subjects, and reinforce the hypothesis that the newborn liver has the ability to undergo a fast recovery after a massive liver cell necrosis.

#### ABS 22

##### IMMUNOREACTIVITY FOR S100B: A NEW MARKER OF HYPOXIA-RELATED CARDIAC DAMAGE IN NEWBORN PIGLETS

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#### AIM OF THE STUDY

This study was aimed at evaluating the expression of S100B protein in the heart of an experimental animal model of neonatal hypoxia and reoxygenation.

#### METHODS

To this end, normocapnic hypoxia was induced in 40 male Landrace/Large White neonatal piglets by decreasing the inspired oxygen to 6-8%. When animals developed bradycardia or severe hypotension, reoxygenation was initiated. Piglets were allocated in four groups of 10, according to the oxygen concentration they were reoxygenated with: group 1, 2, 3, and 4, received 18%, 21%, 40% and 100% oxygen, respectively. The animals were further classified into 4 groups according with the time required for reoxygenation: group A (< 15 min); group B (16-60 min); group C (> 60 min); group D (deceased animals).

#### RESULTS

Immunostaining for S100B protein was detected in 14 out of 40 heart samples (35%), both inside the cytoplasm of cardiomyocytes and as globular deposits in the interstitial spaces. Significant differences were observed among groups 1-4 regarding S100B expression. Reactivity for S100B in cardiac cells was detected in 50% of group 1 and 2 animals, in 10% of group 3 and in 30% of group 4 piglets. Marked differences were also observed among groups A-D: the percentage of newborn piglets showing reactivity for S100B in the heart was 75% in group A, 33% in group B, 12% in group C and 22% in group D.

#### CONCLUSIONS

Our study shows that S100B protein storage occurs in the heart of a percentage of newborn piglets following severe hypoxia. S100B storage in cardiomyocytes correlates with the different oxygen therapeutic regimen utilized during reoxygenation, being higher in piglets reoxygenated with room air (18 and 21%), and lower in animals reoxygenated with 40% oxygen. Intermediate levels of S100B expression were found in 100% O<sub>2</sub> treated animals. The finding of a higher percentage of S100-immunoreactive hearts in piglets with a fast recovery and the detection of a decreased reactivity in animals with a slow and a very slow recovery clearly indicate S100B protein as an early protective factor with a positive prognostic value in asphyxiated newborn piglets.

#### ABS 23

##### COPING AND PARENTAL ROLE COMPETENCE OF MOTHERS OF THE PRETERM INFANT

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#### BACKGROUND

The study explores possible significant statistical differences about coping strategies and self-perceived parental competence, in mothers of preterm children, in reference to the level of gravity of preterm birth.

#### METHODS

The study involved a group of 16 mothers of moderately preterm children (weeks' gestational

age:  $M = 32$ ,  $SD = 2.5$  and birth weight:  $M = 2,000$  g,  $SD = 200$  g) and a group of 14 mothers of severely preterm children (weeks' gestational age:  $M = 29$ ,  $SD = 2$  and birth weight:  $M = 1,700$  g,  $SD = 350$  g). The following instruments were used to investigate focus areas of research: Coping Orientation to the Problems Experienced-New Italian Version, to analyse coping strategies of mothers, and a Q-sort, a self report on maternal competence.

#### RESULTS

The data were analyzed using descriptive and nonparametric statistics. The Mann-Whitney test was used to calculate any differences between the two groups about the coping strategies and the maternal self-perceived competence; the Spearman's rank correlation coefficient was used to detect any possible relationship between coping strategies and maternal self-perceived competence in each group.

No significant differences emerged between the two groups of mothers, both in regard to considered coping strategies (social support, avoidance, problem focused orientation, transcendent orientation, positive aptitude), and the indicators of maternal self-perceived competence (coping, scaffolding, caregiving).

#### CONCLUSIONS

This study highlighting the lack of differences between the two groups of mothers involved, seems to point out that, beyond the levels of prematurity, the condition of preterm birth itself is precisely the main stressor factor for mothers.

#### ABS 24

### REHABILITATIVE TRAINING OF PRETERM CHILDREN'S ATTENTION: A STUDY ON SUSTAINABILITY

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#### BACKGROUND

The study presents a rehabilitative training aimed at severely and moderately preterm children at preschool age, who display impairments of processes of selective attention, self-control and problem solving and, then, who are at risk of Attention Deficit Hyperactivity Disorders. The treatment has planned the involvement of parents, teachers, neonatologists and children's reference paediatricians. The study aimed at investigating the sustainability of the

training path, in terms of impact and transformative valence of the focalised processes.

#### METHODS

The study has been involved a group of 55 healthy preterm children (35 moderately preterm children and 20 severely preterm children) at mean age of 5.2 attending the third year of infancy school; a group of 55 mothers; a group of 15 paediatricians; a group of 5 neonatologists and one of 10 teachers. Specific questionnaires (IPDAG, IPDDAI) have been administered to parents and teachers, before and after the training sessions. Also, the trainer has used techniques of narrative (the critical incident technique) and descriptive (encoding scheme) observation. A check-list to detect the participation of adults has been used. A telephone follow-up has been performed to detect the involved adults' considerations about the stability of promoted changes.

#### RESULTS

The data were analyzed using descriptive statistics (analysis of the visual trend of change) and a repeated measures analysis of variance (ANOVA) to assess the statistically significant differences in the attention and self-regulation performances of preterm children, before and after the rehabilitative training.

The data show a statistically significant decrease in both the processes of inattention and self-regulation of hyperactivity and impulsivity.

#### CONCLUSIONS

Data highlight show good levels of sustainability of the proposed training.

#### ABS 25

### INTERINDIVIDUAL VARIABILITY IN OOCYTE BURDEN AT BIRTH

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#### BACKGROUND

The development of human ovary is a complex and in part unknown process which requires interactions between multiple cell components, eventually

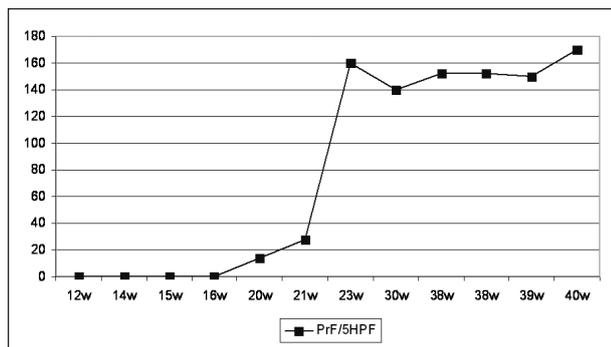
leading to the coordinate development of oocytes, endothelial and stromal cell types within the ovarian architectural complexity. Since few histological and immunohistochemical studies are available on the newborn ovary, particularly in preterm infants, this study was aimed at evaluating ovary differentiation in human fetuses and newborns ranging from 12 up to 40 weeks of gestation.

#### DESIGN

12 ovaries, obtained for legal interruption of pregnancy or at autopsy, were formalin-fixed and paraffin-embedded. Paraffin sections were stained with H&E. For each organ the mitotic index (no. of mitotic figures per 5 high power fields) and the apoptotic index (no. of apoptotic globules per 5 high power fields) were evaluated. Moreover the number of primordial follicles (oocytes surrounded by a single layer of flat pregranulosa cells) per 5 high power fields was obtained.

#### RESULTS

The follicle count evidenced a peak in follicle development around the 23<sup>rd</sup> week of gestation (see **Fig. 1**). As expected, the number of mitotic figures

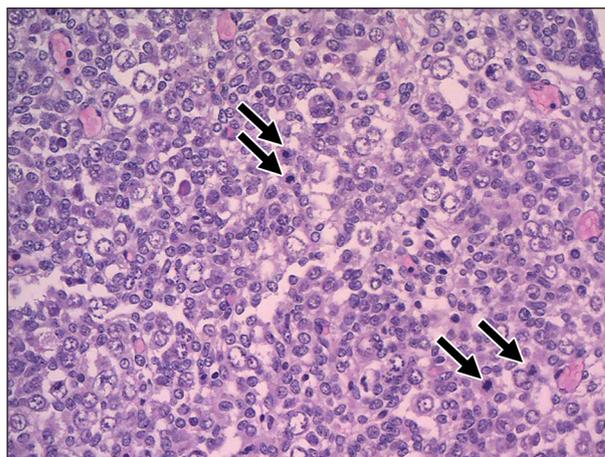


**Figure 1.** Number of primordial follicles (oocytes surrounded by a single layer of flat pregranulosa cells) per 5 HPF.

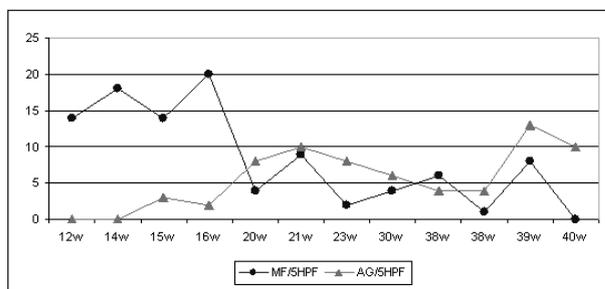
was higher in the earlier weeks of gestation up to 16<sup>th</sup> week (ranging from 14 to 20 mitotic figures/5HPF) corresponding to the great proliferative potential of primordial germ cells and oogonia (see **Fig. 2**). On the contrary, apoptotic rate was very low in the same period. After week 20 of gestation, as differentiation of primordial follicles begins, mitotic and apoptotic count trends cross and overlap accounting for interindividual variability of the number of follicles (see **Fig. 3**).

#### CONCLUSIONS

Our data show that ovary differentiation and in particular oocyte burden is not strictly related to gestational age of fetuses and newborns. We



**Figure 2.** Ovary at 14 weeks gestation: immature oogonia with high mitotic activity (mitotic figures, arrows). H&E, 20X.



**Figure 3.** Number of mitotic figures (MF) and apoptotic globules (AG) per 5 HPF.

hypothesize that, given a similar follicle count, a higher mitotic or apoptotic rate could possibly reflect a trend for following development of oocytes until birth. Fetal environment regulates ovarian development and depends on multiple maternal, nutritional, genetic and molecular variables. Interactions of these complex pathways might determine quantity and quality of oocytes at birth and eventually predict adult life fertility. Further studies are needed in order to better understand these mechanisms that may be at the basis of female infertility.

#### ABS 26

#### UNDIFFERENTIATED NEUROBLASTOMA WITH DISTANT METASTASES AT BIRTH: A CASE REPORT

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## BACKGROUND

Neuroblastoma is the most common malignant tumor of the newborn, representing one-third of all malignancies diagnosed in the neonatal period, 8% being described within the first month of life. Congenital neuroblastoma is mainly localized in the adrenal or extra-adrenal sympathetic structures. It has a very different behaviour as compared to neuroblastoma encountered in older infants and children. Even though fifty-percent of perinatal cases show distant metastases at presentation, they have the capacity to regress spontaneously or to differentiate completely and, in general, carry a good prognosis. Here we report a case of congenital neuroblastoma with an aggressive outcome in a full-term newborn.

## DESIGN

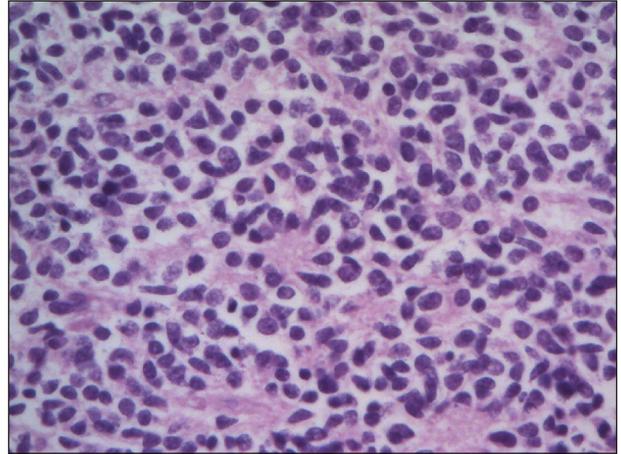
Clinical manifestation at birth was characterized by a hypothalamic deficit, an insufficient respiratory and cardiovascular activity, and a decline of renal and hepatic function. CT scan was performed, revealing a suggestive neuroimaging pattern of paravertebral neuroblastoma. The clinical course was rapidly worsening with a 12 days survival time. Results: At autopsy we observed an extensive paravertebral mass, 5 cm in size, in the thoracic cavity with mediastinic dislocation and without any involvement of the spinal cord. At macroscopic examination the tumor was multilobulated, with a soft consistence and a gray-pink colour and it showed, at cut, a nodular appearance with areas of hemorrhage, calcification and intratumoral necrosis. The liver was increased in size, with multiple white micronodules on the surface. At histological examination the tumor was composed of sheets of small to medium-sized cells with a scant cytoplasm and indistinct cytoplasmic borders. Nuclei were round to ovoid with coarsely granular (salt and pepper) chromatin and indistinct nucleoli (**Fig. 1**). There were thin fibrovascular septa between groups of tumor cells and undifferentiated neuroblasts, in the absence of neuropil.

At immunohistochemistry, tumor cells showed the following phenotype: NSE + (**Fig. 2**), Synaptophysin +, S100 -, Chromogranin A -, Vimentin -, EMA -, Nestin -, Ki67 > 20%.

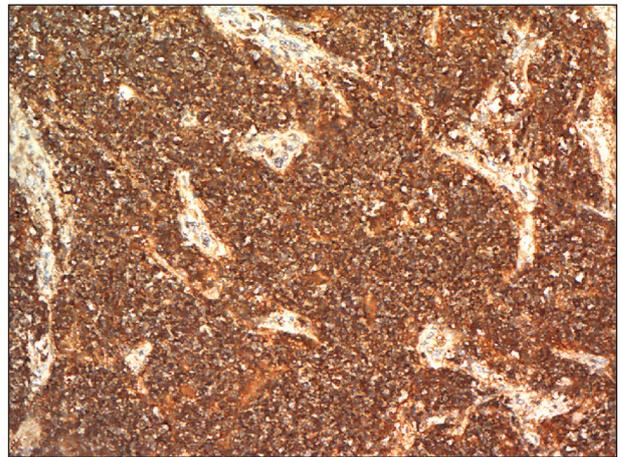
Metastases were found in liver, pancreas, lungs, adrenal glands and bone marrow.

## CONCLUSIONS

Histological and immunoistochemical findings fulfill the diagnosis of undifferentiate neuroblastoma. The negativity for nestin immunoreactivity in tumor cells, paralleled by immunoreactivity for NSE and



**Figure 1.** The tumor is composed of sheets of small to medium-sized cells with a scant cytoplasm and indistinct cytoplasmic borders. Nuclei are round to ovoid with coarsely granular (salt and pepper) chromatin and indistinct nucleoli. H&E, 40X.



**Figure 2.** Tumor cells show a strong immunoreactivity for NSE. IHC, 10X.

Synaptophysin, suggests the absence of neuropil and of maturing neuroblasts, leading to the diagnosis of an undifferentiated form of this neoplasm.

Further studies are needed to better understand the correlation between the undifferentiated form of neuroblastoma and a clinical aggressiveness of this tumor.

## ABS 27

### CHLORAMPHENICOL (ChIA) TOXICITY IN THE NEWBORN INFANT: HISTORICAL PERSPECTIVES

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## BACKGROUND

In the '40s of the last century chloramphenicol (Chl) was originally described by Erlich et al.; it was called chloromycetin. Usually this drug was orally administered, rapidly absorbed from the gi-tract, thus needing frequent administration. Chl has a fairly wide distribution throughout body tissue. It is mainly excreted by the kidney as a nitrocompound previously inactivated by the liver. This drug was widely used in the past in term and preterm newborn infants.

## GOAL

To remind to neonatologists the importance of the Chl toxicity used in the newborn infants, mainly in the preterm babies, as a suggestion for prevention of any drug's toxicity accident.

## METHODS

A research about "Chloramphenicol" and "Toxicity" on Pub Med and in a lot of books published in the second half of the XXth century was performed.

## RESULTS

In 1959 the grey baby syndrome was reported in association with the use of the antibiotic Chl.

Infants developed abdominal distension, vomiting, cyanosis, cardiovascular collapse, irregular respiration and subsequent death shortly after therapy with chloramphenicol was started. Pharmacokinetic studies in the neonate showed accumulation of chloramphenicol in plasma due to impaired drug metabolism. A reduction of the total daily dosage from 100 to 50 mg/kg prevented the development of the grey baby syndrome.

## CONCLUSION

Authors suggest a better understanding of neonatal physiology, and of developmental changes in drug disposition and metabolism, in order to obtain appropriate and more useful formulations for drug treatment in the neonatal period, mainly for safety.

## ABS 28

### BRONCHIOLITIS IN NEWBORNS

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Bronchiolitis is a common paediatric illness, especially under 1 year of age. This disease is typically benign, but in infants younger than 6 months or with underlying risk factors it could be associated also with death.

We considered all the children with bronchiolitis hospitalized between 18 November 2009 and 4 April 2011 in the Paediatric Department of Sant'Orsola-Malpighi, Bologna, to describe the proceedings done and to compare them to scientific evidence. Following guidelines, we excluded more fragile babies.

We found 14 children under 1 month of age in 168 hospitalizations (8.33%). There was male predominance (10 vs. 4; 71.43% vs. 28.57%). In Emergency Room (ER), 10 patients had respiratory distress (71.43%), 5 (35.71%) had oxygen saturation less than 95%. The code was yellow for 9 babies (64.29%) and green for others 5 (35.71%). Chest radiography was performed in ER to 10 babies (71.43%) and to 3 of them (21.43%) were given oxygen. C-reactive protein was positive in 3 patients (21.43%). During hospitalization, 6 babies underwent chest x-ray (42.86%). In the same context patients carried out: physiologic solution aerosol (9 babies, 64.29%), oxygen (8 patients, 57.14%), epinephrine aerosol (13 patients, 92.86%), beta-2 agonists aerosol (1 baby, 7.14%), oral corticosteroids (9 patients, 64.29%), antibiotics (7 patients, 50%), intravenous fluids (10 patients, 71.43%) and nasal washing (10 babies, 71.43%). The mean period of hospitalization was 6.43 days, ranging from 3 to 10 days.

Maybe fewer babies needed hospitalization and the colour code of triage in ER didn't reflect this necessity; only 8 patients have received oxygen therapy and we report 1 infant only treated with physiologic solution aerosol and nasal washing. There are many reasons to hospitalize newborns with bronchiolitis, but we have to consider also negative effects. All infants underwent at least 1 x-ray in hospital or after discharge from it; 4 (28.57%) babies performed 2 radiographies. We think there is an abuse of this diagnostic method, because bronchiolitis has clinical diagnosis.

We emphasize the need for proper hospitalizations for bronchiolitis-affected babies and conscious use of drugs in order to protect them. We remind also the careful use of x-ray.

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## ABS 29

### POSTPARTUM DEPRESSION IN A HIGH-RISK WOMAN AFTER STILLBIRTH

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## BACKGROUND

Postpartum depression (PPD) is a non-psychotic depressive disease with varying severity, from moderate to severe, which according to the DSM IV originates after the 4<sup>th</sup> to 6<sup>th</sup> week from giving birth. The most frequent symptoms of PPD are as follows: moodiness, excessive concern about the wellbeing of the neonate. Co-morbidity is frequent with serious anxiety and attacks of panic.

## CASE REPORT

R. is 31 years woman suffering for attacks of panic, under treatment with paroxetine (20 mg/day) and alprazolam (0.50 mg x 3/day). After various attempts to become pregnant R. makes a choice for an assisted fecundation. The pharmacologic treatment is progressively reduced to avoid drugs exposure in the first trimester of pregnancy. However psychotherapy alone is not enough and drugs are needed again after the first month of pregnancy. R. is informed of the potential toxicity of the treatment, including teratogenic effects and long term cognitive and behavioural long term alterations. After counseling the choice is paroxetine (20 mg/day), which presents no more malformative risks for fetuses when compared to controls: SSRI are considered the first choice in case of attacks of panic and depression during pregnancy. The drugs ensures some periods of well-being during the second and third trimester. However towards the end of gestation R. is very stressed and insists for anticipation of parturition with a planned caesarean section (CS). One week before the programmed day, R. insists again in the request of immediate CS, obtaining the positive answer by her obstetrician. However, the ultrasounds reveal a dead fetus. R. knows this situation and gives birth to a dead newborn. After delivery R. wants to hug her child for some minutes, takes some pictures and decides to personally

organize his funeral. Actually R. continues treatment with paroxetine and alprazolam.

## COMMENT

Loosing a child during gestation is peculiar event with specific psychodynamic implications, including deep wound of self-esteem and self-image. It is a mourning projected into the future (unmet wishes, hopes, fantasies). Many women find again a well-being after a new pregnancy with positive outcome. It is extremely important for physicians offering to the mother a space of Narrative Based Medicine of the experience, allowing a conscious elaboration. Again the empathic hearing is normally underestimated by perinatologists. Relatives and friends improperly believe that it is important to forget the event and to compensate with a new pregnancy. On the contrary, the mothers tends to collect things and material that favour the memory of the dead child. We described a typical case of severe post partum depression, anticipated by a pregress attacks of panic and complicated by a stillborn. In most of cases the history of the woman is more subtle and an active approach is mandatory to early diagnosis of post partum depression and to prevent its dramatic effects.

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## ABS 30

### NEONATAL BRAIN HYPOTHERMIA: THE CAGLIARI EXPERIENCE

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## BACKGROUND

The hypoxic ischemic encephalopathy it is one of the most common causes of cerebral palsy, its effect is 1.6% live births. In serious forms mortality can reach 60% and the neurological

sequelae present in 1 out of 4 of surviving children Hypothermia is currently the treatment of choice for moderate-severe encephalopathy, being capable of reducing the vasogenic edema, the release of excitatory neurotransmitters of free radicals of oxygen, the activation of cytokines and the brain metabolism.

#### AIM

To describe the clinical experience with hypothermia in the Cagliari NICU during the period July 2010-July 2012.

#### METHODS

The treatment was started when the asphyxiated born children presented the inclusion criteria proposed by the group of the Italian Society of Neonatology: Apgar score < 5 at 10 minutes of live, pH < 7 or EB > 16 or presence of neurological signs of suffering severe or moderate (Sarnat&Sarnat), or altered CFM. The target of therapy was a rectal temperature of 33.5 degrees for 72 hours, followed by slow heating (0.5/hour). In our NICU the used method is the selective hypothermia (Cool-Cap).

#### RESULTS

Eleven newborns were treated with hypothermia. Two of them died on the second day of life: they were born at term by emergency caesarean section, for severe fetal distress and placental abruption, respectively. At admission in the NICU the first presented hypotonia and seizures, the second convulsions and coma. Both had started treatment hypothermic before the sixth hour of life. The track CFM was severely impaired only in the second patient, so as the cerebral ultrasound, while in the first there was only diffuse cerebral oedema.

In the nine surviving children, all born at term of gestation except one with GA 36 wks, the mean birthweight was 3,320 g (2,610-3,950), the mean Apgar score was of 1.7 at 1 minute and 3.6 at 5 minutes, the average pH at the entrance was 7.03 and the EB was -15. The hypothermic treatment started on average age of 4.02 hours of birth, the CFM trace was severely pathological in 3 cases (33%) and only moderately compromised in 4 (44%). The EEG examination resulted pathological in 7 (77%) of the nine children tested. The cerebral ultrasonography showed severe injury in 4 children and mild in 2. The MRI reported severe brain injury in 4 cases and only mild in other 2. The follow-up of children (minimal age: 9 months) shows a picture of normality only in 2 cases, 4 require a rehabilitation

treatment for severe psychomotor alteration, 2 are under anticonvulsant treatment.

#### CONCLUSIONS

Our series, although numerically small, confirms the literature data on the results of brain cooling: it is effective only if it is early applied and only if brain injury is not too much serious.

A take-home message is that 1<sup>st</sup> and 2<sup>nd</sup> level centers must send asphyxiated newborns as early as possible to the 3<sup>rd</sup> level NICU, with special care to neuroprotection during the transport.

#### ABS 31

#### DINO GABURRO: A MASTER OF PEDIATRICS

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Dino Gaburro (**Fig. 1**) was born in Castelnuovo del Garda (Verona, Italy) in 1923; he graduated (1948) and obtained his residency in Pediatrics (1950) at the University of Padua. He performed the first investigations in the Padua University on pediatric and neonatal nutrition with the world famous Prof.



**Figure 1.** Prof. Dino Gaburro.

Giancarlo Bentivoglio (the “Bentivoglio rule” for diluting and enriching cow milk for infant nutrition). In 1963 Dr Gaburro obtained a Chair of “Puericultura” in the Ferrara University School of Medicine and after few years the Medical Faculty called him to be chairman of the Pediatric Institute and of the Dept of Pediatrics, performing a very high level clinical activity.

Prof. Gaburro was an excellent investigator performing many important research program on carbohydrate metabolism in thymectomized rats, and on exocrine pancreas function in premature and full term neonates.

In 1970 Prof. Gaburro moved to the Verona University where he was able to founding and organizing the Pediatric University Service: he was Director during 25 years (1970-1995) improving his own Academic impact.

He was elected president of the Italian Society of Social and Preventive Pediatrics (1978-81) and in 1982 he was elected President of the Italian Society of Pediatrics (1982-1985).

In order to organize the best care to premature infants Gaburro sent doctor Paolo Pizzo in Paris (to learn neonatal infantile care, at the Institut de Puériculture, Prof. Lelong and Rossier), doctor Giuseppe Zoppi in Zurich (in the Department of Pediatrics of Prof. Prader) in order to be educated in nutrition of newborn infant and to be able to improve the neonatal growth.

He created a school of Pediatrics, which generated several Professors of Pediatrics and Directors of University Pediatric Departments (Prof. Luciano Tatò, Pediatric Endocrinologist who succeeded him as Director at University of Verona and Prof. Attilio Boner, Pediatric Allergologist who is now Director at the same University), Directors of Hospital Pediatric Departments and Family Pediatricians. One of the lessons of this great open mind Master is that scientific knowledge by itself is not enough for a good Pediatrician, but it must be associated with deep clinical experience and great attention to the child and the parents.

## ABS 32

### THE STERILE VESICoureTERAL REFLUX AS A CAUSE OF CONGENITAL RENAL HYPODYSPLASIA

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## INTRODUCTION

The intrarenal reflux of infected urine may determine (if not properly and quickly treated) the occurrence of renal scarring and it was considered the only cause of reflux nephropathy in the past. Currently also the sterile intrarenal reflux is considered to be a factor in chronic renal disease, can develop renal hypodysplasia during intrauterine life.

We report the clinical case of a newborn in which there is a presence of ultrasound renal hypodysplasia without the patient ever suffered from previous episodes of fever or pyelonephritis. In the subsequent urology follow-up was diagnosed in the kidney affected an high grade of vesicoureteral reflux (VUR), and in scintigraphic examination, a major functional deficit.

## CASE REPORT

A male newborn of 20 days old comes to our attention for fever (39° C), in the absence of other clinical signs of significant physical examination. No history of previous episodes of fever. Laboratory and instrumental examinations: WBC 9,000 with relative lymphocytosis, CRP and PCT negative, e.g. urine sediment and urine culture negative; BUN mg/dl, creatinine 0.6 mg/dl, blood culture negative. Renal-bladder ultrasound shows: “ureterohydronephrosis of the left kidney (2<sup>nd</sup>-3<sup>rd</sup> grade according to the classification of the “Society for Fetal Urology”), small parenchymal (3<sup>rd</sup> centile for age) with poor cortico-medullary differentiation, right kidney size increased (90<sup>th</sup> centile for age), with hypertrophic vicarious aspect”. The patient, after 5 days of intermittent fever presents, in conjunction with the resolution febrile, a widespread rash of the body. Following hospital discharge with a diagnosis of “exanthem subitum.” Included in a follow-up urology, a cystourethrography showed an RVU of 4<sup>th</sup>-5<sup>th</sup> grade in the left kidney, while scintigraphy demonstrated renal secretion by 80% right and 20% left.

## DISCUSSION

The “Big bang theory” of Ramsley and Ridson [1] (which gave prominence to the dramatic effect of the initial infection induce more renal scarring), has been considered for years can explain a reflux nephropathy. Currently it is estimated that a sterile VUR can cause congenital renal hypodysplasia in 29-50% of cases [2].

Several hypotheses, such as the production of the presence of high pressures intratubular fetal factors inducing interstitial fibrosis and renal dysplasia (Angiotensin, TGF, PDGF) or abnormal embryonic development of the ureteric bud, may explain the occurrence of a congenital renal hypodysplasia regardless from any infectious factor acquisition.

#### CONCLUSIONS

We reported a case report of an infant with congenital nephropathy sterile VUR. The evidence that a large part of renal disease by VUR is congenital and not post-infectious (at least in males) and the knowledge that no post-natal medical intervention can influence the clinical course, is changing the diagnostic-therapeutic-prophylactic outlook currently used for the treatment of this important disease.

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#### ABS 33

### INTRAPARTUM ANTIBIOTIC PROPHYLAXIS IN GBS-POSITIVE MOTHERS: EFFECTS ON NEWBORN MICROBIOTA

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#### BACKGROUND AND AIMS

Group B *Streptococcus* (GBS) early-onset sepsis represents an important cause of neonatal morbidity and mortality; however, the introduction of maternal intrapartum antibiotic prophylaxis during labor and delivery has led to a decline of the related death rates. Current literature lacks information about the effects of antibiotic prophylaxis on early bacterial colonization of the newborn gut, which is known to be related to immunity development.

The aim of this study is to evaluate the effect of antibiotic treatment of pregnant GBS-positive women on early bacterial colonization of the newborn gut.

#### MATERIALS AND METHODS

Thirty-four newborns born by natural delivery and breastfed were enrolled; 17 had mothers GBS-

positive treated with 2 g of Ampicillin, 17 had GBS-negative mothers (control group). Two-hundred milligram faeces were collected for each subject and processed for DNA extractions with QIAamp DNA Stool Mini-Kit. The quantification of principal groups of newborn gut microbiota (*Lactobacillus spp.*, *Bifidobacterium spp.*, *B. fragilis* group, *C. difficile*, *E. coli*) was carried out with real-time PCR. Data of microbial counts were subjected to one-way variance analysis, to detect significant differences between treated and control group.

#### RESULTS

Antibiotic therapy reduced *Bifidobacterium spp.* intestinal colonization [5.51 Log(CFU/g) in treated samples again 7.07 Log(CFU/g) in control samples,  $p < 0.05$ ], while no variation was observed in the number of *Lactobacillus spp.*, *C. difficile* and *E. coli*.

#### CONCLUSIONS

Our preliminary results showed a significant decrease of early *Bifidobacterium* count in treated newborns microbiota; clinical meanings or possible effects on immunity need to be investigated with larger studies.

#### ABS 34

### MOTHER'S EMOTIONAL EXPERIENCE AFTER GIVING BIRTH TO A PRETERM INFANT

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#### AIM

Aim of the present research is to investigate the presence of emotional disorders (anxiety and depression) in two groups of mothers of premature children. The first group is formed by mother of premature children without serious pathologies, and the second group is formed by mother of premature children with serious pathologies.

#### HYPOTHESIS OF RESEARCH

The authors want to put in evidence if there are important differences between the two groups about the presence of emotional disorders concerning the anxious-depressive problem.

#### MATERIALS AND METHODS

The sample investigation for each groups of research is formed by 30 mothers of premature children patients in NICU of the hospital "V. Fazzi"

of Lecce, total 60 subjects. The premature children present: an average pregnancy age of 30 weeks (25-34), average weight at the birth 1,435 g (920-1,950), average stay in hospital about 60 days.

Instruments of the research are: a multi-explorative questionnaire, containing personal data and clinic information of the mother and of the newborn, that investigates about prevalent emotional and clinic experiences (medical problems, food disorders, family life) and the Simpton Check List-90 (SCL-90) that gives the possibility to measure the frequency and the intensity of somatic and psychic disorders of the persons of the sample.

In a period of five months we have analyzed 24 mothers of premature children without serious pathologies and 18 mothers of premature children with serious pathologies.

#### RESULTS

The questionnaire demonstrated that the mothers with serious pathologies present superior values for frequency and intensity in the scale relative to anxiety and depression (SCL-90) compared to the mothers of the premature children without serious pathologies. Moreover in the mothers of premature children without serious pathologies the prevalent feelings are sadness and guilty, while in the second group to those emotions you have to add a strong anger respect to the traumatic event suffered.

#### ABS 35

### EFFECTS OF BOLUS FEEDING VS. CONTINUOUS FEEDING ON SPLANCHNIC TISSUE OXYGENATION, CEREBRAL TISSUE OXYGENATION AND APNOEIC EPISODES IN PRETERM INFANTS

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#### BACKGROUND AND AIMS

Tube feeding, either continuous or through intermittent boluses, is a common practice in Neonatal Intensive Care Units, due to the inability of preterm infants to coordinate sucking, swallowing and breathing; however, at present there is no clear evidence if either feeding method is better tolerated. The primary aim of our study was to evaluate and compare cerebral and splanchnic oxygenation trends occurring after bolus and during continuous feeding

in stable preterm infants using Near Infrared Reflected Spectroscopy (NIRS), a non-invasive technique of tissue oxygenation monitoring. Moreover, we aimed to compare the frequency of apnoeic episodes detected with polysomnography between the two enteral feeding techniques.

#### MATERIALS AND METHODS

Eighteen healthy preterm infants with a median GA of 31 weeks underwent a 6-hour NIRS monitoring of cerebral and splanchnic oxygenation, simultaneous to a polysomnographic recording of total (TA), obstructive (OA), central (CA) and mixed (MA) apnoeas episodes. During the monitoring each baby was fed twice through a nasogastric tube: one meal was given continuously over 3 hours, the other as a 10 minutes bolus. Recorded values of cerebral and splanchnic oxygenation, clustered in 5-minutes intervals, and the number of TA, OA, CA and MA were compared between the two techniques using Wilcoxon Signed Ranks Test. Statistical significance was set at  $p \leq 0.05$ .

#### RESULTS

Splanchnic oxygenation significantly decreased ( $p \leq 0.05$ ) during continuous feeding, from 1 h 25' to 2 h 25' after the beginning of feeding administration. No differences between the two techniques were found on cerebral oxygenation and in the number of total, obstructive, central and mixed apnoeic episodes detected.

#### CONCLUSIONS

To the best of our knowledge, this is the first study comparing the effects of different feeding techniques on splanchnic and cerebral oxygenation in preterm infants. Splanchnic oxygenation was significantly lower during continuous feeding than after bolus; further studies are needed to possible physiological mechanisms or long-term effects on mesenteric perfusion.

#### ABS 36

### NATAL AND NEONATAL TEETH: SOME NEW EXPERIENCES

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#### BACKGROUND

If primary teeth erupt during the third to the fifth month of life, this is termed precocious dentition. Such cases involve premature eruption of normal primary

teeth. Teeth erupted at a much earlier age or present at birth have been called fetal, congenital but since 1963 they are called “natal teeth” if they are present at birth and “neonatal teeth” if they erupt during the neonatal period.

In the time, because of the rare occurrence of natal teeth, the phenomenon has been associated with superstition. Natal or neonatal teeth occur with approximately equal frequency among boys and girls (about 1:2,000, 1:3,000).

#### GOALS

1. To review the epidemiology, etiology, clinical presentation, complications and management of natal teeth.
2. To refer the author’s experience or congenital teeth.

#### METHODS

Review of the literature on Pub Med and evaluation of the cases observed during a 48 months period (Jan. 2008-Dec. 2011) in a maternity hospital.

#### RESULTS AND CONCLUSIONS

Natal teeth might resemble normal primary dentition in size and shape, but they are often smaller, conical and yellowish, and have hypoplastic enamel and dentin with poor or absent root formation.

Most natal teeth are mobile. Complications include discomfort during suckling, sublingual ulceration, laceration of the mother’s breasts and aspiration of the teeth. A dental roentgenogram is tooth extraction is indicated if the tooth is supernumerary or excessively mobile. indicated to differentiate the premature eruption of a primary tooth from a supernumerary tooth. Etiology is unknown.

Three syndromes have been associated with natal teeth: Ellis-van Creveld s., Hallermann-Streiff s., and Jadassohn-Lewandowski syndrome.

Over 4 years (Jan. 2008-Dec. 2011) in a 2<sup>nd</sup> level maternity ward 4,697 newborn baby were delivered and 3 newborn having natal teeth were observed (1:1,586).

Natal teeth may also be associated with cleft lip, cleft palate and other anomalies. Histologic investigation has revealed a failure of root formation despite eruption, a large vascular pulp, irregular genesis.

If the tooth does not interfere with breastfeeding and is otherwise asymptomatic, no treatment is necessary. The authors confirm the usefulness of this preliminary contribute: they suggest other larger study should be performed.

#### ABS 37

### INTEGRATION BETWEEN MEDICAL AND NURSING CHARTS AS A TOOL FOR QUALITY

### IMPROVEMENT AND RISK MANAGEMENT IN NICU

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#### BACKGROUND

Clinical documentation is a fundamental tool to get International Standard in terms of patient safety in NICU. Bambino Gesù Children’s Hospital obtained for the first time the accreditation by Joint Commission International (JCI) in 2006 within the project of the continuous improving in quality of care.

Before 2006 the clinical charts were lacking in nursing information, intervention plans, pain and adverse events monitoring. The prescription of therapy was reported in different sheets with consequent high risk for medical and nursing errors and adverse events. After JCI accreditation an integrated nursing and medical documentation was designed and applied, looking specifically at the planning of the procedures and at the individualization of care based on specific patient needs. All these aspects have to be reported and well documented in the patient chart.

#### AIMS AN METHODS

Evaluation of adverse events during the last 18 months in the Department of Medical and Surgical Neonatology (DNMC), through a voluntary communication of adverse events, near miss, or severe events.

#### RESULTS

In the last 18 months 1,005 patients have been discharged by the DNMC. Forty-four adverse events have been documented, of these 8 were near miss events, 36 were adverse events. Eight of these had moderate-severe consequences. The most frequent events (19/44) were therapeutic ones, of whom 16 (84.2%) were due to wrong prescription, that is by far the most risky phase.

Following this analysis a new medical-nursing team organization has been developed with the aim of sharing and optimizing the clinical care for sick newborns and reducing the risk for therapeutic adverse events.

#### CONCLUSIONS

Adverse events and medical errors are relatively frequent in NICU and most of them are related to therapy. The weakest phase seems to be drug prescription followed by administration. In both

cases, the impact of an organization based on a unique medical-nursing team supported by a culture of a continuous improving in health quality is the fundamental instrument to reduce the risks for patients, family and caregivers.

### ABS 38

#### CD44 IMMUNOREACTIVITY IN DIABETIC NEPHROPATHY AND THE DEVELOPING HUMAN KIDNEY: A MARKER OF RENAL PROGENITOR STEM CELLS

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#### BACKGROUND

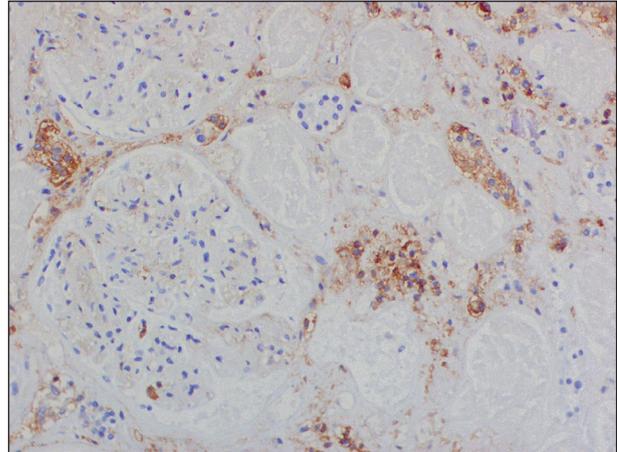
CD44 is a transmembrane adhesion glycoprotein, which functions as a hyaluronan receptor and participates in the uptake and degradation of hyaluronan [1,2]. It is expressed in a wide variety of cell types; recently, CD44 has been proposed in the adult kidney as a marker of activated glomerular parietal epithelial cells, the putative niche stem cells that, in case of damage to podocytes, might migrate inside the glomerular tuft and undergo transition to podocytes [3]. The aim of this study is to investigate CD44 immunoreactivity in a case of diabetic nephropathy compared with nephrogenesis.

#### DESIGN

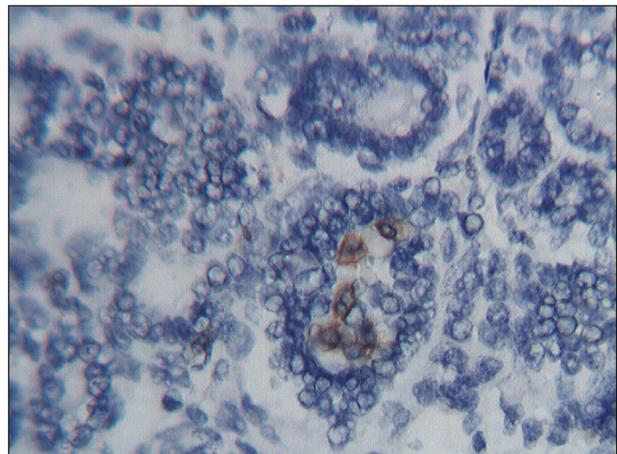
The expression of CD44 was evaluated in kidneys of a 67-year-old diabetic woman and 4 human fetuses, ranging from 11 to 30 weeks of gestation obtained at autopsy, 10% buffered formalin fixed, routinely processed and paraffin-embedded. Immunohistochemical staining were performed using antibodies against CD44 (clone HCAM) on 4  $\mu$ -thick sections, incubated for 30 minutes at room temperature with a 1:50 dilution of the polyclonal anti CD44 primary antibody.

#### RESULTS

Immunoreactive cells for CD44 were detected either in the diabetic adult and in the fetal kidneys studied in the present work. In the diabetic adult kidney, CD44 reactivity was observed in the tubules and in the glomerular parietal epithelial cells (**Fig. 1**). In the fetal kidneys, CD44 was detected in glomeruli, in isolated cells, located inside the immature glomerular tuft, sometimes giving rise to small clusters, and in isolated large cells inside the metanephric mesenchyme surrounding the newly formed renal vesicle (**Fig. 2**), but not in the tubules.



**Figure 1.** 20 week-old diabetic adult kidney: CD44 is expressed in tubules and in glomerular parietal epithelial cells.



**Figure 2.** 20 week-old fetus: CD44 is expressed in small clusters of large cells, inside immature glomeruli.

#### CONCLUSIONS

CD44 reactivity was detected either in glomerular parietal epithelial cells in the case of diabetic nephropathy and in different cells involved in nephrogenesis in the fetal kidneys. In both situations CD44 probably marked a subset of progenitor/stem cells and/or putative niche stem cells involved either in early phases of kidney development and in repairing podocytes damage. Further studies are need in order to better understand the putative role of the CD44-positive cells both in nephrogenesis and in podocytopathies.

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### ABS 39

#### PAS AND WEIGERT METHODS: TWO OLD STAINS FOR A NEW INTERPRETATION OF THE NEWBORN KIDNEY

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#### BACKGROUND

Histochemistry has been abandoned or scarcely utilized in recent years for the interpretation of human kidney samples, including the analysis of the developing kidney, being substituted by immunohistochemistry and electron microscopy. Here we report our preliminary data on the usefulness of two old histochemical methods, PAS-stain for glycogen and Weigert stain for elastic fibers, in the study of the neonatal kidney.

#### DESIGN

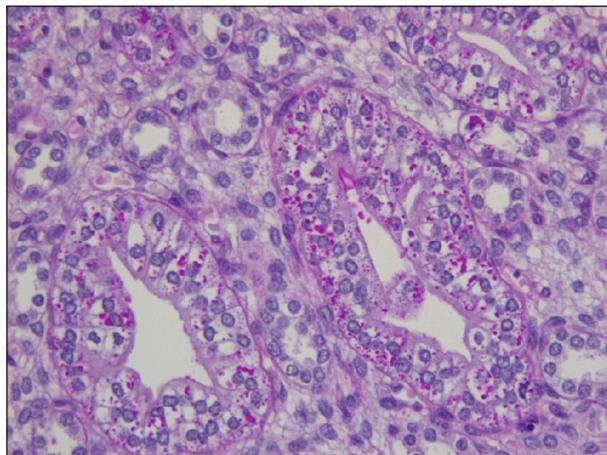
10 well preserved kidney samples, obtained at autopsy from newborns ranging in gestational age from 26 up to 38 weeks were formalin-fixed, routinely processed and paraffin-embedded. Serial paraffin sections were stained with E-E, PAS stain and Weigert methods.

#### RESULTS

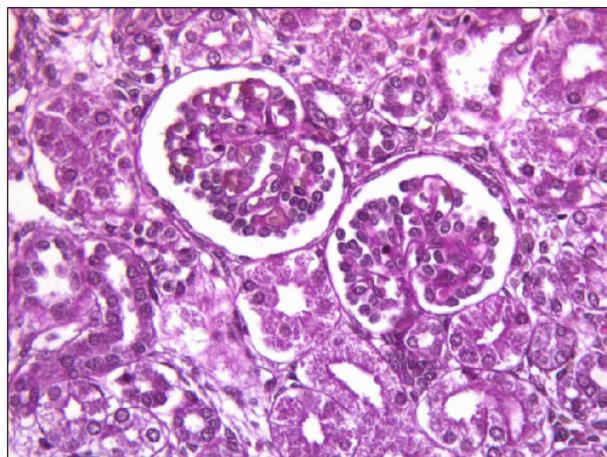
The study of renal sections stained with the PAS method, first evidenced the inability of this method to mark the glomerular basal membrane in the majority of kidneys, particularly in very preterm infants. On the contrary, PAS stain revealed the presence of massive PAS-reactive globules restricted to epithelial cells bordering the collecting tubules (**Fig. 1**). PAS-reactivity of the globules were shown to be diastase-resistant, a finding suggesting their glycogen nature. The study of kidneys stained with the Weigert method for elastic fibers clearly showed the ability of this stain to mark the glomerular basal membranes (**Fig. 2**) that, in preterms, were not evidenced by other histochemical methods routinely utilized in the study of adult kidney biopsies, including Jones method.

#### DISCUSSION

Our preliminary data clearly show that old histochemical methods, probably too early abandoned, may be very useful in the interpretation of the complex scenario characterizing the



**Figure 1.**



**Figure 2.**

newborn kidney. Among the advantages of these two stains, we would like to evidence the very low cost (less than 1 euro/stain), the rapidity of execution (less than 30 min) and the high reproducibility also in low level laboratories worldwide.

### ABS 40

#### SCANNING ELECTRON MICROSCOPY OF THE DEVELOPING HUMAN KIDNEY

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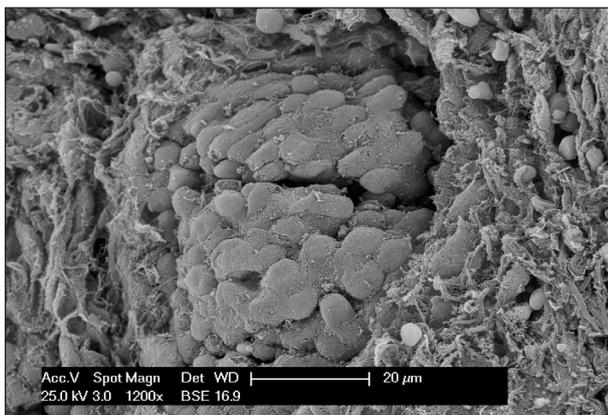
## BACKGROUND

The ultrastructural studies carried out in more than fifty years in the developing kidney of humans and of experimental animals underline the complex of important differentiations taking place in the newborn kidney. It is very important to understand when, how and where all these differentiations occur, in order to provide the whole ultrastructural model of the nephron and discriminate between normal function and disease during the development of these structures. With the present study we suggest a Scanning Electron Microscopy (SEM) approach to show the different phases of renal maturation.

**Design:** The autoptic kidneys of two newborns were fixed in buffered 4% paraformaldehyde and 0.1% glutaraldehyde and postfixed in a solution of 1% osmium tetroxide and 1.25% potassium ferrocyanide for 2 h, furthermore they were reduced in slices 0.2 mm thick with a razor blade, then postfixed again in a solution of 1% osmium tetroxide and 1.25% potassium ferrocyanide for 1 h dehydrated in ethanol, critical point dried in CO<sub>2</sub> and observed in a Philips SEM-FEG XL 30.

## RESULTS

The exposed glomeruli showed differences in the visceral layer organization. The visceral layer could be formed by spherical cells, identifiable as podocyte precursors, but in this early stage of glomerular development, the typical expanded processes of mature podocytes lack (**Fig. 1**). In other glomeruli the visceral layer was made up by flat podocytes spreading elongated processes. Further, inside some glomeruli, adjacent to



**Figure 1.**

undifferentiated globoid podocyte precursors, some podocytes assumed an octopus-like shape, characterized by multiple ramifying processes embracing the underlying capillary loop.

## CONCLUSIONS

SEM allowed to easy collect informations about renal maturation and, in particular, regarding podocytes differentiation. Thanks to these observations, we can summarize the glomerular development in the following steps:

- well defined Bowman's parietal wall enveloping the visceral wall composed by a "mass" of roundish cell. These podocyte precursors are very close to each other and they do not show the typical disposition of mature podocytes resuming the glomerular capillary meshes;
- some podocytes spread processes and pedicels. The capillary network becomes more distinguishable;
- the podocytes are characterized by branched processes and interdigitated pedicels embracing well defined loops.

## ABS 41

### INTERINDIVIDUAL VARIABILITY IN MATURATION OF THE HUMAN THYROID GLAND DURING GESTATION

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## BACKGROUND

Development of thyroid gland during gestation has been analyzed in recent years by many authors. Thyroid primordium first appears as a thickening of the anterior foregut endoderm at the basis of the tongue. Subsequently thyroid progenitors cells move caudally and eventually differentiated into the thyroid follicles, bordered by hormone-producing cells. Later stages of thyroid morphogenesis are characterized by the integration of new progenitors, which ultimately differentiate into C-cells.

## DESIGN

This study was aimed at analyzing the degree of differentiation of the thyroid gland in human fetuses and newborns of different gestational ages. We were particularly interested to verify if thyroid development could be strictly related to the age of

gestation (GA) or influenced by other factors. To this end, we studied 17 well preserved autoptic thyroids from 13 fetuses ranging from 11<sup>th</sup> up to 22<sup>nd</sup> week of GA and 4 newborns (range: 26<sup>th</sup> to 38<sup>th</sup> week of GA).

## RESULTS

The preliminary study of the histological picture of developing human thyroid glands first evidenced a marked interindividual variability regarding the degree of differentiation. Prominent differences were observed regarding the presence and the number of follicle structures among fetuses of the same gestational age. Differences were also found regarding the presence of colloid in the follicular lumen (**Fig. 1, 2**).

## CONCLUSION

Our preliminary data clearly evidenced that thyroid gland development is not exclusively and strictly related to the gestational age. The prominent and striking differences of thyroid development here observed suggests that other factors influence maturation of the thyroid gland during intrauterine life. Further studies at immunohistochemical levels are needed to better characterize the follicular cells differentiation and changes in the migration into the thyroid primordium of C-cells.

## ABS 42

### THE CHILD WITH WOOL STEEL HAIR

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Stefano, 3 and a half months old, came to our observation for vomiting, right upper limb clonuses, deviation of the eye movements and drowsiness.

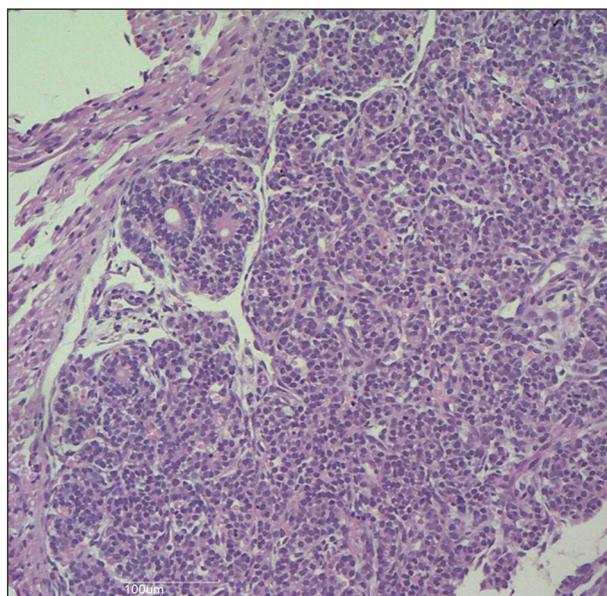
Dysmorphic notes were evident: hypopigmented, curly and frizzy hair, chubby cheeks, abundant and loose skin over main folds, microcephaly.

Spontaneous motor activity and muscle tone liabilities are reduced with inadequate neck and head control. Eye-tracking were absent with numerous episodes of contact's reduction and gaze deviation to the left. DTR were present bilaterally as Moro reflex and symmetric. This raised the suspicion Stefano was affected by neuro-developmental delay syndrome.

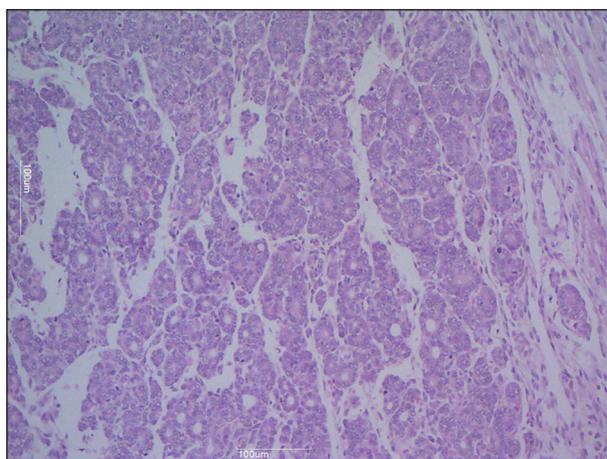
During hospitalization Stefano appeared restless, with poor appetite and has many right upper limb clonuses, episodes of fixity and deviation of eyes.

EEG detected diffuse slow background activity; right fronto-central region produced spindles; continuous discharges of spikes and slow polymorphic SW in the left parietal-occipital region, isolated spikes and SW in the right occipital region were detected. A combined valproate and clonazepam treatment abolishing convulsive episodes was prescribed. After 5 months the infant presented in flexion spasms and ocular revulsion; the EEG was hypsarrhythmic and vigabatrin treatment was added.

Ammonia (162 µg/dl), lactate (5.9 mmol/l) and pyruvate (0.223 mmol/l) were increased; coeruloplasmin (4.5 mg/dl) and copper (7 µg/dl) were reduced.



**Figure 1.** Foetus 12 weeks of GA H&E 10X: thyroid with prevalent solid pattern.



**Figure 2.** Foetus 12 weeks of GA H&E 10X: thyroid with prevalent follicular pattern.

An abdominal ultrasound scan showed lack differentiated cortico-medullary renal zones, calyco-pielic dilatation and a reduced thickness of parenchyma in lower calyces; kidney medulla showed enhanced echogenicity.

Brain MRI showed left parieto-occipital cortical dysplasia with flattening of cortical furrows, and loss of definition between white and gray substance. This data and the evolution of the disease increased the suspicion the child was affected with a metabolic disorder causing psychomotor retardation, the clinical picture recalled Menkes Syndrome, this is caused by an alteration of copper transport protein. Children affected have a typical facies, seizures and low levels of coeruloplasmin and copper. The clinical suspicion was confirmed by genetic tests. These show a mutation of ATP7A gene, responsible for the disease.

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#### ABS 43

### ACUTE RENAL CHANGES IN ASPHYXIATED RATS FOLLOWING THERAPEUTIC HYPOTHERMIA

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#### BACKGROUND

Hypoxic-ischemic encephalopathy (HIE) occurs in 1-3 per 1,000 births in USA and is the primary cause of cerebral palsy. The therapeutic hypothermia is standard treatment for asphyxiated infants.

Several previous studies suggested that therapeutic hypothermia improves survival and neurodevelopment in asphyxiated infants without significant side effects. Little is known about renal changes in asphyxiated infants who underwent therapeutic hypothermia, therefore this study aimed to evaluate renal histology in an experimental model of asphyxia treated with hypothermia.

#### DESIGN

HIE was produced in 7 days old (P7) newborn Sprague Dawley (SD) rats. They were randomized to the following groups: 1) HIE + salin (controls); 2) HIE + salin + therapeutic hypothermia; 3) HIE + adenosine + hypothermia. The rat brain was damaged according to the Vannucci's model. Immediately following hypoxia, animals randomized to receive therapeutic hypothermia were placed on a water-jacketed heating/cooling pad and kept at 34°C for 24 hours. Animals assigned to salin or adenosine treatment received a single (IP) dose of 0.02 ml saline or 0.4 mg/kg of adenosine in saline (0.02 ml) following hypoxia. For evaluating acute kidney injury, rats were euthanatized at 24 hours post hypoxia/ischemia. Mitotic index and apoptotic index were calculated as numbers of mitotic figures and apoptotic globules in 5 fields at x 400.

#### RESULTS

No significant pathological changes were detected in kidneys from rats submitted to hypothermia and to adenosine treatment as compared to control rats. Marked differences were observed among three groups regarding the mitotic activity and the apoptotic index (**Fig. 1**). Hypothermia was associated with a significant decrease in the mitotic index in proximal tubules. In this group, kidney also showed an increase in the apoptotic index in the medulla. The association of adenosine to hypothermia resulted in a higher mitotic activity in proximal and in collecting tubules (**Fig. 2, 3**). Hypothermia also caused an increase in apoptosis in the medulla whereas adenosine association protected against the increase in medullary apoptosis.

#### CONCLUSION

Our preliminary data show that hypothermia does not cause acute pathological changes in rat kidney, but may decrease the mitotic activity in the renal cortex, paralleled by an increase in the apoptotic cell death in the renal medulla. Adenosine treatment plus hypothermia decreased the hypothermia-induced

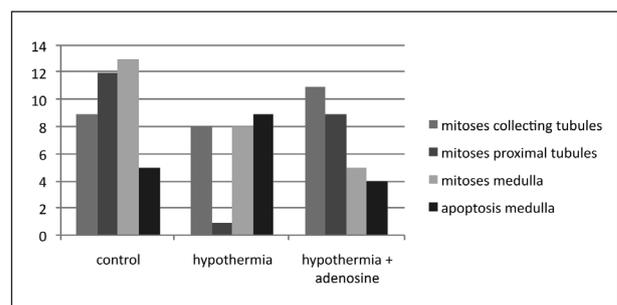


Figure 1.

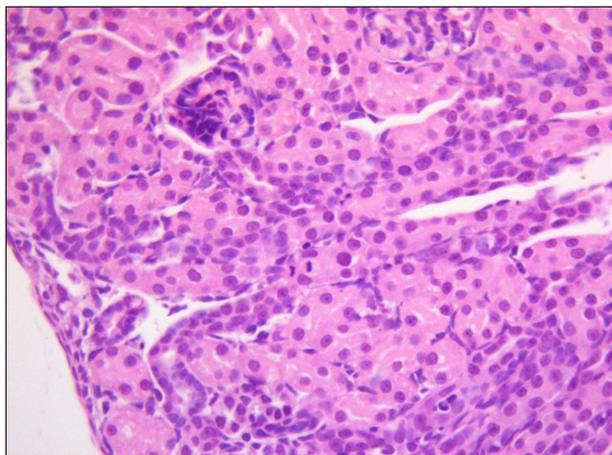


Figure 2.

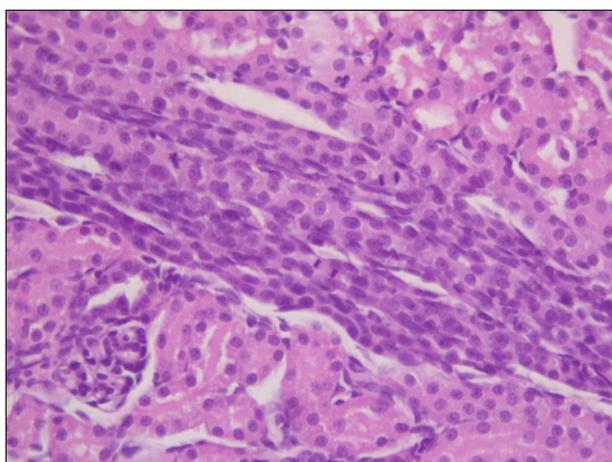


Figure 3.

increase in the medullary apoptosis, suggesting a protective effect against apoptotic cell death in HI.

#### ABS 44

### CHLAMYDIA TRACHOMATIS CAUSING NEONATAL CONJUNCTIVITIS: WHAT KIND OF PREVENTION?

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#### AIMS

*Chlamydia trachomatis* is one of the most common sexually transmitted agents. Infants born vaginally to infected mothers may present with conjunctivitis (20-50%) and/or pneumonia (5-20%) [1]. *Chlamydia spp.* is a frequent identifiable cause of neonatal conjunctivitis, in association with *S. aureus*, *E. coli*, *N. gonorrhoeae*. Topical eye drops such as silver nitrate 1% effectively prevent gonococcal neonatal conjunctivitis; however, antibiotic topical agents are commonly used in the clinical practice in the attempt to prevent also chlamydial infections.

#### METHODS

Topical ocular prophylaxis with fusidic acid was instituted early after birth. Data about new cases of chlamydial conjunctivitis from September 2011 to August 2012 were recorded. Data included length and course of pregnancy, maternal diseases, delivery method, newborn weight, postnatal course.

#### RESULTS

Two cases of isolated chlamydial conjunctivitis were recorded. Both infants (one male, one female) were born by vaginal delivery. They were full term, healthy infants, without ocular malformations, whose mothers were treated with neither systemic nor local antibiotics near delivery. Birth weight: 3,400 and 2,380 g respectively. Pregnancy courses are unknown. Both infants received antibiotic prophylaxis in each eye 20 minutes after delivery. The age of presentation for conjunctivitis was between day 10 and 12 of life. Infants presented with hyperemic conjunctiva, mucopurulent discharge and swollen eyelids. The male infant also had blood-stained eye discharge. Ophthalmological examinations showed follicular conjunctivitis. Definite diagnosis was made by detection of *Chlamydia* DNA by PCR on specimens obtained by swabbing the conjunctiva. Both infants were treated with systemic Clarithromycin at 10 mg/kg/day in 2 doses for 14 days, after an electrocardiogram was performed. No long-term ocular sequelae were found.

#### CONCLUSIONS

In addition to the typical features of chlamydial conjunctivitis, a follicular conjunctivitis was demonstrated at ophthalmological examination in both our neonates. Prophylaxis with fusidic acid did not prevent all cases of chlamydial neonatal conjunctivitis.

In the absence of information about pregnancy, screening for *Chlamydia spp.* may be an effective practice to prevent neonatal infection and related complications.

## REFERENCE

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## ABS 45

**TRANSIENT TACHYPNEA OF THE NEWBORN: WHAT IS NEW?**

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## INTRODUCTION

Transient tachypnea of newborn is quite common but known to be benign and self-limiting; the aim of this talk is to highlight the long term consequences of this illness. Transient tachypnea of the newborn (TTN) was initially described by Avery et al. in 1966. Historically, TTN has been viewed as a “transitory respiratory disturbance resulting from a delay in alveolar fluid resorption”, with an incidence of 5.7 per 1,000 births in term infants. The overall incidence of RD was 6.7%. Preterm babies had the highest incidence (30.0%) followed by post-term (20.9%) and term babies (4.2%). TTN was found to be the commonest (42.7%) cause of RD followed by infection (17.0%), meconium aspiration syndrome (10.7%), hyaline membrane disease (9.3%) and birth asphyxia (3.3%). TTN was found to be common among both term and preterm babies.

## BACKGROUND

Although TTN is considered a self-limited transient condition, there are increasing data to suggest that TTN increases a newborn’s risk for developing a wheezing syndrome early in life [1].

Risk factors for development of transient tachypnea of the newborn period were maternal asthma, birth weight over 4,500 g, male sex, and urban location, and these infants were at significant risk for persistent wheezing later in life [2, 3].

One potential mechanism for the association between TTN and asthma has been the possible genetic predisposition for adrenergic hyporesponsiveness in these infants and mothers with asthma. A genetic factor also was discovered that beta1Gly49 homozygosity and TACC haplotype of ADRB2 gene, both loss-of-function genetic variations, may predispose to TTN. The critical link may be the association of adrenergic response and activation of Na transport in fetal alveolar epithelium required to help clear neonatal lung fluid. Thus, TTN may be the

first manifestation of asthma in these children [4]. It was hypothesized that early life antibiotic treatment may modify the gut flora, and this may predispose the child toward development of allergy and asthma as suggested by the hygiene hypothesis. Thus, there are differences in the intestinal microbial flora between allergic and nonallergic infants. It is probable that the combination of a genetic predisposition and the modification of the environmental exposure from normal gut flora by antibiotic treatment may provide a synergistic risk factor for future asthma [5, 6].

## EXPERIENCE OF THE AUTHOR

We found in our experience that TTN is associated with subsequent respiratory morbidity and may be an early manifestation of “asthma”. We hypothesize that the genetic and environmental interactions synergistically predispose these children for future wheeze. Prospective studies are required to better define these factors.

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## ABS 46

**A 45-DAY-OLD MALE INFANT WITH DISTAL TUBULAR ACIDOSIS ASSOCIATED WITH HYPERAMMONEMIA AND HYPERLACTACIDEMIA**

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A 45-day-old infant was referred to our clinic for growth arrest. He was born at term, by caesarean section for breech presentation. Parents reported

that their baby fed with difficulty and sporadically presented vomiting after meals. He was admitted in bad general conditions with adipose and muscular mass scant trophism, reduced skin turgescence and elasticity, modest plagiocephaly. Anterior fontanelle was normal, with intact sensory functions. Upon hospitalization, complete blood count (CBC) didn't show any significant alterations, whereas a few days later we noticed the appearance of hypochromic anemia.

Blood glucose level was normal, liver and kidney functions were preserved. Inflammatory indicators were negative, and blood tests showed hypokalemia, hyperchloremia, normal natremia, hypercalcemia and a phosphate value of 2.38 mmol/l. At first, blood-gas analysis showed a metabolic acidosis with pH 7.25, HCO<sub>3</sub><sup>-</sup> 13.6 mmol/l, EB -14.4 and 16.4 mmol/l of anion gap. Urine tests detected modest proteinuria, leukocyturia and hemoglobin traces, urinary pH was 7. Urine culture was negative. Some urine electrolytes were modestly increased such as natriuria and kaliuria, while hypercalciuria was quite high. An increase in lactate and hyperammonemia was noted, which made us suspect the presence of an underlying metabolic disease. However, urine organic acids, urinary amino acids, blood amino acids, and pyruvate showed no significant alterations for diagnosis. Transfontanellar and gastroesophageal ultrasonography were normal, while renal ultrasonography showed a marked increase of echogenicity, completely covering all renal pyramids, attributable to medullary nephrocalcinosis. Audiometric screening was negative. Since our patient was strongly considered to have a distal renal tubular acidosis we planned genetic investigations in order to find the mutation type responsible for clinical signs. Additionally, we ordered a corrective treatment firstly by intravenous paediatric hydration and KCl then by oral therapy, that determined an improvement of altered blood values and general conditions (resumption of growth and increase in weight). Sodium and potassium citrate doses were constantly changed during follow-up according to clinical needs, electrolytic modifications and auxological indices. The need of bicarbonate- as well as potassium citrate- had halved over time compared to the initial doses. Upon last control (8 months), the baby weighed 6,950 kg and the metabolic balance was preserved.

#### ABS 47

### METABOLOMIC ANALYSIS OF BRONCHO-ALVEOLAR LAVAGE FLUID IN PRETERM INFANTS

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#### OBJECTIVE

Currently, little is known about the overall metabolic status of preterm infants and only a limited number of metabolites can be measured in their biological fluid by conventional methods. Clinical management of preterm infants could probably be improved if more information about metabolic background of preterm during the development of respiratory distress syndrome (RDS) was available. Metabolomics is a technique used to non-invasively determine a snapshot of the current metabolic status of an organism by analyzing intact tissue or biofluids.

Aim of our study was to analyze bronchoalveolar lavage fluid (BALF) metabolic profiles in preterm infants with RDS in order to identify metabolic assessment of this biofluid and to eventually have an early selection of infants at high risk to develop bronchopulmonary dysplasia (BPD).

#### METHODS

BALF samples were collected from a group of preterm infants at specific time-points (at birth prior surfactant, post-surfactant during mechanical ventilation and at extubation) and analyzed by proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectroscopy and GC-Mass Spectrometry.

#### RESULTS

Statistical analysis of spectral data identified distinct metabolic patterns but only 5 of them had a known molecular structure.

#### CONCLUSIONS

This holistic approach appears to be a promising tool for investigating metabolic assessment newborn with acute lung injury and could lead to a better knowledge and management of respiratory distress syndrome of preterm infants.

This is the first application of this new methods in preterm infants. Our preliminary results provide support for further development and application of

metabolomics technologies and for the utilization of multivariate models for identifying factors involved in BPD development.

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## ABS 48

### TOXOPLASMA GONDII DNA DETECTION IN GUTHRIE CARDS: A RETROSPECTIVE STUDY

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## AIMS

Congenital Toxoplasmosis (CT) in newborns results from primary maternal infection with *T. gondii* (TG). Most infected children show no symptoms at birth but are at great risk of sequelae during the first year of life or in early childhood [1].

Polymerase chain reaction (PCR) analysis of dried blood samples on the Guthrie card has been proposed as a sensitive method to screen for congenital CMV infection, but there are no data about the use for TG screening.

The aim of present study was to assess the utility of PCR analysis of dried blood samples for the retrospective diagnosis of CT.

## METHODS

A retrospective study was performed with 18 infants born between January 2010 and June 2012. Transmitters mothers seroconverted in the second trimester of pregnancy (mean  $23.5 \pm 7.9$  weeks). At birth, serological tests (Enzygnost Toxoplasmosis IgG, IgM, IgA-Siemens Healthcare Diagnostics; Vidas Toxo IgMbioMerieux) as well as IgM-IgG WB (LDBio Toxoplasma WB IgG/IgM-LDBio Diagnostics) were performed in all mother-child pairs.

Nucleic acids were extracted from Guthrie cards with VERSANT kPCR Sample Preparation system (Siemens) and Toxoplasma Q-PCR Alert Kit (Nanogen) was used for the amplification of TG target region AF 146527.

## RESULTS

In 7/18 (38.9%) infants, CT was diagnosed by IgM-WB positivity at birth. The remaining 11 were considered non-infected (61.1%) and became IgG negative within 12 months of life. Infected infants received one-year therapy (pyrimethamine/sulfadiazine) and were followed according to our protocol. Four of these had a pathological neuroimaging (4/4 calcifications, 2/4 ventriculomegaly). None had hearing loss. TG DNA was detected in only one of the Guthrie cards of the infected newborns, while all the others were negative.

## CONCLUSIONS

Although serological methods remain basic in the diagnosis of CT, TG DNA detection in Guthrie cards could be considered a retrospective method to evaluate infants (> 1 year of age) with clinical signs suggestive of CT. More studies with a larger number of infected cases are needed to assess the sensitivity of this method.

## REFERENCE

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## ABS 49

### HYPOTONIA ASSOCIATED WITH RESPIRATORY DISTRESS: A CASE REPORT

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A 7 months old female infant comes to our observation with dyspnea and hypotonia. She was born at 35 wk with low birth weight by twin distocial delivery. The remote pathological anamnesis was characterized by a previous hospitalization for post-vaccination polyradiculoneuritis (3 months of age), treated with intravenous infusion of immunoglobulin without complete benefit. The infant is admitted to our hospital in severe general condition, with dyspnea and paradox breath, tachypnea (respiratory frequency 40-50/min), without fever. She has, in

particular, an important thoracic deformation (bell-shaped arched to the fourth/fifth month of life). The neurological profile is altered and characterized by severe generalized hypotonia and hypokinesia. The remaining physical findings are normal. We start investigations concerning neurological and pulmonary diseases. CBC, inflammatory markers, renal and hepatic functions are normal, as well as CPK. Thoracic x-ray shows an opacification of the upper left hemithorax with modest attraction of the mediastinum framework, compatible with an active pulmonary process. ECG and cardiologic assessment are regular. Neurological evaluations reveal no attention disorder, eyes on axis, preserved ocular motility, no deficit signs of cranial motion nerves, no dysphagia, babbling language phase. Marked hypotonia and hypokinesia are confirmed and interested above all the trunk and limbs (especially lower). Reflexes are bilaterally absent on all landmarks, suggesting an hypotonic syndrome with impaired peripheral motor neuron. Cerebral ultrasound reveals the expansion of lateral ventricles in all coronal scans, interhemispheric fissure flaring with regular subarachnoidal periencephalic spaces. Metabolic investigations, EEG and brain MRI are normal. Electromyography shows neurogenic findings with both axonal and demyelinating aspects. Genetic tests confirm SMN1 (survival of motor neuron 1, telomeric) and NAIP genes deletion in homozygous state (PCR + enzyme digestion with MLPA). This mutation is identified in heterozygous state in both parents. The final diagnosis is spinal muscular atrophy type 1.

## ABS 50

### METABOLOMIC ANALYSIS OF NEWBORN'S URINE WITH CYTOMEGALOVIRUS CONGENITAL INFECTION. FROM THE DESCRIPTIVE TO THE PREDICTIVE ABILITY: PRELIMINARY RESULTS

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## BACKGROUND

Cytomegalovirus (CMV) is a DNA virus belonging to the family of herpes viruses. It is an ubiquitous virus that is found in the saliva, urine, secretions of the female genital tract, blood and breast milk and can be transmitted through the body fluids previously mentioned. Mother to child transmission can result from primary or non primary CMV infection during pregnancy, but primary infection carries the highest risk varying from 14.2 to 52.4%. The gold standard for the diagnosis of congenital CMV infection in newborns are the viral isolation or the use of the polymerase chain reaction in the urine or saliva within the first two weeks of life. Congenital CMV infection may result in multiorgan failure. 10-15% of these babies are symptomatic at birth with a perinatal mortality rate of approximately 10%. 70-80% of surviving babies will present neurological or sensorineural sequelae, namely mental retardation and deafness. However these disabilities can occur in 8-15% of infants asymptomatic at birth.

There are still unsolved questions concerning the prognosis and the follow up of asymptomatic subjects, that is due to the sometime late onset appearance of signs characterizing the disease, and among that those who will develop disabling sequelae [1]. So the detection of virological, laboratoristic or neuroradiological markers is very useful to compose a prognostic picture.

Subsequently the results obtained by Proton Nuclear Magnetic Resonance (<sup>1</sup>H-NMR) "metabolomics" in the characterization of the metabolic states of individuals with overt disease or subclinical disease [2-5].

## AIM

The aim of this study was to explore the metabolic differences between groups of children born with CMV infection which maybe asymptomatic, symptomatic at birth with or without sequelae and a group of children born without CMV infection, as control.

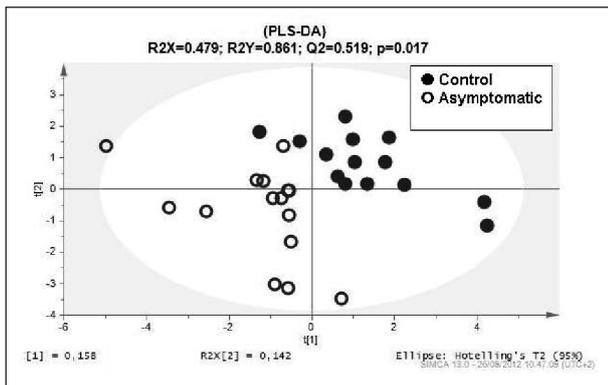
## METHODS

Urine samples were collected from 40 children. The samples were prepared aliquoting 540 µl of urine and adding 60 µl of phosphate buffer (1.5M, pH 7.4 with 10% Trimethylsilyl propanoic acid in D2O). Urines were analyzed using <sup>1</sup>H-NMR Varian 500 MHz (Fig. 1). NMR spectra were subjected to multivariate analysis in order to combine metabolic variables using SIMCA-P+ (version 13.0, Umetrics, Sweden).

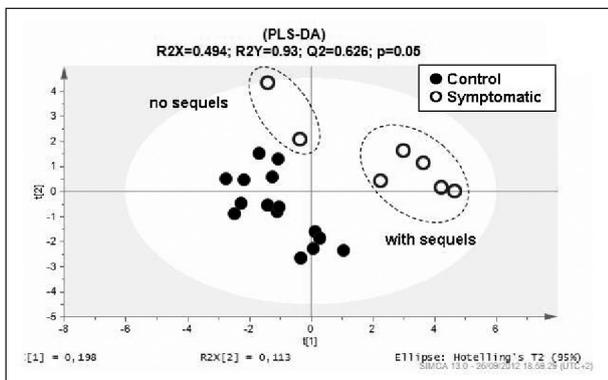
## RESULTS

Using a PLS-DA (Partial least squares discriminant analysis) model, we were able to discriminate

between the group of asymptomatic and the group of control (**Fig. 1**) and the group of symptomatic and the group of control (**Fig. 2**). As a matter of interest is the ability of the technique to characterize in the class of symptomatic those who have had serious complications from those that have an infection without complication. The mathematical models have pointed out the metabolites discriminating the groups.



**Figure 1.** PLS-DA model of CMV infected asymptomatic and control urine analysis.



**Figure 2.** PLS-DA model of CMV infected symptomatic with or without sequels and control urine analysis.

## CONCLUSION

Based on these preliminary results, the metabolomic approach seems to be promising for the study of the congenital CMV infection as well as for the identification of new predictors biomarkers characterizing the severity of the disease.

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## ABS 51

### CONGENITAL DACRYOCYSTOCELE: CASE REPORT

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Congenital dacryocystocele (congenital nasolacrimal sac mucocele) is an uncommon consequence of congenital nasolacrimal duct obstruction. It could be consequent to the obstruction of two sites in the nasolacrimal system: the Rosenmuller valve proximally, the Hasner valve distally. This obstruction is responsible of accumulation of fluid in the drainage system that is clinically evident from birth as a grey-blue cystic swelling just below the medial canthus.

Congenital dacryocystocele is prone to infection causing a form of neonatal dacrocystitis. Such infection at this age can be potentially life threatening due to the risk of sepsis and acute respiratory failure during feeding or sleeping, because neonates are necessarily nasal breathers.

Diagnosis is based on clinical appearance and imaging. It is possible even in prenatal time thanks to ultrasound (US). Other more invasive imaging assessments, necessary for diagnosis confirmation, are nasal endoscopic examination, computed tomography (CT) or magnetic resonance imaging (MRI), especially if the US diagnosis is not conclusive. The differential diagnosis includes haemangioma, encephalocele, glioma, dermoid cysts and malignant processes.

Treatment recommended is intranasal surgery to correct distal obstructions of the nasolacrimal duct, marsupialization of the cyst, balloon dilation, dacryocystoplasty, as first approach or if conservative approach (warm compresses, massage, medications) is inadequate.

We report one case of congenital dacryocystocele in a 4-days old female neonate. She presented

from birth a grey-blue cyst of the medial canthus of the right eye (**Fig. 1**). At nasal endoscopic examination we found a cystic swelling, covered by normal mucosa, obstructing inferior nasal meatus and causing respiratory distress. Diagnosis of dacryocystocele was confirmed by CT scan. The treatment performed was the marsupialization of the cyst, under general anesthesia and endoscopic vision (0° and 45°). During the procedure we observed emission of mucoid material associated with the immediate reduction of the external swelling. Rhinostomy results still canalized after two months after surgery.



**Figure 1.**

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## ABS 52

### A RARE CAUSE OF AIRWAY OBSTRUCTION IN CHILDHOOD: THE PHARYNGEAL TERATOMA. OUR EXPERIENCE

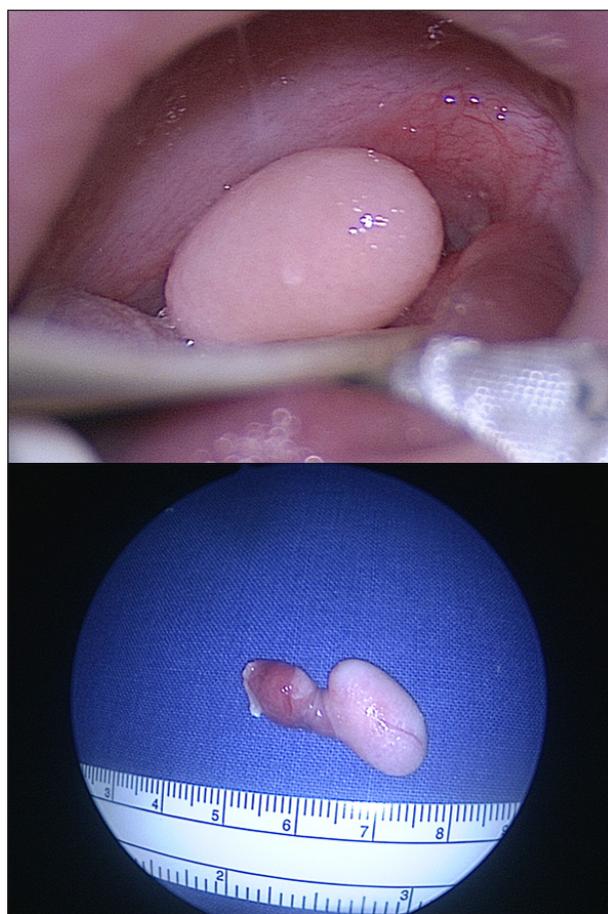
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Teratomas are the most common congenital tumors, but they are rare in the head and neck region (1-9%). The highest incidence is recorded in the neonatal period, although they have been reported rarely appeared in adolescence and adulthood. The symptoms are influenced by the anatomical site in which it develops.

Epipharyngeal lesions may cause unilateral or bilateral nasal obstruction, rhinolalia, snoring and sleep apnea, whereas dysphagia, odynophagia, earache and hoarseness are associated with meso- or hypopharyngeal lesions. Moreover they can cause acute upper airway obstruction and respiratory distress may be dramatic and require emergency measures to safeguarding the airway. The first reported case refers to a male patient of 9 months. The parents of patient reported nasal airway obstruction with mouth breathing, snoring and dysphagia. Physical examination showed an oval and yellow-pink lesion with a diameter of about 3 cm with the planting base on the side wall of the left naso-oropharynx (**Fig. 1**), near the left anterior palatine fold and behind the soft palate, obstructing almost totally breathing space. MRI confirmed the site of the lesion.

The second case refers to a female patient of only 35 days of life. The symptoms reported by the parents were recurrent apnea associated with snoring, perioral cyanosis and bradycardia. The ENT examination showed a lesion oval of the



**Figure 1.**

maximum diameter of 2 cm, implanted on the posterior palatal fold and on the left lateral wall of oropharynx. This lesion was flopping and caused obstruction of the laryngeal vestibule during spontaneous ventilation.

MRI confirmed the presence, in the oropharynx, a nodular oval lesion, about 2 cm in diameter, located postero-inferiorly epiglottis, confined above the glottic plane, with a tendency occlusive and occupying the right pyriform sinus.

Both patients underwent surgical excision of the lesion under general anesthesia, under endoscopic view with a 45° rigid endoscope.

The diagnosis of teratoma was histologically confirmed in both patients who are currently disease free.

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#### ABS 53

### OUTCOME IN CHILDREN WITH VESICO-URETERAL REFLUX: FROM ANTENATAL LIFE TO BLADDER TRAINING

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#### INTRODUCTION AND AIM OF THE STUDY

Vesicoureteral reflux (VUR) rate is around 1-2% (0.4-1.8%) in an unselected population, while it varies from up to 20% to 40% in newborns and in children who develop febrile urinary tract infection (UTI) during the first year of life. VUR may be associated with an impairment of the renal parenchyma, congenital or acquired (post-infective) known as reflux nephropathy. During fetal life, in response to the presence of obstructive impairment, there is an alteration of growth factors and molecules that regulate apoptosis leading to cellular damage. After birth, in course of UTI inflammation activates a mechanism of fibrosis which causes kidney damage and permanent scarring. The aim of this prospective study was to evaluate the validity of the conservative approach to VUR and provide practical guidance to manage the child with VUR and UTI.

#### MATERIALS AND METHODS

This study included 199 pts with primitive RVU aged from 0 to 24 (mean age 9.1 years) referred to our hospital between February 1993 and May 2012.

Of the 199 pts treated initially were enrolled in this study 181 pts (68 females and 113 males) with a total of 260 reflux units investigated. Patients were divided into two groups: group A includes children younger than 2 years, and group B with patients aged between 2 and 24 years. The group A was constituted by 20 pts (70% males and 30% females) while group B consists of 161 pts (61.5% males and 38.5% females). All 181 pts underwent ultrasound of urinary tract and VUR's diagnosis was made through a voiding cystourethrogram (VCUG). Kidney function was evaluated with renal scintigraphy and GFR.

#### RESULTS

Of the 181 pts with VUR, 80 (44.1%) received a prenatal diagnosis. Of the remaining 101 patients, (55.9%), 80/101 (79.2%) had a UTI. During follow-up about 60% of pts had normal renal function, 40% of pts had an initial functional deficit, showed no further reductions in renal function.

None of the 181 pts had chronic renal failure.

None of the pts of group A had episodes of UTI. Only 29/181 patients undergone surgery, mostly made an endoscopic treatment with bulking agents, other patients were treated with a vesicoureteral reimplantation.

The children who have not had prenatal diagnosis and which has not been prescribed prophylactic antibiotics after birth, developed febrile UTI in a percentage by 75% (group A) and 80.6% (group B). In children who have benefited from prenatal diagnosis and to whom has been given antibiotic prophylaxis, UTIs showed a rate of 9% (group A) and 26.7% (group B) (**Fig. 1**). No child had a decrease in renal function and even less developed ESRD. These results show also that the urotherapy allows to avoid recurrent UTIs associated with VUR.

#### CONCLUSION

The prognosis of a patient with VUR is difficult to calculate because it depends on many conditions.

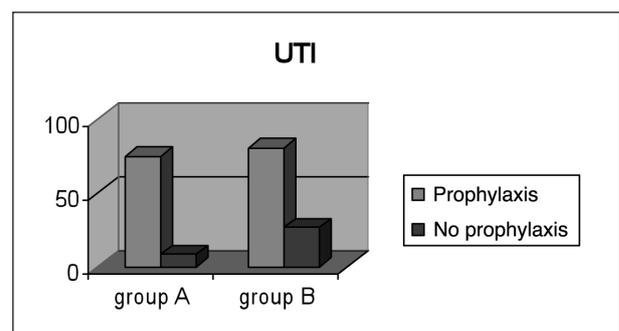


Figure 1.

Only selected cases require surgery. From the results of our study we cannot predict which patients will develop ESRD in adulthood. Prenatal diagnosis has a fundamental role to screen newborns with VUR and doctors may change the natural history of VUR by intervening in appropriate measure on any infections during the follow up in this group of patients and in the older patients by treating the dysfunctional voiding and constipation to prevent recurrent UTI.

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#### ABS 54

#### BLOOD AND MOLECULAR STUDY OF SARDINIAN NEWBORNS

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#### BACKGROUND

The WHO defines screening as the presumptive identification of a disease through the use of tests, examinations or other procedures that can be applied quickly. In the field of neonatal screening is the goal of a identify the conditions responsible for a genetic disease, so as to start an early treatment and prevent complications. Neonatal screening may be useful in identifying congenital blood disorders.

#### OBJECTIVES

The aim of the study was to define the reference values of haematological parameters of Sardinian babies. In addition, we evaluated the incidence of:  $\beta$ thalassemia ( $\beta$ 039); hyperbilirubinemia (S. Gilbert) and deficit of G6PD. The study was approved by the Ethics Committee of the University Hospital of Cagliari.

#### MATERIALS AND METHODS

We recruited 211 infants born at term (115M-96F), apparently healthy, with proper weight for gestational

age. For each patient were taken from 2 samples of peripheral blood, one in first and one in the third day of life. The first sample was performed: blood count, total bilirubin and fractionated, enzymatic analysis of G6PD, the second was measured total bilirubin and molecular investigations have been made.

#### RESULTS

RBC (x10<sup>6</sup>/mm<sup>3</sup>): 5.6 ± 0.6; Hb (g/dl): 19 ± 2.1; MCV (fl): 100 ± 8.3; MCH (pg) 34.4 ± 2.7; WBC (x10<sup>3</sup>/mm<sup>3</sup>): 21.6 ± 5.5; Neutral (x10<sup>3</sup>/mm<sup>3</sup>): 13.8 ± 4.6; PLT (x10<sup>3</sup>/mm<sup>3</sup>): 243 ± 63.

Dosage G6PD/6PGD:

- 14 deficient, 44 intermediate and 153 normal.

Dosage bilirubin (mg/dl):

- Tot 1<sup>st</sup> day: 4.5 ± 1.5; Dir 1<sup>st</sup> day: 0.5 ± 0.1;
- Tot 3<sup>rd</sup> day: 7.7 ± 3.3.

B039 mutation (%):

- Heterozygotes: 6; Normal: 94.

Gilbert's syndrome (%):

- Homozygote: 11; Heterozygotes: 40; Normal: 49.

#### CONCLUSIONS

Our data show no particular conditions of the hematologic phenotype of Sardinians infants. The Gilbert Syndrome showed a % of homozygous mutated lower compared to previous studies on the Sardinian population. The search for the Mediterranean variant of G6PD on the females has highlighted the importance of the test molecular in doubtful cases. With the full study we can have a clearer picture and we can assess the impact of rare diseases such as neutropenia and thrombocytopenia.

#### ABS 55

#### CORRELATIONS BETWEEN ATYPICAL SWALLOWING TRANSVERSE CONTRACTION OF THE MAXILLA AND OBSTRUCTIVE RESPIRATORY DISEASE. MULTIDISCIPLINARY APPROACH

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Several scientific studies have examined causes and effects of abnormalities in respiratory, masticatory, and swallowing function: the pathology of the so-called

functional triumvirate is expressed not only locally but affects the function of organs and systems that at first glance have nothing to do with the breathing, swallowing and mastication.

It has been found a correlation between the occurrence of recurrent otitis media, linked to a reduction of the transverse diameter of the upper jaw as a result of bad habits and breathing difficulties in pediatric patients. Infact, there are often present, in these subjects, changes in palatal morphology and accordingly, the insertions of the internal and external peristaphyline muscles on the palate. These anatomical and functional alterations of the palatal muscles can affect the efficiency of a secondary canal.

It is important to note that the orthodontist, in addition to intervening on the correction of the malocclusion, is able to modify the patency of the upper airway through the use of devices, such as a rapid palatal expander.

The authors will present their wide experience on this topic.

#### ABS 56

### PRESEPSIN VALUES IN HEALTHY MATURE AND NEAR-TERM NEONATES USING A NEW IMMUNOASSAY: A PRELIMINARY STUDY

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#### OBJECTIVE

The purpose of this preliminary study is to measure presepsin levels in plasma from healthy term and near term newborns at birth and at 3 days of life for comparison, using a chemiluminescent enzyme immunoassay (Pathfast®).

#### MATERIALS AND METHODS

Newborns were recruited in the delivery room and in the well baby nursery of the "G. Rummo" Hospital in Benevento, Italy. They were term or near term, apparently healthy. Blood specimens were obtained firstly at birth from cord and secondly from peripheral puncture on day three of life. Samples were processed with Pathfast®.

#### STATISTICAL ANALYSIS

Data are expressed as mean  $\pm$  SD. A Student's t test was used to compare the mean values of presepsin at birth and at the 3<sup>rd</sup> day. P value < 0.05 were considered significative.

#### RESULTS

A total of 64 blood sample were obtained at birth but only 28 samples were available at the 3<sup>rd</sup> day of life for presepsin analysis.

Mean presepsin value in cord blood was 953 pg/ml ( $\pm$  419), with an interquartile range of 661-1,114 whereas a mean value of 741 pg/ml ( $\pm$  316), and interquartile values from 490 to 937, was found in blood samples collected on the third day of life. There was no statistical difference between values (p = 0.12).

#### CONCLUSIONS

Our preliminary study indicates that healthy neonates have a production of presepsin that does not change in the first three days of life. Ongoing study will allow us to develop an age and gestational age related nomogram and to assess eventually a diagnostic role of presepsin in neonatal sepsis.

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#### ABS 57

### TERLIPRESSIN IN THE EXTREMELY LOW BIRTH WEIGHT INFANT WITH REFRACTORY SEPTIC SHOCK: A CASE REPORT

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Terlipressin, a synthetic long-acting analogue of vasopressin, has been investigated as a second line vasopressor in adults and children with refractory septic shock, i.e. not responding to fluid resuscitation and high-dose catecholamine administration. Little experience is available about the safety and efficacy of terlipressin in term and preterm newborns.

We report the case of an extremely low birth weight infant with severe septic shock, unresponsive to fluids, noradrenalin and hydrocortisone, in whom terlipressin was attempted as a rescue drug. Despite three doses of terlipressin, administered 6-hourly, the patient remained profoundly hypotensive and eventually died. Further studies are required before any recommendation on the use of terlipressin in term or preterm newborns with septic shock can be made.

## ABS 58

### PAROXYSMAL SUPRAVENTRICULAR RECIPROCATING TACHYCARDIA (PSVRT) IN THE NEWBORN: THREE YEARS EXPERIENCE IN A NEONATAL CARE UNIT

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## BACKGROUND

Paroxysmal supraventricular reciprocating tachycardia (PSVRT) is an arrhythmia rarely occurring during pediatric age but it is the most frequent heart rate disorder in neonates, characterized by a significant clinical, diagnostic and therapeutic complexity. Pharmacological therapy unresponsiveness and therapeutic side effects lead to a consistent difficulty in management of PSVRT during such a delicate period in life.

## STUDY POPULATION

From February 2009 to February 2012 the Neonatal Pathology Department admitted 10 neonates from 1 to 9 days of age, mean weight 3280 + 160 g, affected by PSVRT. Pregnancy medical history was uneventful in all 10 cases. In three cases a C-section for fetal suffering was necessary. Mean

cardiac frequency at hospital admissions (8 from other hospitals and 2 from home) was > 230/min, without any other symptoms nor cardiac structural abnormalities.

The therapeutic approach in the acute phase was the following: 1<sup>st</sup> step – diving reflex (ice bag). 2<sup>nd</sup> step – pharmacological cardioversion: flecainide (90 mg/mq); in case of unresponsiveness: adenosine IV (start dose 0.1 mg/kg – maximum dose 5 mg/kg). If failure: propafenone IV.

A good therapeutic response with an appropriate control of arrhythmia and absence of recrudescence was achieved in 8 patients, with cardiological follow up and maintaining therapy. Other two patients were transferred to a centre of excellence outside our Region.

## CONCLUSIONS

Our experience shows the high frequency of this form in our region. The complexity in the therapeutic approach of the neonatal PSVRT highlights the need for a close collaboration between neonatologists and pediatric cardiologists.

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## ABS 59

### COUNSELLING IN NICU: WHAT CAN WE DO?

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In a Neonatal Intensive Care Unit (NICU) the care is global, based on the newborn and on its family. This inseparable triad must be supported during the hospitalization both by the medical plan and the aware communication between the staff and those who receive the care.

The birth is physiologically accompanied by considerable expectations from parents, idyllic imagination and positive images. However, when something in birth goes wrong, as in the case of a very preterm birth, the abrupt change of perspective spreads a “grey” veil on the image that parents created. This “veil” makes it difficult to partially perceive ways of improvement. The arrival of a new newborn in the NICU leads to not only the infant’s entry but

the parents' too. It is a very delicate moment, rich in emotional tension: the father is usually the first one to be present at the door. He is very often alone, the entrance in NICU is disorientating, meeting with the baby and its image goes over every expectation. The father's and beyond the mother's reception is a crucial moment, the requested information is so vast, the risk to be misunderstood is very high. On one side, parents' precise requirements do exist, above all to understand what is happening, but also the necessity to get information (and therefore to recognize the right person to ask and to choose the questions). Besides, parents are asked to carry on an important task: to succeed in tolerating the wait (for examinations, checks, answers, etc.) and therefore to bear the uncertainty, also preserving hope. This task is difficult to face up alone: for this reason the project related to counselling at our Intensive Care Unit is called "Not alone".

For these requirements, the difficult objectives of the medical staff are:

- to find the correct words;
- to decide what to say;
- not to use a technical language but not to lose authoritativeness;
- to complete the information given by others;
- to understand if parents have understood or if they need more information;
- to inform without upsetting;
- not to delude, but also not to remove hope.

Counselling arises from the need to satisfy these requirements. Therefore counselling addresses to people (as infant's parents in Intensive Care Unit) who are living a difficult or unexpected situation and they are able neither to modify nor to avoid it. They do not necessarily ask for a psychological intervention, but they have demand for information, orientation, someone who knows how to let them see the resources that they really have, to be able to find sustainable solutions and to face up to their emotions [1-4].

Counselling is developed by all the professionals that intervene in that situation or that are involved to face up to those requirements of information, explanations, facility of decisions, finding of resources, agreement, help, reassurance, attention.

In medicine an important aim of counselling is to act as a "resiliency" factor, which supports parents' passage from the "victim's" to an "active" role. A shocking event (such as preterm birth) physiologically and inevitably produces parents' reactions as incredulity, followed by anger, mistrust and sense of guilt.

If parents are left alone to face up to such a situation, they will inevitably go to search for causes of what

has happened and on this search they do not get over the situation. It is a faulty circle that departs from the emphasis on the past and it stops on the feeling of anger, impotence, report, with the parents' risk to take the role of victims looking for revenge and medical-legal consequences.

Instead, if parents are assisted with support, the passage to confront each other is more probable as the awareness of what has happened and the projection toward the future; they acquire an active role and become responsible in care.

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#### ABS 60

#### COUNSELLING IN NICU: THE "NOT ALONE" PROJECT

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The project "Not alone", put into work thanks to the collaboration of the institute of systemic-counselling "Change", contemplates various aspects, all to let counselling become a shared culture [1].

The first essential aspect is to form the ability of counselling through periodic courses for all professionals of the department (physicians, nurses, physiotherapists). Obviously, the extension of abilities of counselling is useful to other professionals (experts, advisors) and to other departments more frequently involved. In our department a professional counsellor is present assisting the medical staff in direct counselling. Parents are directly contacted in our a department, or on announcement of the medical staff or from the counsellor. Many couples accept the meetings proposed.

In a moment full of emotions, information is sometimes unclear; when a new life breaks into everyday life, the support of this figure, speaking about their living, helping parents to understand the situation, is a kind of well accepted help. The counsellor's intervention has also been helpful also for other professionals: psychologists, neuropsychiatrists, etc., when initially the necessity to be helped was denied.

The counsellor's intervention allows a better parent orientation in the situation. A more effective sharing of these rules also facilitates the communication among parents and medical staff.

Periodic meetings are established among the medical staff, in which the professional counsellor discusses difficult situations in order to share possible communicative strategies.

We wanted to have not only a common communicative style, but also common subjects, independent from the characteristics of each of us. Individuals are often faced with diverse situations. For every setting that we more frequently face in communication (for example the first interview with a parent of a very preterm infant) we have built an "algorithm" that follows a pattern: 1) information always given; 2) frequent questions from parents; 3) frequent difficulties in the communication. The draft of the algorithm has been introduced then to all the professionals, who gave a vote from 1 to 4 to every point of the algorithm, according to their smaller or greater agreement with the proposed solutions. The most critical points have been discussed and we have arrived at a final solution.

To put the communication into effect would also need to record important moments, for instance the "case history of the communication", a tool that we are still studying: in fact it would be desirable to have the case history, a sheet dedicated to important communications that are absolutely to be shared with other professionals. Counselling for us has also been something more. The following phase has been to face the critical moments, the new steps.

In this case counselling becomes "element of development". The decision to open the department to parents 24 h on 24 has been an important moment in our reality.

When a baby is born, its parents bind it to the world. If during the care process there is the possibility of not separating them, the future behavior really changes: the newborn is stronger and the time of hospitalization decreases. To make it happen we must consider parents' life outside our Intensive Care Unit: some couples have got other children at home, the father usually works, moving in the city takes a long time. Based on these requests, the Care group of our NICU proposed to the medical staff and nurses to open of the department 24 h on 24. In the months that preceded the NICU's opening, there have been a series of meetings in which the individual brought to the whole meeting own ideas and fears, absolutely justified. We must not forget that to be under the continuous surveillance of someone leads us to be judged, to lose own

distances and perhaps the own "power". However, this introduction has not weakened the discussion, rather it has made it charming and rich of contents. The counsellor's presence and mediation allowed to find the resources in each individual and in the group and also the correct objective. The rules are written in a brochure that is given to parents by the counsellor that first welcomes them, and then are commented and discussed by the counsellor in the first meeting with parents: it is a questionnaire, including those questions that could be addressed by parents, and the relative answers.

Our group is constituted by imperfect professionals, but this group aims to learn and to look for new solutions for newborns and their families. We try to take advantage of an imperfect result, but we test and try every valid project for the health of those whom we care.

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#### ABS 61

#### NEONATAL MALPRACTICE CLAIMS RELATED TO HYPERBILIRUBINEMIA IN ITALY

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All are the members of the National Commission on Pediatric Risk Management of the Italian Society of Pediatrics

#### BACKGROUND

Medical errors continue to capture the attention of the public, of the policymakers and of the physicians as well. We report the first data obtained in Italy on neonatology regarding a wide population study. Malpractice data can be used to identify problem-prone clinical processes and suggest interventions that may reduce negligence.

#### STUDY DESIGN

We conducted a retrospective, descriptive analysis of a nationwide database on neonatal malpractice

claims, in which patients alleged an error. The Italian Society of Pediatrics (Società Italiana di Pediatria: SIP) has developed a link – through insurance broker Willis Italian SpA – with an insurance company (Carige Assicurazioni SpA) that insure 60% of all pediatricians of the Italian Society of Pediatrics (4,650 over 7,750). We had the permission from Carige Assicurazioni to work on their database that pools information to be utilized.

We asked Willis to perform a query of its database, looking at malpractice claims related to hyperbilirubinemia reported between January 1, 2005 and December 31, 2010 involving perinatologists and neonatologists.

We defined a claim as: any written demand for compensation referred to the pediatrician or the institution where the pediatrician is on charge, with no legal paper having been filed in court, any notice of indictment or notice of investigation by criminal law, any circumstance where the pediatrician is aware of a medical liability action.

#### RESULTS

During a 6-year period (2005–2010), there were 661 claims reported to the Willis insurance of SIP with 4,650 pediatricians insured. The pediatric area was involved in 470 claims; the neonatal area in 191. It must be underlined that each claim interested one or more physicians.

The claims regarding hyperbilirubinemia were 8 and they are reported in **Tab. 1**, as they have been presented by the lawyers.

#### CONCLUSIONS

The neurologic injury is one of the principal drivers of high medical malpractice coverage costs for hospitals and physicians and in many occasions is not related to real negligence of the neonatologist, especially in very low weight newborns. Our data, to our knowledge the first available in Europe for neonatology, provide an update photograph of the situation in Italy, allowing an analysis of main causes and suggesting how to face the problem.

**Table 1.** 8 out of 191 claims in the neonatal area during a 6-year period, related to hyperbilirubinemia.

1.	Exchange trasnfusion not performed (atetosis)
2.	Absence of monitoring with subsequence neurologic damage
3.	Hyperbilirubinemia-associated deafness
4.	Permanent neurological damage and deafness
5.	Breast milk associated hyperbilirubinema
6.	Delayed beginning of phototherapy
7.	Kernicterus
8.	Discharge with hyperbilirubinemia by one Unit and readmission in another Unit

Hyperbilirubinemia represents the cause of claims in 4.2 of patients. This number is probably undervaluated and this percentage will probably tend to increase with time.

Long-term monitoring of the situation will allow to understand the final result of the claim and to definitively inform neonatologist about their daily risks in order to prevent and solve legal problems.

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#### ABS 62

#### DETECTION OF BOVINE ALPHA-S1-CASEIN AND MINOR COMPONENTS IN HUMAN MILK BY PROTEOMICS TECHNIQUES

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#### INTRODUCTION

Cow's milk contains more than twenty five different proteins, but only whey proteins  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, bovine serum albumin (BSA), and lactoferrin, as well as the four caseins, have been identified as allergens [1]. In particular, the casein fraction is composed of  $\alpha$ S1-,  $\alpha$ S2-,  $\beta$ -, and  $\kappa$ -casein, of which  $\alpha$ S1-casein seems to be the major allergen according to IgE and T cell recognition data [2-4]. By now the detection of the above mentioned food allergens in human milk has been achieved by sandwich Enzyme-Linked Immunosorbent Assay (ELISA) and Immunoblotting techniques. [3]. ELISA and Immunoblotting techniques have the disadvantage of possible cross reactions with other proteins that could be present in the sample and don't take into account the possible chemical modification or proteolytic digestion of the proteins [5].

Aim of this study was to investigate cow's milk allergens in human colostrum of term and preterm newborns' mothers, and other minor protein components by proteomics techniques in order to

understand if cow's milk allergens could be a cause of sensitization established through lactation.

#### MATERIALS AND METHODS

Term colostrum samples were collected from 62 healthy mothers delivering at term (36 completed week of gestation).

Preterm colostrum samples were collected from eleven healthy mothers who delivered prematurely (between 25 and 30 weeks of gestational age).

All samples were collected from mothers following a non restricted diet including cow's milk and derivatives, just after breastfeeding their own babies. Samples were gathered up until the 4<sup>th</sup> day after delivery and frozen and stored at -80°C immediately after collection. Before analysis samples were defrosted at room temperature. Two different pools of 245 ml were created, respectively one of Colostrum of mothers delivering Term infants (CT) and another one of Colostrum of mothers delivering Preterm infants (CP). Then in each pool (CT and CP) five complete protease inhibitor cocktail tablets were added.

The sensitive detection of less represented new types of proteins contained in the whey fraction was made through the introduction of an additional pre-fractionation step. This step theoretically increases the concentration of the most diluted proteins and simultaneously reduces the concentration of the proteins present at high concentration (Proteominer Treatment).

Subsequently each sample were subjected to the steps of proteomic techniques (loading on gel, image acquisition by proteomic imaging system, in-gel tryptic digestion of proteins and protein identification by tandem mass spectrometry).

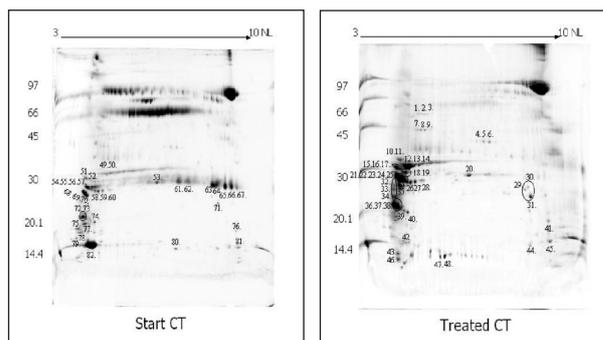
#### RESULTS

The most important result achieved in this study is the detection in human colostrums of one of the major allergens found in bovine milk: the intact isoform of bovine alpha-S1-casein (spot n. 39 in **Fig. 1**).

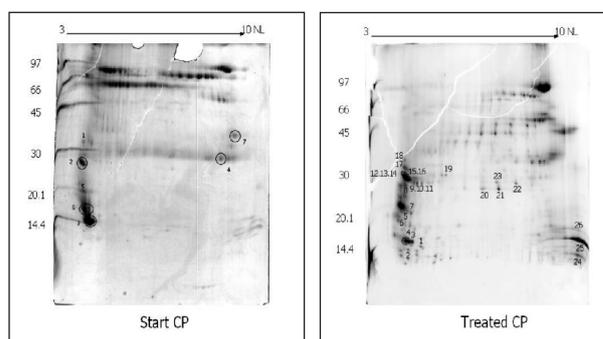
Also in the preterm colostrum sample, most of the proteins identified were different isoforms of caseins not previously reported in literature (spot n. 1 and 18 in **Fig. 2** and **Tab. 1**).

The identification of alpha-S1-casein, the bovine allergenic protein in human colostrum, was possible due to the higher sensitivity, the specificity and the reproducibility of the proteomics technique: for the first time such protein was detected using mass spectrometry based methods, that are more reliable than immunoassay methods, used in the past.

Although the number of unknown proteins detected in human colostrum is not as striking as other



**Figure 1.** 2D-Page map of 120µg of protein in Start and Treated Term colostrum samples (CT).



**Figure 2.** 2D-Page map of 120µg of protein in Start and Treated Preterm colostrum samples (CP).

studies previously suggested, it is possible to say that galectin-7, the different isoforms of the 14-3-3 protein and the serum amyloid P-component are present in human term and preterm colostrum milk at very low concentration (**Fig. 1** and **Fig. 2**).

#### DISCUSSION

The detection of the intact bovine alpha-S1casein in human colostrum was the most relevant finding in this experience. In previous studies  $\beta$ -lactoglobulin, [6] one of the major cow's milk allergen, was detected intact in human milk by immunoassay based techniques which are very sensitive methods of detection of food allergens (the estimated concentrations of nanograms per litre during their peak concentration) [6-8].

However, these techniques have the disadvantage of cross reacting with other proteins that could be present in the sample [5]. Moreover, these methods don't take into account the possible chemical modification or proteolytic digestion of proteins [5] focusing on the detection of the intact protein.

On the contrary in our study, using ProteoMiner techniques,  $\beta$ -lactoglobulin was not detected in human colostrum either in term either in preterm infants. In fact mass spectrometry methods have high

**Table 1.** Identification of alpha-S1-casein from start CP, treated CP and treated CT.

Spot no.	Sample Name	Protein name	UniProtKB/Swiss-Prot Entry name	UniProtKB/Swiss-Prot Accession number	Protein p.I.	Protein sequence coverage
39	Treated CT	Alpha-S1-casein	CASA1_BOVIN	P02662	4.98	14%
1	Start CP					17%
18	Treated CP					26%

**Table 1 (continued).** Identification of alpha-S1-casein from start CP, treated CP and treated CT.

Spot no.	Peptide sequence
39	FFVAPFPEVFGK.E (38-49) EPMIGVNQELAYFYPELFR.Q (148-166)
1	HQQLPQEVNENLLR.F (23-37) FFVAPFPEVFGK.E (38-49) YLGYLEQLLR.L (106-115)
18	HQQLPQEVNENLLR.F (23-37) FFVAPFPEVFGK.E (38-49) YLGYLEQLLR.L (106-115) EPMIGVNQELAYFYPELFR.Q (148-166)

sensitivity, specificity and reproducibility and using a prefractionation step they can be considered more reliable than immunoassay methods in the detection of hidden allergens at very low concentration.

Thus, according to our results bovine alpha-S1-casein could be considered the cow's milk allergen that is readily secreted in human milk and could be a cause of sensitization to cow's milk in exclusively breastfed predisposed infants.

A higher concentration of bovine alpha-S1-casein observed in preterm colostrum compared to term colostrum samples. A possible explanation could be the different membrane permeability observed in mothers who delivered prematurely. However, a definitive explanation of this observation is lacking.

Has alpha-S1-casein a major role in sensitization to cow's milk of exclusively breastfed predisposed infants? Further investigations are needed.

Galectin-7 belongs to the family of  $\beta$ -galactoside binding lectins that are predominantly expressed in stratified epithelia, especially in epidermis. It is involved in the regulation of the normal cell growth (through the regulation of the cell to cell and/or cell to matrix interactions) and presents a pro-apoptotic function and in the regulation of tissue homeostasis.

We assumed that galectin-7 and 14-3-3 protein isoforms are associated with the small cell component present in human colostrum milk that comes from the apoptotic process of milk secretion during lactation [9].

The only previously undetected protein that we only found in colostrum collected from mothers delivering prematurely was the Serum amyloid P-component (SAP), a glycoprotein found in serum and urine: its tertiary structure is similar to the legume lectins. SAP has the ability to bind a variety of ligands including other proteins, glycosaminoglycans and DNA. The functions of lectins are not well known, although recently studies showed that mammalian homologues of the leguminous plant lectins seem to mediate intracellular sorting of glycoproteins in the endoplasmic reticulum and Golgi. In conclusion the functions of the SAP in human milk are not well known, but we could assume that it might have some protective role such as ascribed to lectins.

Further investigations are needed to understand if these families of proteins have specific biological activity in human milk [10], including metabolomics in human milk and formula [11].

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## ABS 63

### IS SOLUBLE CD14 SUBTYPE (sCD14-ST) PRESEPSIN AN EARLY BIOMARKER OF NEONATAL SEPSIS? YES IT IS

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#### BACKGROUND

Neonatal sepsis continues to be one of the most significant causes of neonatal morbidity and mortality. Early identification of neonatal sepsis is a major diagnostic problem because of the nonspecific clinical signs and limitations of the current diagnostic procedures. Recently, it was reported that the soluble fraction of CD14 might be a very early, specific biomarker of systemic inflammation and sepsis due to bacterial infection. CD14 is a glycoprotein expressed on the surface membrane of monocytes/macrophages (mCD14) and serves as bacterial lipopolysaccharides receptor. The complex LPS-CD14 (-LBP) is released into circulation, where plasma protease activity originates the soluble CD14 subtype (sCD14-ST) or presepsin. The commercial availability of a very rapid and accurate analytical method for measuring sCD14-ST presepsin calls for clinical studies investigating the potential role of this biomarker in patients with systemic inflammation, sepsis, and severe sepsis. Moreover, there is the need to assess the potential role of sCD14-ST presepsin in predicting outcome in comparison with traditional sepsis and inflammation biomarkers.

#### OBJECTIVE

The aim of this study was to evaluate the clinical value of sCD14-ST presepsin in critically ill newborns, admitted in Neonatal Intensive Care Unit (NICU).

#### METHODS

This preliminary study was performed on 87 samples

belonging to 26 newborns with gestational age ranging 26 to 41 weeks, admitted on the Pediatric Division, Cagliari. Newborns were divided in two groups: 10 newborns with systemic inflammation/sepsis microbiologically confirmed (group A) and 16 without sepsis (group B). The groups included both term and preterm newborn. In all the samples we measured C-Reactive Protein (CRP) and sCD14-ST presepsin. CRP was measured by immunonephelometry on the BN II (Siemens Healthcare Diagnostics, Milan, Italy); sCD14-ST was measured by a rapid chemiluminescent enzyme immunoassay on the fully automated PATHFAST® immunoanalyzer (Mitsubishi Chemical Corporation, Tokyo, Japan).

#### RESULTS

In group A, CRP and sCD14-ST mean values were 65 mg/l and 2,411 pg/l, respectively. In group B, CRP and sCD14-ST mean values were 5.7 mg/l and 899.0 pg/l, respectively. sCD14-ST seems to be a good marker for risk stratification in preterm patients. As showed in **Tab. 1**. Our preliminary results suggest a potential interesting prognostic value for sCD14-ST presepsin. Here we show data on preterm infants.

**Table 1.** sCD14-ST as stratification risk marker in preterm.

Presepsin as a marker of risk stratification in preterm neonates	
Severe sepsis	1000-2000 pg/ml
Septic shock	2001-5000 pg/ml
Multi Organ Failure (MOF)	> 5000 pg/ml

In particular, sCD14-ST strongly correlated with the severity of sepsis in all preterm babies. More important, sCD14-ST did not significantly increase in non-septic condition.

#### CONCLUSION

These results may support the hypothesis on the high specificity of this new marker in assessing bacterial infections and sepsis.

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