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Selected Abstracts of the 11th International Workshop on Neonatology

FROM THE WOMB TO THE ADULT

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The Workshop has been organized with the patronage of the Italian Society of Neonatology (SIN), the Italian Society of Pediatrics (SIP), the Italian Society of Perinatal Medicine (SIMP), The Italian Federation of Pediatricians (FIMP), the Union of European Neonatal and Perinatal Societies (UENPS), the Union of Mediterranean Neonatal Societies (UMENS), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and lastly the Italian National Observatory of Residents in Paediatrics (ONSP).

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ABS 1

POST-2015 DEVELOPMENT AGENDA: FROM THE MILLENNIUM DEVELOPMENT GOALS TO THE 2030 AGENDA FOR SUSTAINABLE DEVELOPMENT

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At the Sustainable Development Summit on 25 September, 2015, UN Member States adopted the 2030 Agenda for Sustainable Development. The agenda consists of a set of 17 Sustainable Development Goals (SDGs), otherwise known as the Global Goals, to stop misery, fight inequality and injustice, and tackle climate change by 2030. This Agenda is a plan of action for people, planet and prosperity. Each goal consists of targets that have to be reached by a specified time. The most distinctive and innovative attribute of the SDGs is its basic principle of interconnectedness. No goal stands alone, that means: "Every goal is inextricably linked to the rest". Although only one of the SDGs dealt with health (Goal 3: ensure healthy lives and promote well-being for all at all ages), it is clearly understandable that health has a central role. A closer look shows that the single overarching SDG health goal has 13 targets, which cover not only the targets of the MDGs (Millennium Development Goals) health goals, but also health concerns sorely missing from the MDGs in the view of many health observers, such as non-communicable diseases (NCDs), mental ill health, road accident injuries and, most importantly, universal health care, which the SDGs agenda regards as essential for promoting physical and mental health. Looking at the other 16 goals, health is implicitly linked to just about all of them, such as poverty, financial protection, disaster risks, clean drinking water and sanitation, climate change, and so on. They're all linked to health. The most significant targets of goal 3 are: (a) by 2030, reduce the global maternal mortality ratio to less than 70 per 100,000 live births; (b) by 2030, end preventable deaths of newborns and children under 5 years of age, with all countries aiming to reduce neonatal mortality to at least as low as 12 per 1,000 live births and under-5 mortality to at least as low as 25 per 1,000 live births; (c) by 2030, end the epidemics of AIDS, tuberculosis, malaria and neglected tropical diseases and combat hepatitis, water-borne diseases and other communicable diseases; (d) by 2030, reduce by one third premature mortality from non-communicable diseases through prevention and treatment and promote mental health and well-being; (e) support the research and development of vaccines and medicines for the communicable and non-communicable diseases that primarily affect developing countries, provide access to affordable essential medicines and vaccines, in accordance with the Doha Declaration on the Trade-Related Aspects of Intellectual Property Rights (TRIPS) Agreement and Public Health, which affirms the right of developing countries to use to the full the provisions in the Agreement on TRIPS regarding flexibilities to protect public health, and, in particular, provide access to medicines for all; (f) reinforce the capacity of all countries, mostly developing countries, for early warning, risk reduction and management of national and global health risks. A remarkable driver for pursuing successfully these targets is laboratory medicine; it is called to play a strategic role for searching innovative biomarkers for reducing maternal, neonatal and under-5 deaths. Industry should cooperate with laboratory medicine for reducing the gap between bench and bedside with the ultimate goal to make available new diagnostic tools, namely low-cost disposables simply ready/fit for use by everyone worldwide.

ABS 2

MILD RECTAL BLEEDING IN PRETERM INFANTS: A SIGNIFICANT PROBLEM IN NEONATOLOGY?

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Intestinal immaturity in very low birth weight (VLBW) infants causes digestive disorders that can reduce our ability to provide preterm babies with

sufficient amount of nutrients. Postnatal growth has been associated with further cognitive development of VLBW infants. The scope of digestive troubles in VLBW infants is quite wide, ranging from feeding intolerance (gastric residuals, abdominal distension) to severe necrotising enterocolitis (NEC) (≥ stage II) [1]. NEC has been extensively studied and preventive strategies have been proposed. Nowadays the prevalence of this type of digestive disorders is low (3 to 10% depending on gestational age at birth). The other disorders are less severe but are much more frequent. Among these disorders, mild rectal bleeding – either completely isolated or associated with clinical or radiological signs – is a peculiar entity (sometimes considered as "suspected" NEC or NEC stage Ib) [2, 3].

During a 3-year period, we observed that 9% of the 823 VLBW infants hospitalized in our unit had rectal bleeding [4]. About two babies out of three had rectal bleeding associated with clinical or radiological signs. There are discussions about the best way to take care of preterm infants with mild rectal bleeding, and some are proposing to avoid the fasting period. In our experience, when the rectal bleeding is occurring it is often difficult to be sure that it is not the first sign of a NEC. In our unit, the median fasting period was 2.9 days. We observed that maternal hypertension, postnatal growth restriction at onset of bleeding and exposure to ibuprofen were independent risk factors for mild rectal bleeding. Therefore, it could be proposed to monitor carefully the digestive tolerance of VLBW from mothers with treated hypertension, but also to limit the ibuprofen use to the situations where significant benefit is expected from the treatment, and to optimize nutrition and growth.

The altered composition of microbiota has been cited as a risk factor for NEC in premature infants [5]. Anaerobic bacteria like *Clostridia spp.* have been implicated in the occurrence of NEC, but most recent data suggested that the occurrence of a dysmicrobism could be more important than the presence of one or the other microorganism [5, 6]. When we investigated the gut microbiota in VLBW infants with mild rectal bleeding, we rarely found *Cl. difficile*, but we reported significant differences from the usual evolution of gut microbiota [7, 8].

We observed an imbalance in gut microbiota, as the proportion of infants with *E. coli* was significantly higher in infants with rectal bleeding and the opposite pattern occurred for *Staphylococcus spp.* [9]. The significant increase in *E. coli* is of particular interest as there are several genotypes (pathovars) that display particular pathogen behaviors. Classical enteropathogenic pathovars, considered to be strict pathogens, are rarely involved in the infections of infants hospitalized in neonatal intensive care units. However, some genotypes seem to display virulence that differs from the classical acute gastroenteritis associated with most enteropathogenic pathovars. An enterohemorrhagic strain has been associated with a case of NEC [10], and a recently described pathovar, diffusely adherent *E. coli*, harbors genes associated with virulence [11]. Consequently, it could exhibit an opportunistic behavior linked to dysmicrobism, an increased representation in gut microflora leading to symptoms.

Mild rectal bleeding is an entity which is frequent and may have a significant negative impact on the care of VLBW infants. It could be useful to take in account the identified risk factors to prevent mild rectal bleeding. As for NEC, there is probably a dysmicrobism in preterm infants with rectal bleeding.

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ABS 3

SYPHILIS IN PREGNANT ADOLESCENTS: THE CURRENT SITUATION IN THE STATE OF RIO DE JANEIRO

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BACKGROUND
Brazil has high prevalence of syphilis in pregnant women, despite government strategies to reduce syphilis, and for prenatal care and childbirth, as the Rede Cegonha. A recent Brazilian study (2012) points out a national prevalence of 1.02%. The state of Rio de Janeiro (RJ) has the second highest detection rate, 10.8/1,000 live births (NV). Lacking specific studies on syphilis in pregnant adolescents.
AIM
The aim was to analyze the panorama of syphilis among pregnant adolescents in Rio de Janeiro state through the analysis of the state Department database health between 2007-2013, relating to 12 137 cases. They were considered detection rate, skin color, trimester diagnostic, diagnostic classification and partner treatment.
RESULTS
The aged 10-14 detection rate was 16.1/1,000 live births; 15-19 years was 12.1/1,000 live births and 20-49 years, rates ranged from 5.1 to 8.1/1,000 live births. Black pregnant women aged 10-14 accounted for 37.4% of cases, 27.7% of 15-19 years and 20-49 years, 18.6%. The largest proportion of diagnoses occurred in the second trimester: 31.7% for pregnant women aged 10-14 with gradual reduction in other age groups. The stage classification was ignored for more than 50% in almost all age groups. Only 7.4% of partners were treated in pregnant women aged 10-14, 8.5% of 15-19 years and in adults ranged from 6.1 to 7.9%.
CONCLUSION
We concluded that pregnant adolescents are more vulnerable to the occurrence of syphilis, being necessary attention on the programs of prevention, sexual and reproductive education.

ABS 4

HOLDER PASTEURIZATION DOES NOT AFFECT S100B CONCENTRATIONS IN HUMAN MILK

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BACKGROUND
Donor milk (DM) represents an important nutrition source for high-risk newborns. Holder pasteurization (HoP) is, to date, the most recommended procedure for DM treatment, providing a good compromise between microbiological safety and biological quality. HoP was previously shown to affect DM cytokines, growth factors and hormones levels, whilst no data concerning the possible effects of HoP on neurobiomarkers (NB) are available.
AIM
Therefore, our study investigated whether the concentration in DM of a well-known NB involved in brain development/damage, namely S100B, changes due to HoP.
METHODS
We conducted a pretest-test study in 11 mothers, whom DM samples were sub-divided into two aliquots: the first (NO-HoP group) was immediately frozen (-80°C); the second was pasteurized (HoP group) before freezing at -80°C. S100B DM levels were measured in the two groups using a commercially available immunoluminometric assay.
RESULTS
Results showed no significant differences between groups ($P > 0.05$) in S100B levels measured in colostrum, transitional and mature DM.
CONCLUSIONS
Our data confirm the stability of S100B at high/low temperatures and pasteurization stresses and provide evidence that its neurotrophic role is not affected by HoP. These findings support a wider DM use for newborns feeding and HoP as the best compromise between DM safety/quality.

ABS 5

EFFECTS OF HOLDER PASTEURIZATION ON THE PROTEIN PROFILE OF HUMAN MILK

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BACKGROUND

The most widespread method for the treatment of donor milk is the Holder pasteurization (HoP). The available literature data show that HoP may cause degradation of some bioactive components.

AIM

The aim of this study was to determine the effect of HoP on the protein profile of human milk (HM) using a GeLC-MS method, a proteomic approach and a promising technique able to offer a qualitative HM protein profile.

METHODS

HM samples were collected by standardized methods from 20 mothers carrying both preterm and term newborns. A aliquot of each sample was immediately frozen at -80°C, whilst another one was Holder pasteurized and then frozen. All samples were then analyzed by GeLC-MS. The protein bands of interest were excised from the gel, digested with trypsin and identified by nano-HPLC-MS/MS analysis.

RESULTS

The protein profile before and after HoP showed qualitative differences only in 6 samples out of 20, while in the remaining 14 no detectable differences were found. The differences interested only colostrum and transitional milk samples and regarded the decrease of the electrophoretic bands corresponding to alpha and beta-casein, tenascin, lactoferrin and immunoglobulin.

CONCLUSIONS

In the majority of samples, HoP did not cause any modification, thereby preserving the biological activity of HM proteins.

ABS 6

ULTRASOUND AND FETAL MRI CORRELATION IN FETAL NEURORADIOLOGY. OUR RESULTS AFTER TEN YEARS OF EXPERIENCE

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The most common use of fetal MRI has been to evaluate central nervous system (CNS) alterations: in this setting it has been found to influence or change the management and counseling in up to 1 out of 2 cases. MRI is particularly useful in looking at the posterior fossa, corpus callosum, and gray-white matter. Levine et al. found that MRI changed the US diagnosis of CNS anomalies in 26 of 66 (40%) and changed patient counseling in 33 of 66 (55%). Similarly Simon et al. observed that 24 of 52 (46%) fetuses with CNS abnormalities were managed differently after the execution of MRI studies. Our experience matches those results in terms of diagnostic sensitivity of MRI, confirmed by MRI diagnostic imaging after birth or pathologic speciements studies. Thus, this report echoes the conclusions of the others that MRI is an effective adjunct to US in the evaluation of CNS abnormalities and is particularly useful in providing additional information and allowing more definitive diagnoses than by US alone.

ABS 7

10 YEARS EXPERIENCE OF NEWBORN HEARING SCREENING SURVEY

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BACKGROUND

The newborn Hearing Screening Survey is the principal source of information on implementation and coverage of newborn hearing screening in Italy and is one of the major data collection programs of the Italian Institute of Social Medicine (ISFOL).

The Hearing Screening Survey Act of 2003 provided for a bi-annual survey and studies to secure accurate and current statistical information on the amount, distribution, and effects of illness and disability of hearing loss in Italy.

The data are used by the public health research community for epidemiologic appropriate health care, and evaluating Regional health programs.

MATERIAL

All Birth Hospitals were enrolled and newborn screening programs active as of September 30 2014 provided data for this study.

METHODS
Data were collected through a Screening Survey Questionnaire that was sent to all Birthing Hospitals active in Italy in 2014 and were filled in by the chief of the Hospital or by the UNHS program coordinator.
RESULTS
In 2014, the average coverage rate was 90.9%. In twelve out of 20 Italian Regions the coverage was greater than 95%.
Coverage rate was greater in Regions with implemented NHS legislation than in Regions without legislation. As a matter of fact, Regions which passed NHS legislation screened at more than 95% of infants, whereas Regions without legislation reported a mean screening rate of nearly 67% of newborns.
CONCLUSION
In the last 10 years universal newborn hearing screening was implemented in an increasing number of hospitals in Italy.
Current results seem to suggest that legislation might have a positive effect on increase of rate of coverage of newborn hearing screening in Italy.

ABS 8

NEWBORN WITH MULTIPLE BONE FRACTURES

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INTRODUCTION
Osteogenesis imperfecta (OI) is a genetic disease of the connective tissue, characterized by bone fragility, skeletal deformities and variable short stature. The typical phenotypic variability contributes to it's classification in different subtypes.
CLINICAL CASE
We present a case of a female newborn, irrelevant family history and non-consanguineous parents, with the prenatal diagnosis of intrauterine growth restriction and short lower limbs. She was born at 39 weeks by caesarean section with low Apgar score (3/9/10), requiring resuscitation and hospitalization in NICU for hypoxemia and deformity of the face and limbs. She presented multiple bone fractures on radiography and normal phosphocalcic balance and hydroelectrolytic equilibrium. At the fourth day of live, she was transferred to tertiary hospital for proper orientation. Temporary invasive ventilation was needed for sedoanalgesia. The skelectal radiography confirmed multiple fractures in the skull, ribs and limbs in various stages of consolidation (**Fig. 1** and **Fig. 2**). Given the clinical diagnosis of OI type IIA and after multidisciplinary assessment, palliative care was initiated until the death of the newborn on the 29th day of life. Histopathological examination revealed severe pulmonary hypoplasia and skeletal ostochondrodysplasia (lethal form) with multiple fractures, many occurring during intrauterine life, pointing to the diagnosis of OI type II (Sillence classification). No mutations in the COL1A1 and COL1A2 genes was found. Pending genetic study of recessive mutations.
CONCLUSION
The authors emphasize the rarity and severity of the OI type II. Its treatment is merely supportive, due to pulmonary hypoplasia and multiple fractures. The mutational study is important for genetic counselling.

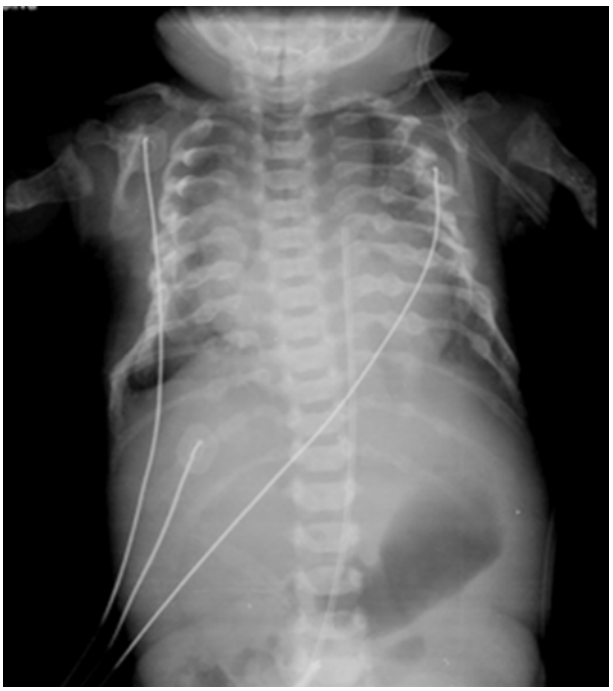

Figure 1 (ABS 8). Thoracic radiography of the newborn on her 13th day of life.

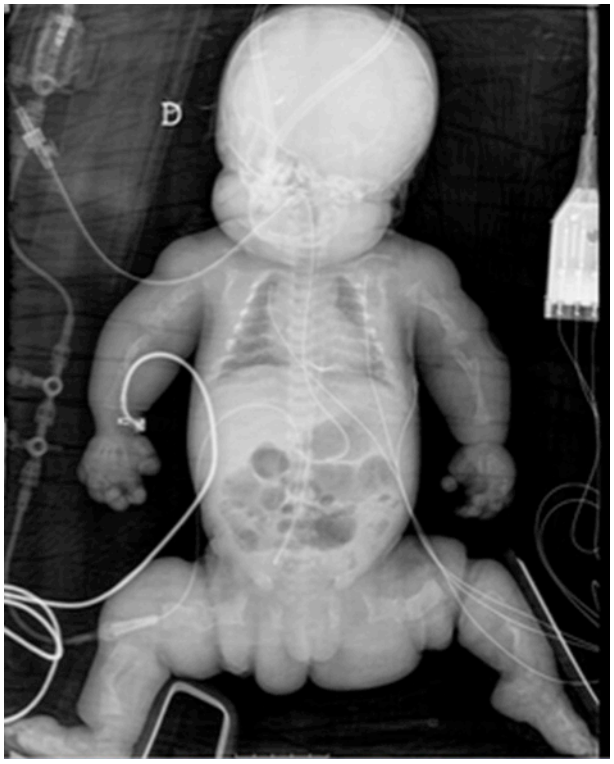

Figure 2 (ABS 8). Skeletal radiography of the newborn.

ABS 9

BIRTHWEIGHT AND MATERNAL LIFESTYLE IN OVERWEIGHT/OBESE WOMEN: A CASE-CONTROL STUDY

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OBJECTIVE

To evaluate the incidence of large for gestational age (LGA) babies, small for gestational age (SGA) babies and macrosomic babies among women undergoing a program of lifestyle, respect to a population of women of the same BMI category.

METHODS

Women with BMI ≥ 25 kg/m^2, enrolled in a trial for the evaluation of the effects of a lifestyle program (hypocaloric, low glycemic index diet and moderate physical activity) were included. Women meeting the inclusion criteria (BMI ≥ 25 kg/m^2, singleton pregnancy, absence of chronic disease) and not undergoing any lifestyle program, were included as controls, retrospectively in a 3:1 ratio. Data on maternal weight, pregnancy complications and newborns' weight were collected from the clinical charts.

RESULTS

Three hundred and sixty women were included: 90 cases and 270 controls. Among the cases there was a lower occurrence of gestational diabetes mellitus (GDM; 18.9% vs. 33.7%, p = 0.008), LGA (1.1% vs. 10.7%, p = 0.004) and macrosomic babies (3.3% vs. 11.9%, p = 0.018). The occurrence of SGA was not different between the two groups, as well as gestational weight gain (GWG) and the percentage of women not exceeding recommended GWG. At logistic regression, after correcting for GDM, BMI ≥ 30 kg/m^2, age ≥ 35 years and Caucasian ethnicity, LGA were still prevented by the intervention (OR 0.104, 95% CI: 0.014-0.784), as well as macrosomic babies (OR 0.282, 95% CI: 0.082-0.961).

CONCLUSIONS

Exposure to a hypocaloric, low glycemic index diet and physical activity started early in pregnancy reduces the incidence of macrosomic and LGA babies among overweight/obese women.

ABS 10

SPECIAL FIXATION FOR TRANSMISSION ELECTRON MICROSCOPY UNMASKS UNEXPECTED STRUCTURAL DETAILS WITHIN THE RENAL STEM/PROGENITOR CELL NICHE

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Samples fixed by traditional glutaraldehyde (GA) solution for transmission electron microscopy reveal that mesenchymal and epithelial cell bodies within the renal stem/progenitor niche are separated by an unremarkable looking interface. In contrast, fixation in GA solution including cupromeronic blue, ruthenium red or tannic acid unmasks in wide areas of this interface earlier not visible filigree extracellular matrix. Further projections of mesenchymal cells cross the interface to contact epithelial cells. Most impressive, the end of a projection does not dangle but is mounted by a special sleeve. At this site the plasma membranes of mesenchymal and epithelial cells are connected via tunneling nanotubes. Regarding this unique ensemble the basic question is to what extent morphogens are operated during induction of a nephron by diffusion, stored for delivery in illustrated extracellular matrix or transported in mesenchymal cell projections via tunneling nanotubes.

ABS 11

DRUG USE AMONG NEWBORNS ADMITTED TO NICUs: A MULTICENTRE STUDY

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BACKGROUND

The large variability observed in the therapies employed by NICUs and the common use of *off-label/unlicensed* drugs are a widespread phenomenon observed both within and between countries [1-3]. This practice is justified by the unique characteristics of the neonatal population and by the lack of evidence-based guidelines and availability of suitable licensed medicines, but may expose newborns to an higher risk of medication errors [4] and adverse drug reactions [5].

PURPOSE OF THE STUDY

To analyse prescription behaviour and to evaluate the extent of *off-label/unlicensed* prescriptions in the neonatal setting.

PATIENTS AND METHODS

A one-day survey was organized on the basis of an on-line questionnaire: demographic data and any information about drug use were recorded for each newborn admitted to 36 Italian NICUs (34% of all Italian NICUs). Every prescription was classified as licensed or *off-label/unlicensed* according to the *Italian Drug Compendium* and compared with a protocol performed by the Neonatal Pharmacotherapy Study Group of the Italian Society of Neonatology (ISN).

RESULTS

Two hundred and twenty newborn infants, 191 preterm (140 VLBW or ELBW infants) and 29 at term, were enrolled in the study. 720 prescriptions were written and analysed, while other 163 treatments applied (parenteral nutrition solutions, vitamins and probiotics, standard intravenous fluids) were only recorded. Antiinfectives were the medicines most commonly prescribed. Only 191/720 prescriptions followed the terms of the marketing authorization, while 73.5% were *off-label* or *unlicensed*. 100% prescriptions of fluconazole, fentanyl, ranitidine and cardiovascular drugs resulted *off-label* in absence of neonatal indications, while antiinfectives were used *off-label* on average in 75% of cases, with differences as regards dose and frequency among NICUs. 100% and 52% prescriptions of respectively folinic acid and caffeine resulted *unlicensed* (galenic preparations). Prescriptions adhered more frequently to the indications contained in the ISN protocol.

CONCLUSIONS

Our data confirm the variability in drug use and the high prevalence of *off-label/unlicensed* prescriptions among newborns admitted to NICUs previously reported [1, 2, 6] despite some recent initiatives to improve drug prescribing in the paediatric population [7, 8]. The high adherence to the ISN protocol suggests that information contained in data sheets rarely reflect clinical practice and need to be updated. The availability of a practical guide could be useful to harmonise drug use in the neonate, particularly if preterm, to avoid medication errors and adverse reactions.

ACKNOWLEDGEMENTS

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ABS 12

ASSESSMENT OF RENAL FUNCTION IN NEONATOLOGY

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Assessing renal function in neonates and infants is difficult. Clearances of exogenous markers iohexol and iothalamate are considered the reference in glomerular filtration rate (GFR) and are accurate, but are expensive, impractical and restricted to research use. The analytical bias of creatinine methods is disappointing in the pediatric range, which leads to an unacceptable variation in the estimation of kidney function in infants.

The availability of the global NIST SRM 967 standard for creatinine is a milestone for estimating GFR. Implementing traceability of creatinine assays to these isotope dilution - mass spectrometry (IDMS) traceable standards has major consequences. In contrast to adults, compensating calibration in Jaffe assays to IDMS results in a underestimation of serum or plasma creatinine due to the lower total protein reference values in infants. As the protein error is smaller in infants, falsely low serum creatinine results are generated. Alternatively, using enzymatic assays overemphasizes the relative proportion of tubular secretion tubular secretion of creatinine which makes serum or plasma creatinine less suited as a GFR marker in children. Due to the restandardisation, the Schwartz equation for estimating GFR in children has been adapted, but only for enzymatically determined values.

In view of the difficulties in uniformly adapting serum creatinine assays to the new calibrators, the low molecular mass proteins cystatin C (Cys C) and beta trace protein (BTP) offer promising alternatives for calculating GFR in pediatrics. Unlike creatinine, serum Cys C reflects renal function in children independent of age, gender, height, and body composition. Cys C and BTP are attractive for assessing GFR since they only require a determination in serum or plasma and are better suited in the blind range of creatinine. Because of its low individuality, Cys C has fewer inherent limitations as a screening test for detecting deteriorating GFR than serum creatinine.

Recently, Cys C based eGFR formulas have been developed. Cys C has fewer limitations for detecting deteriorating GFR than creatinine. Cys C-based GFR estimates show less bias and are a more sensitive and better estimate for GFR in infants. The new international standardisation of Cys C will enable the wide-scale use of these methods.

ABS 13

THE PARENTAL COMPETENCE IN NEONATOLOGY

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INTRODUCTION

The study focuses on the parental competence of mothers in Neonatal Intensive Care Unit (NICU), through the administration of a specific self-observation tool on parental competence, which is called Q-Sort, being validated.

In particular, the research provided for the administration to mothers that live the critical event of the preterm birth, right in the "here and now" of the hospital and in NICU. Such administration allows the possibility to support mothers in the recognition and activation of their parental functions in terms of resources and starting a remapping of themselves and of the own parental competence (Nanetti, 2010). The Q-Sort also allows a different

management of the development of the child. At the same time, this specific tool offers the possibility to create some experience spaces with the mothers of preterm children (Perricone, Morales, 2009), that allow them to reflect, to give a new meaning to this specific condition, through the activation of different relationships (Perricone et al., 2004). In this sense, it is a research tool with a supportive value of the parental competence. The Q-Sort is defined through a theoretical model that provides for the following parental functions: *caregiving*, in terms of adjustment skill to developmental needs of the son and in terms of skill to be a responsible parent; *scaffolding* as the competence of the mother to enable framing, sharing attention and building the routine; *cognitive and emotional coping* as a mother's ability to self-adjust and to recognize her needs and emotions. The cognitive coping refers to the ability of the mother to redefine the critical event of the preterm birth in terms of planning and challenge.

AIM

The aim of the study is to explore parental functions in the NICU; the hypothesis is to verify differences in the parental profile depending on the diagnosis of their child. (preterm, syndromes, chronic diseases, transient pathology in the acute phase).

METHODS

The study involved 75 mothers (average age of 32 years old) with a medium social and cultural level, recruited at the Ospedali Riuniti "Villa Sofia-Cervello" hospital and "G.F. Ingrassia" hospital of Palermo, in remission of the symptom and not in a critical or emergency phase.

The administration procedure of the Q-Sort provides for a "forced" ordering of 90 item, which describe several behaviors of parental functions in the NICU, on a scale from 1 (very different) to 9 (very similar), according to a similarity parameter (similar/different/neither similar nor different)

The collected data were analyzed through descriptive and parametric statistics (MANOVA) depending on preterm birth, syndromes, chronic diseases, transient pathology in the acute phase.

RESULTS

With regard to possible differences between indicators of the Q-Sort, results are not significant ($p > .05$); mothers activate specific parenting behaviors disregarding the diagnosis. Regarding the parental competence, data highlight how mothers perceive themselves as competent especially with regard to scaffolding (mean = 163.2) and caregiving (M = 114.3); lower scores are related to their emotional coping (M = 81.6) of the parent. In this sense, it would seem that the mother is not able to self-regulate emotions, during the hospitalization of her son.

CONCLUSIONS

The data highlight how such monitoring tools of the maternal competence may represent a support for the mother, becoming promotion of strengthening in a Positive Development perspective (Broistein, Davidson, 2003), and therefore, mobilization of specific internal protection factors (Rutter, 1987; Rutter, 2000; Semprini, 2000). This specific new condition could permit to the mother to face (Varni, Katz, 1997) the developmental risk of the preterm birth of the child. In this sense, this tool can allow the mother to act differently, while she evaluates herself, becoming an intervention that wants to transform the mother like a resource.

ABS 14

EPIDEMIOLOGICAL, CLINICAL, AND MICRO-BIOLOGICAL ASPECTS IN NEWBORNS WITH NEONATAL INFECTION (SEPSIS AND UNSPECIFIED NEONATAL INFECTION) TREATED AT THE DEPARTMENT OF NEONATOLOGY OF THE LITHUANIAN UNIVERSITY OF HEALTH SCIENCES DURING 2007-2013

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INTRODUCTION

Neonatal infection (NI) is a worldwide problem – according to the World Health Organization (WHO), infections are the major cause of neonatal deaths worldwide (36%). About 8 out of 1,000 live-born neonates are diagnosed with NI, 1 to 3 newborns are diagnosed with early-onset neonatal sepsis (EONS), and even more neonates (1 to 5) are diagnosed with late-onset neonatal sepsis (LONS). About 5% to 60% of newborns who have received antibiotics to treat NI suddenly die.

AIM

The aim of this study was to analyze the incidence, epidemiology, causative agents, and clinical manifestations of neonatal sepsis (NS) and unspecified neonatal infection (UNI) among newborns treated at the Department of Neonatology of the Lithuanian University of Health Sciences during 2007-2013.

METHODS

In total, 631 medical histories of newborns diagnosed with NS or UNI and treated at the Clinical Department of Neonatology of the Lithuanian University of Health Sciences during 2007-2013 were analyzed in retrospect. The data were analyzed using the statistical software IBM® SPSS® statistics 20.0; 95% confidence intervals were calculated ($p < 0.05$).

RESULTS

The incidence of NS and UNI in Lithuania is 25 out of 1,000 live-born neonates (2.6%). The incidence of NS is 0.8%, and the incidence of UNI – 1.8%. In the study, the percentage of newborns diagnosed with NS in the group of newborns with ≤ 32 weeks of gestation was 39.8%, in those with 33 to 36 weeks of gestation – 38.8%, and in those with ≥ 37 weeks – 24.9%. Concerning birth weight, 46.3% of newborns weighing ≤ 1,500 had NS, and 26.9% of newborns weighing ≥ 2,500 grams had NS. The most common pathogens for EONS were group *B Streptococcus* (12%) and *E. coli* (10%), while for LONS – group *B Streptococcus* (32%) and *S. aureus* (22%). The incidence of NS and UNI were higher in neonates born via vaginal delivery than in those born via Cesarean section. The majority of the newborns with NS had normal body temperature. Newborns diagnosed with NS had higher temperature than those who were diagnosed with UNI. The mortality rates in newborns with NI were 1.4 out of 1,000 live-born neonates. More newborns with NS and UNI were registered during 2007-2009 than during 2010-2013, and the mortality rates were higher as well.

CONCLUSIONS

More cases of NS and UNI were registered during the period of 2007-2009 than during 2010-2013, the mortality rates were higher as well, ($p < 0.05$). Early neonatal infection (NS and UNI) was more common in newborns with birth weight ≥ 2,500 g, ($p < 0.05$). The most common pathogens for EONS were group *B Streptococcus*, *E. coli*, and *S. aureus*, while the most common pathogens for LONS – group *B Streptococcus*, *S. aureus*, and *coagulase-negative staphylococci* ($p < 0.05$).

ABS 15

METABOLOMICS IN OBSTETRICS: FUTURE PERSPECTIVES

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Metabolomics is the science that systematically studies metabolites of an organism in different biological fluids. Different studies shows the effectiveness of this technology in defined metabolic profiles of physiology and pathology. This approach allows to investigate both physiology and pathology in different conditions. In obstetrics it was possible to study the metabolic profiles in plasma, urine, feces, amniotic fluid and placenta obtaining information coming from the mother, the fetus and the placenta. However it's not possible, to date, to have complete profiles of uncomplicated pregnancy vs complicated pregnancy. Many attempt have been made to find particular metabolic profiles or markers of physiological conditions. In a previous study we found that, in subjects before and during labour there were differences in the metabolic pathways. In particular 6 metabolites identified in urine of women in labour vs not labour, hag given a signature of the advanced phase of labour. A recent review showed that different alterations were strictly correlated with very rare diseases such as Smith-Lemli-Opitz syndrome and fetal malformations. Nowadays the major attention regards the profiling of subjects with different pathologies to obtained more large database to be used for comparing large amount of data are going to be obtained. Some data show that particular metabolomic profiles can be found in preterm labour, preterm rupture of membranes, gestational maternal diabetes, into fetal malformations or grow restriction and chromosomal disorders, suggesting the potential use of metabolomics in identifying women at risk of prenatal and maternal pregnancy-related disorders. From this data the main consideration is that other studies must be performed to ascertain wheter other different physiological and pathological conditions show particular profile. The aim of this review is to evaluate what recently happen in this field and in particular to propose new studies concerning the metabolic pathways of fetus, placenta and mother interaction.

ABS 16

SINGLE-BLIND RANDOMIZED CONTROLLED CLINICAL TRIAL TO EVALUATE THE

NUTRITIONAL ADEQUACY OF A NOVEL HUMAN MILK FORTIFIER DERIVED FROM DONKEY MILK FOR THE NUTRITION OF NEWBORNS WITH BIRTHWEIGHT < 1,500 G OR GESTATIONAL AGE < 32 WEEKS

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BACKGROUND

To fulfil the particular nutritional requirements of preterm infants, human milk (HM) is supplemented with human milk fortifiers (HMF). Donkey milk (dM) has been observed to be rather more similar to human milk than the bovine one, so it can be considered more suitable to constitute the base of the protein support for a HMF.

OBJECTIVE

The aim of this work is to evaluate the nutritional adequacy of a multi-component fortifier and a protein concentrate derived from dM for the nutrition of preterm infants with respect to traditional fortifiers derived from bovine milk.

MATERIALS AND METHODS

Inclusion criteria were: GA < 32 weeks and/or birthweight < 1,500 g; feeding with HM > 80% of the total; enteral feeding (EF) ≥ 80 ml/kg/die of HM reached within the first 4 weeks of life. Newborns were randomized 1:1 into two groups: for Group A, standard fortifiers were used; for Group B, other products derived from dM were employed. Alimentary intolerance was evaluated in accordance with the protocol used in our NICU. The primary endpoint was the occurrence of at least one episode of alimentary intolerance, defined as the necessity to interrupt EF for more than 8 consecutive hours during the study period (28 days). Short/long term clinical, metabolic and auxological outcomes were also evaluated. The characterization of the infant metabolic profiles was performed by ^{1}H-NMR spectroscopy, in collaboration with the University of Cagliari, on urines samples collected at three time points: 0, 7 and 21 days of fortification.

CONCLUSIONS

At present there are not preliminary results available being the study still ongoing, except for the metabolomics characterization of the urinary profile of infants. Multivariate statistical analysis of the ^{1}H NMR spectra of urines evidenced differences between the two groups of neonates. In particular, urines from Group B, fed with dM derivatives, were found to be richer in citrate and carbohydrates, such as lactose, galactose and fucose, with respect to Group A. These findings are still under analysis to find correlation with the fortifier composition.

ABS 17

THE STEM CELL NICHE IN THE DEVELOPING CEREBRAL CORTEX

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BACKGROUND

The development of the human cerebral cortex involves a series of orchestrated events that lead to form the 6-layered cortex [1]. During embryogenesis, the ventricular zone (VZ) and the subventricular zone (SVZ) form an active proliferate zone, the site of origin of cortical neurons and glial cells. These zones are considered "stem cell niche" which is constituted by neuronal and glial progenitors and from the surrounding microenvironment including blood vessels that are important for proper patterning of neurogenesis [2]. Two distinct progenitor cell types participate in cortical neurogenesis: radial glia and basal/intermediate progenitors, which produces respectively glial-restricted progenitors and neuron-restricted progenitors.

AIM

On this basis, the purpose of this study was to analyze the stem cell niche in the cerebral cortex during gestation by a histological and an immunohistochemical approach. Define the immunohistochemical markers of stem/progenitor cells in the human cerebral cortex allowing the specification of the different stages of differentiation of the neuronal and glial lineages.

MATERIALS AND METHODS

Cerebral cortices from 4 human fetuses ranging from 11 up to 22 weeks of gestation have been sampled and histologically and immunohistochemically studied. Samples were fixed in 10% buffered

formalin, routinely processed, and paraffin-embedded. Two serial 3 µm-thick sections were obtained from each paraffin block; after dewaxing and rehydrating, one of these was stained with hematoxylin-eosin, the other pre-treated for immunohistochemical analysis, then incubated for 20 minutes with the following antibodies: WT1, Sox2, Vimentin, Nestin, S100, NSE, PAX2, CD117, Ki67 and GFAP.

RESULTS

Histology allowed us to recognize the different zones in which the cerebral cortex is subdivided in these early stages of development (**Fig. 1**) including the stem/progenitor cell niche (**Fig. 2**). As regards immunohistochemichal studies our data show that Vimentin, Nestin and WT1 (**Fig. 3**) are expressed in radial glia fibers extending from the VZ toward the pial zone; nuclear immunoreactivity for Sox2 (**Fig.4**) and Ki67 were detected in the VZ and in the SVZ and in stem/progenitor cells that are migrating in the intermediate zone toward the cortical plate. The SVZ was also evidenced by reactivity for PAX2. The others zones were positive for NSE, S100 and CD117. No reactivity for GFAP was detected in these gestational ages.

CONCLUSIONS

Our preliminary study adds new data concerning the role of different markers in human cerebral

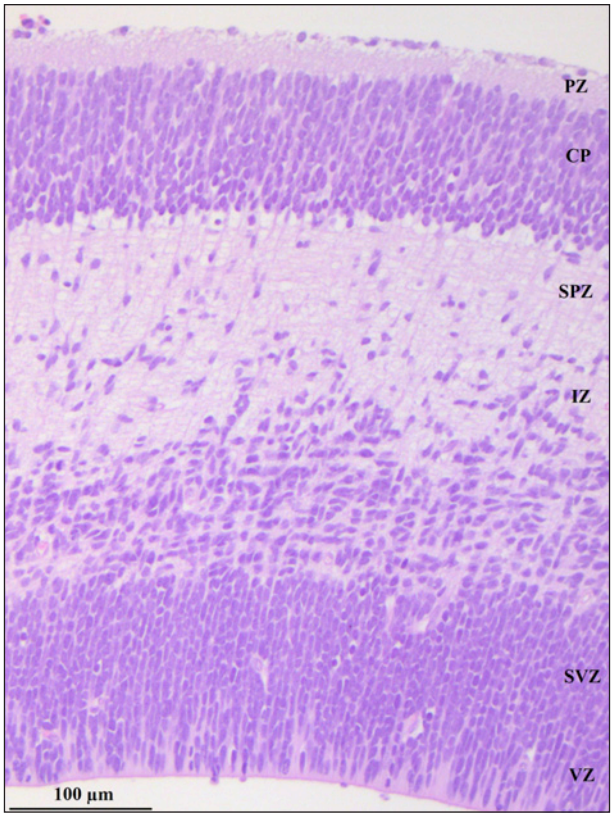

Figure 1 (ABS 17). Different regions of human cerebral cortex at gestational week 11: the ventricular zone (VZ), the subventricular zone (SVZ), the intermediate zone (IZ), the subplate zone (SPZ), the cortical plate (CP) and the pial zone (PZ).

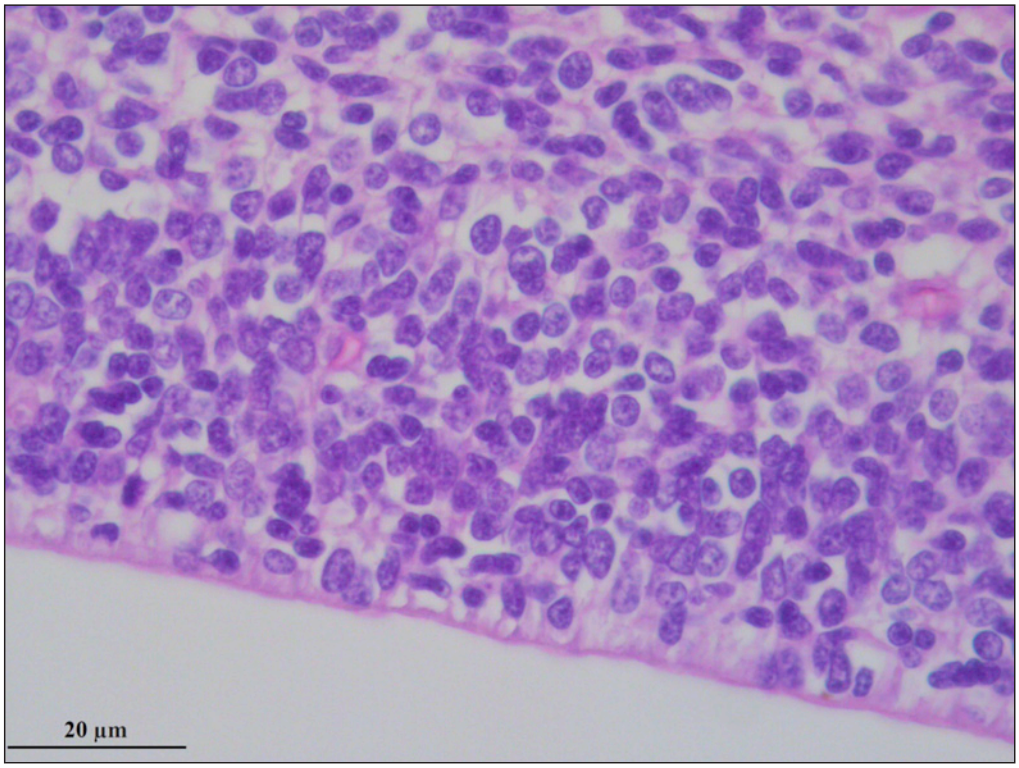

Figure 2 (ABS 17). Stem/progenitor cells niche of human cerebral cortex at gestational week 17.

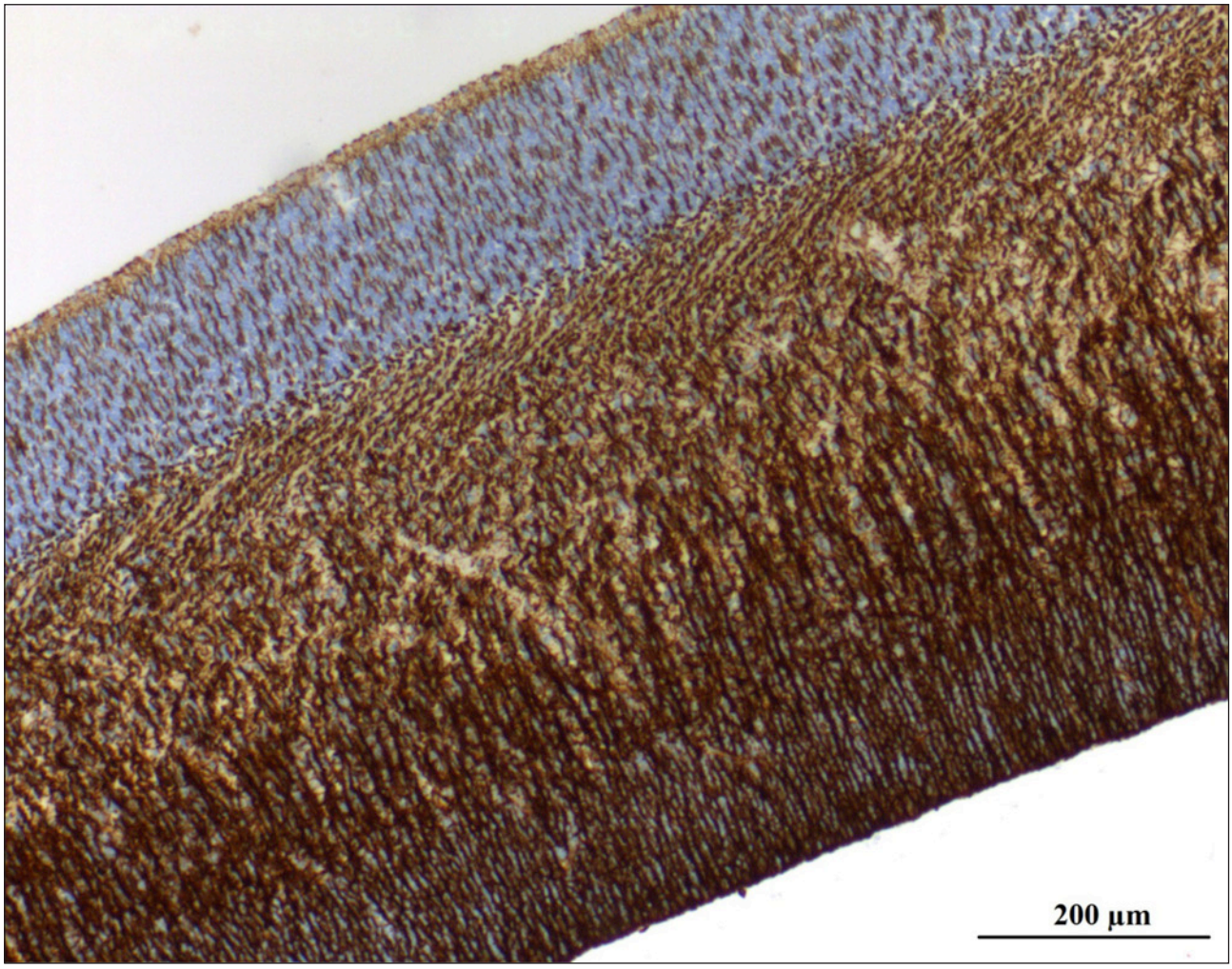

Figure 3 (ABS 17). Immunoreactivity for WT1 in radial glia cells fibers extending from the VZ toward the CP in human cerebral cortex at 12 weeks of gestation.

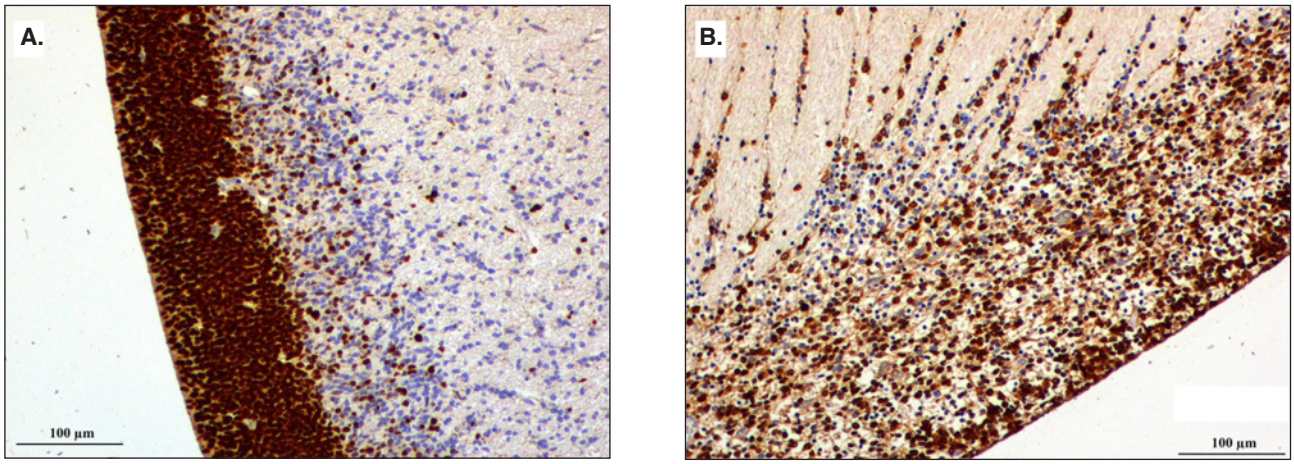

Figure 4 (ABS 17). Sox2 nuclear reactivity of stem/precursor cells localized in the niche and in the IZ in human cerebral cortex at 12 (**A**) and 21 (**B**) weeks of gestation.

cortex development and confirms our previous data regarding the expression of WT1 in radial glia [3]. Identifying the immunohistochemical markers expressed stem/progenitor cells that play a key role during embriogenesis, may be important to develop an "endogenous" target therapy in the

perinatal period. Future studies will be needed to test other important stem/progenitor markers and to better analyze differences in the expression of these markers at different gestational ages.

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ABS 18

PULMONARY ATRESIA: THE IMPORTANCE OF PRENATAL DIAGNOSIS

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CLINICAL CASE

We report the case of a female newborn of 39 weeks, with an uneventful gestation, that 12 h after birth presented with hypoxemia non-responsive to oxygen therapy. Given the possibility of cardiac disease, prostaglandin E_1 was initiated and the newborn was transferred to our center. On admission, she was hemodynamically stable, SpO_2 ~ 80% (FiO_2 50%) and presented a continuous cardiac murmur on auscultation. Echocardiogram revealed pulmonary atresia (PA), moderate intraventricular communication (IC), hypoplastic pulmonary arteries and patent ductus arteriosus (DA). On day 4 (**Fig. 1**), a diagnosis of necrotizing enterocolitis was made and the newborn was submitted to segmental colectomy (transverse and descending colon) with colorectal anastomosis. On day 18, a pulmonary-to-systemic shunt and ligation of DA was performed, complicated by intraoperative desaturation during the mobilization of pulmonary arteries. The patency of the shunt (under heparin) was verified but 2 days after surgery the newborn presented clinical deterioration with severe sustained hypoxemia, poor peripheral perfusion, metabolic acidosis and hyperlactacidemia, later presenting oligo-anuria but maintaining negative markers of infection. Abdominal ultrasonography suggested ileocolic invagination with intestinal necrosis. Considering the clinical severity, surgery and renal replacement therapy were deemed not possible and palliative care were started. The newborn died on day 21 and the autopsy revealed signs of multi-organ failure, kidney multifocal haemorrhagic necrosis and absence of intestinal ischemia. Genetic analysis confirmed the clinical suspicion of DiGeorge Syndrome.

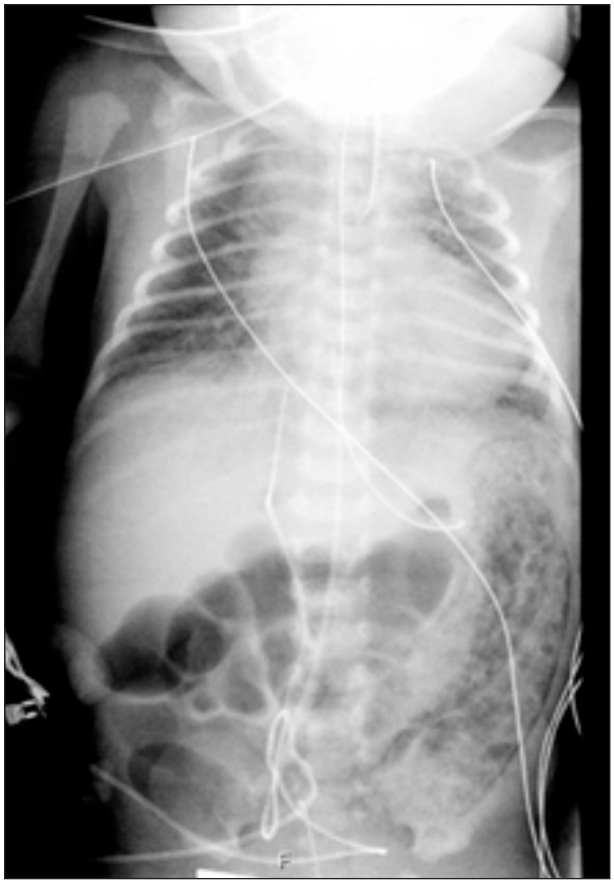

Figure 1 (ABS 18). Toraco-abdominal radiography suggestive of necrotizing enterocolitis on day 4.

CONCLUSION

This clinical case emphasizes the importance of prenatal diagnosis of PA, given the ductus arteriosus dependence until the construction of pulmonary-to-systemic shunt to avoid ischemic complications. The PA with IC is commonly associated with DiGeorge syndrome.

ABS 19

THE USE OF HYPOTHERMIA IN PRETERM ASPHYXIATED NEONATE: NEW RESULTS FROM A METABOLOMICS POINT OF VIEW

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INTRODUCTION

Perinatal asphyxia is a poorly understood event resulted from impairment in the exchange of respiratory gases causing a decreased oxygen levels during the birth process [1]. Oxygen deprivation before or during birth can cause the activation of many different metabolic pathways including mitochondrial failure, hyperaemia, cytotoxic edema, free radicals production, of cell death domains activation and nitric oxide synthesis leading to the brain cell death of newborns [2, 3].

Until few years ago, there was no individual treatment for this occurrence. Nowadays, thanks to the improvement in understanding the pathophysiology, the use of hypothermia (HT) procedure is considered the main treatment for the hypoxic-ischemic encephalopathy (HIE) injury in full term newborns. In fact, lowering the body temperature decreases the cellular response to the injury in terms of inflammatory response, cerebral metabolic rate, cerebral edema, and intracranial pressure. Hypothermia also acts as protection for the brain inhibiting cellular depolarization and therefore the release of toxic by-products.

So far, the use of HT in preterm population less than 36 weeks is still anecdotal, this is probably due to the thought that there are more disadvantages than advantages. In fact the literature about the relationship between HIE and the development of the brain is very poor [4, 5]. In this context, there are new emerging theories based on animal's studies demonstrating the importance of the metabolomics approach for identifying the metabolic fingerprint of HIE conditions [6-8]. However, the relationship between this condition and the hypothermia process on the preterm newborns metabolism has been modestly explored [9, 10].

AIM

The purpose of this study was to perform a longitudinal evaluation of a preterm newborn suffering from HIE and subjected to HT (72 hours) using a metabolomics approach based on high resolution ^{1}H NMR spectroscopy combined with multivariate statistical analysis with the aim of identifying a distinctive metabolic fingerprint compared to a population of term infants suffering from HIE and subjected to HT as reference.

MATERIAL AND METHODS

In this study, 8 newborns were enrolled: Among them 1 was an asphyxiated neonate preterm (33^{+4} w), 7 were asphyxiated term neonates. All the neonates were treated with HT procedure. Urine samples were collected at 3 time points from all pathologic patients admitted to Neonatal Intensive Care Unit of S. Croce e Carle hospital, Cuneo, Italy. The collection or urine included samples at birth, at 72 hours (end of hypothermia), and at 1 month. Urine samples containing an aliquot of sodium azide 1% were stored at -80°C before metabolomics analysis. All chemicals elements used in this study were of analytical grade. Deuterium oxide (D_2O, 99.9%) was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Sodium 3 (trimethylsilyl)propionate 2,2,3,3, d_4 (TSP, 98 atom% D) was obtained from Sigma-Aldrich (Milan, Italy). The urine samples of about 1 mL were thawed on ice and centrifuged at 13,000 rpm for 10 minutes at 4°C. An aliquot of 630 µl was withdrawn from the supernatant and 70 µl of a 1.5 M phosphate buffer solution pH 7.4 with a final concentration of 1 mM TSP was added. The samples were transferred into 5 mm NMR tubes for analysis. The ^{1}H NMR experiments were carried out using a Varian UNITY INOVA 500 spectrometer (Agilent Technologies, CA, USA) operating at 499.839 MHz. Spectra were acquired at 300K using a standard 1D-NOESY pulse sequence for water presaturation. The acquired spectra were processed, zero-filled to 64K, phased and baseline corrected using MestReNova software (Version 9.0, Mestrelab Research S.L.). For all the ^{1}H NMR spectra, the region 0.80-9.30 ppm was segmented into regions (bins) of 0.02 ppm width (bucketing procedure). The integrated area within each bin was normalized to a constant sum of 100 for each spectrum in order to minimize the effects of variable concentration among different samples. The matrix of spectral data was analysed using SIMCA-Pprogram (Version 13.0, Umetrics, Umeå, Sweden). In

order to identify peculiar clusters, anomalies or trends a principal component analysis (PCA) was performed. Later, partial least square discriminant analysis (PLS-DA) was performed with the aim to find the difference of interest that allows a better separation between the classes.

RESULTS AND DISCUSSION

Both unsupervised and supervised multivariate model (PCA, PLS-DA) easily allowed the identification of the preterm metabolic evolution among the term newborns without considering any clinical evaluation (**Fig. 1**). In fact, although the sample of the preterm infant is identifiable at birth, compared with samples collected from full term infants, it shows the same metabolic evolution during the two time points taken into consideration (either at early term during HT, either at long term at 1 month). This result opens interesting discussion about the use of HT in the population of preterm newborns, which is considered still off label for undergoing HT.

CONCLUSION

Metabolomics is a powerful tool for investigating the metabolic profile associated with perinatal asphyxia and follow the metabolic changes related to HT treatment. A larger number of asphyxiated newborns should be analysed using this approach in order to extend the use of hypothermia for the class of preterm newborns.

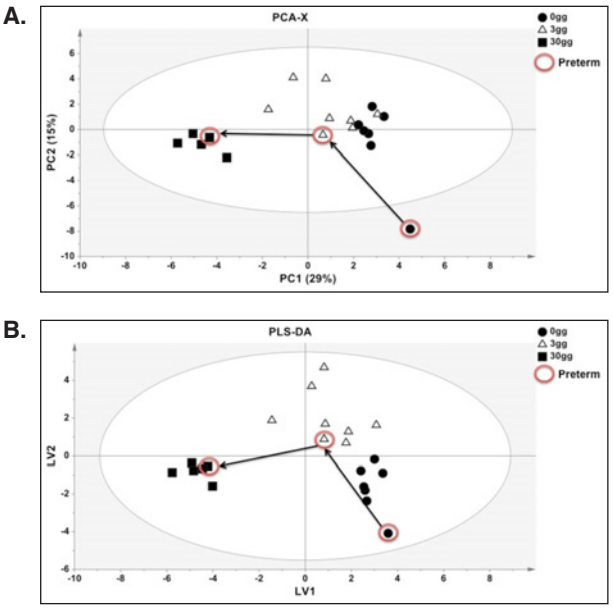

Figure 1 (ABS 19). Unsupervised (**A**) and supervised (**B**) analysis of the metabolic evolution of a preterm infant: black round circle sample collected at birth; triangle: at 72 hours (at the end of the hypothermia process); black square: at 1 month.

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ABS 20

ALOPECIA AREATA IN A CHILD: CASE REPORT, MANAGEMENT AND THERAPY

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INTRODUCTION

Alopecia areata (AA) is an organ-specific autoimmune disease that typically occurs in young adults and children [1]. AA is characterized as a nonscarring alopecia; it can be relatively easy to treat when the disease is patchy and limited; but when children present with long standing extensive scalp and body hair loss, successful management can be challenging. Dermoscopy and thricoscopy may help in the correct diagnosis, when typical signs of AA such as exclamation mark hairs, yellow dots, black dots, breakage, circle hair are present. Dermoscopic findings may also aid in disease monitoring, especially in the more serious cases of AA, and may motivate treatment decision [2].

CASE REPORT

A 8-years-old boy presented to our attention for a AA of the scalp located in the temporal, occipital, parietal region. The overlying skin was normal (absence of atrophy, desquamation or inflammation) (**Fig. 1 A-C**).

Dermoscopy (DermLite I; 3Gen) revealed yellow dots, black dots, breakage, circle hairs, tapering hairs (exclamation mark and coudability hairs), and hypopigmented vellus hairs. (**Fig. 1 D, E**). These dermatoscopic signs are tipical of AA and the child's parents said the occurrence of previous traumatic events. After clinical and dermoscopic diagnosis, the parents were reassured about the benign nature of the disease, and decided together with the physician for an expectant management of the disease. The therapy was carried out with local corticosteroid therapy for 3 months and when the patient was back after this period (**Fig. 2 A-C**) the dermoscopy examination (**Fig. 2 D, E**) revealed the presence of a large number of vellus hair ,more than the previous control and the number of black dots and yellow dots was less than the previous control. The physician decided to continue the therapy (every other day), the patient came back for a second control after 6 months and, dermoscopically and clinically,the hair regrowth appears evident (**Fig. 3 A-E**)

DISCUSSION

In the videodermoscopic analysis the combination of large numbers of yellow dots and short regrowing hairs is a feature of AA. Additional and specific markers of this disease are black dots (or cadaverized hairs), resulting from hair destroyed or broken off at the scalp. Initial clinical severity has been proposed as an acceptable predictor of disease prognosis, although AA is still considered an unpredictable condition [3]. Consequently, in addition to a possible role in prognosis, dermoscopic findings may aid in disease monitoring, especially in the more concealed AA , and may motivate new treatment decisions. For example, black dots and numerous yellow dots are markers

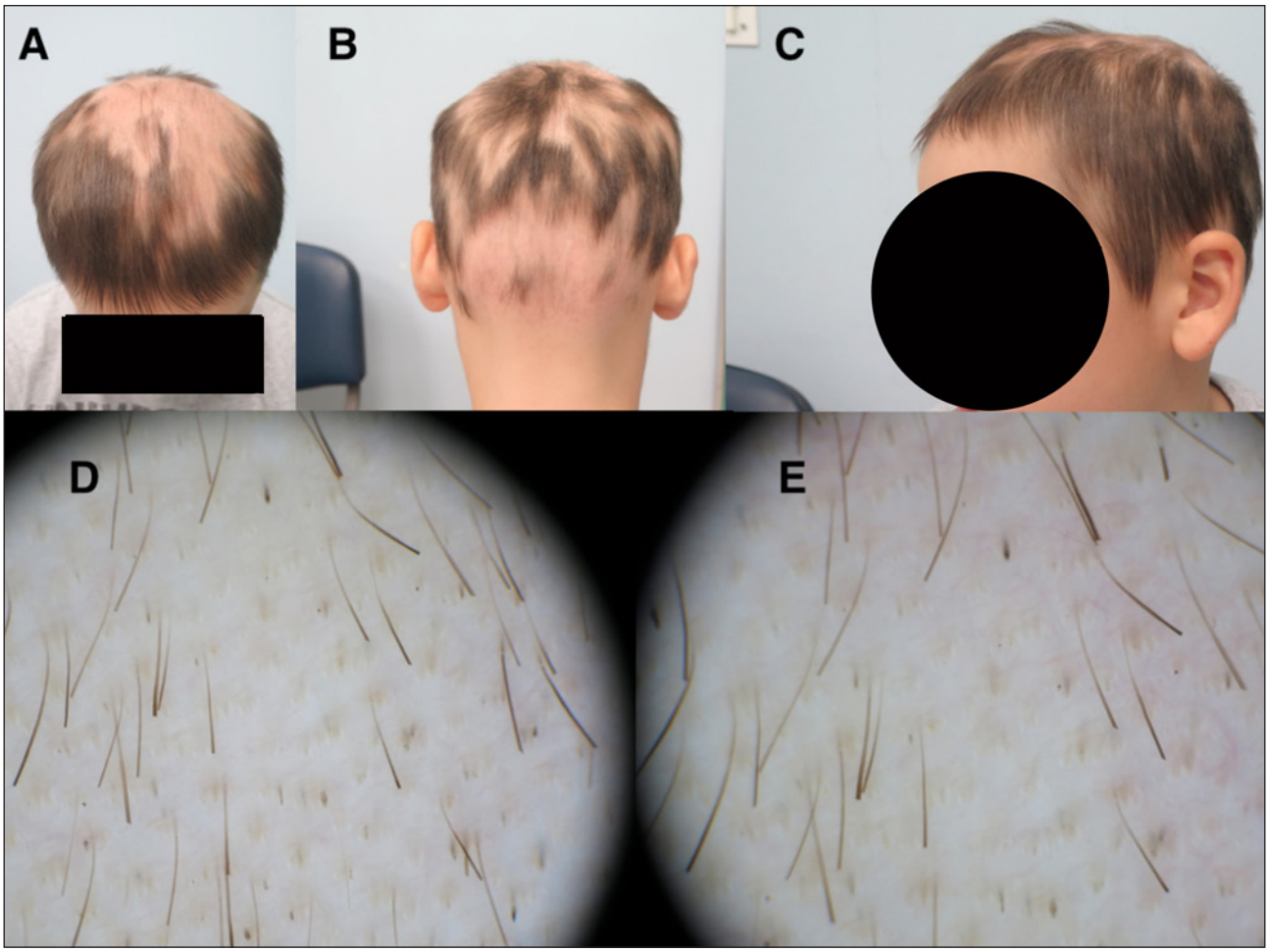

Figure 1 (ABS 20). A-C. Alopecia areata in a child-parietal- occipital-temporal region of the scalp. **D, E.** yellow dots, black dots, hypopigmented vellus hairs on dermoscopy analysis.

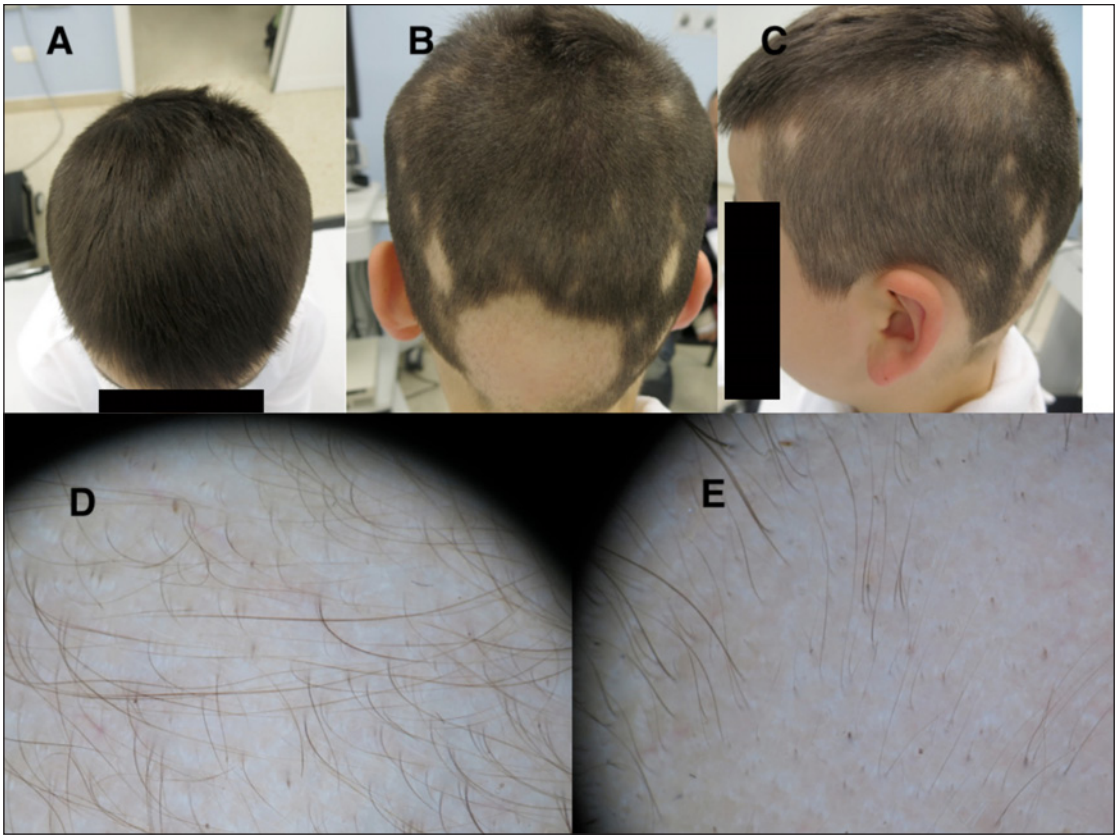

Figure 2 (ABS 20). A-C. Clinical follow-up aftter three months. **D, E.** Hypopigmented vellus hair on dermoscopy analysis (abundant).

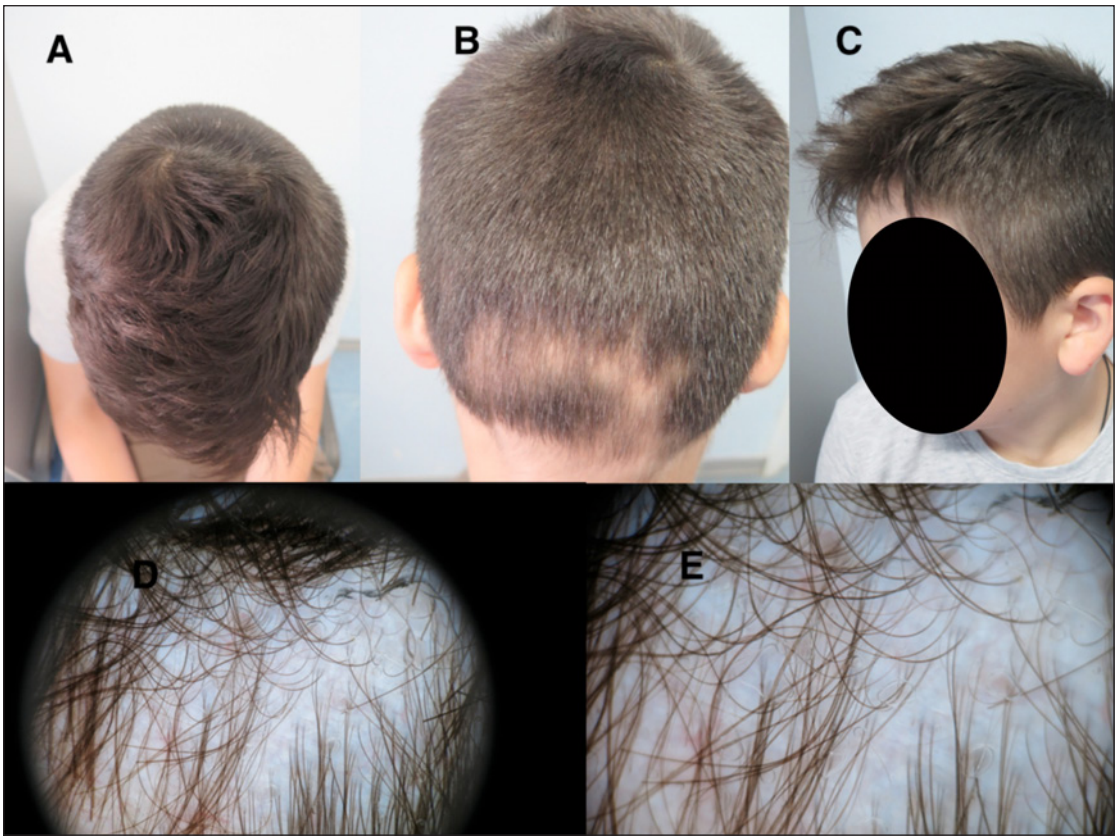

Figure 3 (ABS 20). A-E. Hairs regrowth (clinically – dermoscopically).

of severity. Exclamation mark hairs may reflect exacerbation. The appearance of clusters of short, hypopigmented, regrowing vellus hairs is a possible sign of spontaneous remission or adequate treatment.
In our cases we observed , during videodermoscopy examination: during the frist examiantion a large numbers of yellow dots and black dots, less regrowin vellous air and these is diagnostic for an AA in his initial severity, during the second monitoring control the number of yellow dots and black dots decreased, but the number of vellous air is increased and this is significative for a correct therapy and a regrowing of the air. So our diagnosis is correct and our therapy choice is the right one. This report underline the importance of videodermoscopy analysis in the correct diagnosis of an AA in a child, but also the importance of this thecnique in monitoring the therapy, and it's able to justify the correct therapeutic choice [4, 5].

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ABS 21

PHYSIOLOGICAL AND THERAPEUTIC ROLES OF MESENCHYMAL STEM CELLS. IS THERE A GAP?

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Stem cells are clonogenic cells with the ability to undergo numerous division cycles without losing their cell identity and to differentiate into different specialized cell types. Such unique features, known as self-renewal and multi-potency, allow them to control tissue development and maintenance. Besides hematopoietic stem cells, which give rise to all the blood cell components, a population of mesenchymal stem cells (MSCs) has been identified in the bone marrow, which has the ability to give rise to osteoblasts, condrocytes, adipocytes and other cell types. Following their discovery, MCSs have been isolated from different adult and embryonic tissues and, owing to their remarkable features, including homing capacity to sites of injury and ease of isolation, have been subjected to intensive research for their possible use in various clinical settings. Initially, research focused on their potential use for tissue regeneration and repair; more recently the possible applications of MSCs for therapeutic purposes expanded further because MSCs were found to have immunomodulatory functions, which likely rely on the release by MSCs of various anti-inflammatory and antiproliferative molecules able to act as paracrine factors. Moreover, testing of adult-derived MSCs, compared with embryonic stem cells, is not restricted by ethical implications. So, while a myriad of clinical trials exploring the use of MSCs for the possible treatment of various diseases was rapidly propelled (**Fig. 1**), basic research lagged behind and many fundamental questions on MSCs biology and physiological functions were left unanswered. Probably the main hurdle in MSC research is the fact that no specific surface antigen or antigen combination has been identified for the isolation of a pure MSC population from bone marrow. In fact, the MSCs currently used for therapy are non-clonal heterogeneous populations, which may account for the contrasting results obtained so far with MSCs transplants. Also, although the use of non-clonal populations will probably be unavoidable owing to the low efficiency of clonal MSCs to produce daughter cells, the protocols used for MSCs isolation, culturing and *ex vivo* expansion need to be implemented. Currently these protocols only use the minimal criteria defined by the International Society for Cellular Therapy, whereas it will be important to define and set new criteria, for example for the evaluation of stem/progenitor cell percentage, for the reduction of donor heterogeneity, for the assessment of senescence and potential interaction with cancer stem cells, and also for culturing conditions (such as normoxia/hypoxia and animal/human serum supplements), which could help to preserve *in vitro*

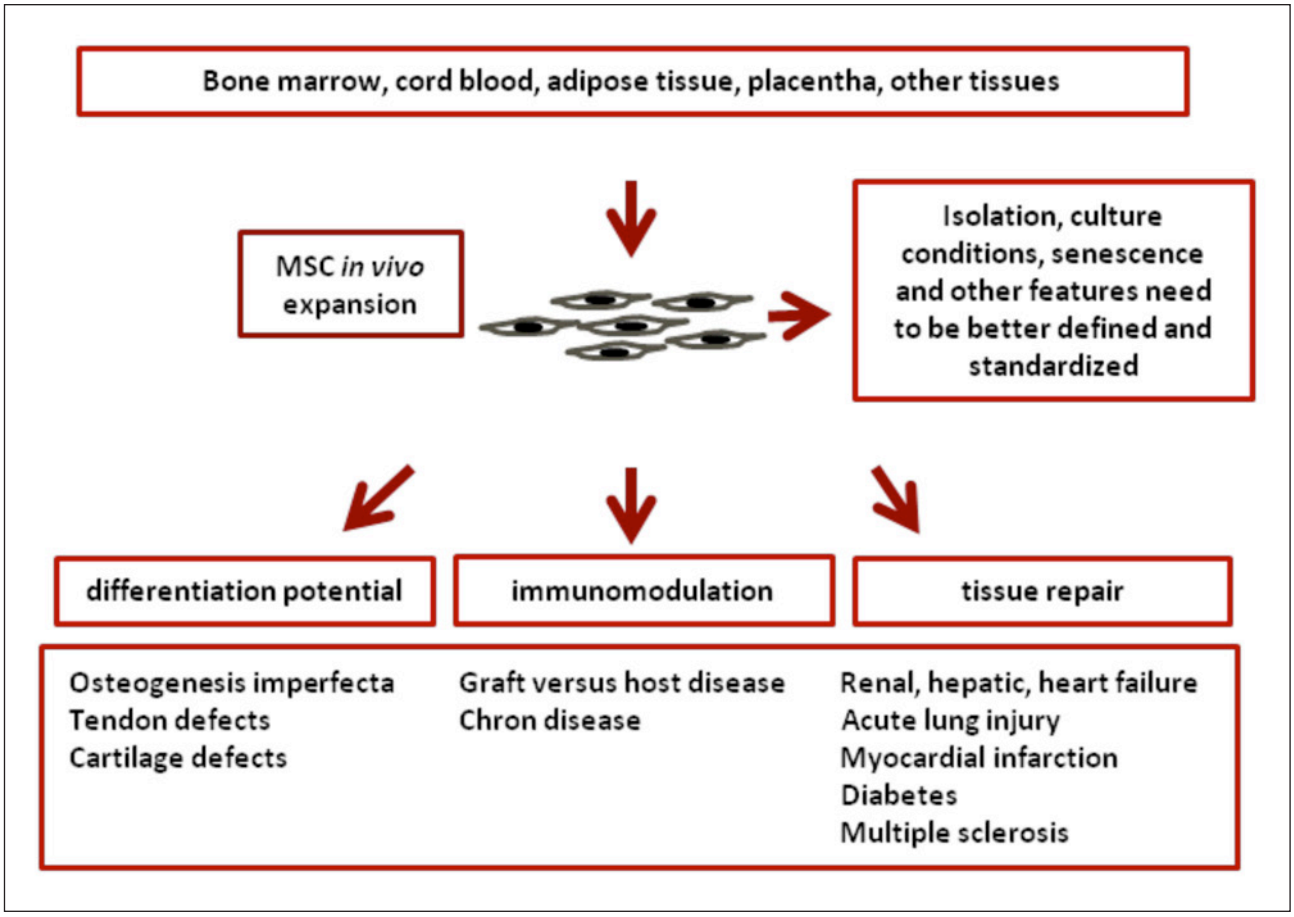

Figure 1 (ABS 21). The figure indicates the main tissues from which MSCs can be isolated and MSC main features, which make them appealing for various clinical applications. In the box at the bottom various diseases are listed for which MSC-based strategies have been attempted.

their natural features. In the past decade our group focused on the characterization of the role of the retinoblastoma protein family, composed of crucial cell cycle regulators, in the biology of MSCs and on the effect of their modulation on MSC ability to differentiate or enter senescence.

ABS 22

ESOPHAGEAL IMPEDANCE BASELINE IS CORRELATED WITH EXPOSURE TO REFLUX IN NEWBORNS WITH GERD SYMPTOMS

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BACKGROUND

Multichannel Intraluminal Impedance combined with pH-metry (MII/pH) monitoring is widely used to detect gastroesophageal reflux events. Esophageal impedance baseline (IB) has been proposed as an indicator of damage to the esophageal mucosa due to gastroesophageal reflux disease (GERD).

AIMS

Our aims were to describe IB in newborns with GERD symptoms and to evaluate the relationship between IB, age, and reflux characteristics.

MATERIALS AND METHODS

We retrospectively evaluated MII/pH tracings in newborns with GERD symptoms (postconceptional age 36-46 weeks). Reflux events and IB were evaluated in the whole MII/pH tracing, in the hour following each meal (postprandial period) and in the subsequent fasting period.

RESULTS

67 newborns (43 at term and 24 preterm) were included and 1,446 hours of tracings were recorded. The IB was lower in the distal channel than in the proximal one (1,824 vs. 2,366 in the whole tracing, 1,763 vs. 2,246 in the postprandial period, 1,831 vs 2,329 in the fasting period; $p < 0.05$). In the distal channel, IB was lower in the postprandial period

than in the fasting one (1,763 vs. 1,831; $p < 0.05$). Age was positively correlated with IB. There was an inverse correlation between IB and reflux index ($r = 0.31$ in the distal channel; $r = 0.26$ in the proximal channel).

CONCLUSIONS

We propose the first IB values in newborns with GERD symptoms. We demonstrate that IB is correlated with age and varies in relation to the time since last meal and to the consequent changes in exposure to reflux.

ABS 23

ANALYTICAL PITFALLS AND FUTURE PERSPECTIVES IN THE MEASUREMENT OF BONE METABOLISM BIOMARKERS

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Bone metabolism is a process of paramount importance, starting from birth and throughout the course of life. The monitoring of this process is not only important in old age, when osteoporotic processes are developed, but it proves very useful also to follow the growth of bone mass from the initial stages of life, as long as the assets of bone reaches its peak and then it begins to be depleted over the years of adulthood.

Alongside the diagnostic techniques based on imaging and physical examination, the laboratory can contribute to this path with the detection of different parameters such as fundamental mineral constituents of the bone matrix (e.g. calcium and phosphorus), enzymes and hormones which modulate bone metabolism, or other molecules that are correlated with the degradation of skeletal bone. Especially the latter often are not considered so significant in clinical guidelines, this is mainly due to the fact that their concentrations interpretation is particularly complex due to the high biological variability and the consequent difficulties in defining effective reference ranges useful for the patients classification.

But, while even the routine laboratories begin to be able to determine molecules which allow to investigate more in depth the bone metabolism (such as, for example, the Fibroblast Growth Factor 23 [FGF23] and Sclerostin), the scientific literature has shown an exponentially growing interest in the study of two molecules: the PTH and, above all, vitamin D.

If on one hand for clinicians PTH and vitamin D represent important sources of information, in particular for evaluation of their interaction and for the role they play (particularly vitamin D) in a large number of pathophysiological processes, for the laboratory the correct measurement of these two molecules is challenging, and the difficulties involved are not always well known.

For PTH difficulties reside mainly on the many forms or molecular fragments that may be present in a sample and which have different meaning and biological activity. The difficulty in interpretation of PTH measurement cannot be separated from knowing what forms and to what extent are detected by the laboratory method used and, therefore, be able to define correctly the reference interval.

On the contrary for vitamin D molecular forms are well defined and those of interest to be detected are perfectly known, but many difficulties are found in harmonizing results obtained with the different analytical methods because of their different sensitivity and specificity performances. Since the wide spread variation in measurement results of total 25-hydroxyvitamin D confounds international efforts to develop evidence-based clinical guidelines, recently a Program for Vitamin D Standardization (VDSP), involving health organizations and assay manufacturers, has been developed.

From the clinical point of view, the renaissance in the interest in the metabolism and biological actions of vitamin D was very noticeable in the last years. Part of this interest arises from the discovery that its active form, 1,25-dihydroxyvitamin D_3, through its nuclear vitamin D receptor (VDR), regulates hundreds of genes around the body including those coding for proteins involved in cell differentiation and cell proliferation as well as calcium and phosphate homeostasis.

Finally, a non-secondary aspect, is how to define which are the desirable and optimal concentrations of vitamin D. Most reports on the prevalence of vitamin D deficiency support that more than 70% of adults and children are vitamin D deficient: but it is difficult to define what are the characteristics of an healthy subject with regard to the vitamin D status and as of bone metabolism it is more appropriate to refer to effects of vitamin D on PTH concentration.

ABS 24

GENETIC ANALYSIS OF SURFACTANT PROTEINS GENES IN PREMATURE NEWBORN

INFANTS WITH SEVERE RESPIRATORY DISTRESS SYNDROME

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INTRODUCTION

Mutations in genes encoding SP-B (*SFTPB*), SP-C (*SFTPC*) and ABCA3 (*ABCA3*) have been identified as cause of progressive respiratory failure in term newborn infants. At present, very little is known about effects of variation in surfactant protein genes in premature infants.

AIM

Analysis of *SFTPB*, *SFTPC* and *ABCA3* in newborn infants with gestational age < 32 weeks presenting unusually severe RDS.

PATIENTS AND METHODS

48 preterm newborn infants with gestational age of 24 to 31 w, affected by unusually severe RDS referring from diverse Italian hospitals from 2000 to 2014, were analyzed for mutations in the *SFTPB*, *SFTPC* and *ABCA3*. Treatments included mechanical ventilation, exogenous surfactant, HFOV, iNO, steroids. Molecular analyses have been performed on genomic DNA extracted from peripheral blood by Sanger sequencing of whole gene coding regions and intron junctions.

RESULTS

Genetic analysis of surfactant genes identified variations in 17 of the 48 newborns. 8 infants deceased at age of 2 to 6 months, 8 are alive, 1 was oxygen dependent at 2 years. 12 infants showed heterozygous variants in *ABCA3*, 5 in *SFTPB* and 1 in *SFTPC*. Only 3 variants have been previously described as pathogenic; a new deletion of 4 amino-acids in *SPTPB* may be suspected as likely pathogenic. For the remaining variants of uncertain significance (missense, intronic variants), we applied web-based prediction softwares: 3 intronic variants resulted to affect splice site, while none of the missense variants was predicted to be deleterious.

DISCUSSION

In this study, variations in *ABCA3*, *SFTPB* and *SFTPC* were found in a population of premature newborns with severe. In 14 out 17 patients the presence of only one variant was observed. Even if we did not demonstrate absence of a second variant, this evidence may suggest that the combination of primitive surfactant deficiency with alteration of surfactant metabolism may have a worsening phenotypic effect. In such families genetic counselling is advisable.

ABS 25

CHILD ABUSE AND NEGLECT: AN INCREASING CHILDHOOD'S CONDITION

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SUMMARY

The pediatric medicine has been considering the problem for a long time.

It is still often not recognized because the symptoms or signs could be: first, missed or mis-interpreted; second, hazy and uncertain; finally, they could appear in many different ways.

The first description of the phenomenon emerged in medical literature in 1962 and it was carefully considered in the following years.

Today, we have a good information and we pay much attention about this increasing social problem, although its identification is difficult. We know criteria needed for recognizing situation at high risk, but a lot of conditions are unexpected, because of the variable presentation.

Maltreatment can be physical, psychological or both of them and there is large spectrum of diseases or abnormal behavior suspected for it. In most cases parents and family are involved, with specific situations and characteristics.

Neglected care, battered child syndrome, shaked baby syndrome, abused child, exposure to domestic violence and other forms of maltreatment need for identification and timely treatment for the child and the family.

It's a social problem and society has an important function in battling this phenomenon, really a crime.

Abnormal behavior or signs could be noted at school, for example, but it isn't simple.

Pediatrician has a very important role, because s/he's aware of the problem and knows the criteria to recognize risk factors and identify phenomenon.

Prevention programs should be implemented urgently: not easy to do, due to complexity and their high cost. In the last years we assisted to an increasing information about the kind of events and to a growing attention from association and social

services. The functioning of association and social services takes root in the American model, in which is present a child abuse-assessment team.
We are at the beginning, but it's the first step.

INTRODUCTION

In the 20th century, international countries began to be interested to protect the childhood and their rights; the Declaration of the Rights of the Child was approved in Geneve in 1924.
In 1959, ONU approved the Charter of Rights of the Child, in which is ratified the right to born, to be instructed and protected from discrimination.
The Convention on the Rights of the Children was approved in 1989 in the US.
WHO defined "maltreatment" in 1991, as a form of physical and psichological neglect treatment, with a real or potential risk for the child's health and his development or dignity.
Already in 1962, during the Annual Meeting of American Academy of Pediatrics, Dr. Henry Kempe gave a lecture on this problem. Indeed, he has been the first to speak about "battered child syndrome", referring to the clinical situations of small children showing unexplained physical injuries and suspicious for maltreatment (fractures, burns, bruises, etc.).
He was responsible for the protection of minors and, in 1972, he replaced the definition "battered child" with "children abuse and neglect", in which it is considered not only physical abuse, but also the psychological mistreatment.
Maltreatment represents the 2nd cause of death in infants between 1 and 6 months of age, after Sudden Infant Death Syndrome (SIDS), and the 2nd cause of death in children between 1 and 5 years of age, after incidents.
Maltreatment can be considered either physical, emotional and sexual abuse, or physical neglect: we are unable to quantify the different forms of maltreatment, because of the lack of registers and, for the same reason, we know only a percentage of sexual abuse and exploitation of children. Many cases are ridden for different reasons as victim's fear or missing diagnosis or the compliant by the doctor.
The SINPIA classification indicate the following forms of maltreatment:

- child abuse (physical maltreatment);
- child neglect (psychological maltreatment);
- disease treatment, when the parent's care is inadeguate;
- sexual abuse;
- exposure to domestic violence.

Some risk factors are identified as cause of maltreatment's situation and they are referred as cultural risk, social risk, risk factors for parents, risk factors for child.

A CLINICAL CASE

The case is about a male infant of three months,with a twin sister, born at term of a normal pregnancy and breast fed. The little boy arrives in pediatric unit of emergency with the parents; while the little sister stays at home, with the brother of nine years old and the grandmother.
The reason for visit is an inconsolable crying, day and night; feeding and growth of the baby was reported as regular.
Parents seem nice, loving to their son, especially the father.
Physical examination of the infant is normal, but it is hospitalized for observation and other possible clinical investigations: the hypothesis, at that moment of recovery, was about abdominal colics or other functional disorders of the infant.
During the first 48 hours of hospitalization, the situation appears regular, infant's behavior and crying are suitable for his age.
Hospitalization continues to observe the situation,but there is something suspicious in parent's behavior, they don't feel comfortable with physicians and request hospital discharge.
On the third day of hospitalization, hypomobility of the left leg during the physical examination of the child, although in the absence of pain during the mobilization, is observed; the following X-ray shows a spiral fracture of the diaphyseal tibial. The case gets complicated, because this kind of fracture is typical of a torsion action to the bone in children. This kind of fracture is usually caused with intention.
We informe the parents about the fracture, they seems surprised and not able to refer any traumatic incident occurred to the infant. Moreover, the mother suddenly asks if someone will decide to take the baby away from the family, this question seems very suspicious and suggests an unclear situation.
A total body X-ray shows other fractures at different stages of consolidation, at chest and at the other leg, not at the head.
Among the social services, the family was not known as a family involved in other situation at social risk.
Later we learned that the nine years old child was supported with a special programme at school, because of his aggressive behavior.

PHYSICAL MALTREATMENT

Physical maltreatment concerns actions in which adult use physical force on a child, causing damage to the health or danger for survival: it can be identified if there are physical signs. There are specific situation and social characteristics, referred to adults and children: for the adults, for example, we know the risks correlated to economic difficulties, depression, unemployment, unwanted pregnancies, abuse of drugs and alcohol, the young maternal age, the single parenthood, history of abuse in childhood. If we consider the child, the warning signs may interest the age usually under 4 years old, physical disability, hyperactivity, preterm baby, cognitive delay and other mental disorders.

Sometimes, also situation absolutely normal for the child's age, are difficult to accept by the parents; in this case these situation could be start a maltreatment behavior.

Schmidt identified, in normal children, "basic personality at risk", and listed the "seven deadly signs" of the child: abdominal colic, night awakenings, separation anxiety, exploratory behavior, opposite behavior, feeding difficulties, urinary and fecal incontinence.

Prolonged cry represents the most frequent trigger of head injury, starting from 1 month old, with the highest incidence between 2nd and 4th month. The "shaked baby syndrome" is the most important situation, at high risk of death or severe neurological damage for subdural and subarachnoid hemorrhages.

The "battered child syndrome" has been descripted in 1962, in medical literature, as a form of maltreatment consisting of a pediatric emergency.

The kinds of actions are a public health problem and an increasing social phenomenon given that they represent the 2nd cause of death among children between 1 and 6 months and between 1 and 5 years old, after only SIDS in the first year of life and incidents in following years.

Other physical damage are represented by fractures, burns, bruising in children under 1 year old: in 75% of cases are intentional and they create suspect when they are located in the upper limbs, present spiral fractures and, finally, discovered in typical positions. For example, finding multiple fractures, at different stages of consolidation, could induce the suspect of a maltreatment's situation, whenever we exclude bone's disease as cause.

Already in 1946, Caffey and Silverman had identified the existence of ill-treatment by the study of X-rays performed on children.

As regards the burns, when intentionally caused, they are usually in atypical position,the delimitation is clear and they are often multiple; they can appear as "glove" or short" , following immersion in hot water.

Bruise is another lesion suspected for maltreatment in child very little, non able to walk, especially if discovered on face, ears, limbs, buttocks.

PSYCHOLOGICAL MALTREATMENT

Psychological maltreatment, missing signs, is difficult to identify: aggressive behavior, silence, isolation, decreasing of performance at school, other abnormal emotions are to be considered as signals of maltreatment. Psychological maltreatment consists of verbal abuse, emotional blackmail, threats, indifference, unjustified accusations, humiliation, denigration and impairment inhibiting the development of cognitive and emotional skills.

There is another form of maltreatment, consisting of pathological and overestimate care as "doctor shopping", as "Munchausen syndrome by proxy" and chemical abuse.

"Munchausen syndrome by proxy" is a well known situation, included in the fourth edition of DSM: the discovery is very difficult and the diagnosis needs a time estimated between 15 and 24 months.

On the other side, we can observe situations of "inadequate care" as "neglect care" in dirty child, missing vaccination, recurring incidents, uncareful feeding.

Sexual abuse is a terrible form of maltreatment and consists of the child's involvement in sexual activity, with or without physical contact: in this situation, the child is in a position of inferiority and not free to decide because of its age.

Exposure to domestic violence is a recurrent form of maltreatment, frequently discovered and continuosly increasing: it's considered a criminal action and, consequently, punishable. This situation, unknown in the past, emerged a few years ago, thanks to groups of women that started asking help. In this case, the child assists at domestic situations of violence and, usually, the mother is the victim.

The data of phenomenon are unknown and the possibility of prevention is difficult, because of a cultural and social structure protecting the family's privacy.

There are situations without evidence of violence, because it's hidden, as mortification, suffering and other negative emotions that are introjected day after day, until something happens, explaining through the body. "Health is in the silence of the organs", wrote Rene Leriche in the early twentieth century: it is healthy if the body doesn't speak.

Negative emotions get the body ill, emotional stress is coded and processed, and after this process, a reaction is followed, the brain speaks through autonomic nervous system, made of mediators acting at central and peripheral receptors. We're talking about Psychosomatic Medicine, that connects symptoms and emotional factors, together involved in the human body and named "somatoform disorders".

Family history and education play a key role.

Somatoform disorders are considered as diseases and placed in the Diagnostic Manual and Mental Disorders (DSM).

The damage following psychological maltreatment is permanent and it can be emotional and/or cognitive, related to disorders of the behavior in general terms.

ITALIAN DATA ABOUT MALTREATMENT IN CHILDHOOD

Fig. 1 is related to the different forms of maltreatment, referring uncorrected data, because of unknown cases.

Italian cases are classified as 57% referred to neglect care, 25% to physical maltreatment, 12% as psychological maltreatment and 6% as sexual abuse.

Fig. 2 is related to the incidence of maltreatment related at the age, showing a peak percentage between 6 and 11 years old, with another peak in the adolescent period. Males are more interested than females, with a little difference (52% vs. 48%), depending on the different areas of Italy.

The first child results at higher risk and it is more frequently the victim, according to statistics.

CONCLUSIONS

We know that maltreatment can be physical, psychological or both of them: and the following damage can be physical or psychological, or mixed. Maltreatment, physical and/or psychological, can reveal itself in different abnormal emotional conditions, as anxiety, depression, social isolation, loss of skills before acquired.

The psychological damage is difficult to repair and require a process of multidisciplinary care, for a long time, often forever. In other characteristic situations, some maltreated children improve their health, after moving away from the condition of maltreatment.

This problem is becoming more relevant and the society has an important function in battling it: suspicious situations must be identify and considered, involving authorities and social services. Teacher and pediatricians are the most important

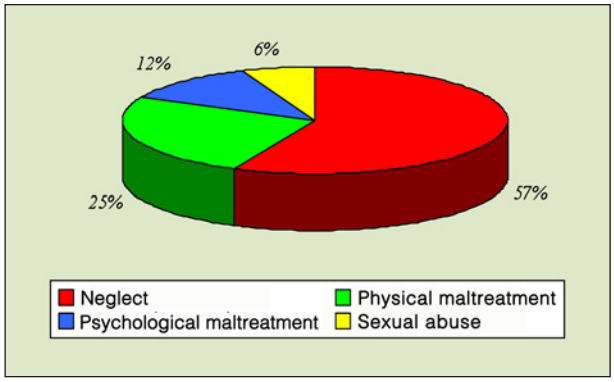

Figure 1 (ABS 25). Italian data about maltreatment in childhood.

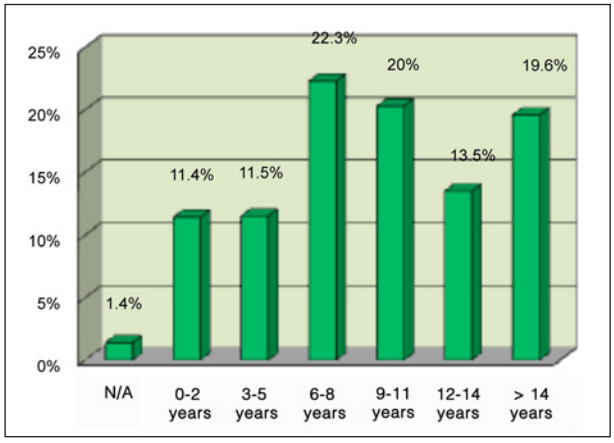

Figure 2 (ABS 25). Incidence of maltreatment in Italy in relation to age.

figures to suspect and to move doubts about children at risk for maltreatment, but everyone have to battle the problem, because it's a social problem.

"*Maxima debetur puero reverentia*" is a line from a Juvenal's satire, ideal of supreme value, everyone must think about it and the society has the duty to be it respected.

Convention on the Rights of the Child, at the 19th article, is about appropriate legislative, administrative, social and educational measures to protect the child from all forms of physical or mental violence, injuries or abuse, neglect treatment, maltreatment, while in the care of parent or any other legal person.

We could consider the mean of "to take care" not just to treat the symptoms, but to care the child as the whole of a body and mind.

In the humanization of children's care, the pediatrician's role is very important, having social function as educator during the child's growth, as guide for the children's family and as "advocate",

too: this last essential function consists in defending the global children's health, when there is a risk of maltreatment.

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ABS 26

WHEN A NEWBORN DIES: EUROPEAN APPROACHES TO RESPONSIBILITY

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In this article we illustrated, from the perspective of three different European countries, how a case of death of a newborn is treated by the Authorities when there is a suspect or complaint from the parents of a death caused by malpractice involving the medical staff.

Two of the countries, Italy and Belgium, are countries of "civil law" traditions: civil law means that the rules are codified. Countries with civil law systems have comprehensive, continuously updated legal codes that specify all matters capable of being brought to a court, the applicable procedure, and the appropriate punishment for each offence.

The third country, UK (treated by the point of view of the English and Walsh system) is a country of "common law", where the rules are generally uncodified: this means that there is no comprehensive compilation of legal rules and statutes which are based on precedent experiences, meaning that judicial decisions have already been made in similar cases.

Despite this fundamental difference, we could see not only the obvious differences but also similarities (such as the possibility of a civil claim even if the authority dismissed the criminal case) between some aspects of the trial for a newborn death in common and civil law countries. Moreover, a few minor differences exist between the trail rules practice between the two countries of civil law, such as the presence of a double path to incrimination in Belgium: one involving the Public prosecutor (like in Italy) and another that can be lead by a "Judge of instruction" that is not a prosecutor but can make investigations on request of the parents, passing then finally back to the Prosecutor.

ABS 27

NMR-BASED SCREENING POSSIBILITIES IN THE NEONATE AND COMPARISON TO MASS SPECTRAL METHODS

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NMR, for many years considered as a tool for structure verification and elucidation on single compounds, has rapidly developed into a widely used mixture analysis technology in the last years, mainly driven by Metabolomics needs. NMR despite its reduced sensitivity compared to different types of Mass Spectrometry is the technology of choice for combined targeted and nontargeted screening in one experiment. This is especially enabled by its unique reproducibility, allowing to precisely monitor even smallest changes of multiple compounds for example in urine and such allows the detection of complex patterns for early disease recognition. Since NMR allows this high reproducibility not only within one instrument, but equally on different instruments operating under the same standard operating procedures and same field strength, it allows researchers to work on the same metabolic problems and integrate data for common statistical analysis. A comparison on ~ 1,000 samples from a paediatric routine lab in Germany for NMR and GC-MS explained in detail in the presentation shows advantages for very small molecules and the precision of quantification, e.g. orotic acid, creatinine/creation ratios and acylcarnitines, for NMR, while GC-MS has advantages in the detection of very low concentrated larger molecules. NMR has minimum needs for sample preparation and allows fast measurements within a few minutes. In untargeted mode, all kinds of deviations from normality can be detected (if visible by NMR),

even if they are so far unknown, like impurities or so far unknown inborn errors of metabolism. In Neonatology it is vital to be able to detect small changes in the body fluids under investigation, be it from the mother or the neonate itself for early detection of developing diseases and risk management for the neonate. Examples are given for example on potential development retardation of the neonate detected by time trajectories on urine over the first days of life.
Another example investigated in detail is the follow-up of administration of dedicated nutrition for PKU patients and comparison to healthy age matched cohorts. This can be used to very precisely to adjust and optimize the dosage and observe other metabolic effects of the treatment. This type of investigation is done as longitudinal study and also benefits in sensitivity and specificity from the high reproducibility of NMR.

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ABS 28

OUR TEAM PROJECT: LEARNING FROM MISTAKES

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Risk management refers to the practice of identifying potential risks in advance, analyzing them and taking precautionary steps to reduce the risk.
Considering that neonates and children are highly exposed to potential risks both in the neonatal and pediatric intensive care unit, in the past three years a priority of our team was to implement the risk management system already present at our institution.
A dedicated neonatal and pediatric multidisciplinary team was created and a dedicated neonatal and pediatric incident reporting system was realized. Previously there was an high incidence of under reporting, as already described by other authors. According to our experience, the creation of a reporting system dedicated to neonates and children and the extensive training of the personnel significantly improved the frequency of reporting.
Frequent audits were held to review the incidents and to identify potential areas of improvement. This process led to the creation of new institutional protocols and guidelines, in particular to reduce the incidents related to drugs preparation and administration.
Finally we wanted to know how parents perceived the risks, so we carried out a survey. We found that only 30% of the parents (50% with babies less than 28 weeks of gestational age) have a perception of risks. Parents think that risks are mainly related to the number of nurses and doctors caring for their babies, concluding that nights and weekends are the most dangerous times.

ABS 29

PERIPHERAL INTRAVENOUS CATHETERS IN NEONATES

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Peripheral intravenous catheters are the most commonly used devices in clinical practise. They are used for therapeutic purposes such as administration of fluids, electrolytes, blood products, medications and parenteral nutrition; therefore achieving vital support and giving nutrients when no significant nutrition is enterally possible. The intravenous line allows a rapid action of the drugs administered, because the required phase of absorption, when given by other routes is bypassed; furthermore a continous infusion can be used and hence allowing to keep a stable and safe level in the blood. The choice of the device should be estabilished upon known parameters such as type and duration of the theraphy, age, weight, diagnosis, clinical condition and veins accessibility. In the last two decades the intravenous device's thecnology has impressively improved and specific attention has been directed to hinder drawbacks.
Nowadays nurses are therefore called for news knowledge, competence and skill in order to choose the most suitable device, use an aseptic procedure, decrease complications and improve healthcare.

ABS 30

NEWBORN INDIVIDUALIZED DEVELOPMENTAL CARE AND ASSESSMENT PROGRAM (NIDCAP)

AS A FAMILY-CENTRED SUPPORTIVE INTERVENTION

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The prematurely born infant is probably the most vulnerable patient in our hospitals due to his or her immaturity. The impact on brain development of the exposure to stressors in the neonatal intensive care unit (NICU) makes premature infants even more fragile. For this reason, over recent years, the importance of developmentally supportive care has become extremely important.

The multidisciplinary-based care philosophy, called developmentally supportive care, has evolved along with the ever-increasing success in treating severely ill or extremely prematurely born infants. With this success, there has been growing attention to long-term medical and mental health, as well as the neurobehavioral and of the survivors. Infant and family-centred developmentally supportive interventions aim at reducing stress and providing pain treatment, support the self-regulation of the infant and promote parental presence and involvement in the care of their baby.

Newborn Individualized Developmental Care and Assessment Program (NIDCAP) is the only infant and family-centred developmental supportive intervention designed to be implemented right from the moment of birth. It is also unique because it promotes a systems perspective of the care of the infant and his or her family, the environment around the infant and the assessment of the needs of the infant and his or her family through naturalistic behavioral observations aimed at formulating recommendations on how to adjust the care or the environment appropriately in a supportive way.

By enhancing the ability to read the behavioral cues of the infant and understand his or her strength and sensitivity, the general knowledge and competence of parents and staff members is enhanced. This approach makes parents and staff capable of facilitating the stability and well-being of the infant and improve his or her coping with stressful situations by supportive actions.

ABS 31

FROM ADULT TO PEDIATRIC RADIOLOGY. ENTROPY-BASED IMAGING ALGORITHMS FOR APPLICATIONS IN PEDIATRIC MEDICINE

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SUMMARY

Ionizing and non ionizing radiation imaging techniques are still under study to increase sensitivity and specificity in the diagnostic process. Specific protocols can be used to reduce the pediatric patients dose in case of ionizing radiation use in the imaging diagnosis process maintaining or increasing the quality of the images; several international task groups are working to improve the application condition of imaging analysis. Performances can be improved both on the side of the radiation properties and on the side of images improvement quality. But there is a third source of performances improvements: the reading of images. Generally specking, images diagnosis is difficult because, despite the basic anatomy being virtually identical from image to image, the degree of natural variation in both normal and abnormal structures is quite high. Radiologists will never see all the possible variations however long they practice or however many images they view. In the last twenty years, much research has been carried out to develop computerized methods to assist radiologists in differentiating benignant from malignant findings in the images. Using a computer aided diagnosis (CADx) scheme, radiologists could benefit from computer output while formulating their opinion. Most promising algorithms for the CAD analysis are based on the concept of entropy. Entropy-based CAD approach produces a great benefit in the Textural Features extraction in the imaging diagnosis process; for example, it is possible to improve Classification of Pediatric Posterior Fossa Tumors. Further, Entropy-based CAD approach in the Diffusion tensor-imaging (DTI) technique could allows to radiologists to create a powerful database with retrospective motion correction for large-scale pediatric imaging. These and others findings suggest an added diagnostic value of quantitative

feature analysis of imaging by entropy-based CAD approach in a particularly sensible field of Medicine like the pediatric neuro-oncology. We report some of the most recent results in the topics to introduce once again the importance of the ICT technology in the modern clinical Medicine.

INTRODUCTION

The diagnosis process in Medicine is complicated by the lack of sensibility and specificity of the criteria adopted for. Also in the powerful and promising imaging diagnostic process several factors affect the evaluation of the findings. Mathematicians, Physicists, Informaticists and Technologists have increased the cooperation in the Medicine applications and all the process have gained by this multidisciplinary approach. Recently, the new automatized process of images reading has produced interesting results in the support to the Physicians in the diagnostic process. These algorithms are able to mining latent informations contained into the images, especially in the multimodal images likes the ones extracted by powerful techniques like the Magnetic Resonance. Further, in the field of the ionizing radiation imaging, the ability to extract more information from images allows the reduction of patient dose. The increase of information from the images for the diagnostic allows the increase of sensibility and specificity for the diagnostic process. A promising class of these algorithms are the entropy-based approaches; particular definition of the 1D or 2D images signals entropy can be used to detect latent findings in the images useful for the diagnosis. Further the automation of this process increase the ability of the "reader" to maintain the higher attention on the single image acquired avoiding the "false negative" errors in the diagnosis process. The concept of entropy has been developed in thermodynamics in order to characterize the ability of a system in changing his status. Measures of system entropy are usually functions defined in the phase space and they reach the maximum or minimum value depending on the contextual definition, whenever system variables are uniformly distributed. It is related to the disorder degree. This concept has been borrowed in communication systems for coding purposes and data compression. Entropy based functionals have been also adapted in image and signal analysis to perform deconvolution and segmentation, to measure the pictorial information and to better define image differences. From the mathematical point of view it is really interesting the studies about the definition of the entropy concept for an image. We have proposed a novel definition of the entropy for the images in our study in the Rx imaging in the breast cancer studies. Properties of this innovative calculation method from the entropy are quite promising for the application in pediatric radiology especially for the neurological applications. Some of these properties have the capability of being correlated with other diagnostic information enhancing the sensibility and the specificity of the diagnosis. We are testing the performances of a possible approach associating the predictive value of a tumor marker as plasma osteopontin with the analysis of entropy in mammograms of patients with microcalcifications (MCs). Osteopontin is a malignancy associated protein detectable in plasma and tumor tissue; in patients with breast cancer high levels are associated with the presence of mammographic calcifications and are correlated with aggressive histology, poor prognosis and shorter survival. Breast cancer (B.C.) represents a major cause of death in women and is exceeded only by that of the lung. The degree of disorder (entropy) of the image is an important indicator; in fact the texture disorder (parenchymal tissue structures) in the suspicious region of the image represents a significant component for a physician in the diagnosis of malignancy or benignancy. In our study [1] we evaluated mammograms and plasma osteopontin levels (immunoenzymatic method) of two women (enrolled at Oncologic Hospital "A. Businco", Cagliari, Italy) with mammographic microcalcifications (MCs) (**Tab. 1**).

Each mammogram was studied separately evaluating the entropy of areas with very limited and connected dimensions. Different 120 x 120 pixel regions were selected in each mammogram so that each region contained healthy parenchyma and the zone with MCs. At least 6 regions were selected for each mammogram. Each region was then subdivided into 15 x 15 pixel contiguous and connected under-regions, thus obtaining 64 tiles. We associated the equivalent entropy value and the standard deviation of the entropy to each tile of the region. Osteopontin levels was 100 ng/ml in patient with malignant MCs and 37 ng/ml in patient with benignant MCs. The entropy trend is showed in **Fig. 1** for patient with malignant MCs and in **Fig. 2** for patient with benignant MCs. Analyzed tiles from each mammogram are reported

Table 1 (ABS 31). Evaluation of mammograms in two women.

Patient	Age	Mammogram	Hystology
D.A.	72	Malignant MCs	DCIS
C.R.	61	Benignant MCs	Normal

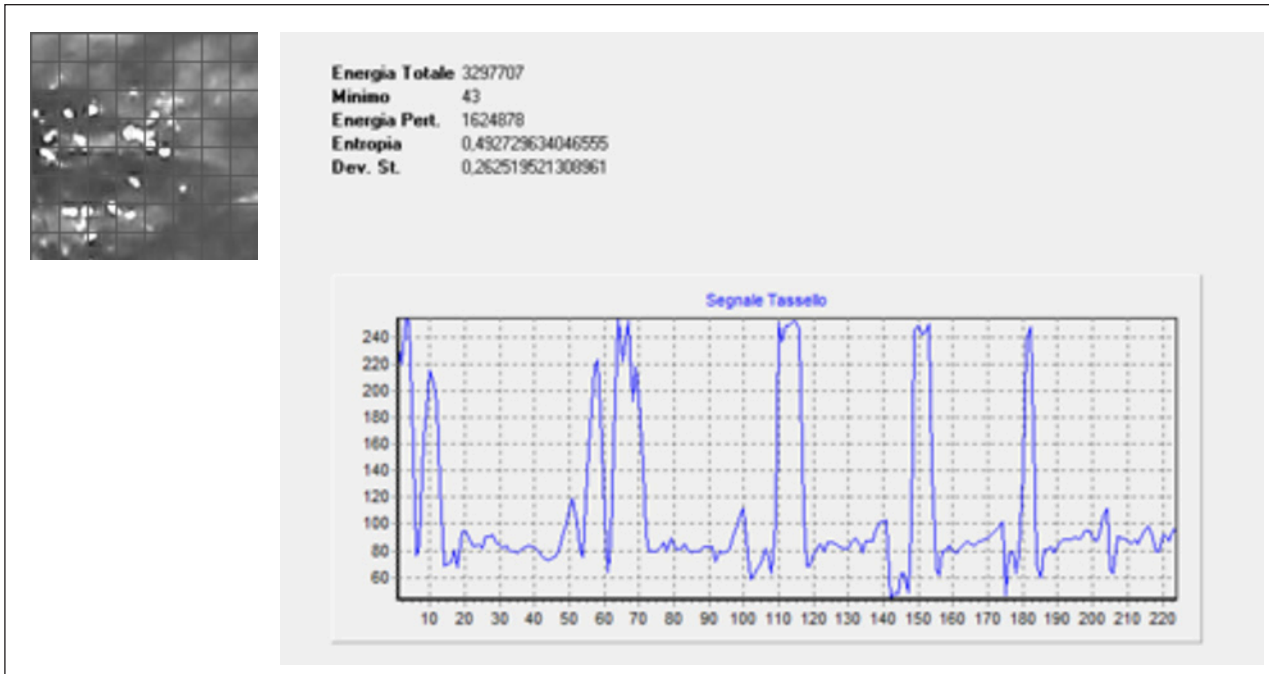

Figure 1 (ABS 31). Entropy trend in patient with malignant microcalcifications (on the right side the mammogram tiles evaluated).

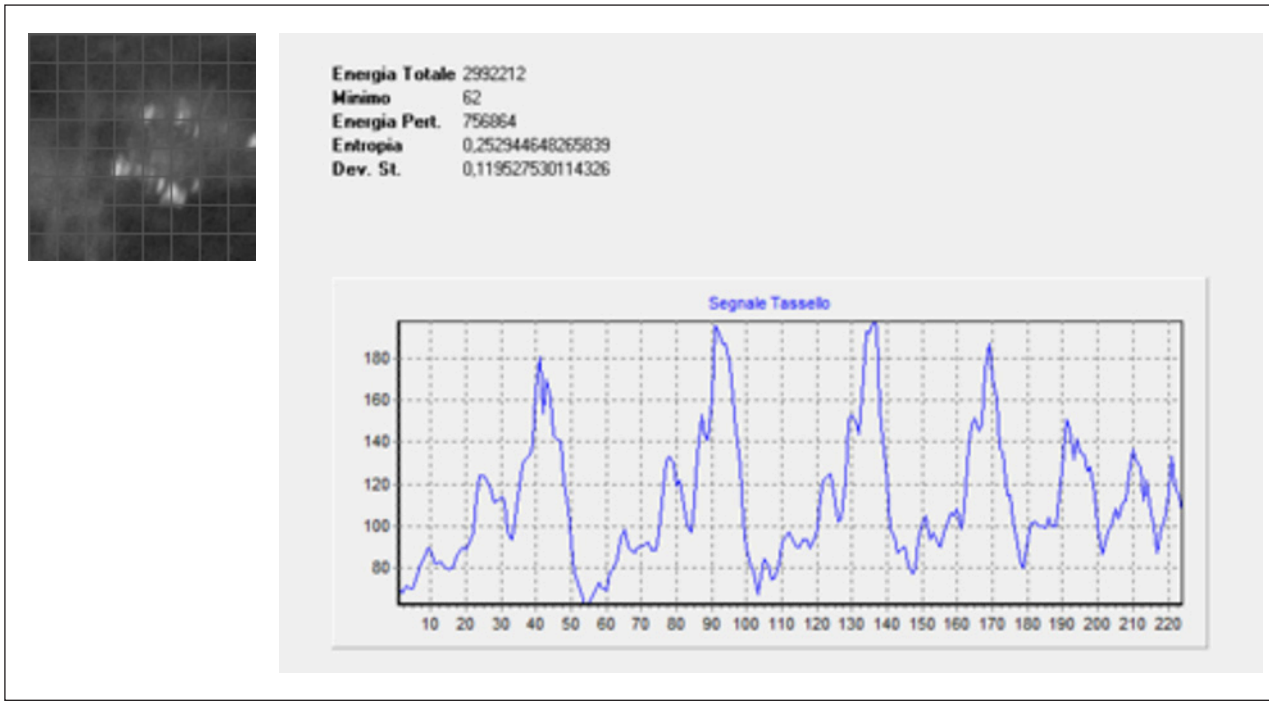

Figure 2 (ABS 31). Entropy trend in patient with benignant microcalcifications (on the right side the mammogram tiles evaluated).

in **Tab. 2**. Signal data of 2 tiles are reported: tile number 36 for malignant MCs and tile number 36 for benignant MCs.

Indeed, it is very difficult to compare the distribution of the grey tones (texture), their value, the possible order or disorder of an area of the mammogram with another area of the same mammogram. Our testing, presented briefly in the previous paragraph, has shown how the entropy measure can be an excellent aid to evaluate and to confront such measures.

Table 2 (ABS 31). Results obtained from signals selected on malignant and benignant areas of MCs in mammograms examined.

	Malignant MCs	Benignant MCs
Etot	3,297,707	2,992,212
Ep	1,674,878	756,864
S	0.049	0.025
Sqm	0.26	0.11

In fact, we noticed that in case of malignant masses, the entropy assumes very high values around the edges of the lesion, while it assumes very low values within the same mass. The entropy standard deviation assumes very high values if compared to a benignant mass. In the case of benignant masses, we observed that there are no major entropy alterations on the whole image taken into consideration, as it also shows a reduced value of the standard deviation. We believe that, with the arrival of the digital mammography, the recourse to entropy measure in different areas, could be a valid aid for the radiologist to formulate diagnosis.

Since the method works in almost real time, the radiologist can choose which and how many areas to confront, the sizes of such areas, the sizes of the areas to evaluate etc.

He can obtain indications as to the nature of the mass under analysis from the trend of entropy measurements. The obtained experimental data show that in the case of benignant masses contained in a mammogram, there are no structural variations, whilst in the case of malignant masses, in the area of the mass, there is a different structure from that of the surrounding parenchyma, defined by areas with high entropy values.

Table 2 shows that it is not important total signal energy (that is strongly affected by image acquisition) as well as perturbation energy, entropy and square deviation. The last are much more high in malignant MCs with a strong increment of the perturbation energy. It is important to notice that the value of the perturbation with respect to the malignant lesion is about twice of the value corresponding to the benignant. In addition the mean square deviation with respect to the malignant MCs is about twice the value corresponding to the benignant MCs. Finally the entropy value of the malignant MCs is higher (0.049) than the corresponding benignant value (0.025).

Evaluation of osteopontin levels in association with entropy analysis of breast radiologic calcification might be an useful aid in the evaluation of microcalcification and provide an additional support for the choice of treatment strategy in women with mammographic calcification.

Texture analysis (TA) is a powerful approach to characterize and quantify the tumor matrix. TA features provide in previously suggested for tumor segmentation. In SVM classifiers, features are represented as n-dimensional vectors and combined to create a model of a particular class by using true and false training examples. Rodriguez-Gutierez et al. [2] proposed a paper for the description of an entropy-based metrics and textural features extraction on MRI Diffusion images to improve Classification of Pediatric Posterior Fossa Tumors. This retrospective study included preoperative MRI in 40 children with posterior fossa tumors (17 medulloblastomas, 16 pilocytic astrocytomas, and 7 ependymomas). Shape, histogram, and textural features were computed from contrast enhanced T2WI and T1WI and diffusivity (ADC) maps. Combinations of features were used to train tumor-type-specific classifiers for medulloblastoma, pilocytic astrocytoma, and ependymoma types in separation and as a joint posterior fossa classifier. A tumor-subtype classifier was also produced for classic medulloblastoma. The performance of different classifiers was assessed and compared by using randomly selected subsets of training and test data. The classifiers SVM coupled to texture analysis procedure has revealed a good level of sensibility for the classification of pediatric posterior fossa tumors. In our opinion the SVM ability to grades the tumors can be increased with a different definition of entropy as the one proposed by Vitulano et al. and applied to the breast tumor study.

The last application of entropy-based algorithm is the EPI-ghost correction derived from a set of T2-w data images applied in iterative calibration scheme. In brief, a constant offset and linearly increasing delay between even and odd bipolar EPI readouts was determined that minimized the total image entropy for each of the volumes analysed. This work was presented by Holdsworth et al. [3].

CONCLUSION

Entropy-based algorithms allow a dramatic improvement in the quality of imaging diagnosis process [4]. Further studies will give to the Radiologists the possibility to get much more informations from the imaging process in the Pediatric Radiology.

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ABS 32

NCAM IS EXPRESSED IN THE METANEPHRIC MESENCHYME UNDERGOING MESENCHYMAL EPITHELIAL TRANSITION IN THE DEVELOPING HUMAN KIDNEY

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BACKGROUND/AIMS

NCAM, also known as CD56, is a transmembrane protein expressed on the cell membrane of neurons, glial cells, neuroendocrine and muscle cells [1]. Recently, up-regulation of NCAM has been reported in renal stem/progenitors in the human adult kidney [2]. This study was aimed at analyzing NCAM expression in the developing human kidney.

METHODS

To this end, kidney samples from four human fetuses, rancing from 11 up to in weeks of gestation, were formalin-fixed and paraffin-embedded. 5 micron-sections were immunostained.

RESULTS

Reactivity for NCAM was restricted to the metanephric mesenchyme and to the early stages of the process of mesenchymal-epithelial transition. Immunostaining for NCAM was mainly observed in undifferentiated capsular cells and in mesenchymal cells located in close proximity of the renal capsule (**Fig. 1**). A membranous reactivity for NCAM characterized cap-mesenchymal cells adherent to

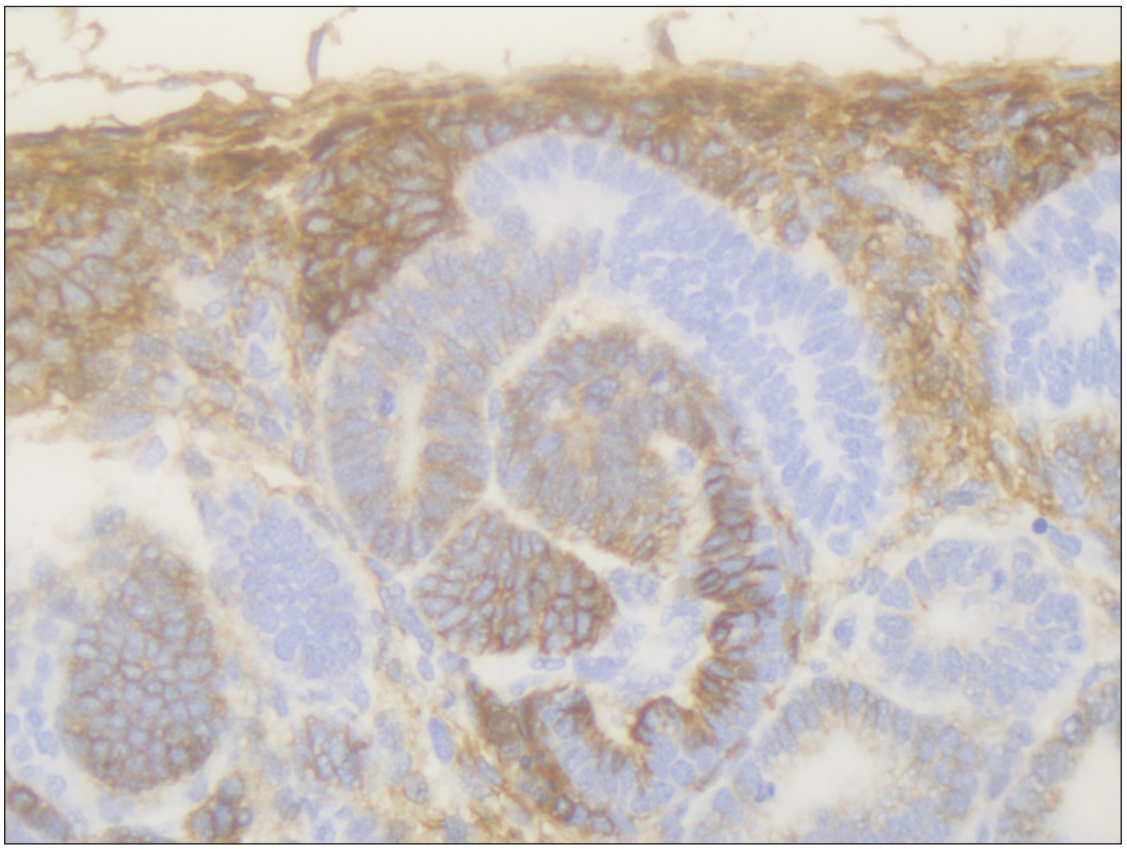

Figure 1 (ABS 32). Immunostaining for NCAM was mainly observed in undifferentiated capsular cells and in mesenchymal cells located in close proximity of the renal capsule.

the ureteric bud tips that were always negative for NCAM (**Fig. 2**). Renal vesicles, the first structures with an epithelial appearance, were strongly reactive for NCAM, whereas developing glomeruli did not express NCAM (**Fig. 3**). Thanks to the absence of reactivity for NCAM of ureteric bud tips, it was possible to better characterize the intimate relationships between mesenchymal and epithelial precursor inside the renal stem cell niche. In some niches, the commistion between the epithelial and the mesenchymal stem/progenitors was evident, with epithelial cells detaching from the but tips and infiltrating the surrounding mesenchyme (**Fig. 4**).

CONCLUSIONS

Our preliminary findings regarding NCAM expression in the developing human kidney lay stress on the major role played by this adhesion molecule in the early phases of kidney development.

An interesting finding emerging from our study is the restriction of NCAM to the mesenchymal component of the renal stem cell niche. The absence of reactivity for NCAM in the ureteric-bud-derived cells allows a better comprehension of the relationships between epithelial and mesenchymal precursors that, at histology, is not evident.

Another interesting finding is represented by strong immunostaining for NCAM of the majority of capsular cells, that confirms the stemness of capsular cells and their major role in nephrogenesis.

NCAM, on the basis of our data, appears a typical marker of undifferentiated mesenchymal renal progenitors and of progenitors undergoing mesenchymal-epithelial transition. NCAM expression is high till the origin of renal vesicle. The absence of reactivity in glomerular cells confirms the significance of NCAM as a marker of mesenchymal progenitors and of their initial transition toward the epithelial world.

Further studied are needed in order to better study NCAM expression in the developing kidney at different gestational ages till birth, in order to better define its role in human nephrogenesis.

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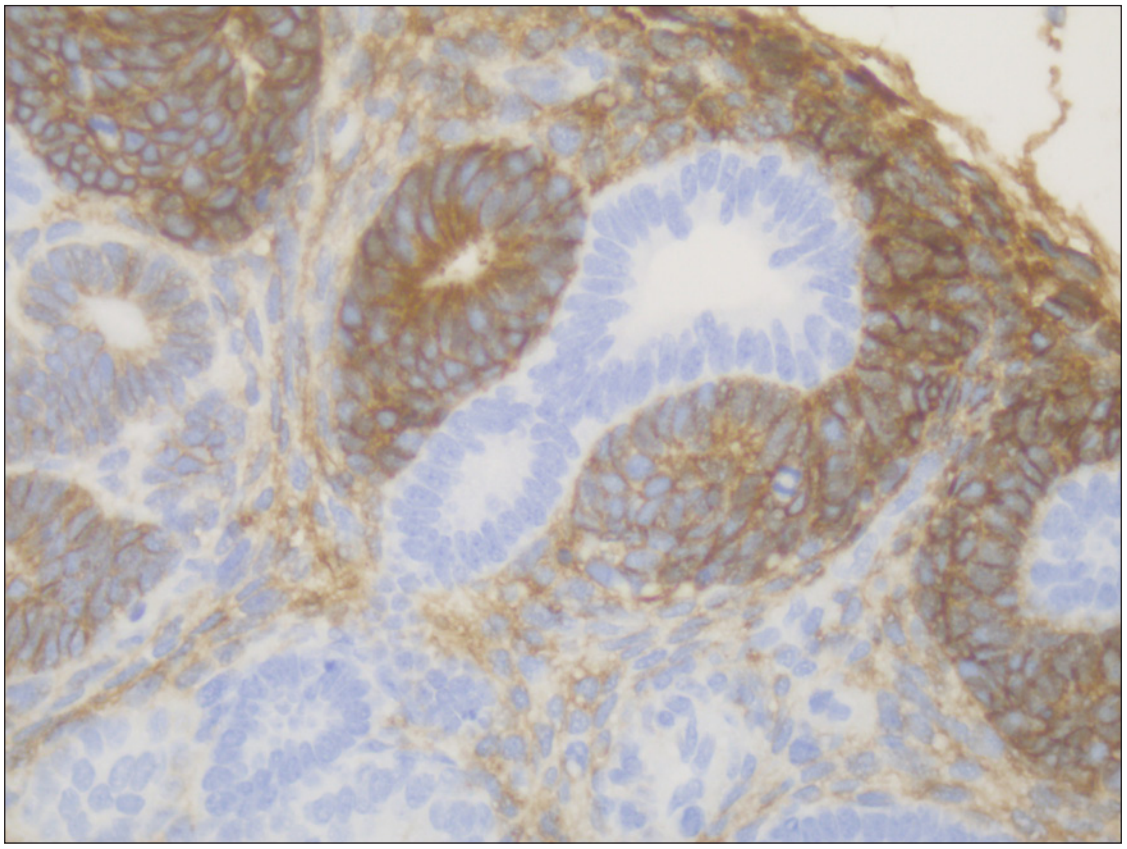

Figure 2 (ABS 32). A membranous reactivity for NCAM characterized cap-mesenchymal cells adherent to the ureteric bud tips that were always negative for NCAM.

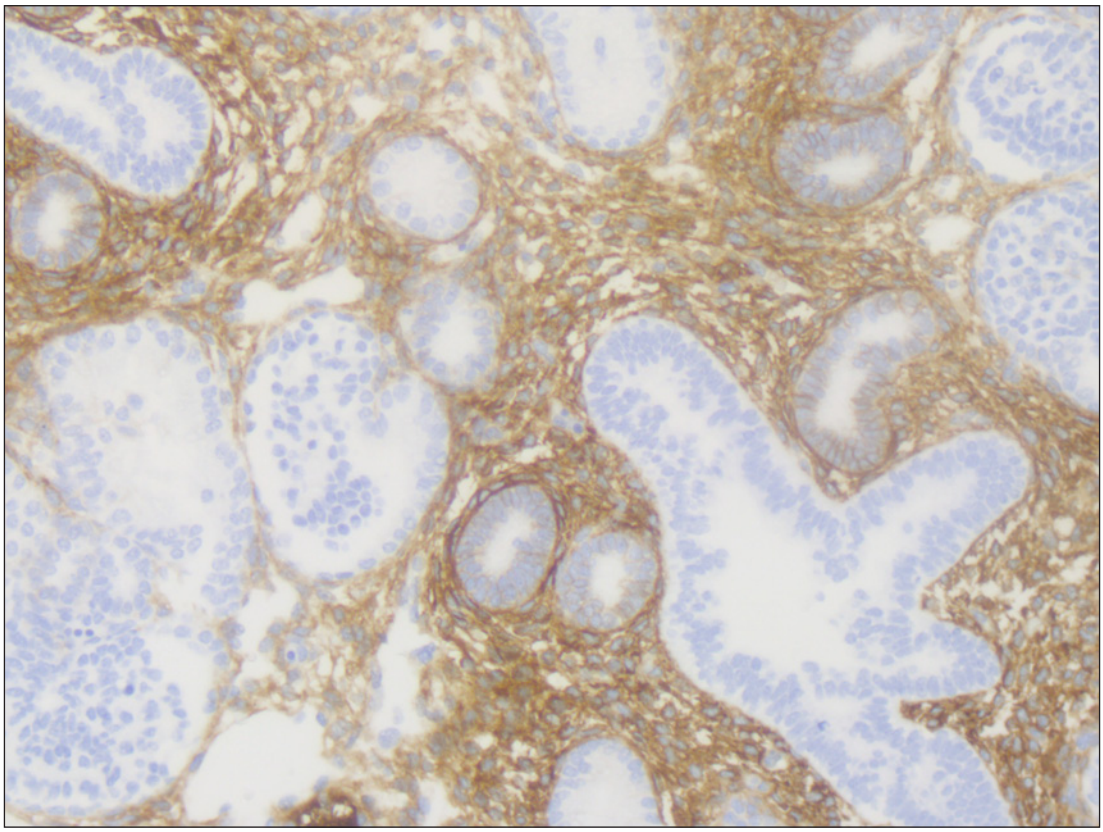

Figure 3 (ABS 32). Renal vesicles, the first structures with an epithelial appearance, were strongly reactive for NCAM, whereas developing glomeruli did not express NCAM.

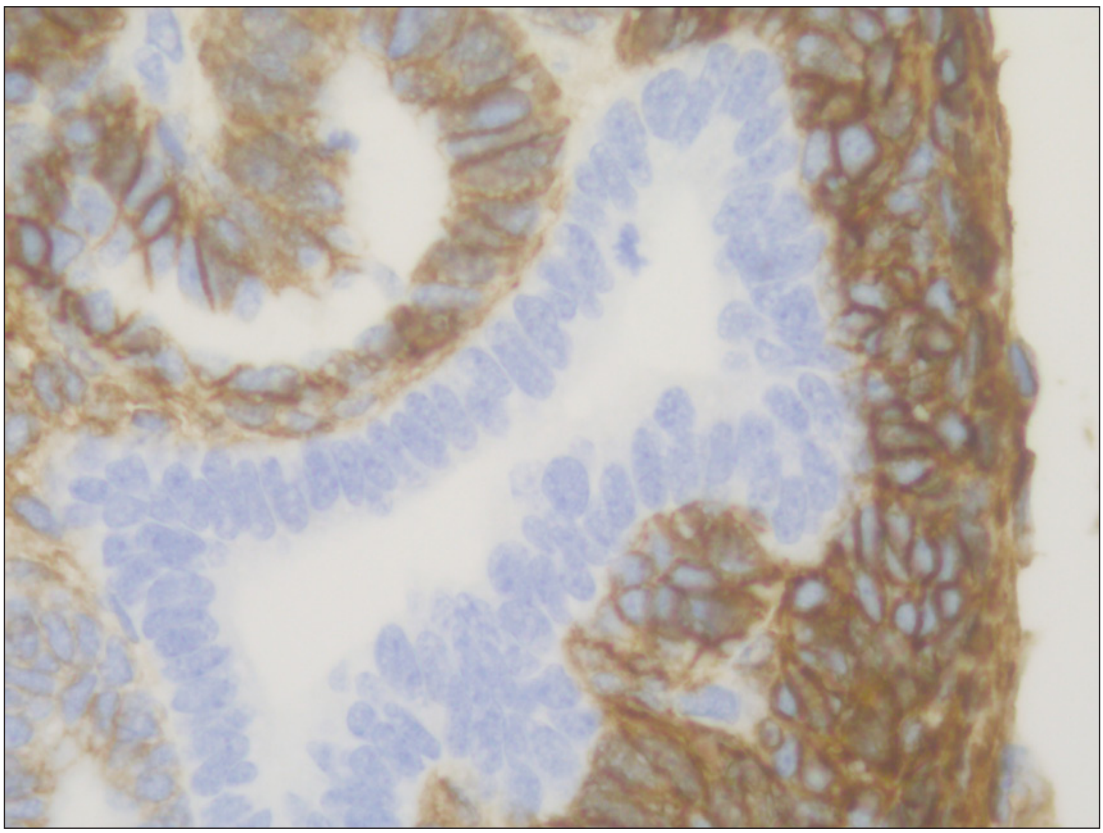

Figure 4 (ABS 32). In some niches, the commistion between the epithelial and the mesenchymal stem/progenitors was evident, with epithelial cells detaching from the but tips and infiltrating the surrounding mesenchyme.

ABS 33

PRIMARY HCMV INFECTION IN PREGNANCY: PRELIMINARY METABOLOMIC DATA ON AMNIOTIC FLUID

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INTRODUCTION

Human cytomegalovirus (HCMV) is the most common cause of congenital viral infection leading to sensorineural hearing loss, and neurodevelopmental delay. Intrauterine transmission may follow either primary or recurrent infection. Primary maternal infection has a risk of vertical transmission of 30% to 35%, within the entire period of gestation.

Recently, transcriptomics, proteomics and metabolomics have been applied on biological samples in order to better understand the physiopathology or the mechanisms of the several diseases. In particular, metabolomics is the study of the entire range of low molecular weight molecules present in an organ, tissue, or biofluid. To our knowledge, only one paper was reported on the metabolic effects of HCMV on congenitally infected newborns. Such study dealt with the analysis of urine samples, collected within the first two weeks of life, applying ^{1}H-nuclear magnetic resonance (NMR) spectroscopy followed by multivariate statistical analysis [1]. In this study we propose a GCMS based metabolomics carachterization of the Amniotic Fluid (AF) in a population of pregnant women, which contracted primary HCMV infection during pregnancy.

A retrospective cohort study was conducted on 20 pregnant women, which transmitted the virus to the fetus ("transmitters"). Institution Review Board was requested for this retrospective study. A written informed consent was obtained from each woman undergoing invasive procedures. Amniotic fluid (AF) was collected from all pregnant women through amniocentesis. Results of prenatal diagnosis were confirmed from urine analysis at birth or from autopsy specimens obtained after the termination of pregnancy. AF not required for virological testing was aliquoted and stored at -80° until metabolomic analysis. In parallel, AF samples from 23 healthy pregnant women who underwent amniocentesis for cytogenetic-based diagnosis were included as controls. At enrolment, median age in the two study groups was 34 for transmitters, and 37 years for controls.

AF samples were thawed at room temperature and vortex mixed to homogenize. 200 µL were transferred in Eppendorf tubes (1.5 mL) and treated with 400 µL of acetone for protein precipitation. The mixture was vortexed for 30 s and centrifuged (1,400 rpm for 10 min). 400 µL of supernatant were transferred in glass vials and evaporated to dryness overnight in an Eppendorf vacuum centrifuge. 60 µL of a 0.24 M solution of methoxylamine hydrochloride in pyridine was added to each vial, samples were vortex mixed and left to react for 17 h at room temperature. Then 80 µL of MSTFA (*N*-Methyl-*N*-trimethylsilyltrifuoroacetamide) were added and left to react for 1 h at room temperature. The derivatized samples were diluted with hexane (100 µL) just before GC-MS analysis.

Samples were analyzed using a Agilent 5975C interfaced to the GC 7820 equipped with a DB-5ms column (J & W), injector temperature at 230°C, detector temperature at 280°C, helium carrier gas flow rate of 1 ml/min. The GC oven temperature program was 90°C initial temperature with 1 min hold time and ramping at 10°C/min to a final temperature of 270°C with 7 min hold time. 1 µL of the derivatized sample was injected in split (1:20) mode. After a solvent delay of 3 minutes mass spectra were acquired in full scan mode using 2.28 scans/s with a mass range of 50-700 Amu.

Each acquired chromatogram was analysed with the free software AMDIS (Automated Mass Spectral Deconvolution and Identification System): each peak was identified by comparison of the relative mass spectrum and retention time with those stored in an in-house made library of 255 metabolites. Other metabolites were identified using the database NIST08 (National Institute of Standards and Technology's mass spectral database) and the

Golm Metabolome Database (GMD). Through this strategy 58 compounds were detected and quantified: 50 identified, 4 unknown compounds matching equally unknown compounds contained in GMD, and 4 unknown metabolites which recurred in every sample.

Multivariate model based on Principal components analysis (PCA) was conducted in SIMCAp12 and used to overview the data variance structure in an unsupervised mode. Data have also been processed with the web tool MetaboAnalyst to confirm the previously obtained results from SIMCAP12+,and to investigate the canonical pathways involved. Subsequently, network mapping was used for the interpretation of multivariate results within a biological context: a biochemical and chemical similarity network was constructed among all the measured metabolites, applying KEGG and PubChem CID identifiers, and using a homemade routines for R.

RESULTS

A partial least squares discriminant analysis (PLS-DA) model was developed to identify important multivariate discriminant variables of control vs. transmitters amniotic fluid metabolomics profiles with a ROC curve (WEB Software MetaboAnalyst) reported in **Fig.1** with area under the curve of 0.9.

The most important identified metabolites of interest with VIP value > 1 for this model include: Serine, Urea and Glutamic acid.

Using these informations the MetaboAnalyst program calculates the most impacted human pathways involved in the discriminating model. Finally, using homemade routine in R and the Cytoscape software we have realized the network of metabolites of interested related to the differences between virus transmitters and control group. The primary contribution of the virus transmission is related to the "Alanine-Asparate" metabolism.

DISCUSSION AND CONCLUSIONS

Since more evidences are necessary to support the hypothesis about the condition for the virus transmission, metabolomics seems to be a suitable, powerful, and promising investigation tool. Indeed, strong correlations between specific metabolites and several gestational conditions (fetal malformations, preterm delivery, premature ruptures of membranes, gestational diabetes mellitus, preeclampsia, and so on) have been reported after the metabolomic analysis of AF, placenta, blood, and urine from pregnant women. To our knowledge, this is the first preliminary study on metabolomics profile of amniotic fluid in relation to HCMV infected fetuses.

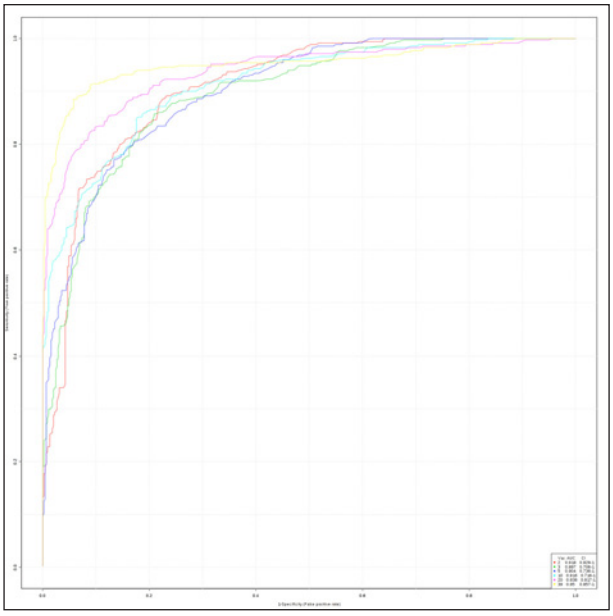

Figure 1 (ABS 33). Roc curve of the model for controls vs. transmitters (AUC = 0.9).

ACKNOWLEDGMENTS

Francesco Palmas gratefully acknowledges Sardinia Regional Government for the financial support of his PhD scholarship (P.O.R. Sardegna F.S.E. Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2007-2013 – Axis IV Human Resources, Objective l.3, Line of Activity l.3.1.). Dmitry Grapov is supported by a NIH Grant: NIH 1 U24 DK097154.

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ABS 34

NMR-BASED METABOLOMICS ANALYSIS OF URINARY CHANGES IN NEONATAL NECROTIZING ENTEROCOLITIS

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Necrotizing enterocolitis (NEC) is intestinal inflammation occurring mainly in preterm or sick neonates after enteral feedings have begun. The

exact etiology of NEC is not clear. It is characterized by variable damage to the intestinal tract, ranging from mucosal injury to full-thickness necrosis and perforation. In these severe cases, the extensive insult may damage the intestinal lining, leading to increased intestinal permeability and leaving the intestine susceptible to bacterial invasion.

We analyzed urine samples obtained from NEC infants (EG < 30 weeks) over a period of 40/60 days from birth and from 10 matched controls, among which 5 infant showing food intolerance but survived free of NEC. The temporal evolution of the urinary profiles was followed by a combined used of NMR spectroscopy and chemometrics tools. The analysis of spectroscopic data by principal component analysis (PCA) evidenced different time-related trajectories of the metabolic profiles for controls and NEC: controls exhibited a overall progressive change; NEC infant metabolic profiles were characterized by a progressive evolution, similar to controls, before symptom onset and during treatment and by a regressive trend at the day of NEC occurrence. Although preliminary, these findings pointed out how metabolic trajectories could help the understanding of NEC causes and facilitate the development of useful therapies for this disease as well as biomarkers for disease and its progression.

ABS 35

PROMOTING THE CONSUMPTION OF FISH IN CHILDREN: A PROPOSAL FROM SAN BENEDETTO DEL TRONTO

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BACKGROUND AND AIM

Italian children eat fish only once a week on average, less frequently than currently recommended (three times per week). Reasons for this feeding pattern may include the following: 1) children are often not used to the taste of fish, as they may have not tasted it during pregnancy through the amniotic fluid. 2) the presence of bones may make chewing and swallowing difficult for children; 3) parents often do not have enough time to prepare meals and cook fish for their children.

This report describes a series of projects and initiatives implemented in San Benedetto del Tronto (AP, Marche, Italy), aimed at the promotion of fish consumption in children through absolutely innovative and original tools.

MATERIALS AND METHODS

The initiative took place in four phases:

1. Analysis of children preferences. A survey to detect children's food preferences, with a particular focus on fish, was launched in July 2014 in San Benedetto del Tronto, in collaboration with the city administration and Assoalbergatori.
2. Presentation and dissemination of results. The results of the survey were presented by Italo Farnetani at a the following conferences: 'Nutrition Forum: the concept of nutrition from 79 AD to 2015', Pompeii (NA) in September 2014; the International Tourism Exchange (BIT), Milan in February 2015 (as part of the initiatives promoted by the Marche region), and Expo, Milan in June 2015 (as part of an event organized by the city of San Benedetto del Tronto).
3. Launch of the project, based on the results of the survey. The city council of San Benedetto del Tronto implemented the 'Sano come un pesce' (healthy as a fish) initiative, featuring educational visits to museums, Preparation Labs and fish taster sessions on a weekly basis during summer 2015. The project included fun activities and games and aimed at encouraging children to get in touch with the marine environment in all its aspects, linking the fun experience of the sea and the beach with food.
4. Collection of results during the 'Laboratory of Taste' event held on 5 August 2015. Italo Farnetani conducted a survey among children taking part to the initiative on fish preferences.

RESULTS

This project showed that anchovies are an ideal type of fish for children. In fact, as bones are easily taken off, this kind of fish is well accepted by children. The small fish is also at a low lead content. The blue fish also provides excellent nutrient supply at a low cost.

Following previous surveys, we have proposed some recipes to cook anchovies which are suitable for weaning. In fact, anchovies can be cooked in different ways that give a different texture to the fish and which can be selected in relation to the ability to chew. For example, boiled anchovies would suit an 8-months-old baby, meatballs with anchovies or anchovy croquettes can be introduced at 12 months, and breaded and fried anchovies can be enjoyed at 18 months. Fried olives stuffed with

fish, 'Olive all'ascolana' as in the variant of San Benedetto del Tronto, can be proposed to children older than 36 months. This recipe is particularly enjoyed by children because they are small and round. Furthermore, this recipe carries nutritional benefits of both the fish and the oil, containing fatty acids of vegetable origin.

The survey concluding the project showed that children like to eat fish. 40% of children claimed to like fish of any kind, provided it is free of bones. This was confirmed by the fact that 60% affirmed to like shrimp or squid, which would not present bones. All children agreed on appreciating breaded and fried fish and they all enjoyed the boneless mackerel cooked by a great chef of San Benedetto del Tronto.

CONCLUSIONS

Fish is an important component of diet in children. Fish should be offered to children three times a week, two times at lunch and one at dinner. Our initiative showed that children were inclined to eat any type of fish, even frozen, provided it was boneless. Creams or sauces were often not welcomed by children. Blue fish and anchovies were especially appreciated, particularly when presented as fillets or breaded and fried meatballs. As fried fish is also palatable to children in other countries of the world, this type of preparation would represent a form of integration and identification with foreigner peers, in addition to nutritional benefits. Our initiative was also in line with the goal of Expo 2015: "feeding the planet, energy for life". Appreciated by the children taking part to the project, the blue fish is easy to find in all the oceans of the world. Thanks to the softness of the fish, when prepared in the way we have shown, the blue fish can easily be eaten even by children with dental problems, common in poor countries worldwide.

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ABS 36

NEW DIAGNOSTIC ALGORITHM FOR THE DIAGNOSIS OF THE α-1-ANTITRIPSIN DEFICIT

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BACKGROUND

Over 100 allelic α-1-antitrypsin (AAT) variants have been described and, among these 30 are considered pathological. The majority of individuals with severe α-1-antitrypsin deficiency (AATD) carries the Z and S mutations. On the contrary, in Sardinian the most frequent pathological allele is the M-Malton variant (also known as Mnichinan and M-Cagliari). In normal subjects the AAT serum concentration is present with values ranging from 90 to 200 mg/dl and represents in a normal Serum Protein Electrophoresis 95% of the α1-globulins; its synthesis is under the influence of IL-6 and of Oncostatin M. The main objective of AAT is to protect alveolar pulmonary by the attach of the elastase released from activated neutrophils by various inflammatory agents.

AIM

The diagnosis of AATD is challenging, particularly in heterozygous carriers of rare allelic variants, that may be asymptomatic. The aim of this study is to verify if it is useful, in the adult and pediatric population of Sardinia, utilize a new diagnostic

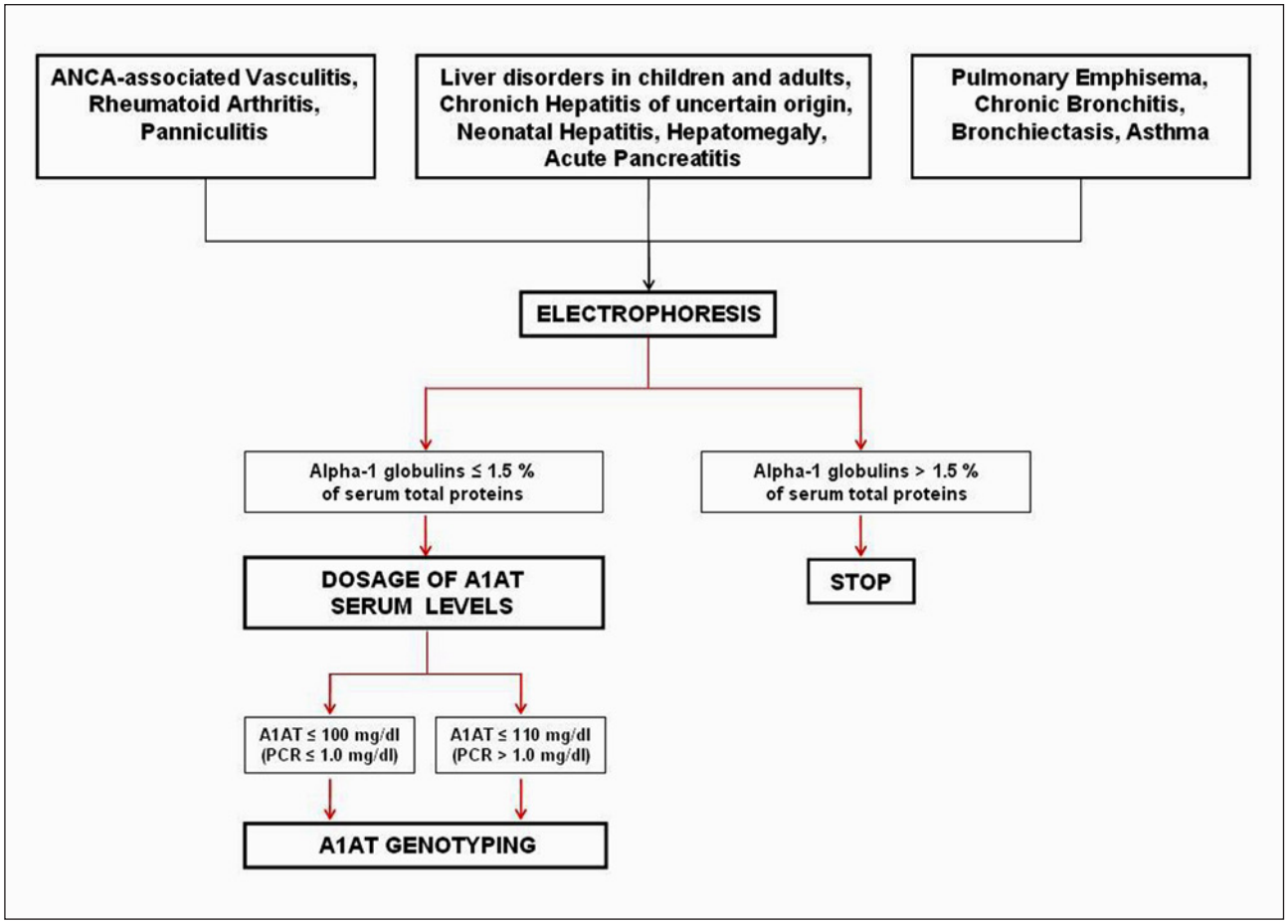

Figure 1 (ABS 36). New diagnostic algorithm for the diagnosis of α-1-antitrypsin deficiency (AATD).

protocol, simple, not expensive and with a better diagnostic sensibility.

MATERIALS AND METHODS

A possible association between serum protein electrophoretic pattern, the values of serum AAT concentration, and the PCR-H levels was examined in 5,305 consecutive serum samples. In all of the samples selected, molecular analysis was also performed.

RESULTS

The comparison of the serum protein electrophoretic patterns with AAT serum concentration and the PCR-H values, suggest that a change of the α1-globulins cut-off peak from 1% to 1.5% is necessary. The analysis of the PCR acute phase protein levels evidenced two groups of patients, one with normal PCR-H values (≤ 1.0 mg/dl) where the best AAT serum concentration sensibility was 100 mg/dl, and another with PCR-H altered levels (> 1.0 mg/dl) and a new cut-off of 110 mg/dl. Out of a total of 5,305 samples examined, 77 (1.45%) presented an alpha-1 fraction peak < 1.5%. Among these, 46 subjects (0.87%) were identified as AATD carriers at molecular analysis; 2 (0.04%) were homozygous for the M-Cagliari allele, 4 were heterozygous for the S variant and 4 for the Z variant. The remaining 36 patients were heterozygous for the M-Cagliari allele.

CONCLUSIONS

Recent developments in the therapy of patients affected by AATD lay stress on the need of a precocious diagnosis of the disease for the prevention of lung and liver pathology. The news diagnostic cut-off determined in this study clearly improves the AATD diagnostic sensitivity in our population. Moreover, for the diagnosis of the AATD we propose the diagnostic algorithm in **Fig. 1** to be used in this disease.

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ABS 37

WHAT NEONATOLOGISTS CAN EXPECT FROM LABORATORY MEDICINE: THE EXAMPLE OF CYSTIC FIBROSIS

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Cystic fibrosis (CF) is a relatively common recessive genetic disease caused by mutations in a gene (CFTR) located on chromosome seven. It has been identified and described over 1,900 different CF mutations, even if some are much more usual than others. Caucasians and Ashkenazi Jews have the highest incidence of CF with 1 in 20-25 individuals being CF carriers. It is estimated that 70,000 subjects are living with CF worldwide and approximately over 1,500 new cases are diagnosed every year.

The gene is encoding a protein called cystic fibrosis transmembrane conductance regulator (CFTR). Changes in this gene lead to reduced, absent, or defective CFTR production and function, giving rise to an imbalance in electrolyte and water interchange between their entry and exit from epithelial cells. Most people with CF are diagnosed in early childhood and require daily therapies to loosen and remove sticky excess mucus, which affects lung function, as well as supplements of pancreatic enzymes to get adequate nutrition. Most of adult males with CF are also infertile because of missing or underdeveloped vas deferens. Early in the life, most of individuals with CF develop respiratory and pancreatic symptoms; however, the severity of symptoms are dissimilar among patients, even in those carrying the exact same mutations. With advances in therapeutic technology and aggressive antimicrobial management, median survival has improved significantly.

CF remains the most common life-limiting inherited disease. An early and accurate diagnosis is basic for an effective treatment and management of the disease. Diagnostic criteria for CF include the presence of one or more typical clinical features, a family history of CF, or a positive newborn screening test result together with laboratory evidence of CFTR dysfunction. Abnormal CFTR function usually is documented by a) sweat chloride test, that remains the diagnostic procedure of choice when carried out in accordance with the Clinical Laboratory Standards (CLSI) guidelines; b) two CFTR disease-causing mutations by genotyping, or an abnormal nasal PD measurement. For the group of patients who do not have the classic CF phenotypes, the term CFTR-associated disease should be assigned.

The recent development of a genotypic CFTR mutation screen has greatly improved diagnostic accuracy. If medically indicated, a complete mutation sequencing can be arranged to provide a more detailed analysis, leading to the recognition of a number of atypical CF disorders. Several newborn screening protocols including CFTR genotyping have increased the likelihood of early diagnosis and identification of carriers in order to plan their families knowing.

Recently, research has been focused in trying to solve the dysfunction of CFTR due to different classes of mutations by agents as correctors and enhancers. A more detailed information on cellular processing and channel gating of CFTR could provide new approaches to treat the underlying defects in patients with CF.

ABS 38

LISTERIOSIS-RELATED NECROTIZING ENTEROCOLITIS: A CASE REPORT IN A PRETERM NEWBORN

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INTRODUCTION

Neonatal listeriosis is a rare but severe illness in the newborn, caused by *Listeria monocytogenes* (L.m.), a small facultatively anaerobic gram-positive bacillus, producing incomplete β-hemolysis. The severity of listeriosis is mainly due to the ability of L.m. to cross three host barriers: the intestinal, blood-brain and placental barriers [1]. Clinical presentation in neonates is often characterized by signs of meningitis or septicemia. The disease may present in main two forms: the early-onset sepsis syndrome, usually associated with prematury and probabily acquired in utero, and the late-onset meningitis that occurs about two weeks postpartum in full-term babies who are most likely infected by organisms present in the vagina at birth [2]. Here a case of neonatal listeriosis is reported, characterized by a severe necrotizing enterocolitis (NEC).

CLINICAL DATA

A 2,100 g female neonate was born prematurely at 34 weeks of gestation by Cesarean delivery. At birth, the APGAR score of the neonate was 0, 2, 3 at 1, 5 and 10 minutes, respectively. She was admitted to the NICU center. Blood culture was positive for L.m. and the infant died after 3 days.

PATHOLOGICAL FINDINGS

At autopsy, macroscopic examination of the peritoneal cavity, first evidenced the typical features of NEC (**Fig. 1**). Some adjacent ileal loops showed an homogeneus dusky colour. Moreover, small dark lenticular areas were observed unevenly distributed throughout the ileum. At histology, the study of the affected ileal tracts showed major changes in the architecture of the whole intestinal wall. The luminal surface appeared covered by necrotic material mixed with inflammatory cells, giving rise to the typical pseudo-membranes (**Fig. 2**). The major part of the submucosa and the muscular layer showed a dense inflammatory infiltrate, formed by lymphocytes, polymorphonucleates and monocytes. Necrotic areas were also found in the deeper layers of the intestinal wall (**Fig. 3**). No clear evidence of peritonitis or perforation was found.

DISCUSSION

Necrotizing enterocolitis is a severe disease, mainly affecting premature neonates, characterized by a worse prognosis. NEC is the most common acquired gastrointestinal disease of premature neonates and is one of the leading causes of death in neonatal intensive care units [3]. Historically, it was believed that NEC arose predominantly from ischemic injury to the immature gastroinstestinal (GI) tract. Alternate plausible hypotheses indicate that many factors are likely to be involved. The aetiological factors may include issues related to the introduction and advancement of enteric feeding, alterations in the normal bacterial colonization of the GI tract, bacterial translocation and activation of the cytokine cascade[4]. Despite many advances in the management of neonates affected by NEC, unfortunately the overall survival for this poorly understood and complex condition has not improved during the last years. Here we report a case of NEC insurging in a premature infant. The association between L.m. infection and NEC has not, at the best of our knowledge, previously reported in the perinatal period. There are sporadic cases with L.m. infection among pregnant women in this country,

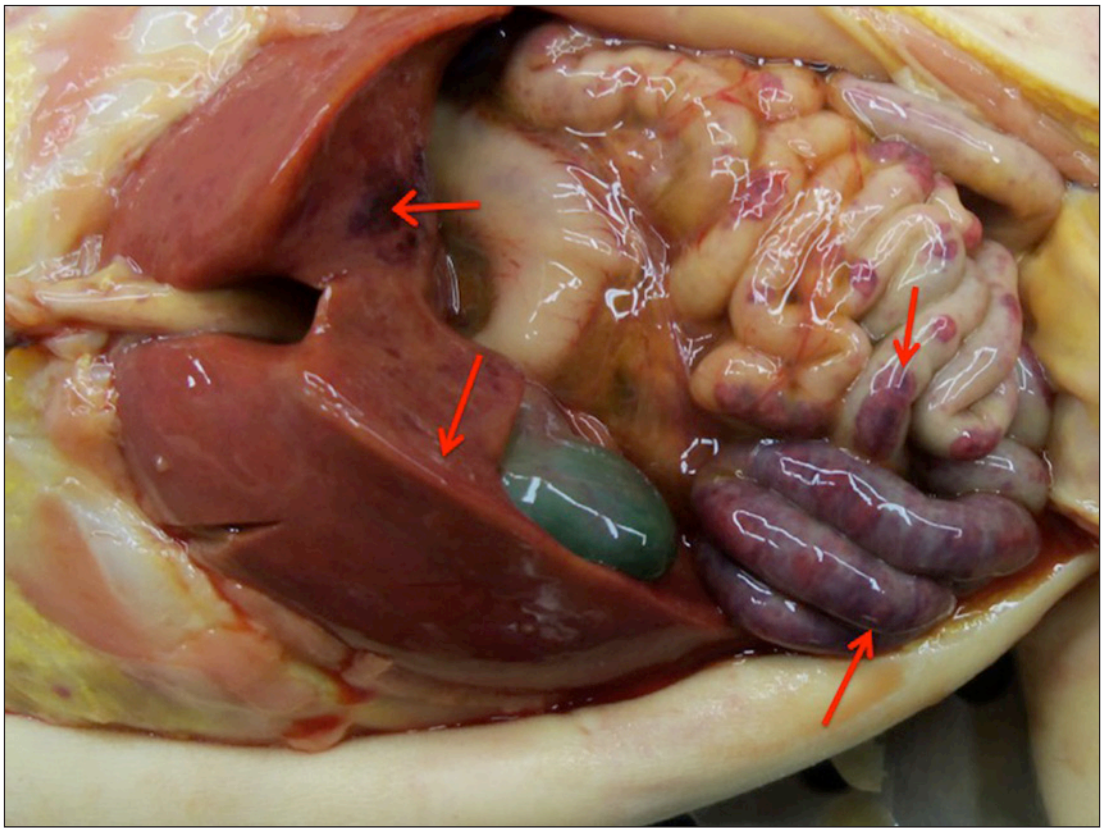

Figure 1 (ABS 38). The peritoneal cavity, evidenced the typical features of NEC: adyacent ileal loops showed an homogeneus dusky colour and the small dark lenticular areas (red arrow).

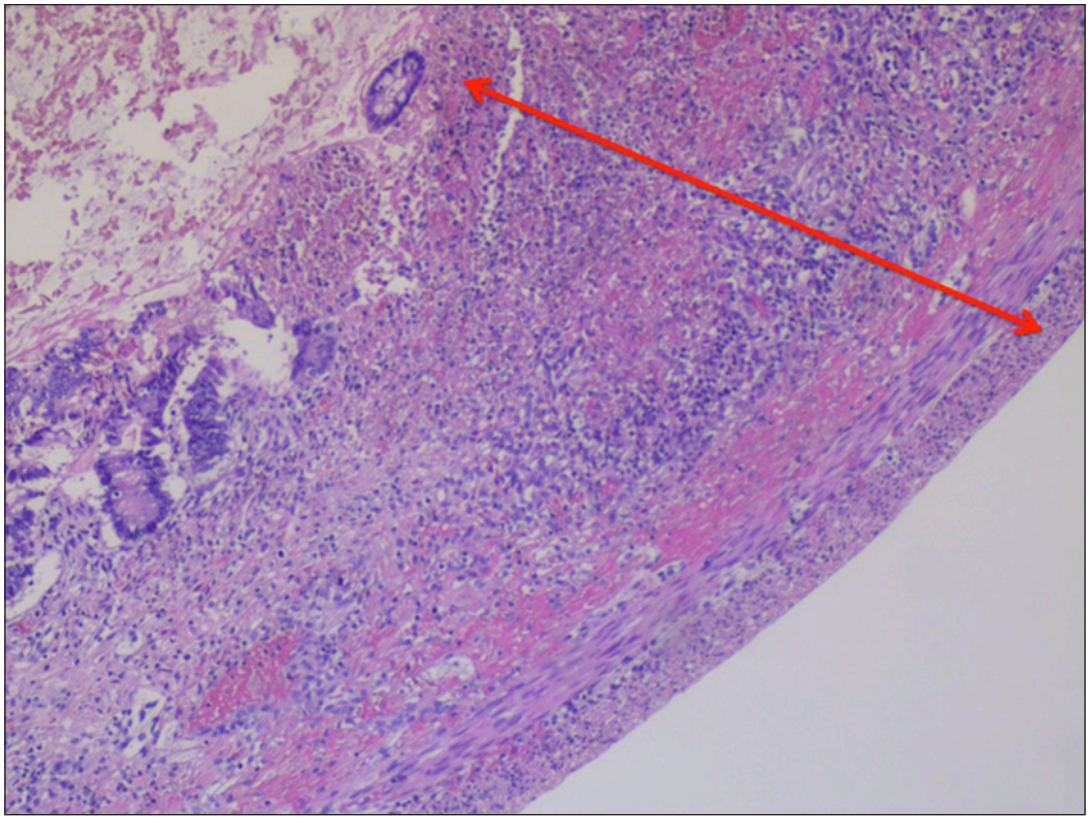

Figure 2 (ABS 38). The luminal surface appeared covered by necrotic material mixed with inflammatory cells, giving rise to the typical pseudomembranes (red arrow). H&E. OM400x.

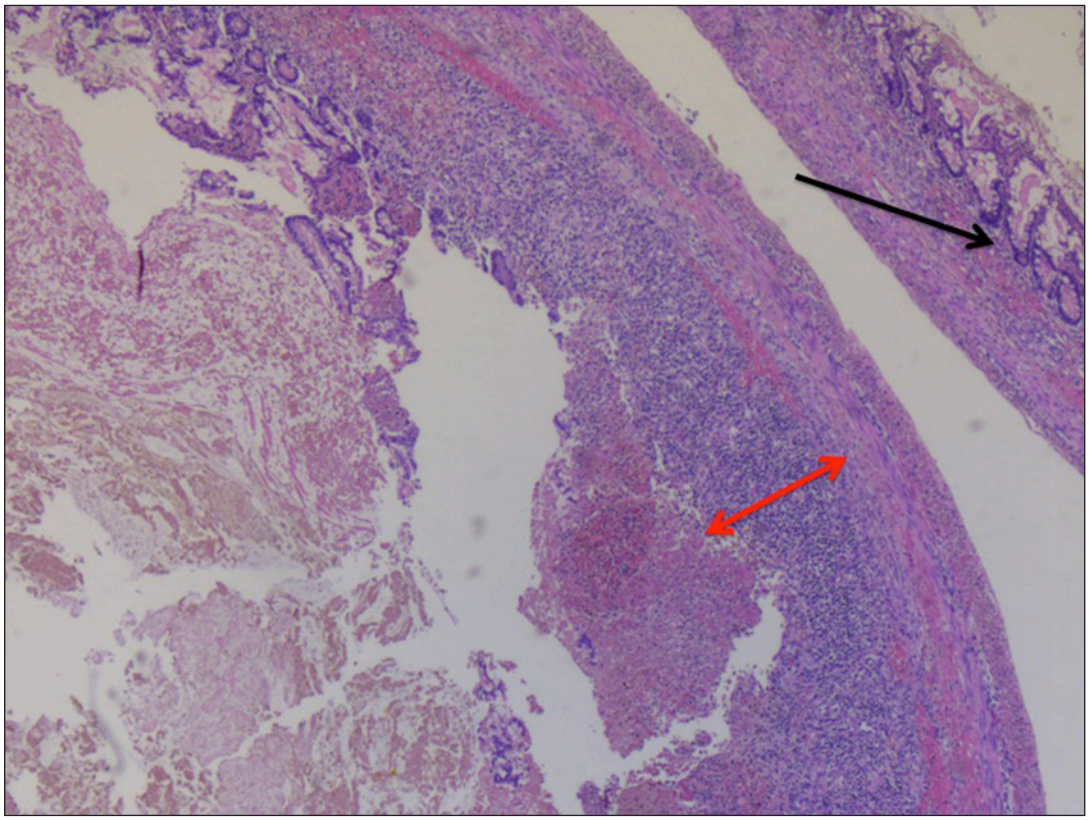

Figure 3 (ABS 38). Normal architecture and the presence of an intact intestinal barrier (black arrow). Necrotic areas (red arrow) were also found in the deeper layers of the intestinal wall; no clear evidence of peritonitis, not of perforation was found. H&E. OM200x.

resulting in severe illness of their newborn infants. Early differential diagnosis, early detection of causative organisms, especially in newborn infants infected with L.m., early treatment with sensitive antibiotics can decrease the mortality rate and improve neonatal outcome. In conclusion, the case here reported lays stress on the necessity to enhance nationwide surveillance for listeriosis.

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ABS 39

TREATING PAIN IN NEONATES: HISTORY YET TO BE WRITTEN

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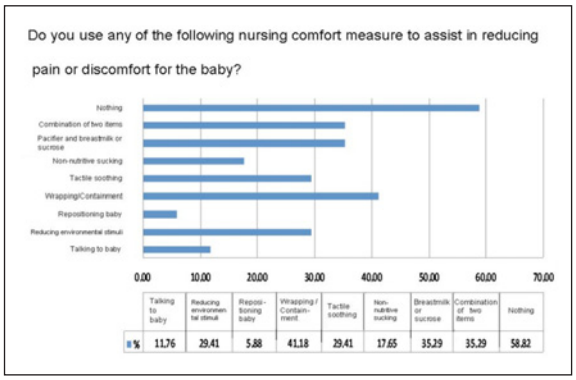
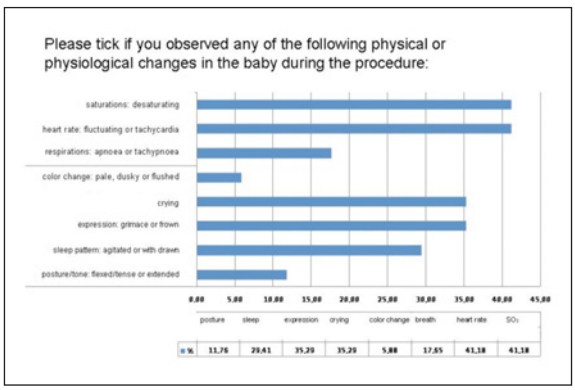
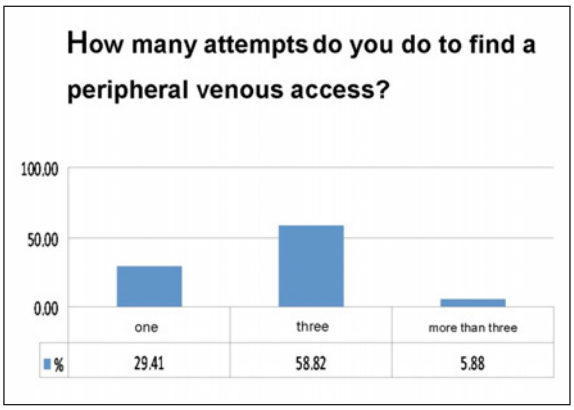
The study of the pathophysiology of sensorineural development of the infant demonstrate that the experience of pain that occurs during the neonatal period and childhood may determine the final architecture of the nociceptive system of the adult [1].

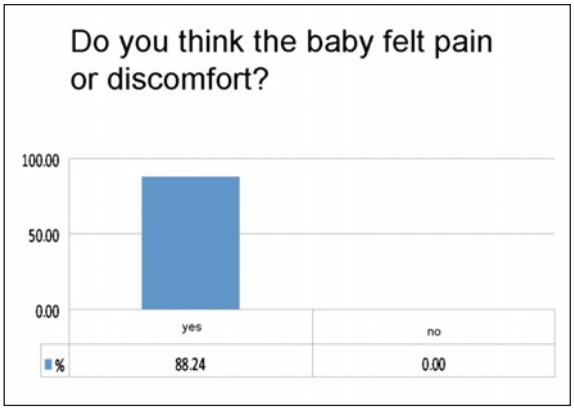
The Joint Commission on Accreditation of Healthcare Organizations launched the "Pain as the 5th Vital Sign" [2].

It is essential, in order to recognize the pain and therefore its rating, to know the development sensorineural, reactions to pain, the different types of the same.









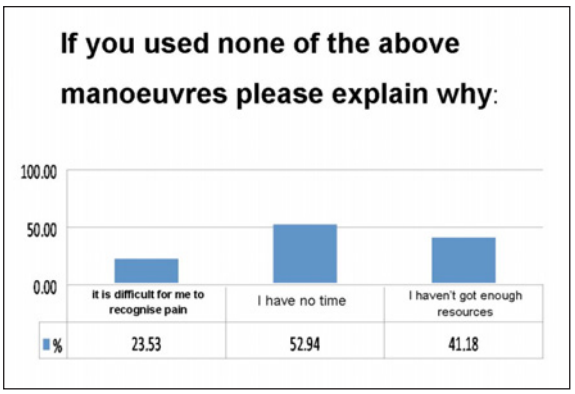


Figure 1 (ABS 39). Results of the questionnaire.
The questionnaire was distributed in different nursery and NICU to 24 pediatric nurses.

This, however, does not guarantee the presence of procedures of treatment of pain in all the Italian facilities. The problems related to the implementation of procedures, depends on several factors: lack of personnel, mindset, difficulties in recognition of pain, lack of knowledge of the development of sensorineural newborn, as well as non-pharmacological techniques for treatment, poor work organization department and improper distribution of tasks.

A questionnaire, in different NICUs, showed that nurses who are used to treat pain, do not find difficulties nor complain "waste of time", in the implementation of invasive procedures with pain treatment before and/or during procedures, compared to centers that do not provide, in their organization, procedures for the treatment of pain, with a reduction of the attempts for the positioning of peripheral venous catheter.

Results of the questionnaire are presented in **Fig. 1**. This shows that everyone can contextualize the different treatment techniques and become a promoter of changes that might make it more functional procedures.

Obviously, changes need to be tackled step by step, starting from the simplest procedures to those that require more complex interventions, in order to improve even more the quality of care.

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ABS 40

WHAT IS THE FUNCTIONAL BACKGROUND OF FILIGREE EXTRACELLULAR MATRIX AND CELL-CELL CONNECTIONS AT THE INTERFACE OF THE RENAL STEM/ PROGENITOR CELL NICHE?

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Development of a nephron is induced by a reciprocal exchange of morphogenetic proteins between epithelial and mesenchymal cells within the renal stem/progenitor cell niche. For sustaining concentration of diffusing proteins high, it is believed that an intimate contact exists between involved cells. However, actual morphological data show that both types of stem/progenitor cell bodies are separated by an interface. To analyze details of this arrangement, neonatal rabbit kidneys were fixed in traditional glutaraldehyde (GA) solution for transmission electron microscopy. For an enhanced contrast fixation of samples was performed in GA solution including either cupromeronic blue, ruthenium red or tannic acid. To record always the same perspective, embedded blocks of parenchyma were cut in orientated vertical and transverse planes to the lumen of lining collecting duct tubules. Screening of samples fixed by GA solution demonstrates a constant separation of stem/progenitor cell bodies by an unobstrusively looking interface. In contrast, improved fixation of specimens in GA solution including cupromeronic blue, ruthenium red or tannic acid unveils between them earlier not visible filigree extracellular matrix. Further projections of mesenchymal cells covered by this matrix cross the interface to contact epithelial cells. The end of a projections does not dangle but is mounted by a special plug connection. At this site the plasma membranes of mesenchymal and epithelial cells are connected via tunneling nanotubes. Regarding this unique arrangement the principal question is to what extent illustrated extracellular matrix and cell-cell connections are involved in the exchange of morphogenetic proteins during induction of a nephron.

ABS 41

SEVERE NEONATAL ANEMIA: THE SPECTRUM OF CAUSES IN OUR UNIT

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INTRODUCTION

Neonatal anemia is defined by hemoglobin and hematocrit concentration below two standard deviations from the mean of postnatal value and can be consequent to blood loss, decreased red blood cells (RBC) production or increased RBC destruction; a significant fall in hemoglobin and/or hematocrit usually requires RBC transfusions.

It can be a life threatening condition, because it could present with respiratory distress and, sometimes, neurological alterations. That's why it's important to threat it promptly.

The prevalence of neonatal anemia requiring transfusion is relatively high in preterm infants and is often dependent to blood loss due to sampling for laboratory tests and to a suboptimal erythropoietic response. In infants reaching the term age, a wide group of cause may contribute to the development of severe anemia, requiring careful clinical investigations in order to obtain an etiological diagnosis.

We report the clinical course of five full term newborn who presented requiring transfusion anemia as principal clinical manifestation.

PATIENTS AND RESULTS

Our outpatient unit takes care of children with hematological diseases.

Recently, 5 full term newborn were referred to our unit for follow-up of anemia which required RBC transfusions at birth.

Patient 1: male, presented with macrocytic anemia (Hb 9 g/dl), respiratory distress, jaundice, splenomegaly and acral dysmorphism. Postinfancy, he still required RBC transfusions and blood test showed chronic hemolytic anemia, with a negative Direct Coombs Test. A bone marrow smear suggested a congenital dyserythropoietic anemia type I (CDA-I), but genetic testing is still ongoing.

Patient 2: female, presented with hyporegenerative isolated anemia (Hb 8,8 g/dl) and facial dysmorphism. Her X-ray showed accessory bone in the thumbs bilaterally with thenar eminence hypoplasia. Post infancy, the patient required regular blood transfusions for anemia with a gradual reduction during the subsequent months. DNA testing of RPL5 gene allowed the genetic diagnosis of Blackfan Diamond Anemia.

Patient 3: female, black African, presented with progressive microcytic anemia (Hb: 6 g/dl), jaundice resistant to phototherapy and reticulocytosis. Hemoglobin HPLC didn't show hemoglobinopathies. A peripheral blood smear test revealed pyropoikilocytosis confirmed by detection of spectrin mutation on gene analysis. The patient required monthly transfusions for 5 months; actually is transfusion free.

Patient 4: female, presented with severe normo-macrocytic anemia (Hb: 5.5 g/dl), reticulocytosis, jaundice, hypotonia, respiratory distress, dysmorphic features and skeletal anomalies. Blood test showed a hemolytic disorder, but hemoglobinopathies, G6PD deficiency and spherocytosis were excluded. A bone marrow smear showed morphologic alterations compatible with CDA-I, confirmed by omozygous mutation in C15ORF41 gene. During the first 9 months of life she required twice a month transfusions, then monthly transfusion.

Patient 5: female, presented with poor general conditions, pallor and severe anemia (Hb 3.1 g/dl). Maternal history reported vaginal bleeding during the third trimester of pregnancy. She needed 2 RBC transfusions and then, after starting therapy with iron and folic acid, Hb reached stable values of hemoglobin (11.5 g/dl).

DISCUSSION AND CONCLUSIONS

Severe neonatal anemia in full term newborn can be a relatively benign condition or the first sign of a wide range of hematological disease; an etiological diagnosis is critical in order to establish the appropriate treatment.

A careful clinical history and physical investigation associated with peripheral blood smear and a complete blood count are the most important diagnostic tools in the majority of cases. A long-term hematological follow-up, eventually including bone marrow smear and specific genetic tests allow to obtain etiological diagnosis even in presence of very rare conditions.

ACKNOWLEDGMENT

This work is dedicated to the memory and in honor of Prof. Renzo Galanello.

ABS 42

ORTHOGONALIZATION METHODS IN METABOLOMICS. HYPOTHESIS AND TESTS FOR VALIDATION

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Metabolomics is a powerful tool for the System Biology applied to medicine. The mathematical

methods applied to data analysis allows to the researchers to investigate about hypothesis in the metabolome characterization (phenotype). The metabolome is a complex multidimensional signal with many components related to different contributions. The human metabolome can be affected by alteration induced in several systems apparently detached. But propagation of this alteration can occur and can interest in different ways all the systems. This makes complicate the description of altered metabolome.

Our methods of analysis are organized in order to try to separate the components and contributions by means of analytical ways named "Orthogonalization methods" (OM). Separation means filtering of data in order to remove the noise in the signal and in this framework it is important the definition of noise and this task is related to the definition of the perturbation of interest in the metabolome. OMs are techniques that allow testing hypothesis important in the new biomarkers discovery in metabolomics and, for these reasons, OMs can be used as powerful strategy for Metabolomic Data Analysis.

Generally speaking, those are methods based on Partial Least Squares projection to latent structures, or PLS methods. There are a lot of advantages in the orthogonal inspection of data in metabolomics, but the most important vantage is related to the interpretation of projection-based latent variable models that is frequently not straightforward because of its confounding of different systematic variations in the model components.

The possibility proposed recently by the bioinformatics to realize algorithms to study these problems suggested to the researchers to shift their attention away from the traditional emphasis of metabolomics on the model predictive capacity toward optimizing mining information, towards to the model interpretation of metabolomics data in the contest of Systems Biology. Metabolomics connectivity emerging by statistical analysis defines the so-called "network-driven" analysis of the metabolome: perturbation of interest (POI) produces modification of the behaviour of the metabolites, defined as nodes of the "network", strictly related to the pathology under study. Changing the properties of the single metabolites affects consequently the properties of the network and vice versa; this is the emerging property of the metabolites in a network.

In a recent paper we postulated that the increase in plasma and consequently urinary inositol could constitute a marker of altered glucose metabolism during fetal development in both IUGR and LGA newborns. This is an emerging functional connectivity in the metabolome of these two classes of intrauterine fetal developments apparently different.

The paradigm of systems biology emerged with the diffusion of systems-level experiments: understanding *complex* biological systems requires understanding and modelling characteristics that are fundamentally determined by the *organization* of their constituent parts, emergent phenomena created by the interactions of those elements defined as hubs and spokes depending on the level of connection and interconnection. Especially for the metabolites defined as "hub nodes" there is an increased interest in medicine because they importance in the comprehension of the pathologies.

Network driven approach is a powerful theoretical technique to analyse metabolome, to unveil the underlying hierarchical structure and to predict their behaviour under different conditions. Each metabolite gives contribution to several canonical pathways. In the metabolome some metabolites can exhibit a co-variation stronger than others. These correlations can have different influence on different metabolic pathways depending on the "position" of the metabolites. These co-variations can be described as different level of "connectivity" between metabolites. This connectivity is the expression of the metabolome dynamic that results in a pattern of statistic dependencies (functional connectivity) of some metabolites in order to realize a "functional connectome". Hub nodes are among the most intriguing structural features of metabolic networks. Hubs have attracted much attention in network science since they often correspond to nodes that have special integrative or control functions. It is likely that neuronal hubs have a privileged role in organizing network dynamics and exert strong influence on the state of more peripheral nodes. Due to their structural and functional connections, hub nodes integrate a highly diverse set of signals and are in a "position" to control the flow of information between relatively segregated parts of the metabolic network. So we can have a modular structure in the metabolites "community" (secondary approach to the metabolites functional). Since much of the "between-modules" (modularity property) information flow travels through hubs, the rate at which they relay signals would have a large impact on system-wide communication. Criteria for hub identification vary across different studies. In some cases, hubs are identified as "highly connected nodes," that is, primarily on the basis of node degree

or strength or on the clustering index. Because of their position on many of the network's shortest paths, any perturbation of the state of a hub node would be able to spread quickly across the network. Because of their position on many of the network's shortest paths, any perturbation of the state of a hub node would be able to spread quickly across the network. For example, considering the following reactions:

2-oxoglutarate + 1 glycine -> 1 glyoxylate + 1 glutamic acid

glyoxylate + L-alanine -> glycine + pyruvate

Those mechanisms create a competition in the biological pathways and it is possible to reveal an unbalance in the metabolites flows. The internal variables of a biological system are rarely independent of each other, as the interactions between the system's components induce systematic interdependencies between them. Hence, a well-selected subset of variables can contain sufficient information about the rest of the variables, allowing us to reconstruct the system's complete internal state, making the system observable (network medicine). With this approach we have asserted the hypothesis of a common metabolic behaviour for the fetal development for the IUGR and LGA fetus. Using the OPLS-DA approach we have created a two groups classification for the samples: controls and (IUGR + LGA). After the Orthogonalization of the signal (OSC) a new PLS-DA model has been calculated. Partial least squares discriminant analysis (PLS-DA) maximizes the covariance in independent variables (metabolites) and a dependent variable (class labels, e.g. IUGR+LGA, AGA). To capture the maximum variance between clinically defined groups in the first dimension of the PLS-DA model, we operate the OSC on the data matrix. In this way we produce a corrected data matrix Xc:

$$Xc = X - t_o^T p_o$$

with t_0^T and p_0 are calculated from the regular PLS-DA model.

$$\omega_\perp = p - \frac{\omega^T p}{\omega^T \omega}\omega$$
$$T_\perp = X\omega_\perp$$
$$p_\perp = \frac{X^T t_\perp}{t_\perp^T t_\perp}$$

Usually these components can be calculated by the loadings and weights of the PLS-DA. The matrix Xc can be used in a new PLS-DA model. Several orthogonal components can be extracted but it is claimed that for univariate Y only one PLS component is required in the final model in other to avoid the overfitting of data. The new variables are examples of what often is called latent variables (LVs). The appropriate Y function adopted for the PLS-DA model is the discrete-values function:

$$y = 0 \; \forall \; samples \in AGA$$
$$y = 1 \; \forall \; samples \in (IUGR \cup LGA)$$

and the metabolic hypothesis may be tested with this function. Assumption made with the previous assignment is strictly related to the metabolome alteration induced by the external conditioning; in this case the maternal metabolism and the "placenta filtering". This is the critical point of the model and OSC-PLS-DA allows us to get into the metabolic mechanism of fetal malnutrition. Xc is the matrix of the model; crossvalidation of the PLS-DA model is referred to data filtered by OSC and the same transformation should be applied to external data set for validation. The PLS-Da model calculated on the matrix Xc has separation graph as in **Fig. 1**: the new latent variable t(1) has a strong power of separation strictly related to the hypothesis. In case of fault of this hypothesis we could not achieve a good descriptive model. For this reason when we perform the analysis on the new matrix Xc we can achieve a so powerful result (**Fig. 2**).

Using the ROC curve calculated by the MetaboAnalyst tool and the boxplot related to the Inositol metabolite we can see that the weight of this

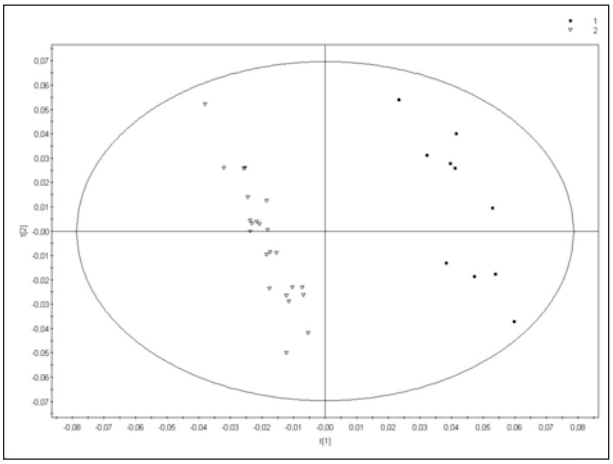

Figure 1 (ABS 42). OSC-PLS-DA model.

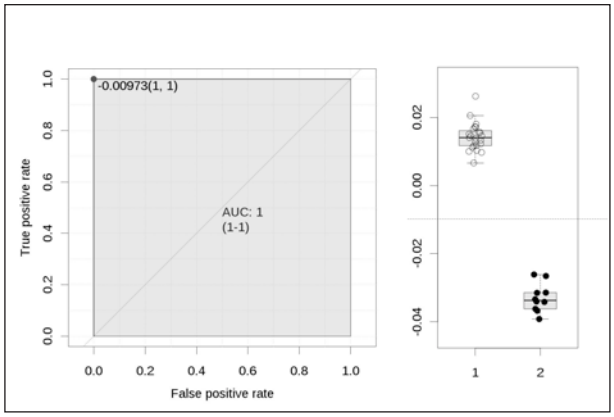

Figure 2 (ABS 42). Properties of the metabolite contribution after OSC correction.

sugar in the model is really impressive. Filtering operation has good results if the separation between "noise" and signals of interest is not negligible. This is not so frequent in metabolomics except for the situation of strong perturbation. The model has the maximum informative content to realize the network of the interactions emerging from the pathology description, For example, in a recent our paper we established, with an OSC-PLS-DA model, the network of autism syndrome diseases population [1].

CONCLUSION

We present and explain a method for the data mining in metabolomics. Results can give the representation of the power of the metabolome signal filter. This is the new way to move from the "ancient" Chemiometry to a modern Computational Metabolomics.

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ABS 43

TRAINING NURSES AND SPECIALTY DOCTORS ON CENTRAL LINES MANAGEMENT IN NEONATES IMPROVED SKILLS IN A THIRD LEVEL NEONATAL UNIT

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BACKGROUND

The central lines improvement team of the level III Neonatal Unit of Verona, composed of four nurses and two doctors, organized and run a training course on central lines management dedicated to neonatal nurses and specialty doctors. First aim of the course was to update our team to the new guidelines of management of central lines in order to decrease catheter-related blood infections.

MATERIALS AND METHODS

Two editions of a 2-day course were held in May and June 2015. Frontal lectures were held on the first day, practical skill stations and simulated neonatal scenarios were run on the second day. The course was based on the EPIC 2013, SHEA 2014, CDC 2014 and PICC 2012 guidelines. Participants were assessed before and after the course. Mannequin-based simulation was used to assess theoretical and practical skills. Chi-quadrate test was used. $P < 0.05$ was considered significant.

RESULTS

24 nurses and 5 junior doctors were trained. Skills assessment before and after the course showed an overall improvement from 63% to 79% ($p < 0.05$). Detailed results are shown below:

- correct sterile field preparation improved from 62 to 72% ($p < 0.05$);
- correct catheter placement improved from 42% to 73% ($p < 0.05$);
- correct dressing improved from 72% to 82% ($p < 0.05$);
- correct drug and infusions administration improved from 62 to 85% ($p > 0.05$);
- correct removal improved from 78 to 83% ($p > 0.05$).

CONCLUSIONS

The course was very appreciated by the participants although significant improvements in performance on practical skills seem achievable only with additional training.

ABS 44

TRANSFERT OF NEWBORNS WITH CRITICAL DUCTAL-DEPENDENT CONGENITAL HEART DISEASE

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INTRODUCTION

Congenital heart diseases (CHD) are the most common congenital disorders in fetuses and newborns with a reported prevalence of 20:1,000 and 10:1,000 respectively.

Ductal-dependent CHD requires a patent ductus arteriosus (PDA) for adequate pulmonary or systemic circulation or to allow adequate mixing between parallel circulations. In critical right heart obstructive lesions such as pulmonary stenosis, pulmonary atresia with intact ventricular septum (PAIVS) or associated with inter-ventricular defect (IVD) and Fallot's Tetralogy, the PDA is necessary to supply blood flow to the lungs. In critical left heart lesions such as Aortic stenosis and coarctation and left heart hypoplasia, PDA supplies systemic circulation. In in parallel circulations such as transposition of the great arteries (TGA), bidirectional flow in the PDA allows mixing between oxygenated and deoxygenated circuits.

Clinical features may vary from cyanosis to heart failure symptoms, on the basis of the underlying CHD.

Due to the increased morbidity and mortality of CHD infants, a initiation of prostaglandin E_1 (PG-E_1) to re-open or maintain the ductus arteriosus (therapeutic range 0.02-02 mcg/kg/min) and a rapid referral to a tertiary center is imperative in order to guarantee the highest level of care.

PATIENTS AND METHODS

We reviewed all neonates admitted to the NICU of the University of Cagliari from January 2010 to February 2015. Data collected from the medical records included patient demographics, gestational age, birth weight, birth place, type of CHD, age at diagnosis, prenatal diagnosis, previous diagnosis.

RESULTS

Among 1,500 admission to the NICU, 23 neonates presented ductal dependent CHD. Aortic coarctation was present in 9 patients (39.1%), pulmonary stenosis in 5 (21.7%), TGA in 5 (21.7%), and pulmonary atresia associated with IVD, univentricular heart, PAIVS, Anomalous pulmonary venous return (APVR) respectively in 1 patient (total 17.3%). The diagnosis of CHD was performed within 24 ours from birth in 16 patients (69.5%) and within 10 days from birth in all patients. PG-E_1 was administered in all patients and almost all (95.6%; n = 22) were referred to a tertiary center and are currently alive. One patient deceased consequently to subendocardic ischemia in prematurity (27 week of gestational age).

CONCLUSIONS

Ductal dependent congenital heart diseases represent a frequent and frightening clinical condition in neonatology. Since cardiac surgery is the definitive therapy of these conditions, a quick diagnosis is crucial in order to rapid refer CHD patients to tertiary level centers. Time saving measures includes patient's admission to NICU and immediate instauration of support therapy including PG-E_1 infusion under constant cardiology monitoring.

ABS 45

WNT1 EXPRESSION IN ONTOGENESIS OF HUMAN FETUS

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INTRODUCTION

Wnt1, a member of an expanding of cysteine-rich family, is a glycosylated signaling protein that mediate several developmental processes including control of cell proliferation, adhesion, cell polarity, and establishment of cell fates [1]. Intracellular transduction of Wnt signals can activate at least three pathways, of which the canonical involved β-Catenin and the two non canonical Wnt pathways are the Wnt/Ca2+ and Wnt/planar cell polarity (PCP) pathways [2].

MATERIALS AND METHODS

Three human fetuses of 12, 20 and 39 weeks of gestation, have been completely sampled and histologically and immunohistochemically studied. Samples were fixed in 10% buffered formalin, routinely processed, and paraffin-embedded. Two serial 3 μm-thick slices were obtained from each paraffin block; after dewaxing and rehydrating, one of these was stained with hematossilin-eosin; the other, on a pre-coated slide for immunohistochemical analysis, has been incubated for 20 minutes with anti-Wnt1 rabbit polyclonal antibody.

RESULTS

At 12 weeks of gestation, Wnt1 was expressed in the cytoplasm of superficial epithelium of

oropharynx, esophagus, stomach and intestine, myoenteric plexus, superficial and glandular tracheal epithelia, hepatocytes and biliary ducts, central nervous system (CNS) and retinal neuroepithelium, conjunctival epithelium, muscle cells (smooth, skelethal and cardiac, with higher positivity in atrial walls than in ventricular ones), superficial epithelium of ovary and oogonia, epithelium of the Bowman's capsule and renal tubules, urothelium, cells of adrenal glands "fetal zone" with ascending gradient from external to internal side. At 20 weeks of gestation, Wnt1 was also expressed in the cytoplasm of "blue strip" elements of the kidney and in lung epithelium. At 39 weeks of gestation, Wnt1 was expressed only in the cytoplasm of gastric and intestinal epithelia, tracheal epithelium, hepatocytes, renal tubules, glomerular zone of the adrenal glands, tubal epithelium, myoenteric plexus and ependymal cells; no reactivity was found in muscle cells and CNS neurons.

DISCUSSION

Our study shows that Wnt1 is involved in the development of several tissues and organs. As a signal protein, immunostaining for Wnt1 is always cytoplasmic. Its expression varies at different gestational ages during the ontogenesis of the organs; its cytoplasmic positivity of several epithelial cells at 39 weeks of gestation suggest that it is involved in epithelial homeostasis, as it does in adult tissues. Future studies will be needed to understand its role in cancer development.

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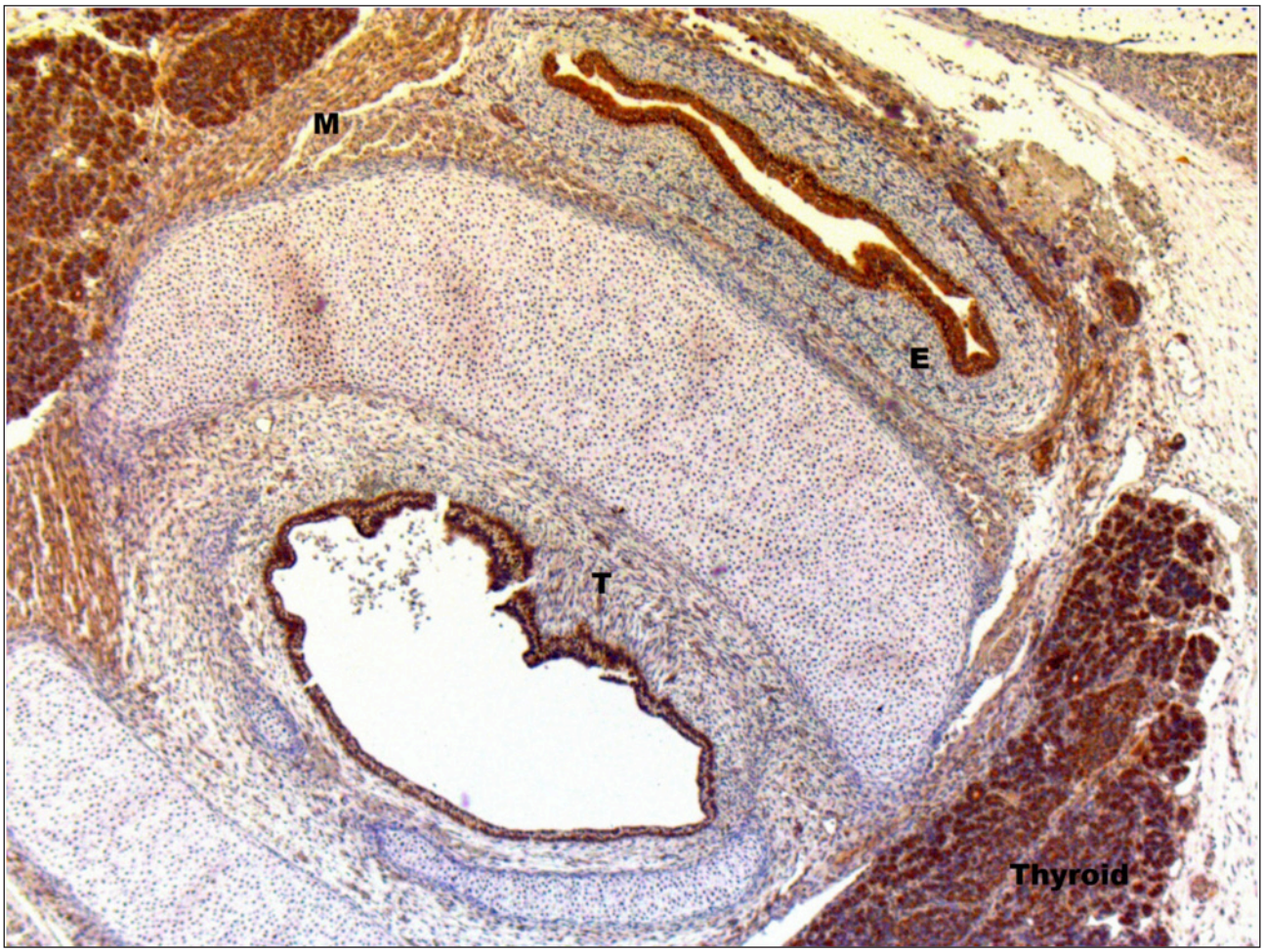

Figure 1 (ABS 45). Wnt1 immunoreactivity was detected in the cytoplasm of superficial tracheal epithelium (T), esophageal epithelium (E), thyroid epithelium and striated muscle cells (M).

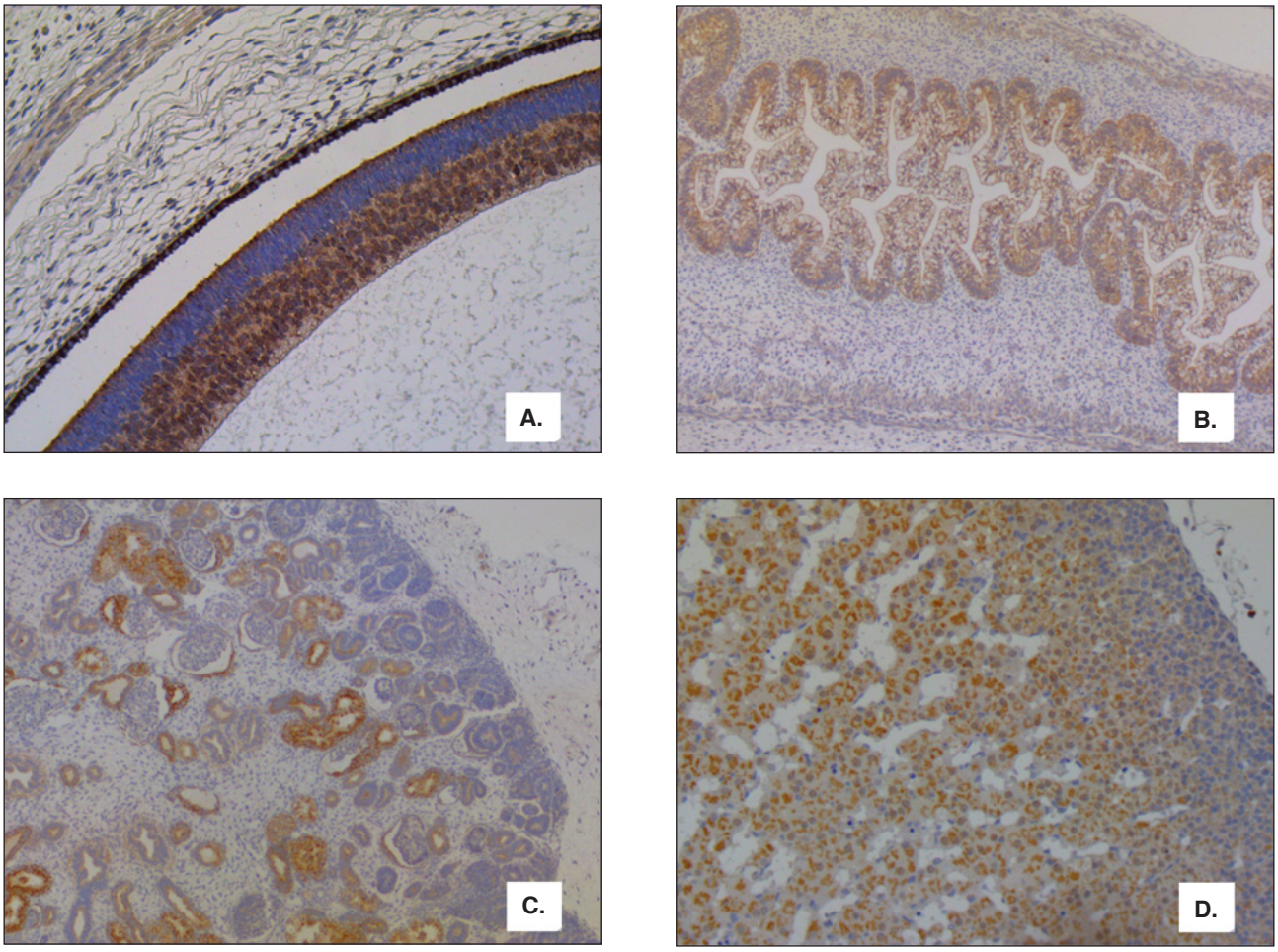

Figure 2 (ABS 45). Wnt1 immunostaining. Wnt1 was expressed in the cytoplasm of retinal neuroepithelium (**A**), intestinal epithelium (**B**), epithelium of the Bowman's capsule and renal tubules (**C**), cells of adrenal glands "fetal zone" with ascending gradient from external to internal side (**D**).

ABS 46

'GREEN FLAGS': ONE HUNDRED ITALIAN BEACHES FOR CHILDREN. A SELECTION BY PEDIATRICIANS

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BACKGROUND AND AIM

The study aimed to 1) describe frequency of children and adolescents in a scientific perspective of health promotion, 2) help families to choose the best beaches for children. There are currently limited studies on this subject. This research was performed with the aim to improve the seaside experience for both parents and children for them to gain greater benefits from the holiday, also in terms of well being and growth.

MATERIALS AND METHODS

The 'Green flags' have been awarded each year since 2008, through two methods.

- From 2008 to 2012, locations were selected by indication, following the results of a questionnaire delivered to pediatricians. The survey was administered to an adequate sample of pediatricians specialized in health and growth of children and working in different settings (university, hospital and local surgeries). The distribution of participating pediatricians throughout the national territory not only ensured the direct knowledge of the seaside locations, but also allowed the possible detection of disorders and diseases contracted in specific localities. Since 2011 the research is conducted in collaboration with the Italian Society of Preventive and Social Paediatrics (SIPPS).
- From 2013 to 2015, the list of places awarded a 'Blue flag' issued by the Foundation Environmental Education (FEE) was used.

The seaside location requirements for the award of a 'Green flag' to a beach resort are: the presence of sand, the allowance of sufficient space for children to play between umbrellas, a gradual increase in sea water depth to ensure safe bathing for children, the presence of beach attendants, the availability of equipment for children, and the presence of recreation opportunities for parents (shops, restaurants, bars, sports facilities).

RESULTS

The list of 'Green flags' awarded from 2008 to 2015 is presented in **Tab. 1**.

CONCLUSIONS

The study showed that the most lively beaches were favored in receiving the 'Green flag' awards. The awards reflect the fact that acoustic and visual stimulation that children are likely to receive in such locations (eg new sounds, new voices, new faces) are important for children since birth, as they are necessary for the development of the nervous

Figure 1 (ABS 46). Vatican City, September 9, 2015. Italo Farnetani (first from right) presents the Green flag project to Pope Francis, explaining the characteristics of beaches selected by pediatricians, focusing on the needs of children and parents.

Table 1 (ABS 46). Green flags awarded from 2008 to 2015 [1].

Abruzzo: Giulianova (Teramo), Montesilvano (Pescara), Roseto degli Abruzzi (Teramo), Silvi Marina (Teramo), Tortoreto (Teramo), Vasto Marina (Chieti)

Basilicata: Maratea (Potenza) and Marina di Pisticci

Calabria: Bova Marina (Reggio Calabria), Bovalino (Reggio Calabria), Cariati (Cosenza), Cirò Marina-Punta Alice (Crotone), isola di capo Rizzuto (Crotone), Melissa-Torre Melissa (Crotone), Mirto Crosia (Cosenza), Nicotera (Vibo), Praia a Mare (Cosenza), Roccella Jonica (Reggio), Santa Caterina dello Jonio Marina (Catanzaro), Soverato (Catanzaro)

Campania: Centola-Palinuro (Salerno), Marina di Camerota (Salerno), Positano-Spiagge: Arienzo, Fornillo, Spiaggia Grande (Salerno), Santa Maria di Castellabate (Salerno), Sapri (Salerno)

Emilia Romagna: Bellaria-Igea Marina (Rimini), Cattolica (Rimini), Cervia-Milano Marittima-Pinarella (Ravenna), Cesenatico (Forlì Cesena), Gatteo-Gatteo Mare (Forlì-Cesena), Misano Adriatico (Rimini), Riccione (Rimini), Ravenna-Lidi Ravennati

Friuli Venezia Giulia: Grado (Gorizia), Lignano Sabbiadoro (Udine)

Lazio: Formia (Latina), Gaeta (Latina), Lido di Latina (Latina), Montalto di Castro (Viterbo), Sabaudia (Latina), San Felice Circeo (Latina), Sperlonga (Latina), Ventotene-Cala Nave (Latina)

Liguria: Finale Ligure (Savona), Lerici (La Spezia)

Marche: Civitanova Marche (Macerata), Gabicce Mare (Pesaro - Urbino), Porto Recanati (Macerata), Porto San Giorgio (Fermo), Numana (Ancona), San Benedetto del Tronto (Ascoli), Senigallia (Ancona)

Molise: Termoli (Campobasso)

Apulia: Ginosa - Marina di Ginosa (Taranto), Ostuni (Brindisi), Otranto (Lecce), Rodi Garganico (Foggia), Vieste (Foggia), Marina di Pescoluse (Lecce), Marina di Lizzano (Taranto), Gallipoli (Lecce)

Sardinia: Alghero (Sassari), Bari sardo (Ogliastra), Cala Domestica (Carbonia-Iglesias), Capo Coda Cavallo (Olbia), Carloforte - Isola di San Pietro: La Caletta - Punta Nera - Girin - Guidi (Carbonia-Iglesias), Castelsardo-Ampurias (Sassari), Is Aruttas - Mari Ermi (Oristano), La Maddalena-Punta Tegge-Spalmatore (Olbia Tempio), Marina di Orosei-Berchida-Bidderosa (Nuoro), Oristano - Torre Grande (Oristano), Poetto (Cagliari), Quartu Sant'Elena (Cagliari), San Teodoro (Nuoro), Santa Teresa di Gallura (Olbia Tempio)

Sicily: Casuzze-Punta secca-Caucana (Ragusa), Cefalù (Palermo), Ispica-Santa Maria del Focallo (Ragusa), Marina di Lipari-Acquacalda-Canneto (Messina), Marina di Ragusa, Marsala - Signorino (Trapani), Porto Palo di Menfi (Agrigento), Pozzallo - Pietre Nere (Ragusa), San Vito Lo Capo (Trapani), Scoglitti (Ragusa), Torretta Granitola (Trapani), Tre Fontane (Trapani), Vendicari (Siracusa)

Tuscany: Camaiore - Lido Arlecchino - Matteotti (Lucca), Castiglione della Pescaia (Grosseto), Follonica (Grosseto), Forte dei Marmi (Lucca), Marina di Grosseto (Grosseto), Pietrasanta - Tonfano, Foccette (Lucca), Monte Argentario - Cala Piccola - Porto Eercole (Le Viste), Porto Santo Stefano (Cantoniera - Moletto - Caletta) - Santa Liberata (Bagni Domiziano - Soda - Pozzarello) (Grosseto), San Vincenzo (Livorno), Viareggio (Lucca)

Veneto: Caorle (Venice), Lido di Venezia, Cavallino Treporti (Venice), Jesolo and Jesolo Pineta (Venice)

system. Furthermore, parents may choose more isolated beaches until their children reach 6 years of age, as up to this age children mostly seek the company of their parents. For children older than 6 years and adolescents, livelier beaches allow more opportunities of relationship with peers.

'Green flag' locations are present throughout Italian regions with access to the sea. To date, Italy is the only country in the world to have a tourist map for children made by pediatricians.

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ABS 47

ULTRASTRUCTURAL STUDY OF FRESH HUMAN BREAST MILK CELLS

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BACKGROUND/AIM

The most recent studies related to breast milk reported the presence of multipotent stem cells. Human milk contains a heterogeneous population of multipotent stem cells, able to proliferate preserving their stemness. Maternal mammary stem cells is now considered as one of the main cell sources in breast milk [1]. A common origin between mammary glands and the nervous system has been well proved. Therefore, common regulators are involved in the expression of a group of embryonic stem cells both in the mammary gland and in the neuroepithelium [2]. Accordingly, breast milk, might be utilized as a valuable and a new easily available source for regenerative medicine, in particular for the therapy different neurodegenerative diseases [3, 4]. This study was aimed at better characterized at cytological and ultrastructural level stem/progenitor cells in the human breast milk.

METHODS

Fresh milk was centrifuged with normal colture medium DMEM was centrifuged and the pelle twas fixed in a mixture of 3% formaldehyde and 0.1% glutaraldehyde in 0.1 M cacodylate buffer and processed by standard methods for embedding in epon resin. Ultrathin sections (90 nm thick) collected nickel grids and was observed and photographed in a transmission electron microscope (JEOL 100S model, Jeol, Tokyo, Japan) operatin at 80 KV.

RESULTS

Breast milk cells appeared, at low power (3,500 X) as large cells, with abundant cytoplasm often rich in vacuoles. Some vacuoles were electron-trasparent, whereas other were moderately electron-dense microvilli were frequently observed on cell surface. Nuclei were mainly formed by open chromatin, and showed an irregular shape with nuclear/cytoplasmic indentations (**Fig. 1**). At higher power (7,000 X) it was possible to better analize the breast milk cell cytoplasm: dense vacuoles were surrounded by few shall mitochondria. Smooth and rough endoplasmic reticulum was less evident. The irregularity of the nuclear shape was confirmed (**Fig. 2**). Apoptotic cells were occasionally observed. Cells undergoing apoptosis showed a dark cytoplasm, chromatin condensation and nuclear fragmentation (**Fig. 3**).

CONCLUSION

Our preliminary data show that breast milk stem/progenitors are characterized by active metabolism, clearly indicated by the irregularity of nuclear shape with cytoplasmic infoldings. Moreover, these cells maintain their ability to secretion, well evidenced by the high number of dense vacuoles observed in the majority of breast milk cells. Further studies at immunoelectron microscopy are needed, in order to

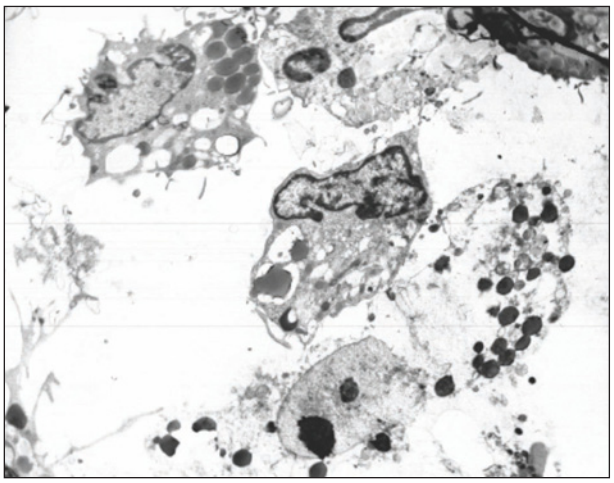

Figure 1 (ABS 47). Human breast milk fresh cells at transmission electron microscope (3,500 X). Nuclei are formed by open chromatin, and show an irregular shape.

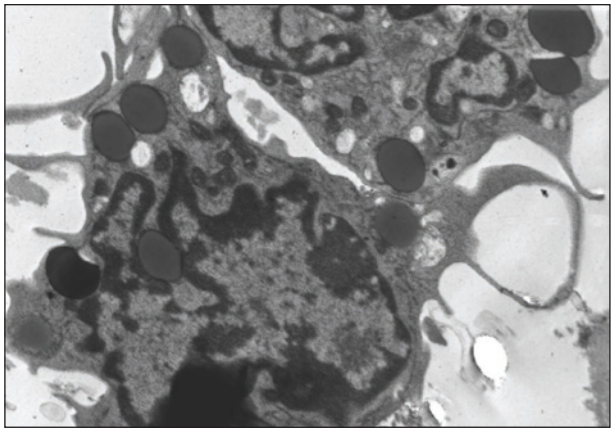

Figure 2 (ABS 47). Human breast milk fresh cells at transmission electron microscope (7,000 X). Vacuoles are surrounded by few shall mitochondria.

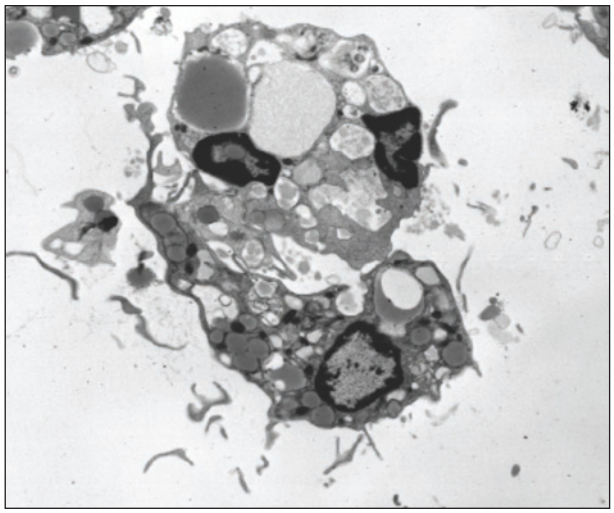

Figure 3 (ABS 47). Human breast milk fresh cells at transmission electron microscope. A cell undergoing apoptosis: it presents a dark cytoplasm, chromatin condensation and nuclear fragmentation.

better characterize the stage of differentiation or of stemness of human breast milk cells.

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ABS 48

EMERGING BIOMARKERS FOR METABOLIC DISEASES

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Metabolic diseases present as an extremely diverse group of inherited genetic conditions with clinical phenotypes that are variable and frequently hard to distinguish from one another [1]. Diagnosis relies heavily upon analytical measurement which can be used effectively to identify disease-specific biomarkers. The advent of gas chromatography-mass spectrometry, and tandem and other advanced forms of mass spectrometry has allowed for the rapid development of novel and sensitive disease-specific biomarkers which are central to today's diagnostic approach.

Currently, more than 500 single gene defects that are associated with metabolic dysfunction have been identified. New metabolic disorders are currently being identified based upon next generation sequencing studies which identify novel metabolic pathways. Biomarker measurements are going to be an essential part of the understanding of all metabolic disorders as both diagnostic tools and tools to monitor the effectiveness of therapeutic intervention as therapies become available.

Metabolic diseases may sometimes be diagnosed pre-symptomatically in the newborn period by a process known as newborn screening. This process began in the 1960's with a simple assay for the diagnosis of phenylketonuria using a small blood spot collected onto filter paper. Currently, newborns are diagnosed with some metabolic diseases analysis of either amino acids or acylcarnitines using the same small blood spot and flow-injection tandem mass spectrometry. The American College of Medical Genetics proposed a cohort of around 30-40 metabolic disorders that can be diagnosed in this manner simultaneously [2]. Many parts of the world have adapted this protocol with variations sometimes dependent on the frequency of different metabolic diseases in that population.

The process of newborn screening is designed to be sensitive so that the fewest positive cases are missed during the process. This drive for great sensitivity results in a number of screens that are false positives. Therefore it is incumbent on

the metabolic disease laboratory to develop biomarker testing that has greater specificity and rules out the false positive screens. This is a process known as confirmatory testing for which guidelines have been written [3]. Confirmatory testing may include analysis of urine organic acids by gas chromatography-mass spectrometry, a process that can identify over 100 genetic conditions and is also used for the investigation of the symptomatic non-screened population. Urine organic acid analysis is an excellent example of how untargeted metabolomics can be valuable in disease diagnosis.

As mass spectrometric devices become increasingly sensitive, novel biomarkers are being identified. One of the largest growth areas in the metabolic disease field involves the diagnosis of a group of conditions known as carbohydrate-deficient glycoprotein disorders for which time of flight mass spectrometry for abnormal protein glycosylation products provides the optimal diagnostic technology. In my own laboratory, we have developed an untargeted process for the diagnosis of metabolic diseases by measurement of the full range of intracellular coenzyme-A species which has promise for the diagnosis of mitochondrial disorders for which there are currently no distinctive biomarkers [4].

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ABS 49

RETINOPATHY OF PREMATURITY: INCIDENCE AND RISK FACTORS

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INTRODUCTION

According to the WHO data, ca. 3.9 million children are diagnosed with retinopathy of prematurity (ROP) worldwide. This disease is one of the main causes of blindness in children.

AIM

To evaluate the incidence of ROP in the Department of Neonatology of the Lithuanian University of Health Sciences (LUHS), to identify the most common risk factors of this disease, and to evaluate the influence of these factors on the development of ROP.

METHODS

This retrospective study included 753 neonates (born at ≤ 32 weeks of gestation, weight ≤ 2,000 g) who were treated during 2003-2012 at the Department of Neonatology of the LUHS. The distribution of ROP and the frequency of its surgical treatment was evaluated in the subjects studied. We evaluated the influence of sex, gestational age (GA) (the subjects were distributed into the following groups: ≤ 25 GA, 26-27 GA, 28-30 GA, and 31-32 GA) and birth weight (the subjects were distributed into the following groups: 500-999 g, 1,000-1,499 g, and > 1,500 g) on ROP, and the distribution of this condition in groups.

RESULTS

ROP was diagnosed in 16% (n = 120) of subjects: 15.8% males and 16.1% females. A statistically significant correlation was found between the incidence of ROP and gestational age groups: ≤ 25 GA – 48.2%, 26-27 GA – 24.5%, 28-30 GA – 11.5%, and 31-32 GA – 4.4% ($p < 0.05$). The highest incidence of ROP (36.3%) was found in the group of neonates with birth weight of 500-999 g, while the incidence of this condition in other groups was much lower (1,000-1,499 g – 11%, and > 1,500 g – 0.6%); however, the difference was not statistically significant ($p > 0.05$). A statistically significant correlation was found between the development of ROP and congenital heart defect, hypoxia, congenital/acquired infection, bronchopulmonary dysplasia, anemia, and encephalopathy ($p < 0.05$). Surgical treatment was applied in 3% (n = 20) of the subjects, most frequently – in the groups with birth weight of < 1,000 g (18%) and > 1,000 g (14%) ($p < 0.779$).

CONCLUSIONS

ROP statistically significantly correlated with the neonates' gestational age: the incidence of ROP decreased with increasing gestational age. Birth weight did not have any significant influence on the development of more severe stages of ROP or the application of surgical treatment. The incidence

of ROP was statistically significantly influenced by perinatal hypoxia, congenital heart defect, infection, anemia, bronchopulmonary dysplasia, and encephalopathy.

ABS 50

TRANSIENT HYPERTRANSAMINASEMIA IN A MALNOURISHED NEWBORN

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INTRODUCTION

Isolated hypertransaminasemia can be highlighted in newborns and children associated with hepatic and extrahepatic diseases. The most frequent hepatic causes are viral, bacterial, protozoan infections. Then, metabolic and autoimmune diseases or iatrogenic causes should be also considered in childhood. Obesity, celiac disease, irritable bowel diseases can seldom be associated with hypertransaminasemia. Diagnosis may be difficult and sometimes unsuccessful. In literature, we could not find examples of hypertransaminasemia linked to the nutritional status of the newborn, as occurred in this clinical case.

CLINICAL CASE

A., cesarean delivery born baby. No syndromes known in her family. At birth, normal parameters were found: weight 2.910 kg (50^{th}-75^{th} percentile), length 51 cm (50^{th}-75^{th} percentile), head circumference 34 cm (50^{th}-75^{th} percentile). Discharged in the 4^{th} day of life, in good conditions, with human milk feeding only. 15 days later (on the 20^{th} day of life [d.o.l.]), she was hospitalized once again, because of a pathological weight loss (more than 10%). At the admission, we found these parameters: weight 2.4 kg, pale and dehydrated skin, peri-horbital dark circles, dystrophy (weight/length rate less than 5), hard hyporeactivity and hypotony. Laboratory analysis showed a sharp elevation of AST (979 IU/l) and ALT (494 IU/l), despite of a normal liver enzymatic pattern (BUN, creatininemia, gammaGT, ALP, albumin, protein amount, direct and conjugated bilirubin were at physiological levels). We did not found any alteration in electrolytes nor in blood glucose. To evaluate a possible hemolitic syndrome or a rhabdomyolysis, we also tested LDH, CPK, blood panel, which were normal. In order to analyze the most common causes of an isolated hypertransaminasemia, we made also an infection screening (PCR, blood culture, urine culture, TORCH, HBV, HCV), a metabolic one (EGA, urea) and the coagulative pattern (PT, PTT, fibrinogen), and everything was negative. We also asked for a cardiac ultrasound evaluation, from which no congenital defects emerged, and an abdominal one, which could exclude hepatosplenomegalia and other biliary abnormalities.

According to these data, she started an intravenous re-hydratation with glucose and saline solutions, which lasted 6 days, and also human plus formula milk feeding. During this therapy, we noticed a weight increase together with AST and ALT decrease. At the discharge, on the 34^{th} d.o.l., she presented AST 44 IU/l, ALT 53 IU/l and an appropriate weight (2.970 kg). She came back for an ambulatory evaluation on the 50^{th} d.o.l., and the clinical conditions resulted completely restored (4 kg, AST 39 IU/l, ALT 34 IU/l).

CONCLUSIONS

This case shows for the first time an episode of transient and isolated hypertransaminasemia in newborn, in absence of common causes of elevated transaminases. In literature [1-5], there is not any other clinical case reported about neonatal transient hypertransaminasemia due to malnourishment and dehydratation, although hypertransaminasemia is described in malnourished adults affected by anorexia nervosa. In conclusion the hypertransaminasemia observed in our patient probably is caused only by malnutrition. Further studies are needed to confirm this association.

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ABS 51

HEPATIC LYMPHANGIOMATOSIS IN A 2 YEAR-OLD CHILD

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INTRODUCTION

Hepatic lymphangiomatosis is a rare disorder characterized by cystic dilatation of the lymphatic vessels in the liver parenchyma. This disorder becomes symptomatic secondary to compression or replacement of the normal parenchyma, and may lead to liver failure [1]. Clinical diagnosis may be difficult, due to the rarity of this disorder and changing clinical manifestations [2]. Lymphangiomatosis in childhood is mainly localized in the head and neck regions in 50% of cases. Less than 5% are intra-abdominal, while 10% of pediatric patients present with visceral disease including the thorax involvement [3].

AIM

The aim of this study consists to show the histological and immunoistochemical findings of hepatic lymphangiomatosis diagnosed in a two year-old child.

MACROSCOPIC FINDINGS

Clinically, the child revealed a markedly protuberant abdomen. We observed a voluminous liver cyst, weighing 189 grams and with a volume of 10.5 x 8 x 4 centimeters, characterized by thin wall and smooth outer surface, with clear serous content and internal surface with multiple smooth thin septa. All the tissue samples of the liver cyst were fixed in 10% buffered formalin, routinely processed and paraffin-embedded. Five-micron-thick paraffin sections were stained with Hematoxylin and Eosin (H&E) and examined under an optical microscope.

Histological findings. At histology, liver architecture was preserved with focal reactive inflammatory infiltrate with presence of eosinophils; the wall of the liver cyst appeared adherent to the liver parenchyma, with coating consisting of endothelial cells (**Fig. 1**). Inside the portal tracts, we observed hyperplasia of the lymphatic vessels (**Fig. 2**). At immunoistochemistry we showed intense reactivity for the marker D2-40 in the endothelial cells lining the cyst and in lymphatic vessels of the portal tracts (**Fig. 3**). These data, taken together, confirmed the diagnosis of liver cystic lymphangiomatosis.

CONCLUSIONS

This case-report shows some features that deserve some consideration. First, the pathological lesions of lymphatic vessels were restricted to one organ, the liver, in the absence of other localizations. Moreover, the pathological changes of lymphatic vessels were predominantly located inside portal tracts, in the absence of major lesions of the surrounding liver parenchyma. Portal discrete changes were associated with a voluminous liver cyst, showing a thin endothelial lining. Our findings lay stress on the existence of a spectrum in lymphangiomatosis:

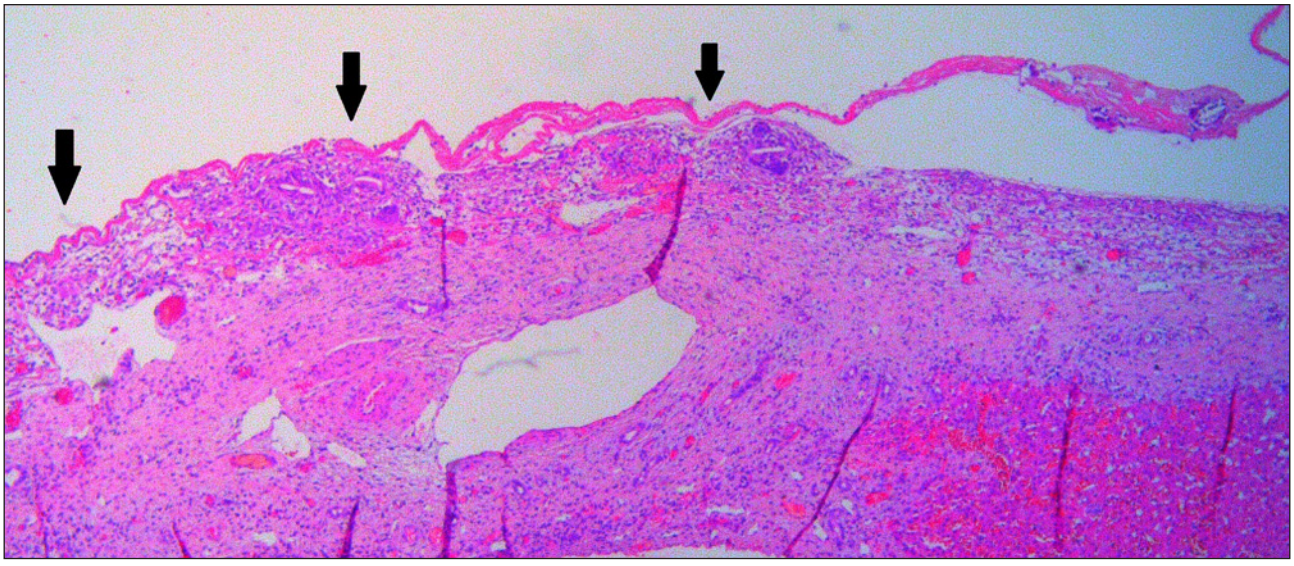

Figure 1 (ABS 51). Liver cyst showed a thin endothelial lining (black arrows).

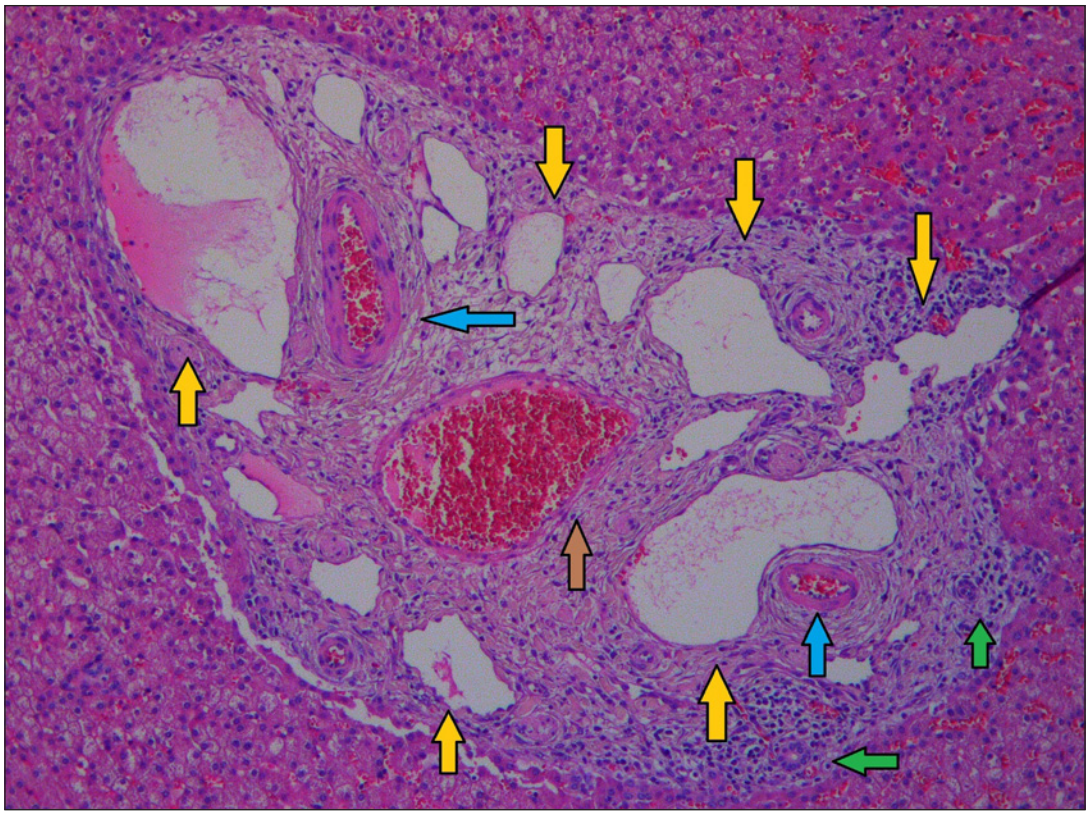

Figure 2 (ABS 51). Hyperplasia of the lymphatic vessels inside the portal tracts (yellow arrows); inside the portal tract we observed branches of the hepatic artery (blue arrows), portal vein branches (brown arrow) and septal ducts (green arrows).

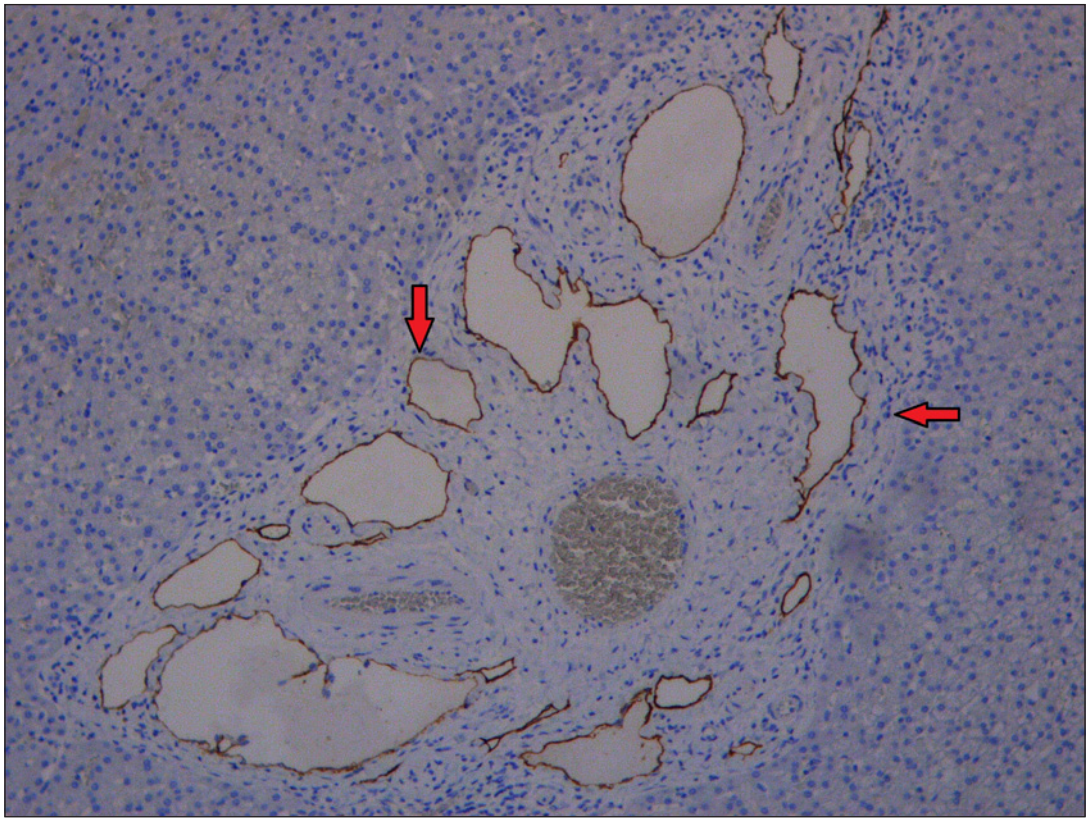

Figure 3 (ABS 51). At immunoistochemistry we showed intense reactivity for the marker D2-40 in the endothelial cells lining the cyst and in lymphatic vessels of the portal tracts (red arrows).

the case here reported probably represents one of the extremes of this spectrum, characterized by the involvement of a single organ, the liver, and by minor pathological changes, without modifications of the liver architecture. These findings allow to perform a good prognosis for our patients.

ACKNOWLEDGEMENTS

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ABS 52

CONGENITAL REMNANTS AS A CAUSE OF AIRWAY OBSTRUCTION IN NEWBORNS

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INTRODUCTION

Airway obstruction is the most common life-threatening condition among newborns, representing a diagnostic and therapeutic challenge for the physicians especially when it is caused by rare pathologies. Head and neck congenital remnants are rare benign congenital neoplasms rarely observed in neonates [1, 2]. Teratomas are the most common congenital tumours in childhood, with an incidence approximately of 1/4,000 newborns [3]. Head and neck epithelial and mesenchymal hamartomas are uncommon, with an incidence of 2-3/30,000 newborns. We report three cases of pharyngeal congenital remnants presented with neonatal airway obstruction.

RESULTS

Case 1

A 9-month-old male child was referred to our Department for recurrent airway obstruction. Flexible scope examination of the upper airways showed an oval and yellow-pinkish lesion of 2 cm of diameter, originating from the left wall of the pharynx, confirmed by Magnetic Resonance Imaging (**Fig. 1**) and intraoperative examination (**Fig. 2**).

The excision was performed by trans-oral laser CO_2 technique under microscopic magnified vision. The postoperative course was uneventful and the patient was discharged after 48 hours. The polypoid lesion (**Fig. 3**) was composed by a chorion of fibroadipose and fibro-connective tissue, pilosebaceous units and serous and mucous gland, covered by skin with a focal hyperkeratotic layer.

Histology allowed the diagnosis of a mature teratoma completely removed.

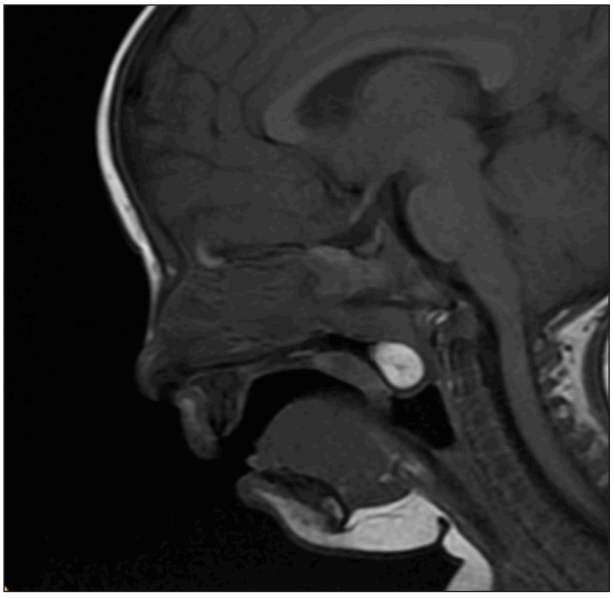

Figure 1 (ABS 52). Case 1. Magnetic Resonance Imaging (MRI): oval lesion of 2 cm of diameter, originating from the left wall of the pharynx.

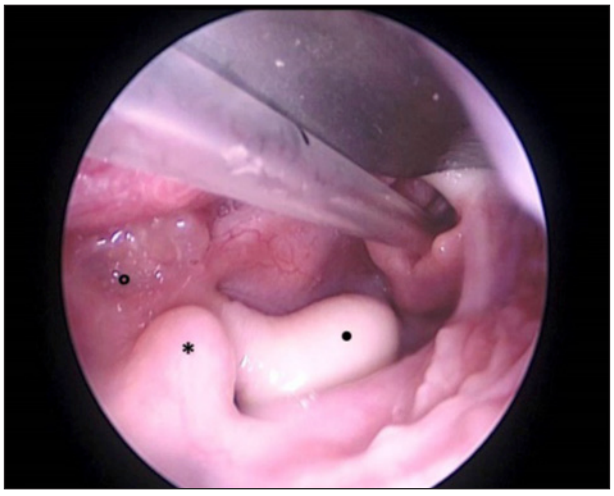

Figure 2 (ABS 52). Case 1. Intraoperative examination of the lesion.

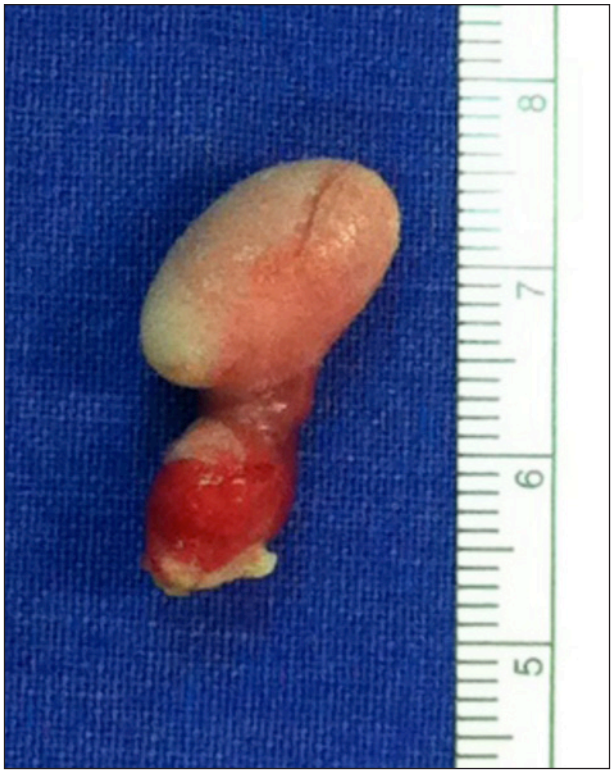

Figure 3 (ABS 52). Case 1. The polypoid lesion was composed by a chorion of fibroadipose and fibro-connective tissue, pilosebaceous units and serous and mucous gland, covered by skin with a focal hyperkeratotic layer.

Case 2

A 35-day-old female was transferred in our Department from a Paediatric Department as an emergency for respiratory distress, cyanosis and bradycardia related to upper airway obstruction. Intraoperative examination showed an oval flopping lesion of 2 cm of diameter originating from the left wall of the pharynx. Surgical treatment consisted in a microscopic-assisted laser CO_2 excision under general anesthesia. During the induction of general anesthesia, the lesion becomes firmly obstructing the laryngeal vestibule requiring an urgent mechanical dislodging from the larynx. Histology confirmed the diagnosis of a mature teratoma completely removed (**Fig. 4**).

Case 3

A 15-hour-old female was referred to our Department from the Neonatal Intensive Care Unit for a lesion originating from the base of tongue, causing cyanosis and intermittent severe respiratory distress. Radical and timely trans-oral laser CO_2 surgery was performed under general anesthesia. The diagnosis of "fibrovascular" hamartoma was confirmed after histologic analysis.

In all cases intraoperative bleeding was minimal and haemostasis was achieved with the use of bipolar diathermy (20 W). There were no surgical complications and at the present time the patients are well and free of disease.

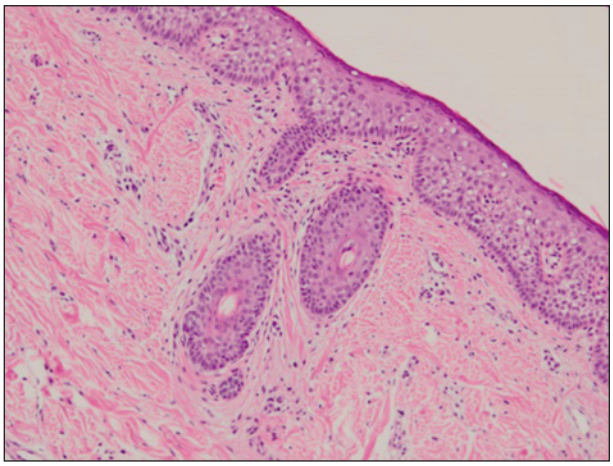

Figure 4 (ABS 52). Case 2. Mature teratoma.

CONCLUSION

Newborns airway obstructions are a challenging condition and must be managed, when possible, by a well-trained paediatric team and, when clinicians face obstructive airway congenital remnants, a timely and radical surgical excision is required to avoid potentially lethal asphyxia.

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ABS 53

INVOLVEMENT OF THE FAMILY IN THE PROCESS OF CARE IN PEDIATRIC WARDS PERCEPTION OF NURSING AND MEDICAL STAFF

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BACKGROUND AND AIM

A recent definition of family-centered care is as follows: "The professional support to the child and the family through a process of involvement,

participation and sharing, supported by the empowerment and negotiation" [1].
From this definition, we tried to explore, through a survey in pediatric hospitals in Cagliari, if the process of parental involvement in childcare evolved, what they perceive health workers to the problems present in the daily, and what it is proposed for possible improvement.

METHODS

Through an observational study was administered a survey to nurses and doctors of three hospitals of Cagliari in pediatric wards.

RESULTS

Of a total of 150 questionnaires were returned delivered 145.
Our sample was aware that the presence of parents, during hospitalization is beneficial for parents, for the child but also for the staff that takes care of the dyad.
The need for adequate training and communications staff that takes care of children and parents, to improve the role of practical and emotional support to participate in the care.
Adequate training of staff, to keep up with scientific evidence.
Have more time available, which could be achieved by enhancing the staff.

CONCLUSIONS

The results of the study will provide guidance for organization and training relevant to the FCC interventions, to increase the quality of care and in addition the results provide evidence based outcomes for benchmarking the improvement clinical practices.

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ABS 54

INVESTIGATING THE METABOLOME FOR MONITORING PKU PATIENTS UNDER TREATMENT USING HIGH RESOLUTION NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMRS) IN URINE

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INTRODUCTION

Monitoring of treatment in PKU patients is based on blood phenylalanine. Fluctuation of phenylalanine is often not well understood. Progress in NMRS technology may offer a new horizon to monitor metabolic diseases. We used urinary analyses of treated patients with PKU as a model to demonstrate the advantage of treatment monitoring.

METHODS

60 urines of patients (age 1-35 years) with dietary (47) or sapropterin (13) treatment were measured using a Bruker Avance IVDr 600 MHz System. Multivariate statistical analysis against a reference of 311 healthy children using Hotelling's T-squared statistic and principal component analyses (PCA) were performed.

RESULTS

The urine profiles of PKU patients did not show differences to the healthy reference group (95% CI). However, PCA analyses in patients under dietary treatment revealed 5 outliers. All Sapropterin treated patients had an excellent metabolic profile under a higher natural protein supply compared to the other patients.

DISCUSSION

Investigation of the metabolome in urine using NMRS and statistical analysis is a promising approach to further monitor the quality of and patient compliance to treatment. Other than PKU, the monitoring of treatment of e.g. organic acidurias, urea cycle disorders and mitochondrial defects may profit from this approach.

ABS 55

ADMINISTRATION OF SURFACTANT IN SPONTANEOUSLY BREATHING PRETERM INFANTS: LISA IN VERONA

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BACKGROUND AND AIM

Early NCPAP in delivery room associated with early administration of surfactant in spontaneously

breathing preterm infants with RDS decreases the risk for intubation. LISA (Less Invasive Surfactant Administration) procedure allows the administration of surfactant during nCPAP.
Our aim is to evaluate efficacy and safety of LISA and compare these results with a historical group of patients treated with INSURE in our NICU.

PATIENTS AND METHODS

21 spontaneously breathing preterm infants (mean GA 31.5 weeks; birth weight 1,650 g) admitted to our NICU between July 2014 and August 2015 were included in LISA group; 21 preterm infants (mean GA 30.75 weeks; mean birth weight 1.525 g) stabilized with CPAP and treated with INSURE composed control group. All infants presented RDS at birth. Surfactant was administered if $FiO_2 >$ 40% or > 30% and Silverman score > 6 on nCPAP, through a 4-6 Fr orogastric tube within 2 minutes after premedication with Atropin and Remifentanyl.

RESULTS

Reduction of FiO_2 was observed in all cases after LISA; complications verified in 28% of them but there was no need to stop the procedure in any case; Global time of mecchanical ventilation (MV) of LISA group was lower ($p = 0.043$) compared to control group. No differences at 72 hours from the procedure ($p = 0.65$) in term of MV or in O_2 dependence at 28 day of life ($p = 0.49$) were observed.

CONCLUSIONS

LISA treatment is safe and effective; it allows to reduce global time of MV compared to INSURE one.

ABS 56

POSTERIOR QUADRANT DYSPLASIA (PQD) AND EPILEPTIC ENCEPHALOPATHY WITH ONSET IN THE FIRST MONTH OF LIFE: PRESENTATION OF A CLINICAL CASE

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INTRODUCTION

Posterior quadrant dysplasia (PQD) is a rare variant of cortical dysplasia involving the posterior regions of a single hemisphere; this condition is always associated with very early onset epilepsy (most frequently before the first year of life), psychomotor developmental delay and visual deficits [1-3]. The most frequently reported seizures are epileptic spasms (SE) and focal seizures, which often have a very high frequency (pluri-daily) and are early drug-resistant, with a consequent bad evolution in the majority of cases [3]. In view of this the option of surgery should be kept in mind in order to avoid a deterioration in the clinical picture [2].
We present a case of a child with PQD and epileptic encephalopathy with onset in the first month of life.

CLINICAL CASE

Male, born at term (38th week) by normal delivery after an uneventful pregnancy. No perinatal sufferance was reported. At birth presence of macrocrania (head circumference 38.5 cm) with normal brain ultrasound. A few days after the birth onset of focal seizures, followed around the 15th day of life by clusters of asymmetrical tonic spasms at awakening. The EEG showed a severe epileptic encephalopathy with repetitive paroxysmal activity of spikes, poly-spikes and spike-waves on the posterior and temporal regions of the right hemisphere. In sleep presence of an atypical burst-suppression pattern.
Neurologic examination: presence of a "poor" motricity, with cramp-synchronized movements. Slight left hemiparesis and buccal dyskinesia. Lack of eye contact and presence of nystagmus in the left eye.
Brain MRI: presence of a PQD of the right hemisphere. Visual evoked potentials: cortical response not evocable bilaterally.
At 23 days of life start of therapy with Phenobarbital.
Last examination (1 month 15 days of life): no more seizures; at EEG presence of sub-continuous epileptiform activity with bilateral abnormalities.

DISCUSSION

Actually, only few data from the literature are available with regards of children affected by PQD and operated during the first year of life [2]. Despite the short follow-up, the current clinical situation of our patient (no seizures) may lead us to postpone the surgical option in case of the development of a drug-resistance. However, based on the data in the literature, the high probability of worsening and the presence of an epileptic encephalopathy with bilateral abnormalities, we believe that early surgical option should be considered despite the absence of seizures, in order to try to avoid (or reduce) not only a probable recurrence of epileptic seizures, but also a progressive impairment of psychomotor development.

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ABS 57

HCMV INFECTION IN PREGNANCY: FROM CLASSIC DATA TOWARDS METABOLOMICS

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Human cytomegalovirus (HCMV) is the most common cause of congenital infection worldwide. Primary maternal infection has a risk of vertical transmission of 30 to 35%, considering the entire period of gestation. In detail, the transmission rate is 0-10% for preconceptional maternal infections, 30-45% for periconceptional infections, 34-42% for first trimester infections, 43-44% for second trimester infections, and 64-73% for third trimester infections. Congenital HCMV infection can also follow recurrent maternal infection. About 1% of children born to women who are HCMV seropositive before pregnancy acquire the virus in utero. Symptoms develop in about 10% of HCMV-infected fetuses, with a fatality rate in 5% and neurological handicap, visual impairment or hearing loss in 90% of survivors. Among the asymptomatic newborns, 10 to 15% develop sensorineural hearing loss, and/or learning disabilities. Diagnostic criteria for establishing or excluding a primary HCMV infection in pregnancy are: i) HCMV-specific IgG seroconversion; ii) presence of virus-specific IgM antibody and low IgG avidity index (AI); and iii) presence of viral DNA in maternal blood. Timing of maternal infection is determined in the great majority of pregnant women based on a HCMV-specific IgG seroconversion and/or the presence of specific-IgM, low AI and *de-novo* appearance of neutralizing antibodies on human embryonic fibroblasts, associated with presence of clinical signs and symptoms. When signs/symptoms were not reported, kinetics of IgG and IgM and AI are considered for determination of the onset of maternal infection.

HCMV transmission is documented antenatally by quantitation of viral DNA in and virus isolation from amniotic fluid. Amniotic fluid collected at proper time points in pregnancy (> 6-8 weeks after maternal infection and at 20 to 21 weeks' gestational age) can be examined for diagnostic testing of fetal HCMV infection to provide obstetricians and pregnant women with important information for decision making about pregnancy management. Sensitivity of PCR ranges between 70 to 90% but, when correctly timed, it approaches 100%. When PCR is positive, congenital infection can be diagnosed with 100% certainty. On the other hand, when PCR is negative, fetal infection can be ruled out with a high degree of certainty (negative predictive value, 97.9%). False-negative culture and PCR results have occasionally been reported and may be a result of delayed transmission of HCMV to the fetus.

As far as concern prognostic significance of quantitation of viral DNA in amniotic fluid, whereas a low viral load is consistently found in asymptomatic fetuses, high viral load in amniotic fluid is associated with either symptomatic or asymptomatic congenital infection. Gestational age at maternal infection, timing of HCMV intrauterine transmission, and timing of prenatal diagnosis are variables that may account for the lack of an association between HCMV DNA quantification in amniotic fluid and fetal prognosis.

The use of the metabolomics in amniotic fluid offers a new challenging approach to investigate the magnitude and quality of HCMV replication in utero and to identify new biomarkers of fetal disease that have not been predicted with the limited knowledge currently available.

ABS 58

BASIC LIFE SUPPORT TRAINING FOR A SARDINIAN SCOUT GROUP

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BACKGROUND
Several studies show the importance of introducing the teaching of first aid in primary and secondary schools. The American Heart Association recommends such a training in school-age children as mandatory. Many European schools have introduced LSFA (Life Supporting First Aid) programs. In Italy, scientific societies and voluntary organizations are often the only ones spreading the culture of first aid among boys.
AIM
Show that boys are able to learn and put effectively into practice the First Aid maneuvers they are taught.
MATERIALS AND METHODS
Descriptive study in which a Scout group "AGESCI" (Association of Italian Catholic Guides Scouts) participated in a session LSFA. Children aged 12 to 16 years were included. The initial theoretical knowledge was assessed by a questionnaire given to participants before the training. An instructor nurse of the Emergency System taught theory and first aid maneuvers on instructional mannequins. The evaluation was performed one week after the training by the administration of a post-test questionnaire and the simulation of emergency scenarios.
RESULTS
25 scouts were observed (m = 16, f = 9), including 9 aged 13 and 8 aged 12. Only 32% (n = 8) had previously received a training from associations and Emergency Service. After the teaching, the percentage of correct answers was 66% compared to 49% pre-intervention. In the practical evaluation, 58% of the boys identified the correct sequence of actions to perform cardiopulmonary resuscitation.
CONCLUSIONS
These results show that boys are able to learn Basic Life Support manoeuvers with a very short training. The spreading of cardiopulmonary resuscitation knowledge to school-aged boys could become a resource for the community, provided its teaching is included in study programs.
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ABS 59

DEVELOPMENTAL TRENDS IN PRETERM CHILDREN: AN INTEGRATE MODEL OF FOLLOW-UP IN THE 1ST YEAR OF LIFE

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BACKGROUND
The study focuses on the developmental process of preterm children through an assessment and a monitoring path from 3 to 12 months of the corrected age. It is a study based on outpatient path that provides a follow-up within an experimental program of a neonatologist-psychologist integrated intervention according to a model of the co-management and the co-construction of instruments according to a multidisciplinary and between professionals relationship. The theoretical model is in line with the guidelines of the National Institute of Child Health and Development (NICHD, 2002) and of the National Institute of Neurologic Diseases and Stroke (Follow-up Care of High-Risk Infants, Pediatrics, 2004; Browne, 2004). In fact, it is highlighted as the modern neonatal intensive care is associated with an improved survival of very preterm children compared to the past (< 28 weeks with birth weight < 1,500 g and < 1,000 g). On the other side, the survival seems to be characterized by significant neuorodevelopmental impairments in the long term. Several studies have highlighted that extremely preterm children have cognitive (Kleine et al., 2003; Morales et al., 2013), language, (ibidem; Sansavini, Guarini, 2014) and motor impairments as well an atypical socio-relational development (Allen, 2008; Salt, Redshaw, 2006). A few researches were carried out outside the hospital, starting from the first year of correct age and providing intelligence and/or performance texts (WISC, behavior scales, CBCL, etc.) (Perricone, Morales, Polizzi, 2012; Perricone, Morales, Anzalone, 2013). In this sense, the study is in discontinuity with the literature, anticipating the age of the monitoring, through the use of a specific development scale in accordance with the integrate approach of physician-psychologist. In the light of such considerations, the aim of the study is the early exploration of several developmental dimensions of preterm children starting at 3 months of the corrected age. The hypothesis of the study is to assess developmental impairments or their own precursors, as suggested by the literature of the field.

METHOD

The study involved 30 extremely preterm children without neurological sequelae, genetic syndromes (G.A. mean = 31.3; birth weight mean = 1.300, SD = .253) collected at the follow-up of the Neonatology and Intensive Neonatal Care Unit of the "Ospedali Riuniti Villa Sofia-Cervello" Hospital of Palermo. The used tools were the Griffiths Mental Development Scales Revised 0-2 (Griffiths, 1996), at 3-6-9-12 months of the corrected age respectively; the tool is structured by six subscales (locomotor, personal-social, hearing and language, hand-eye coordination and performance). The choice of this specific tool is related to the possibilities to make an early analysis of the development, that could be focused on the child's skills with regard to several neurodevelopmental dimensions (locomotor, personal-social, hearing and language, hand-eye coordination and performance). The administration also provides the involvement of the parents in the reading of the development trends of their own children.

In addition, the Griffiths Scales allows to found the adaptive profile of the child in terms of performance, not only of the "what" but also of the "how", with regard to the locomotor development, language, coordination and socio-interpersonal skills, and problem-solving competences. The Griffith Scales are also characterized by a playful and a daily dimension, offering simple activities that parents could repeat at home.

RESULTS

The collected data were analyzed through descriptive and parametric statistics (ANOVA).

The results, both on the descriptive and statistic level, show the stability of the Development General Quotient (GQ) during the first year that is in line with the mental age (GQ 3-month, GQ 6-month, etc.). Therefore, results highlights an increase and a significant growth trend in the social skills in daily activities of the child, in the receptive and expressive language, in the visual-spatial performance skill ($p < .001$). Moreover, data show a decrease of some specific dimensions: grosso-motor skills are still below of corrected age, and also the hand-eye coordination (fine motor) decreases at 6/9 and 12 months ($p < .05$).

CONCLUSIONS

The data suggest several interesting considerations on the importance of the follow-up oriented by the physician-psychologist integrated model and by the use of tools that make it more effective in terms of a positive impact. This integrated model, in fact, allows to understand not only developmental impairments but also resources, and allows to promote a new reading of prematurity for the physician, a new reading of their child in terms of future projects for parents, in according to the Positive Semiotics perspective (Viziello, 2008; Righetti et al., 2013) and a Positive Parenting (Sanders, 1999).

ABS 60

TARGETED ANALYSIS IN URINE BY HIGH RESOLUTION NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMRS) AND GAS CHROMATOGRAPHY/MASS SPECTROSCOPY (GC/MS) IN THE SELECTIVE SCREENING PROGRAM FOR INBORN ERRORS OF METABOLISM

C. Cannet[1], L. Beedgen[2], F.K. Trefz[2], J.G. Okun[2], C.D. Langhans[2], H. Schäfer[1], M. Spraul[1], G.F. Hoffmann[2]

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INTRODUCTION

NMRS has shown to be a highly reproducible and quantitative method capable of high throughput analysis. Lower sensitivity and higher instrument costs may have limited the widespread introduction into the metabolic laboratory. GC/MS is more sensitive but less quantitative, sample preparation and analysis time is longer. The purpose of this study was a comparison of both methods in view of quantification of relevant metabolites and of general efficiency to correctly diagnose disorders in the routine metabolic laboratory.

METHOD

700 urine samples which were sent to exclude metabolic disorders were split for GC/MS and NMRS analysis using a quadrupol GCMS Trace/DSQ II (ThermoScientific) and a Bruker Avance IVDr 600 MHz system respectively.

RESULTS

Correlation was calculated for 28 substances. Detection limit was higher for most substances in NMRS than in GC/MS. However, in some pathologic conditions quantitative results are 2-5 times higher in GCMS than in NMRS. Diagnoses of Isovaleric, Propionic, Methylmalonic acidemia and MCAD deficiency could be confirmed in the patient urines by both methods.

CONCLUSION
NMRS is similar efficient as GC/MS but is more quantitative for higher concentrations. However, for some metabolites GC/MS or HPLC/MS methods are necessary to quantify low concentrations.

ABS 61

EXPLORING THE ROLE OF DIFFERENT FETAL AND NEONATAL NUTRITION DURING THE FIRST WEEK OF LIFE BY URINARY GC-MS METABOLOMICS

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BACKGROUND
Nutrition plays a key role in the human life and can affect the quality of life in several ways. By the time is became a common knowledge that obesity and the risk to develop metabolic syndrome are correlated with insufficient and excessive fetal malnutrition. It is also important how the nutrition in neonates could affects their life quality or could prevent the risk of chronic pathologies [1]. A scientific method useful and promising for the study of these problems appears to be metabolomics approach. Metabolomics is a new field of research in the panorama of "-omics" sciences, and it focus on the quantitative measurement over time of the metabolic response of a living system to pathophysiological stimuli and genetic modifications. The use of this approach allows evaluating the effects that occur in the metabolism of the neonates and those strictly related to the type of nutrition received in the first period of life [1, 2].
SCOPE
The aim of this study was to uncover the ability of metabolomics approach to identify neonatal urine metabolites linked to different neonates classes (based to birth weight) and to assess the influence of diet on metabolite excretion in the first week of life.
METHODS
The study population included 36 neonates, exclusively either breastfed or formula milk fed, in a seven days timeframe. Urine levels of low molecular weight of polar metabolites were investigated by GC-MS obtained from intrauterine growth retardation (IUGR) and large for gestational age (LGA) neonates and compared to appropriate gestational age neonates.
RESULTS
This study showed a distinctive urine metabolic profile at the first day with an overlapping metabolic pool between IUGR and LGA samples while after seven days samples were statistically different based on different nutrition approaches. In agreement with our previous studies [1, 3], alterations in the content of myo-inositol was found to be characteristic of IUGR and LGA, whose urinary level was higher with respect to controls at birth, while higher contents of long chain fatty acids, organic acids, amino acids and mono- and disaccharides were noted in newborns exclusively breastfed (at 7 days of life).
CONCLUSION
In this study, metabolomics has proven to be an efficient tool helping in establishing the metabolic parameters which artificial neonatal feeding must take as a reference. The results here reported could give an important contribute to the wider project of improving the quality and the life expectancy of newborns that cannot be breastfed.
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ABS 62

INFORMATICS IN HOSPITAL. INFORMATION TECHNOLOGY (IT) IN THE PHARMACY SAFE MANAGEMENT FOR PEDIATRIC APPLICATION

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SUMMARY

The introduction of the automation process in the Hospital Pharmacy Service regards the mechanical processes of handling and distribution of medications. The entire pharmacy tasks chain may be monitored and realized by the informatics control, including patient information, medical history of patient, tracking and updating drugs submission during the hospitalizations, drug interaction and allergies risk detection, and finally the inventory management.

Modern Informatics and constant developments in technology make the dispensing of prescription medications safer, more accurate and more efficient especially in the critic situation like in the pediatric Units.

Robots and informatics procedures in NICU and in Pediatric Units can help the Physicians in the safe monitoring of the pharmacological treatments in order to reduce, for example, the risk of inappropriate drugs submission. Further, Information Technology (IT) can help in the optimization of the drugs management by means the costs reduction for the Pharmacy Service in the Hospital.

In this paper we present our experience in the AOUCA with this technology installed in the Neonatal Intensive Care Unit, Puericulture Institute and Neonatal Section of the "Azienda Ospedaliero-Universitaria" and University of Cagliari.

INTRODUCTION

Over the last decades, the development of the health care system proceeded with higher expectations from medicine, and with higher attention toward clinical practice. Therefore, hospitals and health professionals, exploiting new technologies and subsequently revising former outdated procedures, applied novel practices in order to reduce the incidence of clinical errors. Errors regarding drug management (prescription, storage, administration) constitutes 15-20% of the total clinical errors. This is a considerable responsibility from both clinical and an economic perspective. Errors over the administration of the drug depend on several factors such as similar packaging, prescription misinterpretation, the use of abbreviations, inappropriate dosage. The risk involves the entire drug management and it often origins from supply, planning, and storage of the products.

The AOUCA, the Universitary Hospital of Cagliari, designed a project on the employment of the most important technologies that guarantee safety and efficacy of storage, prescription, delivery, and traceability of the drugs. This project aims to reduce clinical risks, and to lower expenses and costs. Due to Information Health Technology, healthcare institutions/hospitals are focusing on technologies that may limit clinical risks. Such technologies serve to improve and trace processes, and to optimise certain pivotal aspects: active molecule, drug expiration date, storage, logistics, etc. Among these, the most important are computerised prescription, unitary dose, and computerised distribution systems by means of cabinets, and carts.

CRITICISM ON PHARMACOLOGICAL CYCLE

Clinical risk is defined as the probability of a patient to be subjected to adverse events, i.e. discomfort or damage, derived, even involuntarily, from the medical treatment, hence extending hospitalisation period and causing health impairment, or death. In Medicine, errors, and prevention of adverse events are important topics from both clinical, and economical point of view. The former case regards the quality of the therapies, while the latter concerns the costs. Errors, which are inevitable in a real world, may originate from human behaviour or from the system of human, technical, and relational network. Although errors cannot be completely avoided, they may be significantly limited by behavioural protocols together with technological instruments. In literature it is reported that 18% of medical treatments led to adverse events for the patients, and half of them could be prevented through the application of a clinical risk management policy. It has to be notice that this intervention is strongly recommended by several Italian, and International scientific associations. Among damages occurred during hospitalisation (national mean: 7.2 days per hospitalisation), adverse drug events (ADE) are of particular interest, constituting 20% of the total adverse events. Therefore, circa 2 patients out of 100 are subjected to damages from health professionals during therapy. Within a policy that aims to avoid the occurrence of adverse events, those derived from improper drug use are significantly easier to prevent due to the possibility of a massive use of technologies in the premises, hence a more careful risk management compared to other error types (e.g. surgical errors). In particular, ADEs

are easily evitable through the introduction of robotic systems for the automatisation of drug management in the wards.

Five error categories may be identified/distinguished:

- prescription errors;
- trascription/interpretation errors;
- preparation errors;
- distribution errors;
- administration errors.

CURRENT DRUG SYSTEM MANAGEMENT IN HOSPITALS

Italian hospitals employ a traditional/"stock" distribution system which is based on order-delivery process. In this context, Pharmacy Service supplies the so called Ward Cabinets or Stock Cabinets in each hospitalisation area. Subsequently, these cabinets may often contain a dramatic number of drugs. According to this traditional system, physician's prescriptions are transcribed into clinical record; in turn, nurses check drug availability in the ward cabinets and, in case of unavailable items/pieces, fill a form for the Pharmacy Service to supply the cabinet. Therefore, since drug orders are not dependent on a single patient, but on the hospital needs, the role of the hospital pharmacist is limited to the verification of what is ordered, and the qualitative and quantitative control over deliveries. In the case of drug availability, the nurses proceed to preparation and administration of the treatment to the patient. Although virtuously followed by the diverse health professionals, this protocol inevitably leads to affect the expenses of the hospital.

THE SOLUTION: ROBOTS AND INFORMATICS PROCEDURES

Given these issues, the AOU of Cagliari designed a novel approach for the computerised management of drugs, and for the application of a different distribution and administration process. Such approach is based on a different concept of Pharmacy Service – Operative Unit – patient relations, providing computerised cabinets for the management of the drug therapy. This organisational change reduces criticisms related to the traditional distribution system. As a concrete result, this strategy would save economic resources.

Due to this technological aid, the simplification of management and administration of the drug would benefit nurses, which may spend more time to the care of the patient. Regarding safety, this structure possess high levels of "computerisation and automatization", exploiting computerised prescription system, and barcodes, hence lowering error incidence. Among the institution that applied an automatized drug management strategy, administration errors decreased of 24%. Not only the employment of a computerised system leads to several advantages but it also constitute a fundamental organisational change that may deeply modify pharmacist-physician-nurse-patient relations.

BUSTERSPID SYSTEM

Busterspid is a robotic system that allows for the automatization of the entire drug management within hospitals. This system is able to integrate prescription, administration, supply, and storage of the drugs. It is constituted by a robotic dispenser that stores and automatically delivers the drugs in the wards, aided by management and administration software.

Busterspid may manage all the steps regarding the drug supply, allow the central pharmacy to monitor and interact with all the hospital ward systems through a control platform. This technical-organisational model is based on the computerised prescription at the bed on the patient, and on the real time communication between prescription software, administration, robotic cabinet, and central pharmacy.

Further, BusterMed, the software suite, realizes an integrated pharmacy management system with a stronger reduction of human errors in all the phases. The form "therapy-patient-drug" has instruments for the automatic management control. All the devices are wireless connected and the software drive the physicians in the proper drug submission process.

Administrative control is activated at all level for the storage of drugs and new orders, in automatic way by means the wireless hospital network connections with central pharmacy and all the medical departments.

Table 1 (ABS 62). Increase of efficiency in the therapy management.

The adopted technology allows for:
• proper prescription;
• proper drug selection;
• proper patient selection;
• proper timing;
• proper submission;
• automatic operationals transcription.

CONCLUSIONS

Although we are still in the start-up stage in the Department of NICU, the main advantages of robotized and informatizioned technology for Drugs management are highlighted, especially for the reduction of risk, generating better conditions for nurses and doctors operations in NICU, without removing professionalism and delegating to the information technology management only the control aspects of medical routine. The system manages the entire pharmaceutical supply chain and contributes to costs containment and reduction of clinical risk.

ABS 63

PULMONARY ATRESIA WITH INTACT VENTRICULAR SEPTUM: A CASE REPORT

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INTRODUCTION

Pulmonary atresia with intact ventricular septum is a rare cyanotic congenital heart defects (1.9% of all congenital heart defects) [1]; it is a morphologically heterogeneous lesion characterized by absence of communication from right ventricle (RV) into pulmonary artery due to atresia of pulmonary valve or infundibulum. The size of RV varies and correlates to survival. Based on the presence or absence of the three portions of RV (inlet, trabecular and infundibular), there are three types of pulmonary atresia (Bull's classification, 1982):

1. tripartite type (all three of these portions are present and the RV is almost normal in size);
2. bipartite type (inlet and infundibular portions are present);
3. monopartite type (the only inlet portion is present and RV is hypoplastic).

The RV size is highly correlate with tricuspidal valve size. Abnormalities in coronary circulation, such as sinusoids, fistulae, coronary stenosis, or atresia, are common. The patient survival depends on the presence of interatrial communication (atrial septal defect or foramen ovale) and patent ductus arteriosus (PDA). Pulmonary blood flow is usually provided through PDA. Without adequate management (PG_1 infusion, and surgery), the prognosis is extremely poor: about 80% die by six months of age and the death usually occurs because of the spontaneous closure of the ductus arteriosus [2].

CASE REPORT

M.C., female, is the second born by multiple pregnancy. One of the triplet died in the 14th week of gestational age. At the 26th week of gestation, the mother threatened preterm labour. Soon after, a fetal ultrasound revealed the presence of a heart defect in the female fetus: the tricuspid valve was particularly hypoplastic and dysplastic, the RV was monopartite with absence of trabecular and infundibular portion; the pulmonary valve was atretic with annulus size of 4 mm; the pulmonary branches were perfused retrogradely from the ductus arteriosus; forward flow from the right ventricle was absent. Several sinusoids were present in the ventricular septum. A diagnosis of pulmonary atresia with intact ventricular septum and markedly hypoplastic RV was made. The infants were born at the 29th week of gestational age in our hospital. During pregnancy, three doses of betamethasone were administered to the mother. The APGAR of our patient was 4 at the 1st minute and 4 at the 5th minute; in the delivery room, she was intubated and ventilated with Ambu-device to reach SaO_2 of 75-80%. At the admission in NICU, physical examination revealed cyanosis (SaO_2 50% without oxygen administration), respiratory distress, poor reactivity, bilaterally breath sounds decreased; heart murmur 2/6; the remaining physical parameters were normal. Hemogasanalysis revealed mild respiratory acidosis; the chest X-ray revealed RDS. Endotracheal surfactant was administered and HFOV was started. The echocardiography (**Fig. 1** and **Fig. 2**) confirmed the diagnosis of pulmonary atresia with intact ventricular septum of monopartite type; it also showed a PDA with sn > dx shunt and PFO with sn > dx shunt. Because of low saturation levels (70-75% with FiO_2 0.1), the administration of PGE_1 was started (0.02 mcg/kg/min); then, it was observed a rapid reduction of FiO_2. Because of clinical improvement, at 12 hours of life, the infant was extubated and support ventilation with BiPAP (FiO_2 0.21 and SaO_2 98-100%) was started. The subsequent onset of several episodes of apnea and desaturation and the respiratory acidosis made reintubation necessary and HFOV was restarted. The administration of caffeine was introduced in the therapy. Then, the echocardiography showed a large PDA (2.8 mm) with exclusive shunt sn > dx (**Fig. 3**). Given the normal values of SaO_2

(SaO_2 98-100%) and diastolic theft proven by other examinations (brain ultrasound: RI = 1.5), we decided to gradually reduce the dosage of PGE_1 (0.01 mcg/kg/min): the subsequent severe episode of desaturation made it necessary to increase again to the previous dosage.

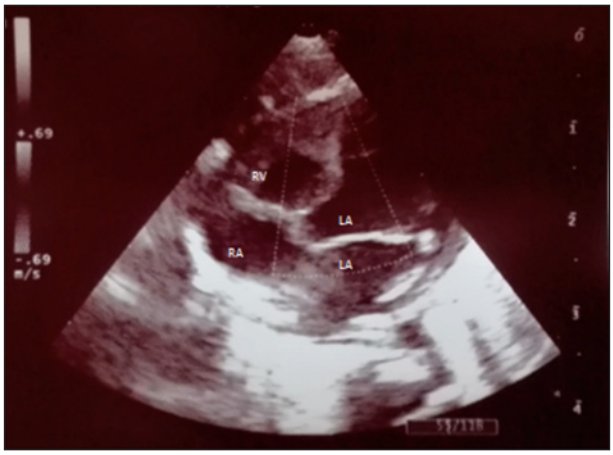

Figure 1 (ABS 63). Hypoplastic right ventricle (RV).

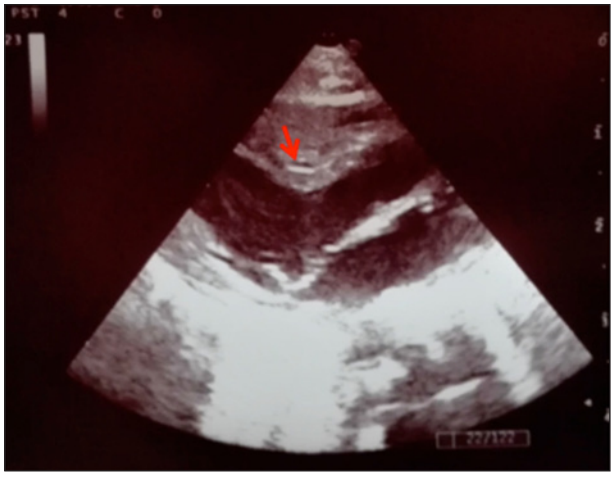

Figure 2 (ABS 63). Sinusoids (red arrow).

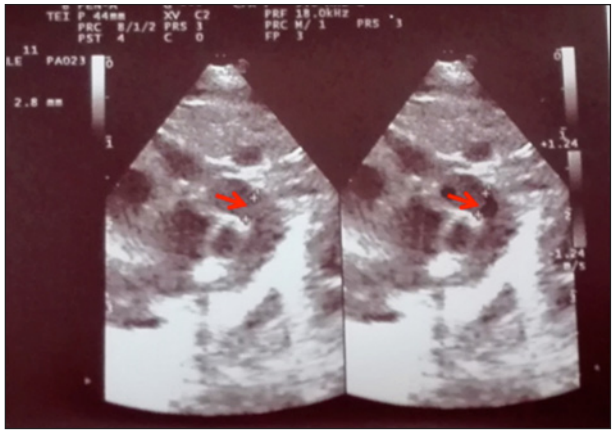

Figure 3 (ABS 63). Large PDA (red arrow).

At 3 days of age, because of the clinical worsening (increased FiO_2, severe respiratory acidosis, worsening of respiratory distress), a second administration of endotracheal surfactant was made; quick improvement followed. To reduce the size of the ductus and the systemic hypoperfusion, we tried to gradually reduce the PGE_1 dosage (down to 0.007 mcg/kg/min, at 7 days of age). At 8 days of age, after a severe bradycardia, she underwent to cardiopulmonary resuscitation and adrenaline administration. The echocardiography showed then a mild hypokinesia and a PDA still open. Three hours later the infant died.

The autopsy confirmed the diagnosis of pulmonary atresia with intact ventricular septum and showed several myocardial infarctions (cause of death), massive necrosis of both adrenal glands, acute tubular necrosis, severe hepatic stasis with focal hemorrhages and extensive necrosis of splenic pulp.

CONCLUSIONS

Despite cyanotic heart defect, the infant early presented normal saturation values (98-100%) without need for supplemental oxygen. In this case, the exitus of the infant was not due to the closure of the ductus arteriosus (the echocardiography performed just before the death revealed a ductus still open) but to the coronary anomalies associated to the congenital heart defect together with the hemodynamical alterations. The multiple pregnancy together with normal fetal ultrasound findings on the other twin contraindicated the option of fetal valvulotomy on our patient, while the low birth weight of the infant contraindicated the surgery at birth (packing of systemic pulmonary shunt): it can only be performed in infants weighing more than 1,500 g.

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ABS 64

PRE-LABOR RUPTURE OF MEMBRANES (PROM) AND MODERN METABOLOMICS

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INTRODUCTION

There is a growing interest in the application of metabolomics to the study of normal and complicated pregnancies [1, 2]; metabolomics claims to give a great amount of informations about the mechanisms of the fetus developing along all the pregnancy period, about the mechanisms inducing normal delivering in the labour and in case of deliveries with complications for the fetus and for the mother. Pathologies and complications induced during the labour are frequently causes of dead for the newborn and for the mother. It is mandatory for the modern society to investigate about all the possible mechanisms to improve the outcome for the babies and the mothers. Metabolomics methods are applied for the characterization of the biological tissues of interest, like amniotic fluid, blood, plasma, cord blood, placenta, urine, and vaginal secretions. Our group is developing methods and techniques of data analysis for the description of the pregnancy process, essentially moving towards the application of the systems biology methods. Last our application regards the premature rupture of membranes (PROM) condition during the pregnancy. PROM, or pre-labor rupture of membranes, is a condition defined as rupture, or breakage, of the amniotic sac more than one hour before the onset of labor. In order to study the conditions of PROM mechanism activation we applied metabolomics techniques to investigate about it. We realized a preliminary model characterising the canonical pathways involved.

MATERIALS AND METHODS

Urine samples from term pregnant women with PROM before labour onset were collected and stored at -80°C. 52 urine samples analysed from 11 subjects, 11 samples selected for a preliminary modelling (first samples after PROM alert). Urine samples were thawed at room temperature and vortex mixed to homogenize. 100 µL of each sample were collected to form a pool sample to use for quality control and to form an average composition sample to analyse among the others. 150 µL of urine were transferred in glass vials (2 mL) with PTFE lined screw caps and evaporated to dryness overnight in an Eppendorf vacuum centrifuge. 30 µL of a 0.24 M solution of methoxylamine hydrochloride in pyridine was added to each vial, samples were vortex mixed and left to react for 17 h at room temperature. Then 30 µL of MSTFA (N-Methyl-N-trimethy lsilyltrifuoroacetamide) were added and left to react for 1 h at room temperature. The derivatized samples were diluted with hexane (600 µL) just before GC-MS analysis.

GCMS technique was applied to characterize the maternal urinary metabolome. Samples were analyzed using a Agilent 5975C interfaced to the GC 7820 equipped with a DB-5ms column (J&W), injector temperature at 230°C, detector temperature at 280°C, helium carrier gas flow rate of 1 ml/min. The GC oven temperature program was 90°C initial temperature with 1 min hold time and ramping at 10°C/min to a final temperature of 270°C with 7 min hold time. 1 µL of the derivatized sample was injected in split (1:20) mode. After a solvent delay of 3 minutes mass spectra were acquired in full scan mode using 2.28 scans/s with a mass range of 50-700 Amu.

We performed Multivariate analysis (MVA) on the GCMS data matrix. The data processing of GCMS results generated a matrices imported into SIMCA-P+ software (version 12.0, Umetrics, Umea, Sweden). Principal Component Analysis (PCA), was performed. Subsequently, Partial Least Square-Discriminant Analysis (PLS-DA), a supervised classification method, was applied to realize a model from a dendrogram analysis in the PCA space. Model performance was evaluated using the coefficients R^2 and Q^2. The model was validated by cross validation and permutation test. The importance of the discriminating variables has been indicating as VIPs (variable of importance on the projection). This list generates values having a corresponding scores which is considered significant if higher than 1.0. The VIPs list was used to realize the Pathway Analysis.

The Pathway Analysis module of MetaboAnalyst (ref. http://www.metaboanalyst.ca/) was used to identify the most relevant pathways involved in the

conditions under study; MetaboAnalyst is a web-based pipeline for metabolomics data processing, statistical analysis and functional interpretation. The pathway topology analysis uses the betweenness centrality as well-established node centrality measures to estimate node importance: the betweenness centrality measures number of shortest paths going through the node. A Google-map style interactive visualization system is available to allow pathways data exploration.

RESULTS

A MVA model has been realized using a dendrogram measure to identify inner grouping between samples and related similarity of the metabolome. We report the PLS-DA models for a two classes clustering in **Fig. 1**.

Parameters of cross validation for the first and the second PLS-DA are:

M3 (two cluster) $R^2X = 0.9$ $R^2Y = 0.99$ $Q^2 = 0.91$

These two classes are characterized by their clinical status: not in labour and labour, with two outliers.

This two classes model is characterized by a good PLS-DA cross-validation parameters and it seems to be a good model to apply for the classification of the intermediate states of PROM in order to support clinicians' decision about the proper treatment to apply. It is possible to get an inside of the metabolome related to the PROM condition studied. It is important to underline that this is a preliminary model. Using the MetaboAnalyst Web Tool we can identify five important pathways involved with statistical significance: Aminoacyl-tRNA biosynthesis (1), Citrate cycle (TCA cycle) (2), Nitrogen metabolism (3), Alanine, aspartate and glutamate metabolism (4), D-Glutamine and D-glutamate metabolism (5), with a graphical representation (**Fig. 2**).

CONCLUSION

GC-MS-based metabolomics analysis of the urinary metabolome seems to have the requested sensibility and specificity to get more insights of the PROM phenotypes and to investigate about he inner mechanism of PROM activation.

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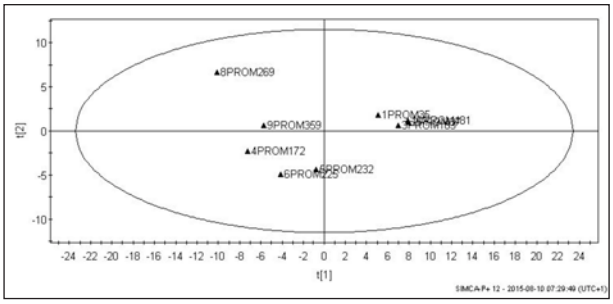

Figure 1 (ABS 64). PLS-DA model for PROM states.

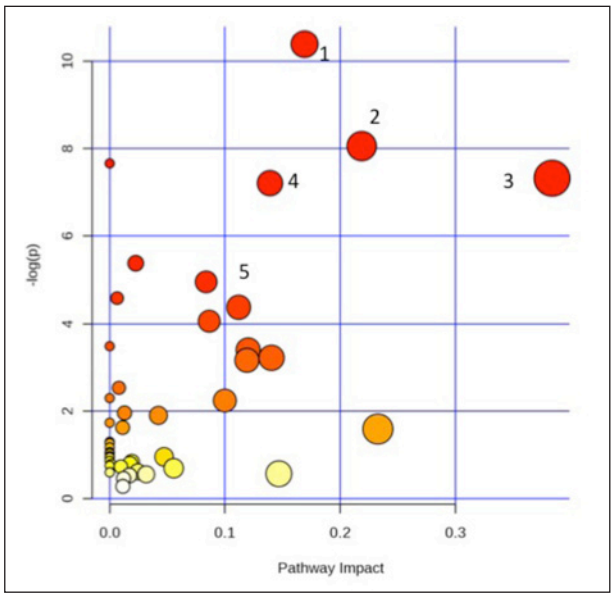

Figure 2 (ABS 64). A Google-map style interactive visualization system implemented to facilitate data exploration.

Using the MetaboAnalyst Web Tool we can identify five important pathways involved with statistical significance: Aminoacyl-tRNA biosynthesis (1), Citrate cycle (TCA cycle) (2), Nitrogen metabolism (3), Alanine, aspartate and glutamate metabolism (4), D-Glutamine and D-glutamate metabolism (5), with a graphical representation.

ABS 65

CHECKLIST AIMING TO DECREASE CLINICAL RISK OF NASAL INJURIES IN PREMATURE NEWBORNS ON NON-INVASIVE VENTILATION

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BACKGROUND

The interface used in non-invasive ventilation (NIV), if not properly managed, may lead to nasal injury and eventually to the formation of nasal scars.

OBJECTIVE OF STUDY

- To decrease the clinical risk of nasal injury in premature babies into NIV;
- to evaluate if the application of a check-list improves the management of the NIV interface.

MATERIALS AND METHODS

The study was conducted at the Neonatal Intensive Care Unit of AOUI Verona from May to December 2012.

We enrolled 31 premature newborns in NIV (23-36 weeks, 500-2,050 g).

We elaborated a checklist to assess the right position of the interface, the onset, type and development of nasal lesions.

We categorized the lesions according a classification based on the severity:

- first stage (irritation, erythema with intact skin);
- second stage (destruction with partial thinning of skin);
- third stage (skin necrosis – transmural).

At the early onset of a first stage nasal lesion we switched from nasal prongs into nasal mask ventilation.

RESULTS

In the 25.8% (n = 8) of subjects we did not find lesions. Instead we found lesions in the 74% (n = 23): the 87% of them (n = 20) had lesion at 1st stage and the 13% (n = 3) of them at 2nd stage.

We did not find lesions at 3rd stage.

The checklist significantly reduced the skin abrasions incidence at 1st stage from 83.3% to 42.9%.

CONCLUSIONS

The check-list represents an effective method to monitor the early onset of nasal lesions in order to switch the interface, once the first signs of a skin lesion appear.

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ABS 66

POINT-OF-CARE ULTRASOUND PERFORMED BY PEDIATRIC PROVIDER IN A COMMUNITY SETTING PRACTICE

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INTRODUCTION

Bedside ultrasound (US) can be performed parallel to physical examination for rapid assessment of ill children presenting a variety of symptoms. The sonographic findings have to be correlated with the clinical picture to make critical decisions.

AIM

Review of our experience over the last 15 years of US point-of patient-care in pediatric ambulatory setting.

MATERIAL AND METHOD

Observational review study of our experience:

- a total of 3,225 US examinations were performed between years 2000 and 2015;
- age group: 0-14 years;
- US was done using multipurpose ultrasound machine with convex, linear and sector probes.

RESULTS

A wide clinical spectrum of applications was observed:

- in acute illness (chest pain, abdominal pain, acute scrotum...);
- most relevant gastrointestinal application of focused assessment with US in acute illness (hypertrophic pyloric stenosis, appendicitis, intussusception, gallbladder stones, urinary tract infection and renal abnormalities);
- focused assessment with US for heart and lung applications in acute illness (mainly for cardiac functional evaluation, valve dysfunction, pericardial effusion, pleural effusion, infection – pneumonia): periodic control with echo-Doppler is crucial to observe worsening of valve or ventricular dysfunction;
- focused assessment with US for testicular abnormalities in acute illness (acute scrotum – testicular torsion): again echo-Doppler provides valuable information for early recognition of this condition;
- focused assessment with US for soft tissues in acute illness (cellulites [swelling/edema], abscess, foreign body);

- late detection of congenital anomalies among infants (heart defects [ASD, VSD, PDA, etc.], urogenital system [monolateral renal agenesis, hydronephrosis, funicolar spermatic cyst, hydrocele, cryptorchidism, etc.], congenital masses in the neck);
- follow-up of repaired heart complex defects: for these patients close follow-up is very important for decision making to determine the appropriate timing of redo surgery.

CONCLUSIONS

US system are becoming more affordable and easy to use, however, US results depend on the operator's training and experience.

Appropriate training and educational programs are mandatory to ensure that physicians can accurately perform and interpret bedside US examination and learn how to incorporate US findings in the clinical management of children [1].

US bedside is a new model of health care and has potential role to enhance and improve the effectiveness of interventions in children and should be integrated into routine pediatric practice.

Despite challenges associated with integrating point of care US into pediatrics, major efforts should be done to make more pediatricians familiar with diagnostic US tool and give them sufficient skills in their workplace setting practice [2].

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ABS 67

STEM CELL NICHES IN THE DEVELOPING HUMAN UTERUS

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BACKGROUND

The uterus, during the initial phases of development, is characterized by the presence of a huge amount of stem/progenitor cells.

The hypothesis that stem/progenitor cells, capable of self maintenance and of originating multiple epithelial cell types, might be detected in the mature endometrium was first advanced in 1978 [1].

Stem cell niches have been reported to be located in close proximity of the superficial epithelium of the developing endometrium (**Fig. 1**).

AIM

This study was aimed at studing, at morphological level, the localization and organization of human uterine stem cell niche.

One of the key functions of niche cells is to sense the need for tissue replacement and communicate proliferative and differentiation signals to resident stem cells [2].

METHODS

To this end, the uterus of two fetuses aged it and 20 weeks were formalin-fixed, paraffin embedded.

Sections were stained with HeE.

RESULTS

At histology, stem cell niches were detected in close proximity to the superficial epithelium of the endometrial cavity (**Fig. 2**).

Stem/progenitor cells showed a large irregular nucleus were surrounded by an eosinophilic stroma (**Fig. 3**).

Nuclei were oval and stem cells were in strict contact with the basal membrane of the developing endometrial epithelium (**Fig. 4**).

In some niches, multiple small blood vessels were found in termingled among stem cells (**Fig. 3** and **Fig. 4**).

CONCLUSIONS

Our data show that stem cell niches may be easily identified in the developing human uterus.

Further studies are needed in order to characterize these progenitors at immunohistochemical level.

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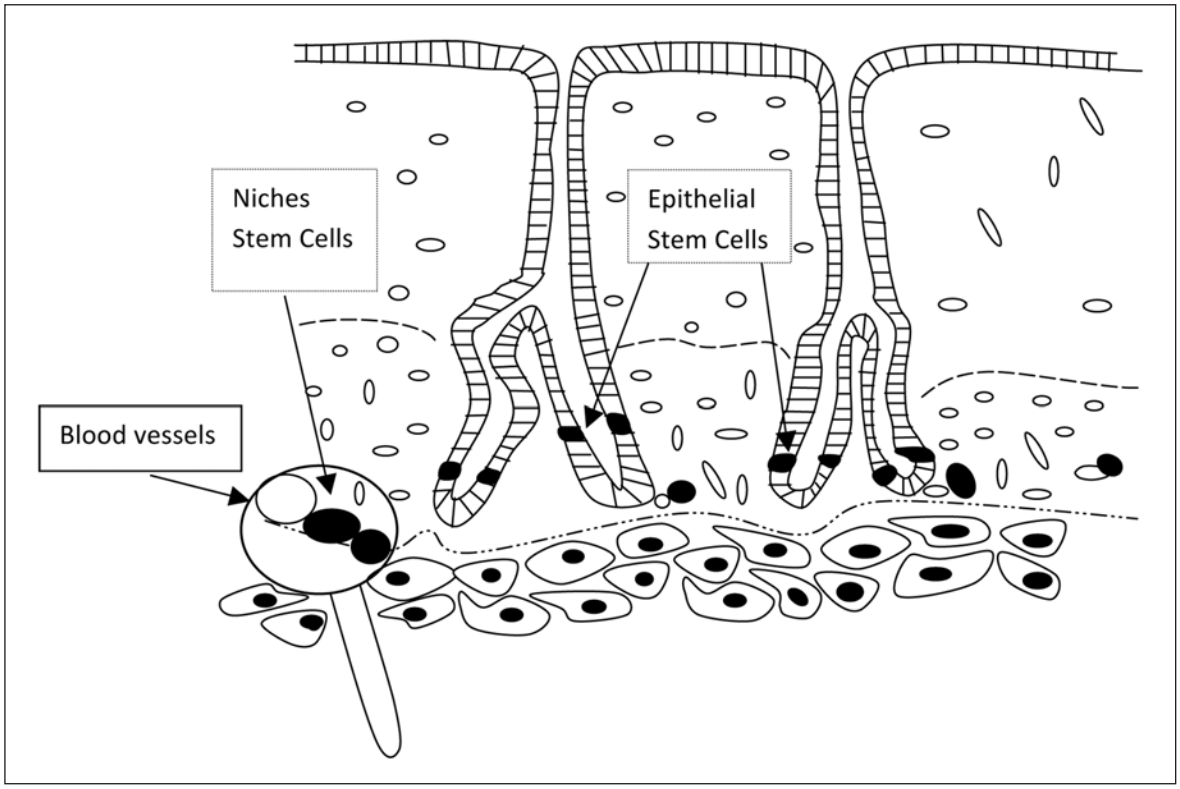


Figure 1 (ABS 67). Stem cell niches have been reported to be located in close proximity of the superficial epithelium of the developing endometrium.

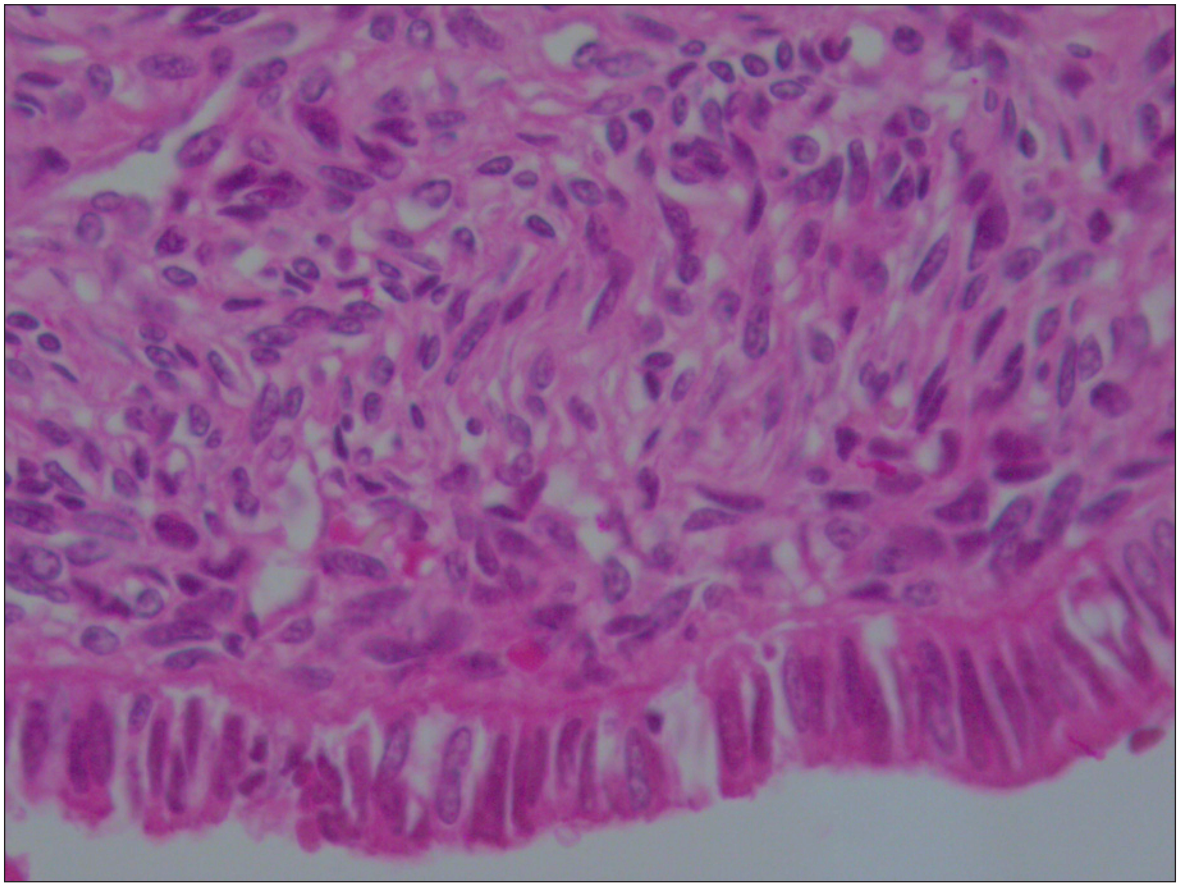
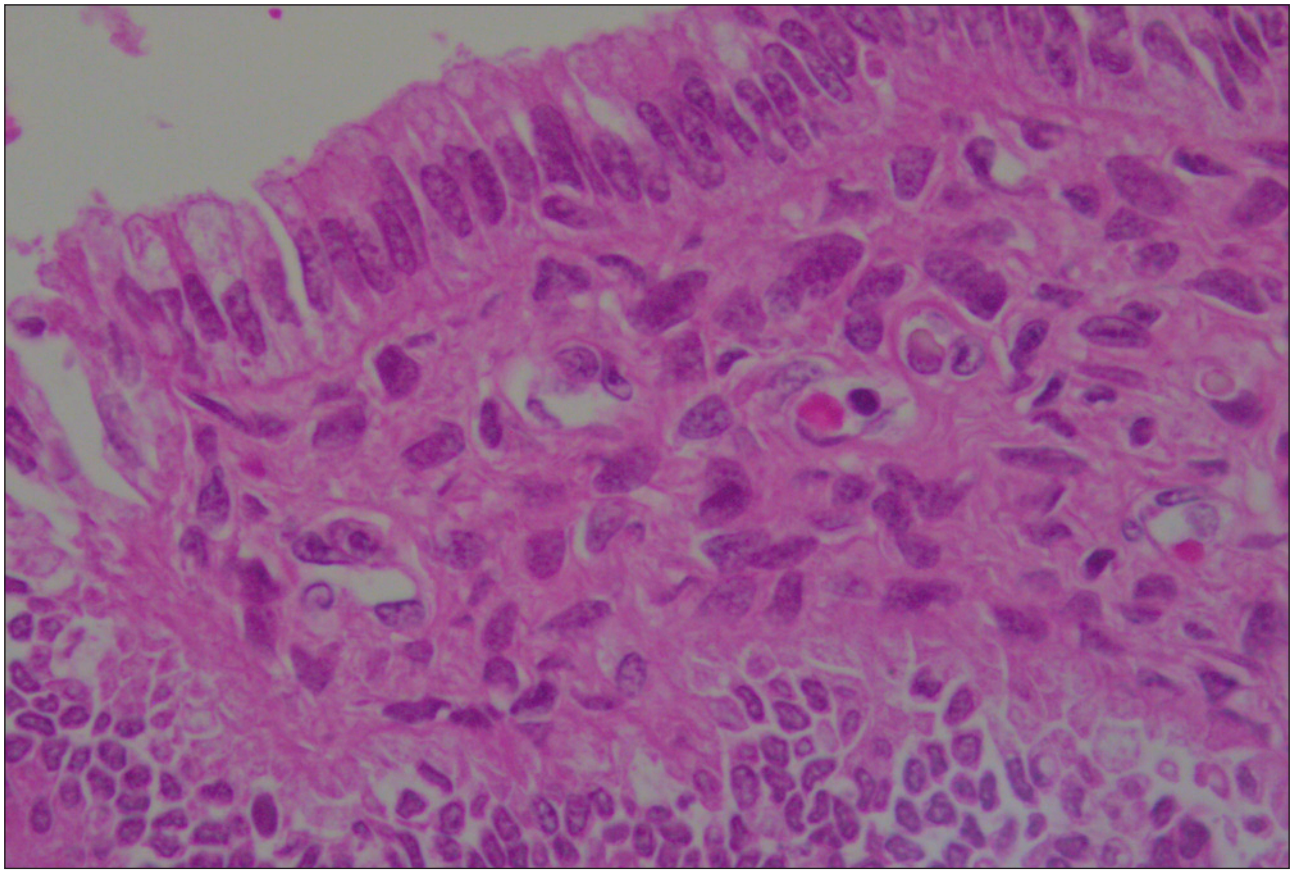


Figure 2 (ABS 67). Stem cell niches are detected in close proximity to the superficial epithelium of the endometrial cavity.

A.



B.

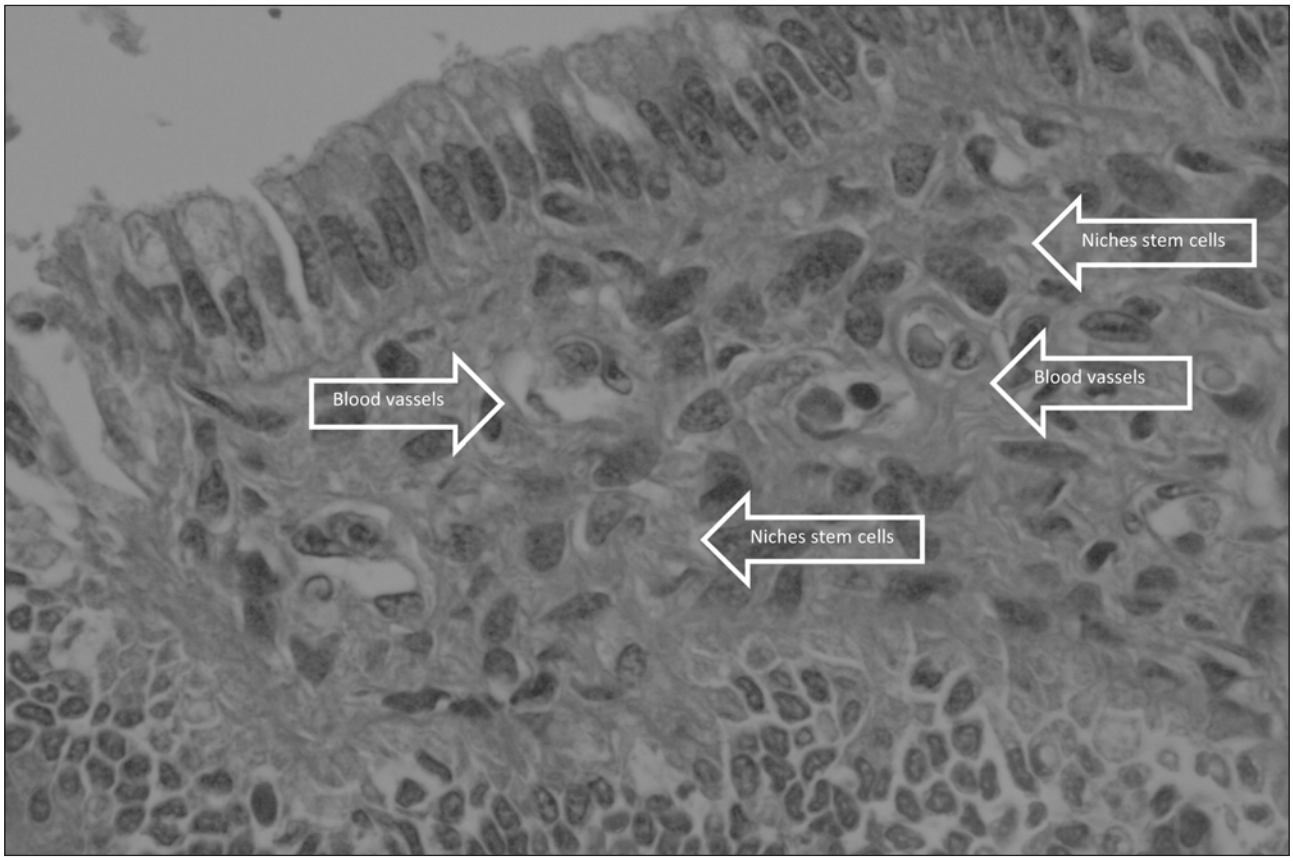


Figure 3 (ABS 67). Stem/progenitor cells showed a large irregular nucleus are surrounded by a eosinophilic stroma.

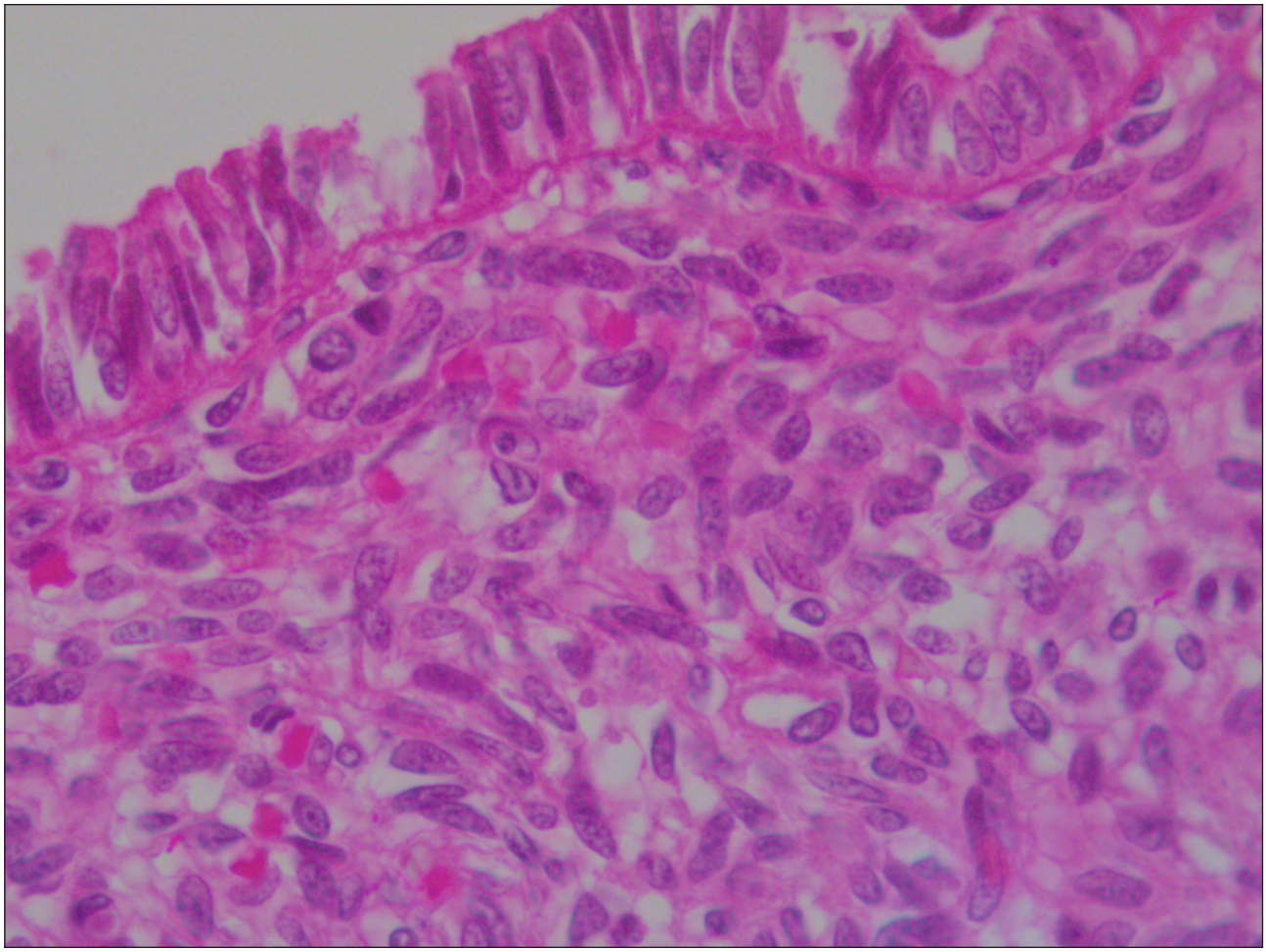


Figure 4 (ABS 67). Nuclei are oval and stem cells are in strict contact with the basal membrane of the developing endometrial epithelium.

ABS 68

MATERNAL PERCEPTIONS ABOUT BREAST-FEEDING: EVALUATION OF 40 MOTHERS WITH LATCH SCORE

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BACKGROUND

The benefits of breast milk both for the baby and the mother are well known. WHO and UNICEF recommend exclusive breastfeeding for the first six months of life and the LATCH score is one of the usual evaluation tools of breastfeeding. This rating scale was developed by Jensen, Wallace and Kelsay in 1994 and explores five areas of interest: the attack on the breast, the spontaneity of audible swallowing, the shape of the nipple, the comfort of the breast and nipple and the mother's skills about supporting and positioning. However, it is important to note that beyond the information and assessments, it is needed also to provide a practical and psychological support to the mothers.

AIM

To evaluate the attitudes of mothers to breastfeed and their perception of the support received during the counseling on breastfeeding. In addition, this study evaluated breastfeeding through the LATCH score.

METHODS

This study was conducted in the Neonatal Section, Azienda Ospedaliera Universitaria, University of Cagliari (Cagliari, Italy). The study population consisted of 40 healthy women with a mean age of 33.8 years, mothers of healthy term infants. The sampling was carried out in the period between September 2014 and July 2015. Each mother received a counseling on breastfeeding, in the second day postpartum, by a medical specialist (pediatrician). After each counseling, the LATCH

score was compiled and the mothers were asked if they needed further counseling on breastfeeding. The answer to this last question was recorded on its LATCH score and then a questionnaire index structured in nine closed questions was proposed to the women. This questionnaire was administered anonymously and the compilation was voluntary.

RESULTS

In this study population, most of the women was primipara and all those multiparae had previously breastfed. The 77% of mothers had previously received information about breastfeeding during pregnancy and assessed very positively the possibility to breastfeed. The 70% of women of the same sample has a very positive assessment about the counseling received in the ward. Into the questionnaire were proposed six options to investigate the need for additional supports: 30% of mothers requested the advice on breastfeeding and pediatric nutrition; 24% advice on minor illnesses of the newborn and educational counseling to child development; 14% training on techniques for childcare and 4% group discussion and sharing with other parents. Regarding the scores obtained by LATCH score: most scored a rating of 8 (28%); none scores of 10 or less than or equal to 2; 55% has obtained LATCH scores ≥ 7. Among mothers with a LATCH score ≥ 7 a large part (55%) required another breastfeeding counseling while half of those with a score < 7 did not request.

CONCLUSIONS

The results of this study show that the majority of the mothers who had obtained a score ≥ 7 (i.e., not at risk for a non-exclusive breast feeding) needs to receive further advice. In conclusion, the LATCH score is a good rating scale for breastfeeding, particularly useful in the first approach with the mother-child, however the insecurities and needs of mothers must be taken into account.

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ABS 69

ABNORMALITIES IN THE KIDNEY OF FETUSES WITH DOWN SYNDROME

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INTRODUCTION

A variety of renal and urological abnormalities have been reported in subjects with Down syndrome (DS). With increased longevity, it appears that a growing number of these subjects presents chronic renal failure [1, 2]. Definition of underlying cause of renal failure could lead to the prevention of progressive renal dysfunction in these patients. The aim of this work was to evaluate morphological renal differences between DS fetuses and normal fetuses. We hypothesized that DS might be associated with variations in structure of the fetal kidney, such as glomeruli, in agreement with what it has been observed in adults [3].

PATIENTS AND METHODS

Kidney samples were obtained from twenty-five fetuses with gestational age ranging from 9 to 22 weeks. Subjects were organized into two groups: fetuses with Down syndrome (DS-fetuses, n = 11) and fetuses with normal karyotype (N-fetuses, n = 14). Portions of the kidney were formalin-fixed and embedded in paraffin, sectioned with a microtome at 5 μm and collected onto glass slides. Complete kidney sections were selected and stained with hematoxylin and eosin. For each kidney, one section was examined. An observational histological analysis was performed in order to evaluate differences in renal architecture, and a quantitative analysis was performed in order to evaluate differences in total glomerular area (TGA) and blue strip width. We also investigated area enclosed by Bowman's space, that we defined functional glomerular area (FGA), and the ratio between FGA and TGA. We defined this ratio as functional ratio (FT), because it provides information about the percentage of functional glomerulus compared to total glomerulus, where the functional glomerulus represents the part of glomerulus that allows filtration [3]. All the quantitative analyses were performed using a specific algorithm based on segmentation techniques developed with Matlab software.

RESULTS

The histological analysis of kidney samples from the 11 subjects affected by DS showed

multiple changes in glomerular architecture. Large glomeruli irregular in shape, with a pseudopapillary pattern, were frequently observed (**Fig. 1**). Even the quantitative analyses showed differences between DS-fetuses and N-fetuses; in particular we observed bigger glomeruli and a lower functional ratio (FR) in DS fetuses than normal fetuses (**Fig. 2**); moreover, blue strip was wider in DS carriers than in normal subjects (**Fig. 3**).

DISCUSSION

This study comprehensively examines nephrogenesis in the kidney of DS carriers, and their morphological renal differences as compared to healthy subjects. We found an increased nephrogenic zone width, which suggests delayed renal maturation. Of concern, there was an increase in glomerular area and several glomeruli were morphologically abnormal. Moreover, analyses concerning the functional ratio showed that the ratio between internal and external glomerular areas is significantly higher in N-fetuses as compared to DS-fetuses. This finding was associated with a greater glomerular Bowman's space in fetuses with DS as compared with normal fetuses. An enlarged Bowman's space and shrunken glomerular tuft have been correlated with a functional scarcity of the glomerulus. Together, these harmful changes in the glomeruli may result in a nephron deficit. These findings, therefore, have significant implications for renal health of subjects with DS [4].

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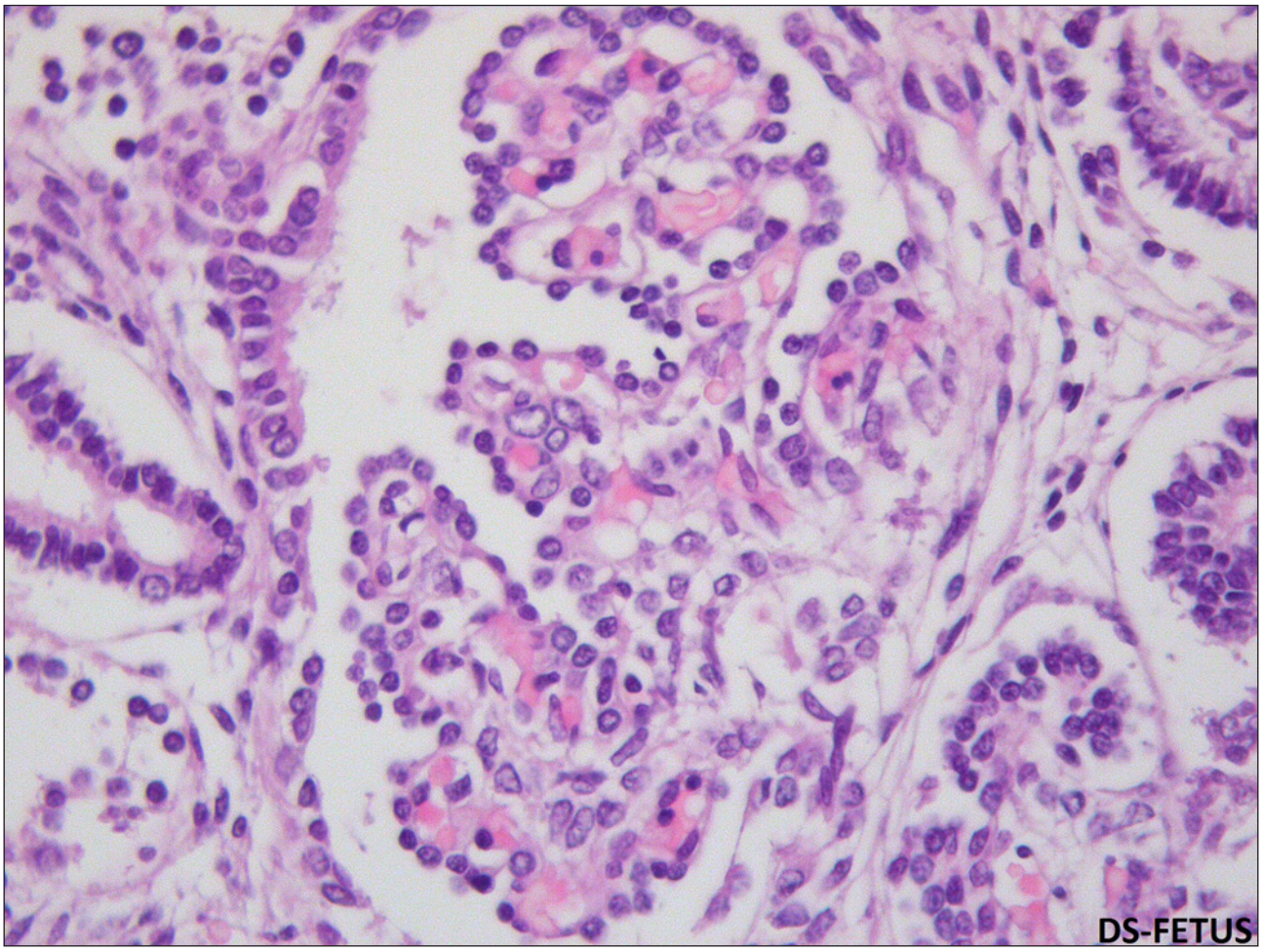

Figure 1 (ABS 69). Large glomeruli irregular in shape, with a pseudopapillary pattern.

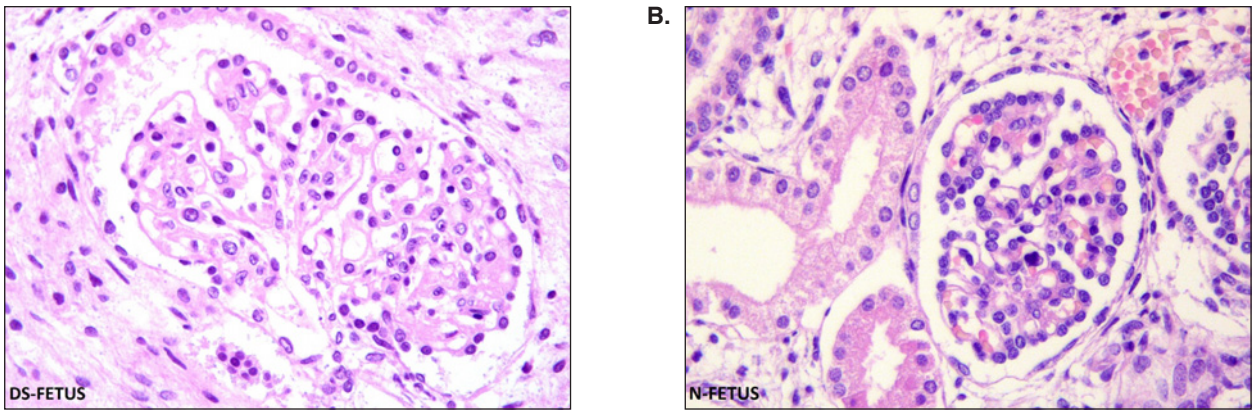

Figure 2 (ABS 69). Bigger glomeruli in Down syndrome (DS) fetuses (**A**) compared with normal fetuses (**B**).

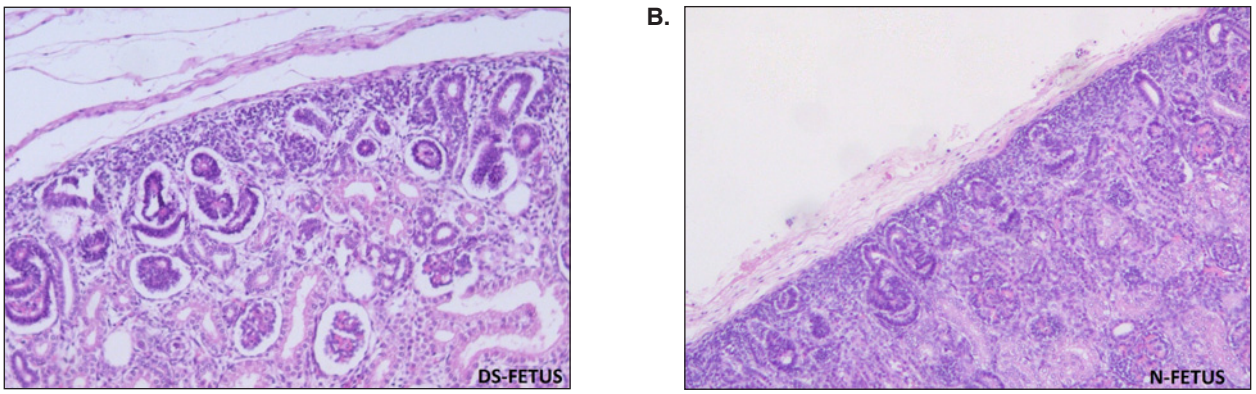

Figure 3 (ABS 69). Wider blue strip in Down syndrome (DS) fetuses (**A**) than in normal subjects (**B**).

ABS 70

PRIMARY FETAL PLEURAL EFFUSION IN ASSOCIATION WITH DIFFUSE CAPILLARY MALFORMATION: A CASE REPORT

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INTRODUCTION

Fetal pleural effusion is a rare condition that carries a high rate of perinatal morbidity and mortality, especially in the presence of hydrops fetalis and premature delivery.

Capillary malformation (CM) is defined as a pink to dark red macular stain first evident at or recently after birth.

We report a case of a recently born baby with both of this clinical manifestations.

CASE REPORT

A 33 years old pregnant mother who had been diagnosed to have left pleural effusion on routine fetal ultrasonography was referred to our center.

Antenatal history of the mother was uneventful There was no consanguinity between the parents.

Fetal anemia was ruled out by demonstration of normal middle cerebral artery-peak systolic volume and congenital heart defects were excluded by fetal echocardiography.

Because of mediastinal shift, fetal pleural effusion was drained at 34 weeks of gestation under ultrasound guidance. But after 4 days the large collection reappeared.

So at 34.4 weeks of gestation, a male infant (3,000 g, 35 cm head circumference) was born by an urgent cesarean section, with APGAR scores of 7 and 8, at 1st and 5th minutes, respectively. The patient did not require any resuscitation in the delivery room. On physical examination he had erythematous-purplish stains, with a narrow network morphology and with a diffuse distribution involving the skin overlying the trunk, both anterior and posterior (**Fig. 1** and

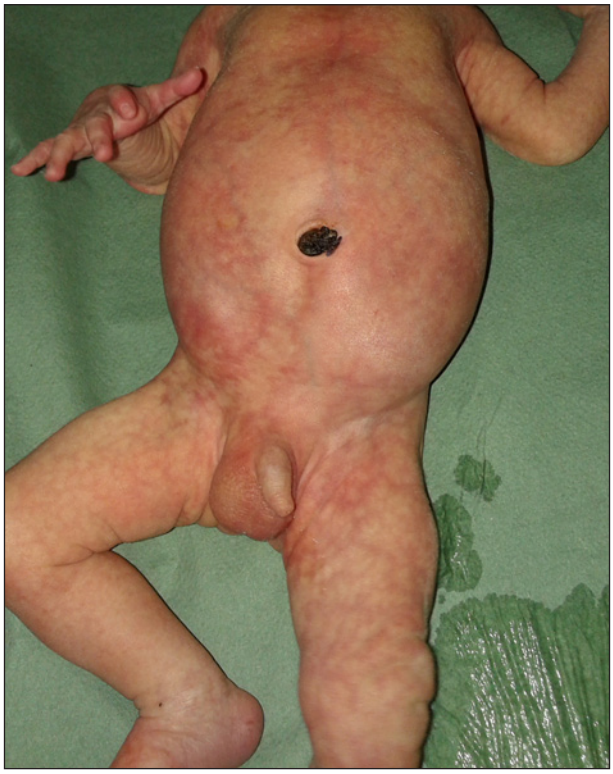

Figure 1 (ABS 70). Diffuse distribution of the capillary malformation.

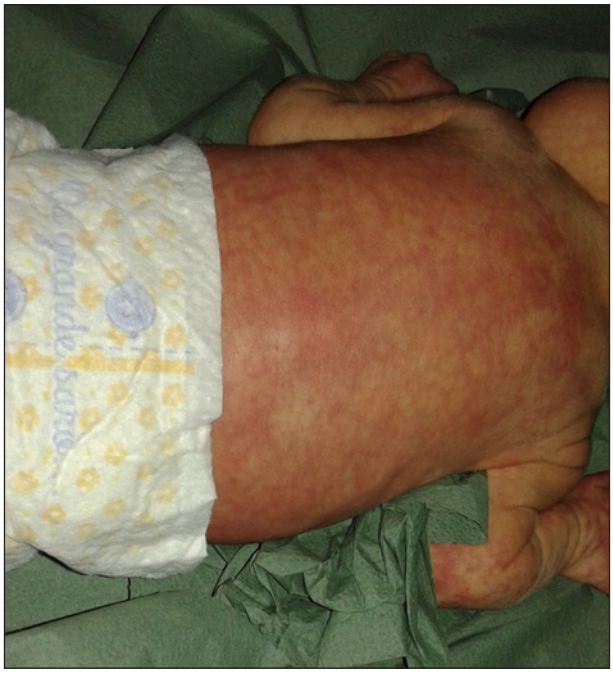

Figure 2 (ABS 70). Capillary malformation involving the posterior trunk.

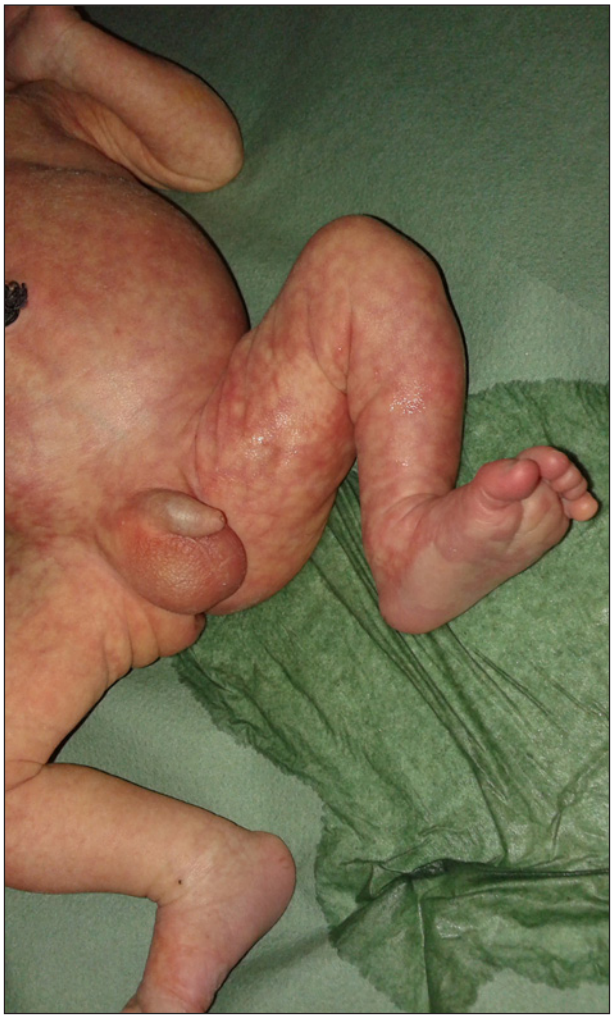

Figure 3 (ABS 70). Capillary malformation in the left leg spreading along the plantar surface.

Fig. 2), the limbs, especially the right arm and the left leg spreading along the plantar surface (**Fig. 3**). No signs of cutaneous atrophy, ulceration and prominent veins were present. Later on, he was admitted to the NICU due to mild respiratory distress. Tachypnea and decreased breath sounds on the left side of the chest were detected on physical examination. SpO_2 was normal in room air. The chest X-ray demonstrated left sided pleural effusion with mediastinal shift. The thoracic ultrasonography detected the left pleural collection. Results of the abdominal, transcranial and cardiac USGs were unremarkable. He was breast feed initially. At seven days of life there was a worsening of the respiratory distress. Upon thoracentesis, 130 ml of chylous fluid was drained. The pleural fluid was considered chylous because of 90% lymphocytes and 280 mg/dL triglycerides. But, as in fetal period, after few days it has reformed the collection. So a chest tube drainage was positioned and he was feeded with a special formula extremely low in fats. There was no drainage from chest tube after five days. Currently, he is 1 month old and on discharge planning.

DISCUSSION

The etiology of fetal pleural effusion is often unknown even after detailed postnatal evaluation. A detailed discussion has been reviewed [1-3].

Primary pleural effusions, unilateral or bilateral, are most commonly a chylothorax caused by abnormal circulation of chyle in the central conducting lymphatic channels, particularly the thoracic duct. Secondary pleural effusions represent a more generalized state of fluid retention consistent with nonimmune hydrops fetalis that can occur from a variety of etiologies. CM can be an isolated finding or can occur in association with syndromic vascular anomalies.

CONCLUSION

We described the rare case of a patient with fetal pleural effusion and diffuse CM. In such case follow-up is mandatory because vascular anomaly syndromes should be considered, especially with isolated primary effusion, and because potential pulmonary sequelae, such as reactive airway disease and pneumonia.

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ABS 71

PAROXYSMAL SUPRAVENTRICULAR TACHYCARDIA: A TEN-YEAR EXPERIENCE IN THE NICU OF CAGLIARI

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INTRODUCTION

Paroxysmal supraventricular tachycardia (PSVT) is the most frequent arrhythmia in newborns and infants. Most of these forms affect structurally healthy hearts. The chronic tachycardia associated with PSVT can cause a secondary form of dilative cardiomyopathy. Treatment of acute episode usually has an excellent outcome. Vagal manoeuvres are effective in patients with AV reentrant tachycardia. Adenosine Triphosphate (ATP) is the drug of choice at all ages for tachycardias involving the atrioventricular node. Antiarrhythmic prophylaxis of PSVT recurrence is recommended in the first year of life, because the diagnosis of tachycardia may be delayed up to the appearance of symptoms and class I C drugs such as propaphenone and flecainide are the first choice, despite the fact that amiodarone has the greatest antiarrhythmic effect. In fact this drug but should be used with caution due to the high incidence of side effects. The objective of this work is review the incidence rate and management strategies of PSVT in our Neonatal Intensive Care Unit (NICU).

PATIENTS AND METHODS

We reviewed all 0 to 3 months neonates admitted to the NICU of the University of Cagliari from January 2004 to September 2014. Data collected from the medical records included patient demographics, gestational age, birth weight, Apgar scores, diagnosis of PSVT, onset of PSVT, vagal manoeuvres, ATP treatment, maintenance therapy.

RESULTS

A total of 5,259 from 0 to 3 months neonates were admitted to the NICU from January 2004 to September 2005. 106 experienced arrhythmia, 16 (0.3%) of whom had PSVT. Male sex was predominant (n = 10; 62.5%); 12 neonates reached the full term age (75%), although all presented a 5 minute Apgar Score > 7. The onset of PSVT was within the first day in 5 neonates (31,2%), within the first month in 10 (62.5%) and after the first month in 1 neonate (6.3%). Overall 9 neonates (56.2%) required vagal manoeuvres, 10 (62.5%) required ATP in six patients of the latter group the drug was administered after vagal manouvres. No adverse side effects or hemodynamics changes occurred after ATP administration. Electrical cardioversion was performed in 1 patient. Maintenance therapy was based on flecainide in 13 patients (81.2%), while propafenone, digoxin and amiodarone were used in the remaining patients (respectively 6.2%).

CONCLUSIONS

Paroximal Supraventricular Tachycardia (PSVT) is an uncommon event in the NICU. ATP is a safe and reliable antiarrhythmic in the newborn, including the preterm infant. Our experience indicates that flecainide is well tolerated and effective as first-line maintenance treatment for PSVT in newborns without structural heart disease. Considering the rarity of this condition, further studies are needed in order to improve the management and improve knowledge and long-term outcome of TPSV in this cohort of patients.

ABS 72

PAI OR NOT PAI

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INTRODUCTION

Pai syndrome (PS) is a rare disorder characterized by combination of three congenital anomalies including midline cleft lip (MCL), cutaneous polyps and lipoma of the corpus callosum [1].

Congenital cutaneous nasal polyps of the facial skin and nasal mucosa appears to be the distinctive characteristic of PS, it has been reported in all cases of PS described and is not associated with any other syndromes [2].

Pericallosal lipoma is seen in 85% of PS cases and 80% have MCL [3]. Hypertelorism, ocular anomalies and normal neuropsycological development are other signs that can be present [2].

Intracranial lipomas are rare lesion that represent less than 0.1% of intracranial lesion.[4] They usually occur secondary to the abnormal differentiation of meninx primitive layer, arachnoid and the inner of dura mater [5].

Two morphological types of pericallosal lipoma have been described: tubulonodular (appearing round and measuring > 2 cm) and curvilinear (elongated, measuring < 2 cm in diameter and usually more posterior). Demaerel et al suggested that interemispheric lipoma can be considered as a unique entity, with variable expression depending on the time and on the degree of the insult [6]. The tubularnodular subtypes most likely the result of a more severe and earlier insult than the curvilinear subtype, and is commonly associated with other cerebral especially callosal, and frontofacial abnormalities [4, 6, 7].

CASE REPORT

We report a patient with only skin polyps of the nasal dorsum and lipoma of the corpus callosum; these two very rare features are associated with very few syndromes.

The baby (male, 3,460 g, 38 weeks GA) borns by vaginally delivery with a normal Apgar score in a peripheral center. He referred to our neonatal patology unit at 8th day of life for the presence of skin polyps on nasal dorsum. MCL or other dysmorphic features were absent.

Cranial ultrasonography showed an hyperechogenic structure above corpus callosum's splenium, vascolarized at doppler examination.

MRI confirmed the presence of the lesion of spindle shaped (size mm: 13 AP, 2.5 CC, 3.5 LL). Ventricles, basal ganglia and white matter didn't show abnormalities. Laringoschopy didn't show any polips and other findings were normal.

Psycomotor development and neurological status were normal at 5th month of life.

DISCUSSION

The classic triad to define PS (central nervous system lipomas, median cleft of the upper lip and facial skin polips) [1] wasn't present in our patient. However the phenotype may be variable and when an intracranial lipoma is coupled with a facial polyp it may be suspicious for PS [4]. In the last year new diagnostic criteria for this syndrome were proposed by Lederer et al. The presence of congenital nasal polyps plus one of three between MCL, midanterior alveolar process congenital polyp and peri-callosa lipoma were sufficient [3].

CONCLUSION

Our case seemed to be a varierty of PS; we can support Lederer's proposal to modify diagnostic criteria for PS with the presence of congenital nasal polyps plus one of three following: MCL, midanterior alveolar process congenital polyp and peri-callosal lipoma.

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ABS 73

IMPACT AND MANAGEMENT OF THE NURSING DYSFUNCTIONAL TURNOVER IN NICU

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The term *turnover* refers to the "number of employees hired to replace those who left or were fired during a 12 month period". Depending on the underlying reason/s, turnover can be functional (or natural) or dysfunctional (due to job dissatisfactions). The process of *functional turnover* happens by planned and expected decisions by the organization that include hiring, retirements and lay-off. The main cause of *dysfunctional turnover* is job dissatisfaction that induces high skilled employees to leave the organization. The reasons might vary including: mistakes by the human resources personnel increase overtime hours, lack of collaboration, difficult relationships with coworkers, and relationships with supervisor, stressful work environment, poor safety of workplace, lack of training and education, lack of developmental opportunities and promotion opportunities. Dysfunctional turnover has a significant impact when it affects nursing in the Neonatal Intensive Care Unit, being potentially harmful to the organization because leading to negative consequences. The high replacement costs and the exit of a high number of skilled nurses negatively affects the efficiency of the NICU. The management of turnover in the NICU needs deep understanding in order to develop strategies for avoiding and/or managing the dysfunctional turnover.

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ABS 74

STEM/PROGENITOR CELLS IN THE HUMAN DEVELOPING OVARY

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BACKGROUND

The ovary is the female organ in which germline and somatic cells are arranged in functional units, called follicles, adapted to maintain gamet cells. Recently the ovarian surface epithelium (OSE) has been proposed as the source of ovary stem/progenitor cells, giving rise to pregranulosa cells (pGC) [1, 2] and probably to germ cells in postnatal life [3].

The aim of this work was to characterize ovarian stem/progenitor cells by immunohistochemistry in order to define their role in the complex development of the ovarian structure, adapted to ensure survival and growth of fertilizable gamet cells in women.

DESIGN

This study was carried out on 14 fetal ovaries from human fetuses ranging from 12 up to 22 weeks of gestation. Ovarian samples were formalin-fixed and paraffin-embedded. Sections were stained with H&E.

RESULTS

At 13 weeks, the fetal ovary was formed by a fibrovascular medulla and a cortex, rich in primordial germ cells (PGCs) intermingled with rare somatic cells, including stromal (SC) and pregranulosa cells. At this gestational age the developing ovary was covered by a stratified epithelium (**Fig. 1**). In the further weeks, cells from the OSE proliferated into the inner cortex, giving rise to pregranulosa cells that progressively encircled oogonial clusters, forming the ovigerous cords. These appeared surrounded by mesenchymal cells originating from the medulla (**Fig. 2**); subsequently, their fragmentation gave rise to primordial follicles, composed by a single oocyte encircled by a flattened pregranulosa cell layer. At the 21st week, the ovarian cortex was

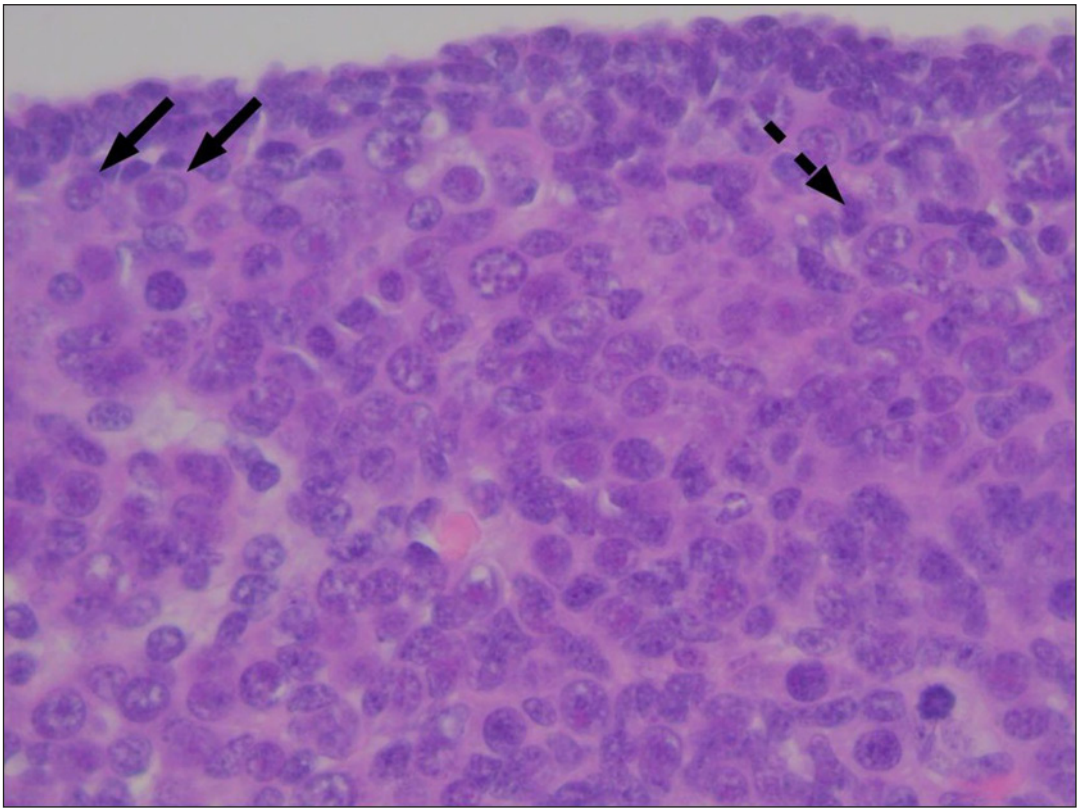

Figure 1 (ABS 74). Human fetal ovary at 13 weeks of gestation: primordial germ cells (arrows), intermingled with pregranulosa cells (dashed arrow), occupied the greater part of the ovarian cortex (H&E, 630x).

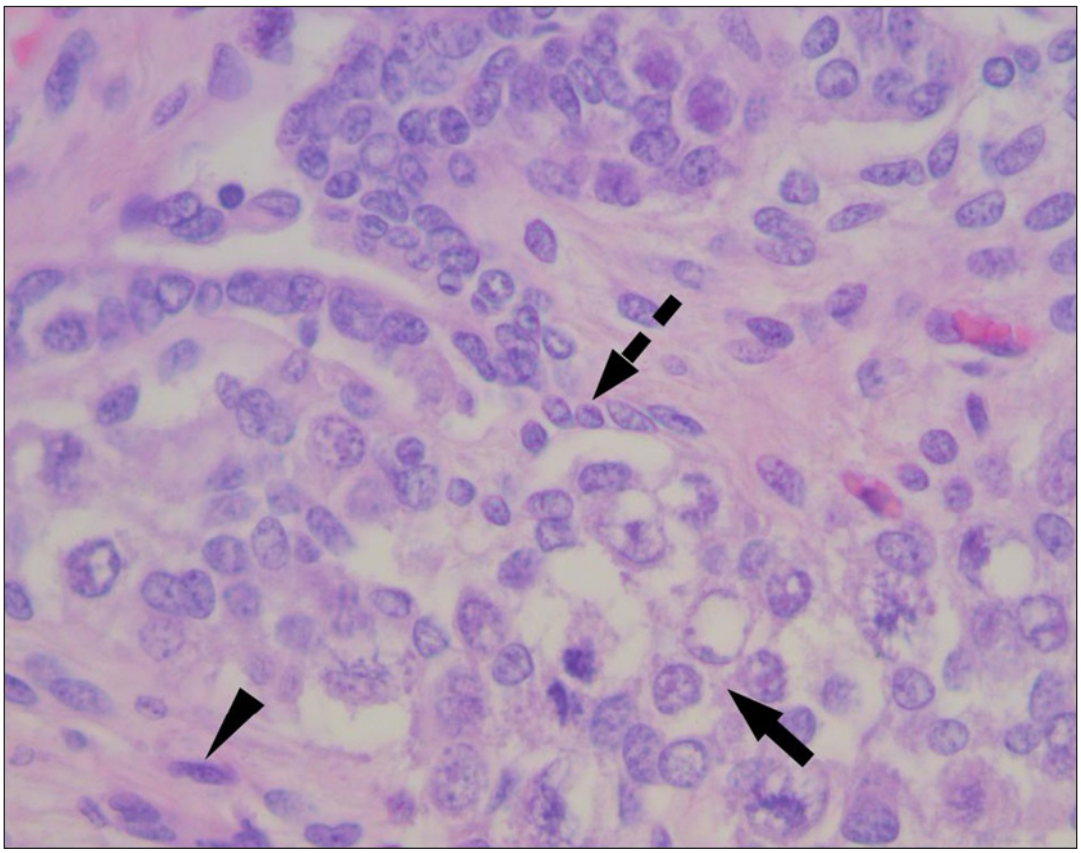

Figure 2 (ABS 74). Cells originating from the OSE proliferated, giving rise to pregranulosa cells (dashed arrow) that encircled oogonial clusters (arrow) forming the ovigerous cords, surrounded by stromal cells (arrowhead) (H&E, 630x).

mainly organized into follicles and stroma and appeared covered by a monolayered epithelium (**Fig. 3**).

A schematic representation of oogenesis is shown in **Fig. 4**.

DISCUSSION

Our preliminary data clearly show that the developing human ovary represents an interesting model for the study of ovarian stem/progenitor cells. These were found in huge amounts, particularly in the early phases of gestation, allowing further studies aimed at better clarifying the multiple stages of differentiation of germ and somatic lineages, with the hope that a better knowledge of the mechanisms regulating ovarian embryogenesis might improve the attempts of regeneration in the ovary of adult human, including a possible treatment of female infertility.

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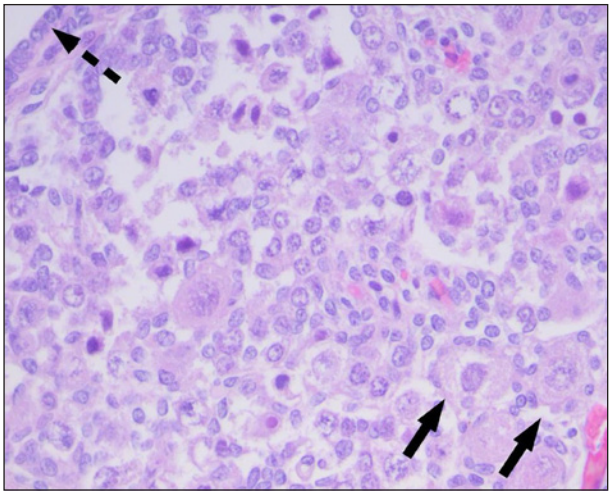

Figure 3 (ABS 74). At 21 weeks of gestation the ovarian cortex was formed by follicles (arrows) and appeared covered by a monolayered epithelium (dashed arrow) (H&E, 630x).

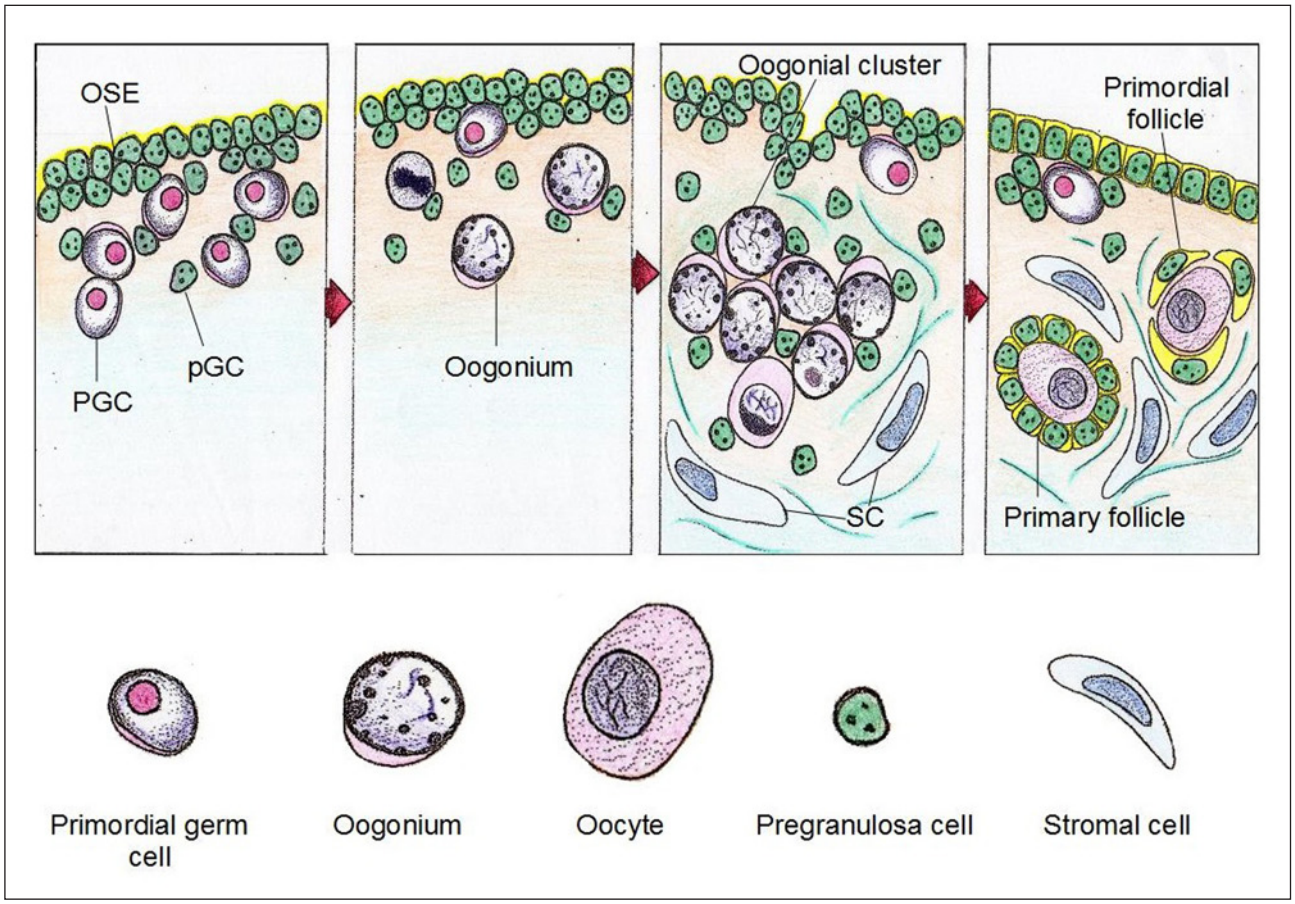

Figure 4 (ABS 74). Schematic summary of human oogenesis.

ABS 75

NON-INVASIVE URODYNAMICS IN CHILDREN WITH BLADDER AND BOWEL DISFUNCTION

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INTRODUCTION

Lower Urinary Tract Symptoms (LUTS) are very common in school-age children (15-20%). Functional constipation (FC) affects over 50% of children with LUTS and is also responsible of the 25% of paediatric gastroenterology visits. It is not uncommon the development of chronic constipation in over one third of these children. In order to improve and regulate diagnosis in gastroenterology, the Rome III criteria for paediatric constipation and fecal incontinence were presented in 2006.

This study aims to investigate, through a non-invasive urodynamic study (according to ICCS), the emptying parameters in children with LUTS and constipation, following the Rome III criteria.

MATERIALS AND METHODS

In our study we observed 25 patients (16 males and 9 females), from 4 to 14 years old (mean age 8.28 years) with LUTS and constipation, in the period between January and December 2014.

This non-invasive approach included a collection of frequency and volume chart and the gastroenterological approach with Rome III criteria and Bristol Stool Chart. To complement, all the patients were subjected to dynamic uroflowmetry with ultrasound (US) of the bladder and measurement of the diameter of the rectum. The cut-off value of the rectum is 3 cm for the transverse diameter.

The natural filling of the bladder has been achieved by taking adequate fluid in a hospital setting. At the first need to void, the bladder wall thickness was assessed by ultrasound to get the capacity of the bladder and to calculate roughly the post-void residual (PVR). The second need to void was evaluated at the Maximum Cystometric Capacity (MCC). The uroflowmetry was carried out twice for each patient. For optimal performance of the exam, it was chosen a comfortable place and recreational activities to entertain the child (in all the examinations there was the collaboration of volunteers for playing with children).

RESULTS

Out of a total of 25, 17 (68%) children, 12 male and 5 female, had constipation (Bristol Stool Chart score < 3). Out of children with constipation, 4/17 pts (23.5%) had encopresis, 13/17 pts (76.5%) had the diameter of the rectum > 3 cm, and in only 2/17 pts (11.75%) the measurement obteined was between 2.5-3 cm.

In the 13 children with dilatation of the rectum > 3 cm, the MCC was 112% higher than expected ($p = 0.03$).

Of the 17 children with constipation, 10 pts (59%) had a bladder volume above normal for age.

Out of the total of 25, 9 pts (36%) had a detrusor-sphincter dyssynergia; of these 9, 7 pts (77.7%) had a PVR higher than 10%. In a total of 25 pts, 12 had a low Bristol stool form scale (score 1-2). In this group of pts, 8/12 pts (66,6%) had a abnormal voiding frequency (< 4 times a day) ($p = 0.024$).

Finally, 10/25 children (40 %) had recurrent urinary tract infections (UTIs) and the 40% of them had a dilatation of the upper urinary tract ($p = 0.017$).

CONCLUSIONS

We got a direct correlation between dilation of rectum (> 3 cm) and abnormal MCC ($p = 0.03$).

This result is confirmed by literature studies. There is also a strong correlation between LUTS and constipation ($p = 0.024$). Children with bladder and bowel dysfunction can be examined with the non-invasive urodynamic approach, which is economically affordable, sensitive and well accepted. This study, together with the urological and simultaneous gastroenterological approach, in the future could better describe the etiology and physiopathology of elimination of dysfunctional disorders, from childhood to adulthood.

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ABS 76

WT1 MARKS INDUCED MESENCHIMAL PROGENITORS INSIDE FETAL HUMAN LUNG STEM CELL NICHES

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BACKGROUND/AIM

Wilms' Tumor 1 gene (*WT1*) firsty cloned in 1990 is a gene located on chromosome 11p13, which encodes an embrionic zinc-finger transcription factor involved in development of multiple human organs, including kidney [1, 2]. Recently, a study from our group revealed that *WT1* is involved in development of a large number of human tissue, included lung [3]. The aim of this study was to better analize *WT1* expression in the fetal human lung, in order to better characterize the lung progenitors mainly involved in *WT1* expression.

METHODS

Lung samples were obtained from four human fetuses ranging in gestational age from 11 to 13 weeks. Tissue samples were fixed in 10% formalin routinely processed, and paraffin-embedded. Two 5 µm-thick sections were obtained from each paraffin block; one of these was stained with hematossilin-eosin, the other pre-treated for immunohistochemical analysis, then incubated for 20 minutes with anti-*WT1* mouse monoclonal antibody (DAKO North America, CA, clone 6FH2) at 1:100 dilution.

RESULTS

At low power, *WT1* expression appeared restricted to the mesenchymal components of the developing lung, in the absence of any significant reactivity in the brancing tubular structures (**Fig. 1**). At higher power, the highest levels of *WT1* expression were observed at the periphery of the developing lung, in close proximity to the tips of the branching endoderm derived tubular structures (**Fig. 2**). The study of *WT1* immunostaining, inside the stem cell niches, clearly evidenced *WT1* expression in the cytoplasm of induced mesenchymal precursors surrounding the epithelias precursors of the tips of branching tubules (**Fig. 3**).

CONCLUSIONS

Our preliminary data confirms previous data on a major role played by *WT1* in lung morphogenesis. An interesting finding emerging by this study is the restriction of *WT1* expression to the mesenchymal components of the fetal lung. Another intriguing finding is represented by the preferential immunostaining for *WT1* inside the stem cell niches, in the mesenchymal progenitors surrounding the

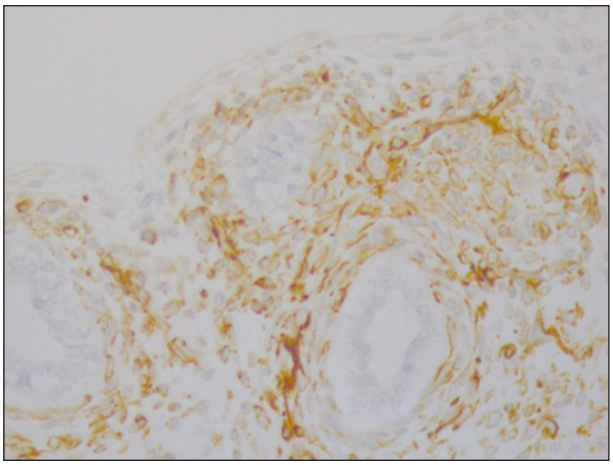

Figure 2 (ABS 76). *WT1* higth reactivity at periphery of lung (600X).

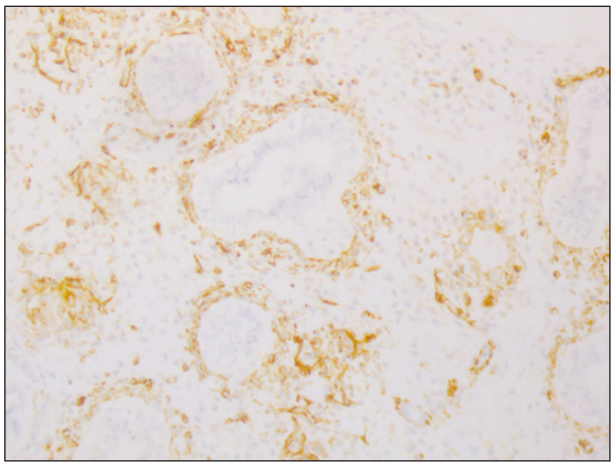

Figure 1 (ABS 76). *WT1* expression of mesenchymal cell (200X).

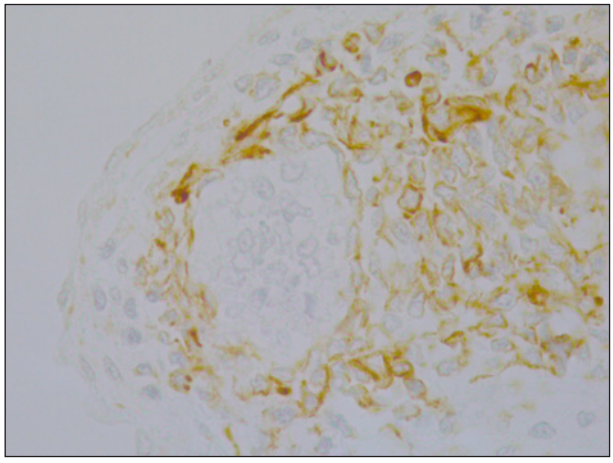

Figure 3 (ABS 76). *WT1* expression inside the stem cell niches (600X).

epithelias tips. The high levels of *WT1* expression indicate a major role for this transcriptional factor in the architecture of pulmonary stem/precursor cell niches and, as consequence, in human lung development. Further studies on *WT1* expression during development are need, in order to a better understanding of the role played by *WT1* in lung cancer in which targeting *WT1* might represent an important tool for new alternative therapeutic strategies [4].

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ABS 77

SEVERE SAS IN A CASE OF PRO-OPIOMELANOCORTIN (POMC) DEFICIENCY

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BACKGROUND

Congenital deficiency of proopiomelanocortin (POMC) is a genetic disease that causes a syndrome characterized by early onset severe obesity, hypoadrenalism and altered hair and skin pigmentation.

METHOD

We describe the case of a female infant of 18 months old of indian origin diagnosed with POMC deficiency, with severe obesity. She was admitted to the pediatric ward for phase delayed sleep patterns, irritability and hyperactivity with respiratory distress during sleep.

A full-night polysomnographic was performed by means of cardiorespiratory – Embletta®, manually analysed with software Somnologica Studio®, in order to assess the cardiorespiratoty function during sleep and identify any alterations. Furthermore circadian melatonin profile was perfomed through withdrawal of salivary sample every 2 hours for a total of 13 samples.

RESULTS

The recording showed many episodes of phasic desaturation, (ODI 30.7) and reduced levels of oxygen saturation (mean SpO_2 93.3% – min 72%), mean desaturation of 6.4%, time with SpO_2 under 90% of 14.4% of the registration time and under 80% of 1.8% of registration time, revealing a condition consistent with severe sleep apnea syndrome, likely obstructive.

DISCUSSION

Obesity, even in infants, although less than in adults, is an important risk factor for obstructive sleep apneas (OSA), that may result in adverse physiological effects that impact on health and development.

CONCLUSIONS

It could be useful programming a polysomnographic study in infants affected by POMC deficiency associated with obesity in order to early diagnose and properly treat a condition of OSA.

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ABS 78

MESENCHIMAL STEM CELL IN FRESH HUMAN BREAST MILK CELLS

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BACKGROUND

Recently it was reported that breast milk contains mesenchymal stem cell. These stem cells are distinguished from other cell types because they have the ability to self-renew and to develop into a more differentiated cells. Therefore, when a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell.

For this reason, it was suggested a possible use of these multipotent stem cells in human milk as an alternative source for autologous therapy although the real significance of these cells needs to be determined.

AIM

Only few data are available regarding the human stem cell composition in the fresh maternal human milk [1-3]. Almost all information regarding the functions and the characteristics of these cells coming from in vitro and animals experimental studies with a large inter- and intra-individual variations.

In order to obtain more detail about stem cell in fresh isolated human milk, using several stem cells markers, we tried to standardized sampling and immunocytochemical protocols.

METHODS

Fresh human milk was centrifuged and then the pellet was stored in commercial Cytological ThinPrep solution (Hologic Inc.). Microscope cytological slides were obtained using the automatic ThinPrep processor (Hologic Inc.) and these slides were used for standard immunoistochemical reactions performed with an automated stainer (Dako).

RESULTS

Even if the results obtained in this study are preliminary we can make some considerations about the stem cells in the fresh human milk. Among different mesenchimal stem cells markers, the CD44 positivity seems to give the best positivity in order to identify stem cells in fresh isolated human milk (**Fig. 1**). The number of CD44 positive cell seems to be influenced in particular by the days of lactation and by the weeks of gestation. In fact a decrease during lactation was found and an increase in the stem cell concentration seems to be associated to more weeks of gestation. The CD133 is present only during the first 3 days of lactation and the use of Ki76 could be important in order to clarify the role of cell proliferation and apoptosis in these cells (**Fig. 2**).

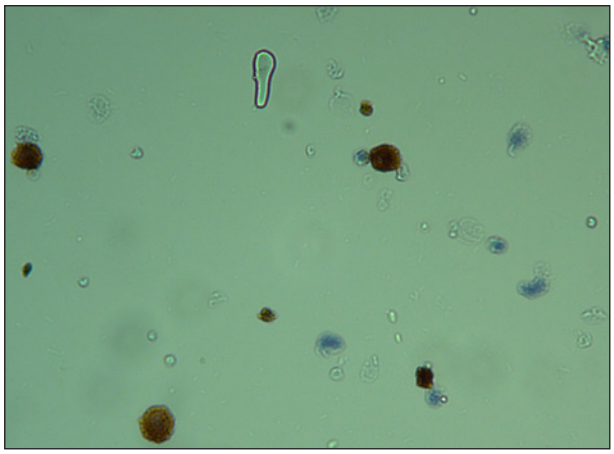

Figure 1 (ABS 78). CD44 positive cells isolated from human breast milk.

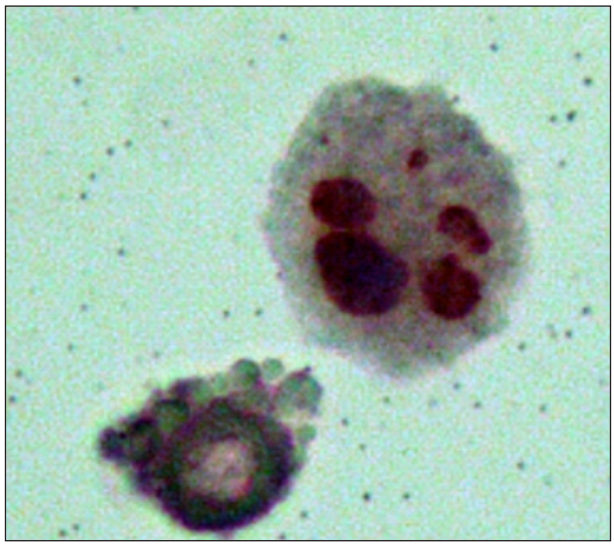

Figure 2 (ABS 78). Freshly human breast milk cells positive for the Ki67 immunoistochemical reaction. Apoptotic cell with several nuclear fragments Ki67 positive.

CONCLUSIONS

More details need to be determined about mesenchimal stem cells in fresh isolated milk. In this study we standardized the experimental conditions and our preliminary data indicate that, thank to the cooperation of perinatologists, biologists and molecular pathologists, we might obtain new data on the complex relationship between the mother and the lactating newborn after birth.

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ABS 79

NEURAL TUBE DEFECTS: NOT JUST CUTANEOUS ANOMALIES

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INTRODUCTION

Neural tube defects (NTDs) are congenital malformations of the central nervous system secondary to lack of closure of the neural tube [1, 2]. Their incidence in Italy is 0.5-2 per 1,000 live births. Anencephaly and Spina bifida (SB) are the majority of cases. SB can also be characterized in *aperta* (SBA) and *occulta* (SBO). SBA is not covered with skin and it includes *meningocele* and *myelomeningocele* (MMC) [3]. Sensory and motor deficit, compromised sphincter function, limb deformities, associated anomalies can be present. SBO is always covered with skin and it may be recognized by cutaneous stigmata such as clefts, tufts of hair, angiomas, pigmentation skin defect. It includes lypomyelomeningocele, dermal sinus, split cord malformation, thickened filum terminale and some rare malformations. Symptoms may often be classified as a *Tethered Cord Syndrome* which is characterized by motor and sensory deficits in the lower limbs, incontinence and scoliosis [4]. Antenatal screening for MMC can be done by determining maternal serum alfa-fetoprotein (AFP), acetylcholinesterase and AFP levels in the amniotic fluid and by ultrasonography (US). After birth additional US and Magnetic Resonance Imaging (MRI) are required. Periconceptual folic acid can prevent 60-70% of cases of neural tube defects [4].

CASE DESCRIPTION

Three clinical cases of NTDs identified in our Neonatal Pathology Department are shown below.

- M.T. (male, 3,560 g, 41 W, normal Apgar score). He came to our attention for a tender swelling with a little appendix on top, covered by depigmented skin, in the middle of the sacral region. Vital signs and neurological functions were normal. US examination showed a lesion extended from subcutaneous region to spinal cord. MRI also was performed and showed a lumbar lipomyelomeningocele that compress and dislocate spinal cord.
- M.F. (female, 2,920 g, 37 W, normal Apgar score). During physical examination a soft swelling was founded in the sacral region, it was covered by normal skin and associated with a cutaneous appendix. An undetermined dysraphism was suspected after US examination. Therefore an MRI was performed that showed a congenital lumbosacral lipoma associated to a tethered spinal cord.

These two cases were both sent for a surgical treatment.

- V.S. (male, 3,070 g, 40 W, normal Apgar Score) came in our unit because he had a pit in coccygeal region. US examination revealed a fistula of 3.7 mm that extends from skin to bone level. MRI wasn't performed and we opted for a conservative treatment.

CONCLUSIONS

All patients that present any anomalies suggestive for a NTD have to be carefully examined. NTDs can have various degree of seriousness and it is essential to identify it in order to an appropriate treatment. SBO requires surgical treatment if there are neurological symptoms, generally caused by a tethered spinal cord, instead prophylactic treatment in asymptomatic patients has not been settled in all cases and depends on the type of the congenital anomaly involved. When SBA is identified surgery must be performed usually within 48 hours to reduce the risk of complications such as meningitis and sepsis that can be very dangerous causing mental impairment.

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ABS 80

PREVALENCE OF CONGENITAL HEART DEFECTS AND PERSISTENT PULMONARY HYPERTENSION OF THE NEONATE WITH DOWN SYNDROME

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INTRODUCTION

Down syndrome (DS) is a common chromosomal abnormality associated with congenital heart disease (CHD). Cardiac malformation is the main cause of mortality in the first 2 years of life.

The aim of this study was to assess the prevalence of (CHDs) and persistent pulmonary hypertension (PPHN) in children with Down syndrome (DS) and to assess its impact on neonatal factors.

PATIENTS AND METHODS

In a 10-year period 56 patients, admitted to the department of Neonatology of the University of Cagliari, from January 2006 to December 2014 were diagnosed with DS.

Diagnosis was based on echocardiogram and genetics.

Data collected from the medical records included patient demographics, gestational age, birth weight, presence or absence of a CHD, type of CHD, age at diagnosis, prenatal diagnosis, previous diagnosis, presence or absence of persistent pulmonary hypertension.

RESULTS

A CHD occurred in 39/56 (69%) children with trisomy 21. Atrioventricular septal defects was found in 41% (16/39), ventricular septal defect in 28.2 % (11/39) and patent ductus arteriosus in 23% (9/39).

The incidence of PPHN in DS was 48.2% (27/56), which is significantly higher than the general population.

The presence of CHD in children with DS had no influence on their birth weight and mean gestational age.

CONCLUSIONS

This is the first study to document the types, distribution and frequency of CHDs in Sardinian children with DS. Atrioventricular septal defect was the most common single cardiac lesion in DS.

In conclusion, we have demonstrated a 69% prevalence of CHD in our population of neonates with DS and a significantly increased and elevated incidence of PPHN in neonates with DS (48%) compared to the general population.

ABS 81

SOX2 EXPRESSION IN THE HUMAN LUNG DURING DEVELOPMENT

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BACKGROUND/AIMS

SOX2 is an embryonic transcription factor that plays a relevant role in development of multiple organs, including foregut, esophagus and lung [1]. Regarding lung development, SOX2 has been show to be involved in two crucial processes: epithelial progenitor differentiation and branching morphogenesis [2]. Recently, the role of SOX2 in mouse lung development has been better clarified, showing that SOX2 induces differentiation and proliferation in the respiratory epithelium into cells of the conducting airways. The aim of this study was to verify the role of SOX2 in the human developing lung.

METHODS

To this end, lungs from five human fetuses, ranging from 11 up to 14 weeks of gestation, were formalin-fixed, routinely processed and paraffin-embedded. Tissue section were stained with hematoxylin and eosin and immunostained with a commercial anti-SOX2 antibody (Santa Cruz Biotechnology Inc., Clone E-4, Dilution 1:50).

RESULTS

Histology showed the typical features of the immature lung, characterized by two main components: branching endoderm-derived tubular structures surrounded by mesenchymal pulmonary precursor. At low power, immunoreactivity for SOX2 was restricted to the epithelial precursor, in the absence of any immunostaining in the lung mesenchyme (**Fig. 1**). When SOX2 reactivity was better analyzed at higher power, SOX2 appeared mainly expressed in the proximal tracts of branching structures, staining for the transcription factor decreasing toward the peripheral and distal branching tubular structures (**Fig. 2**). When SOX2 immunoreactivity was analyzed inside the sub pleural stem cell niches, no significant immunostaining was found (**Fig. 3**).

CONCLUSIONS

Our preliminary data suggest a major role for SOX2 in human lung development, confirming previous data obtained in experimental models [1, 2]. The role of SOX2 appears restricted to branching morphogenesis of endoderm-derived tubular structures that will differentiate into bronchi, bronchioles and alveoli. An interesting finding emerging from our preliminary study is the

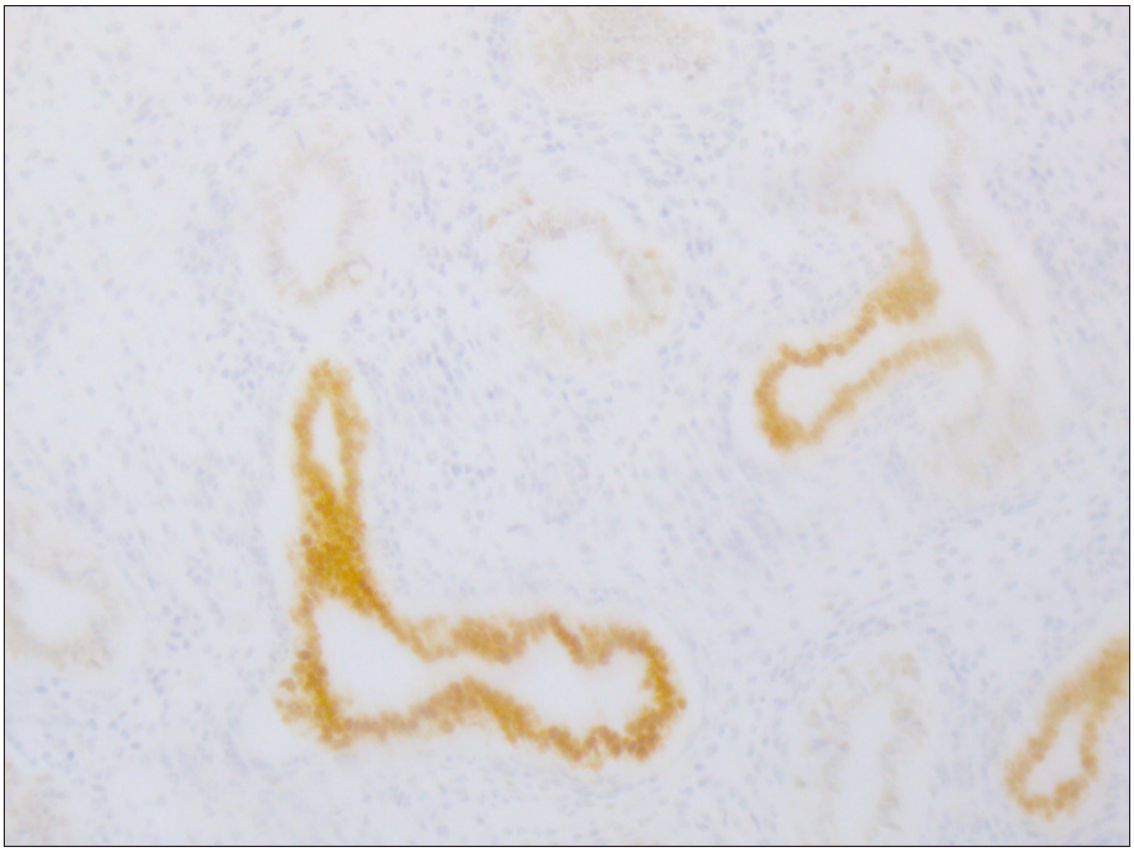

Figure 1 (ABS 81). Immunoreactivity for SOX2 is restricted to the epithelial precursor (200×).

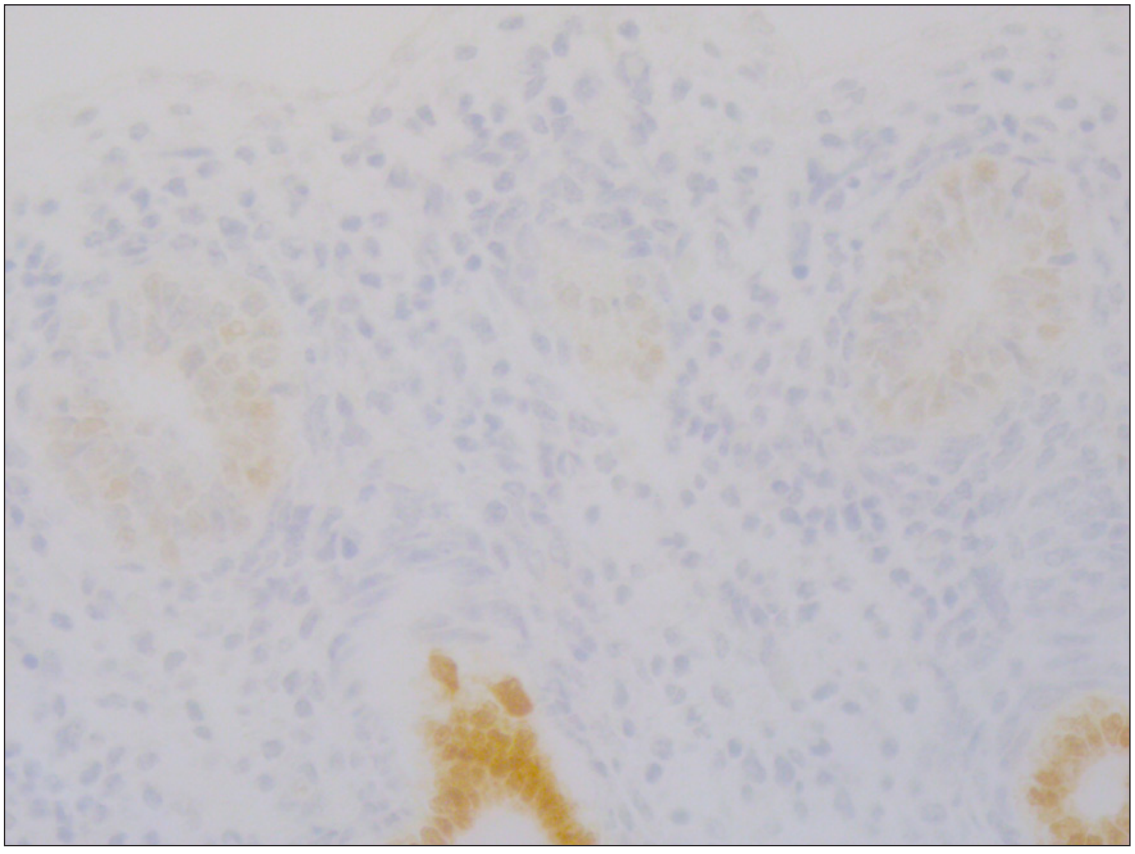

Figure 2 (ABS 81). SOX2 appears mainly expressed in the proximal tracts of branching structures (400×).

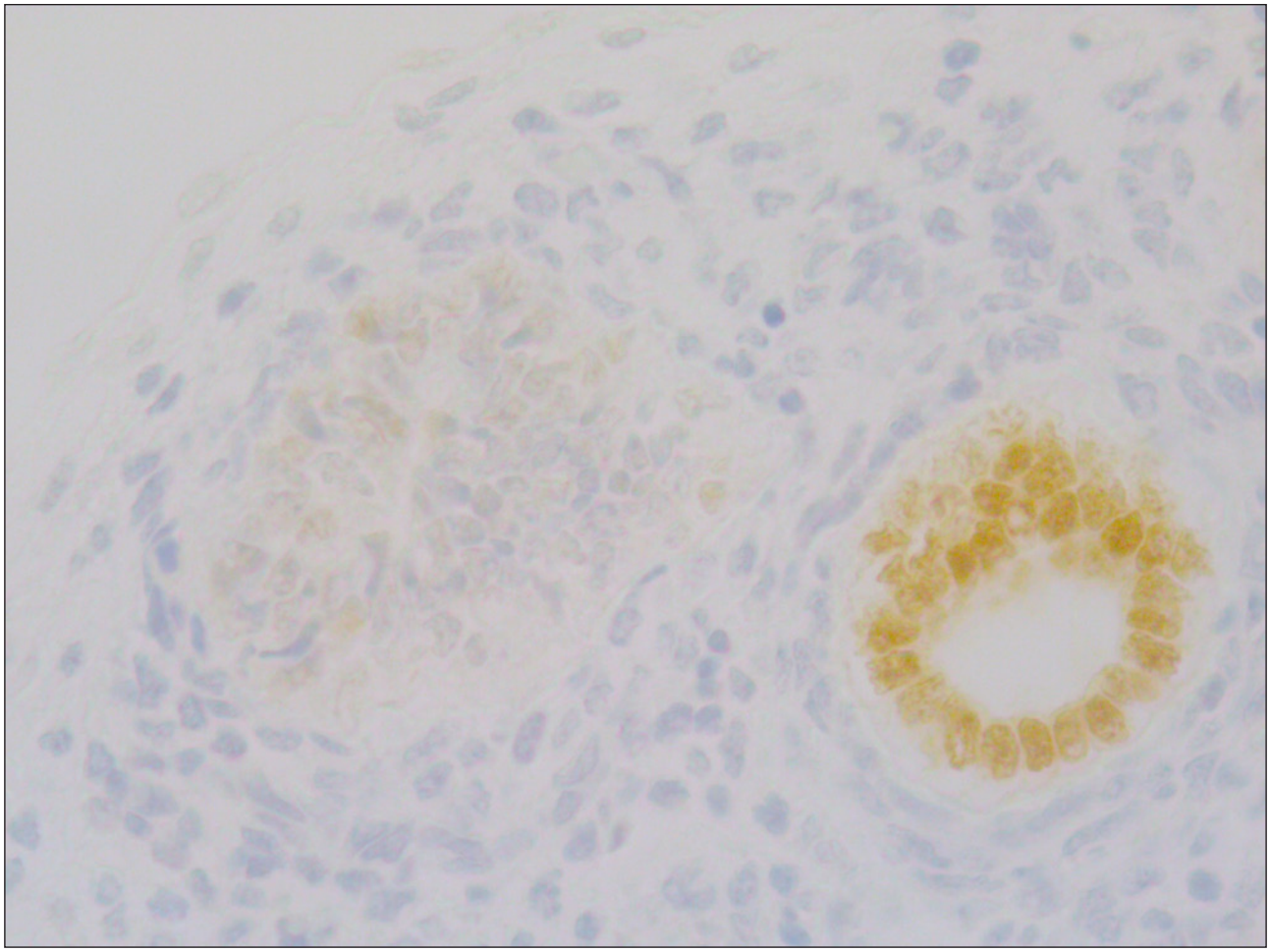

Figure 3 (ABS 81). No significant immunostaining for SOX2 was found inside the sub pleural stem cell niches (630×).

restriction of the SOX2 expression to the proximal tracts of the developing bronchial tree, that contrasts with the absence of SOX2 expression in pulmonary stem cell niches located at lung periphery in the zone in the sub pleural areas. These findings underline the need of further studies to better characterize the role of SOX2 in lung development. Moreover, these studies might have relevant consequences even in oncology, give the role for SOX2 in self-renewal and vascular mimicry of cancer stem cells in non-small cell lung cancer [3].

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ABS 82

CD34 IMMUNOSTAINING REVEALS A HIGH NUMER OF VASCULAR PROGENITORS IN THE STEM CELL NICHES OF THE DEVELOPING HUMAN LUNG

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BACKGROUND/AIM

In recent years, the importance of blood vessel to the function, persistence and differentiation of stem/progenitor cells has been highlighted by many authors [1]. Ravealing the role of blood vessels to the function of stem/progenitor has been

a major focus of stem cell niche resarch over the last decade [2]. Since previous studies on stem cell niche morphology did not show any significant sign of vascularization in pulmonary stem cell niches [3]. This study was aimed at analyzing by immunohistochemistry the presence of vascular structures in stem cell niches of the human lung during development.

METHODS

Lung samples were optained from four human fetuses of gestational age ranging from 11 to 13 weeks. Specimens were fixed in 10% formalin and embedded in paraffin. 5 micron sections were immunostained for CD34, a typical endothelial marker, with a commercial antibody (Daropatt, Clone, Dilution).

RESULTS

At histology, stem cell niches did not show a clear evidence of a vascular component (**Fig. 1**). Immunostaining for CD34 revealed a previously unreported neoangiogenesis in the lung of all four cases analyzed (**Fig. 2**). Multiple small vessels were particularly evident inside the stem cell niches, where they gave rise to a circular plexus formed by small CD34-positive blood vessels that delimited each niche (**Fig. 3**). Moreover CD34 reactivity was detected in mesenchymal progenitors in the stroma (**Fig. 4**).

CONCLUSIONS

Our preliminary study shows the relevance of a immunohistochemical approach in revealing the vascular components of the developing human lung. First, our data evidence the high number of vessels throughout the whole pulmonary parenchyma. Moreover, immunohistochemistry allowed a better knowledge of the significant vasculature of the lung stem cell niche, confirming previous definition of a vascular stem cell niche [1]. Further studies will better clarify the changes in vascular organization of the human lung at different gestational ages, till birth.

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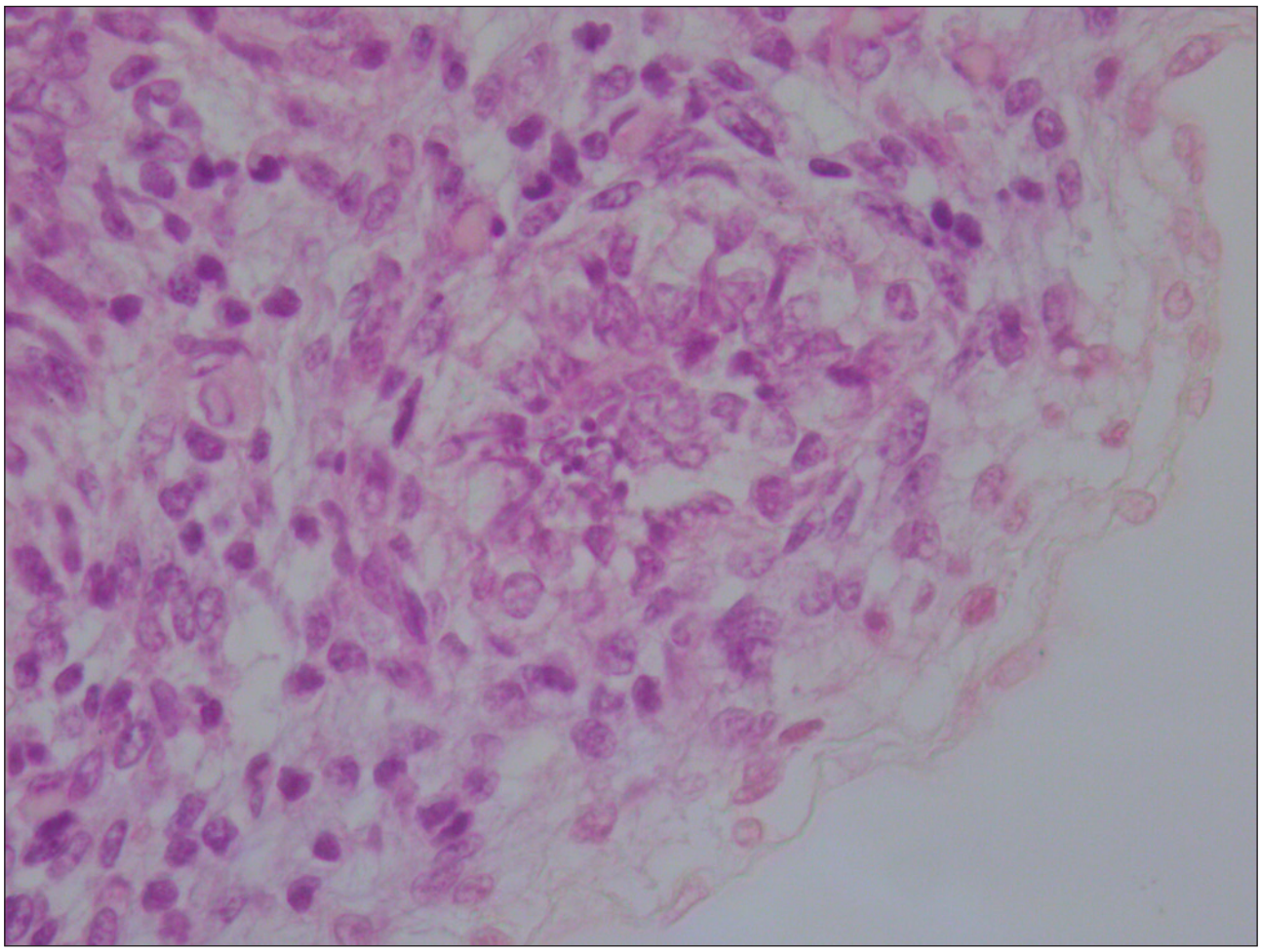

Figure 1 (ABS 82). At histology, stem cell niches did not show a clear evidence of a vascular component.

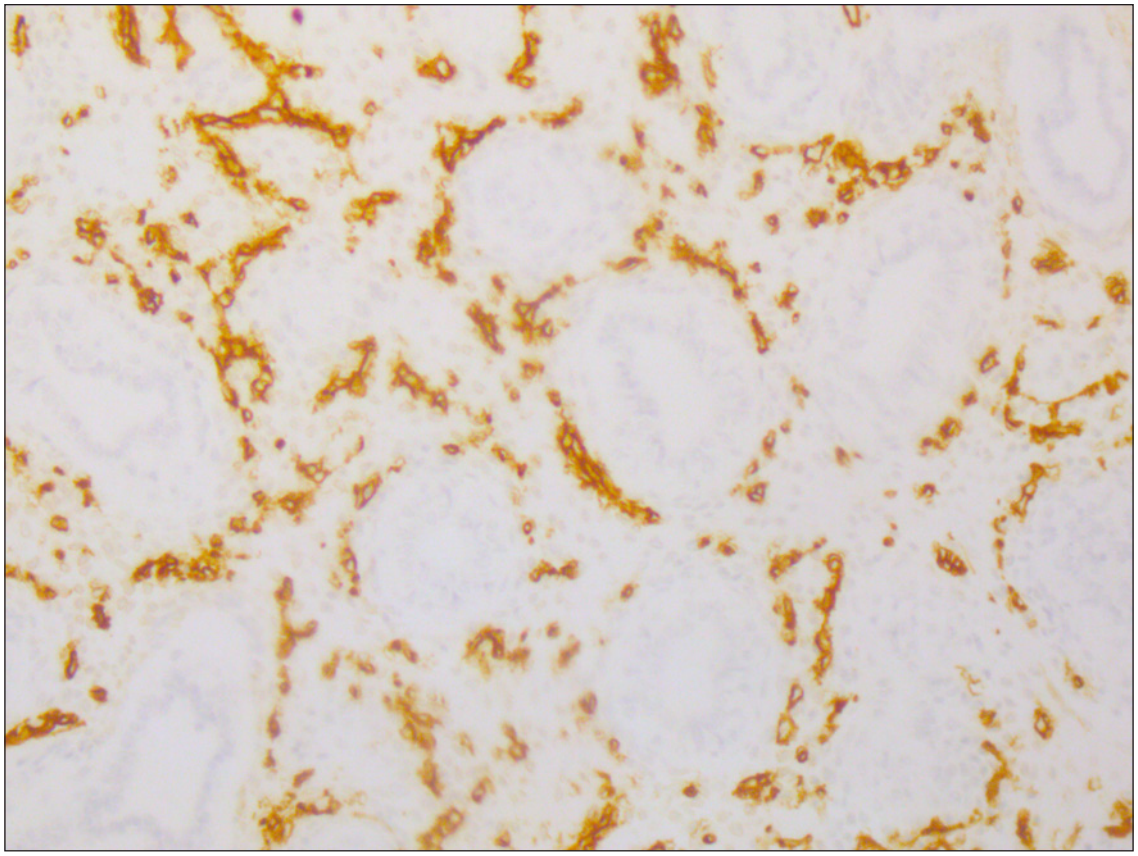

Figure 2 (ABS 82). Immunostaining for CD34 revealed a previously unreported neoangiogenesis in the lung.

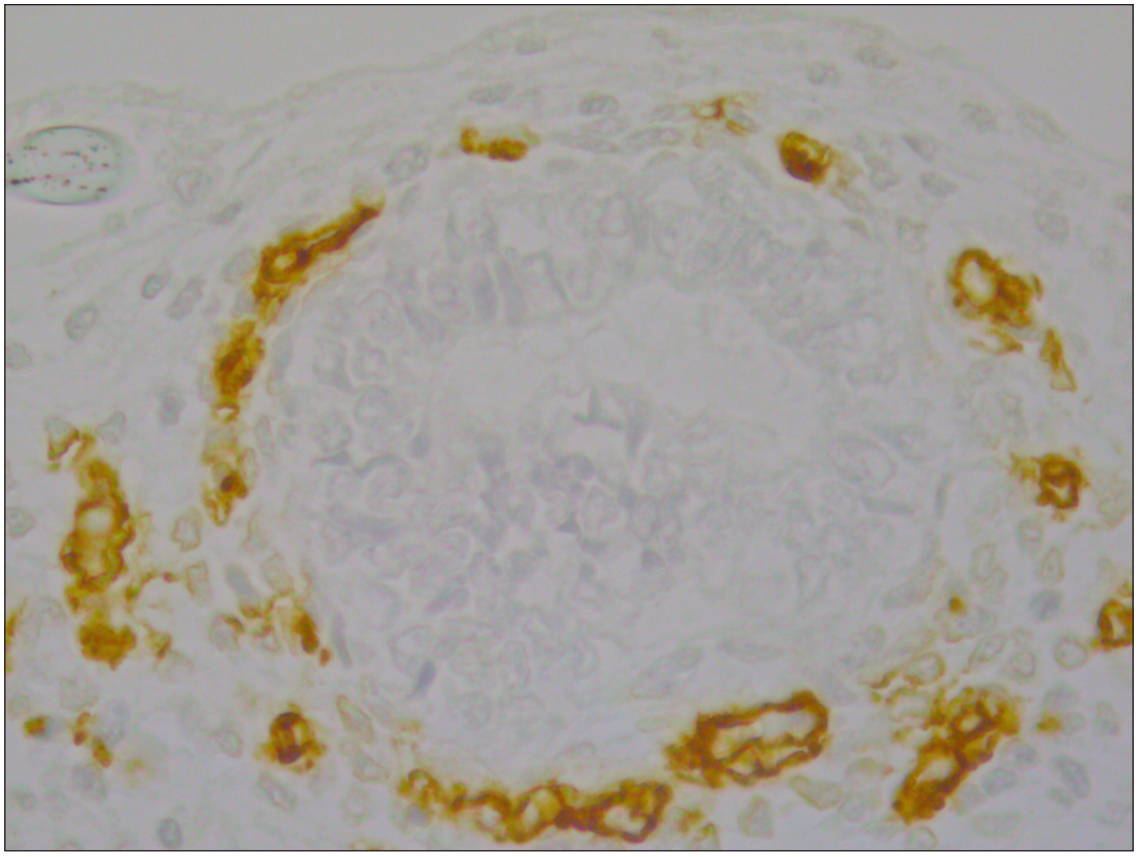

Figure 3 (ABS 82). Circular plexus formed by small CD34-positive blood vessels that delimited each niche.

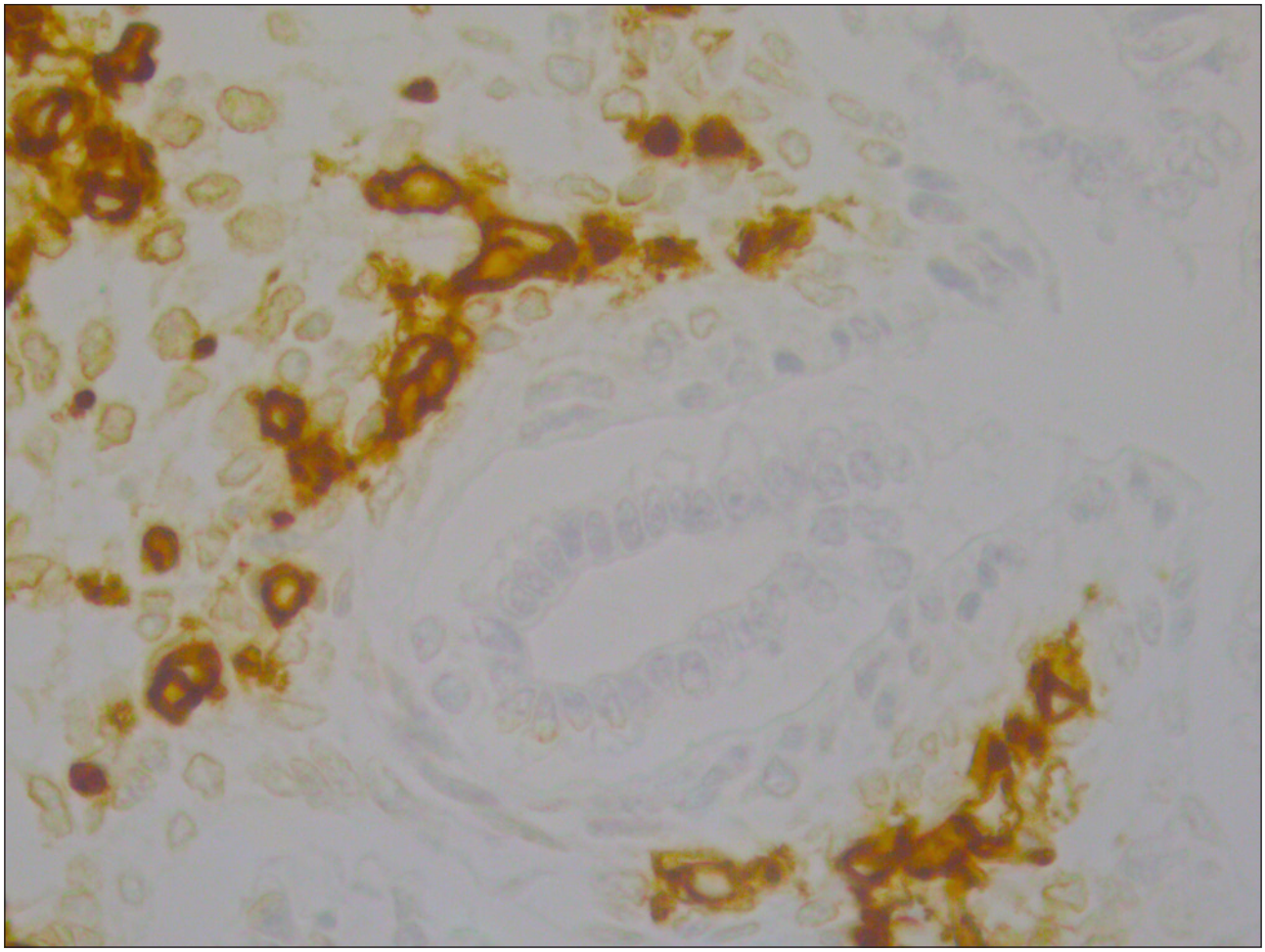

Figure 4 (ABS 82). CD34 reactivity detected in mesenchymal progenitors in the stroma.

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ABS 83

TTF1 EXPRESSION IN THE DEVELOPING HUMAN LUNG

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BACKGROUND/AIMS

Thyroid Transcription Factor-1 (TTF-1) is an important transcriptional regulator that plays a key role in embryonic lung development and lung cell differentiation [1, 2]. TTF-1 is essential for branching morphogenesis, epithelial cell proliferation and development of distal lung structures [3]. This study was aimed at investigating where TTF-1 is expressed throughout the developing human lung.

MATERIALS AND METHOD

4 human fetuses of 11-12 weeks of gestation were completely sampled and histologically and immunohistochemically studied. Samples were fixed in 10% buffered formalin, routinely processed, and paraffin-embedded. Two serial 3 µm-thick sections were obtained from each paraffin block; after dewaxing and rehydrating, one of these was stained with hematoxylin-eosin, the other pre-treated for immunohistochemical analysis, then incubated for 20 minutes with anti-TTF-1 mouse monoclonal antibody (Dako, clone 8G7G3/1, dilution 1:100).

RESULTS

TTF-1 expression was restricted to the nuclei of stem/progenitor cells of the epithelial component of the developing human lung. No reactivity was found in the mesenchymal pulmonary stem/progenitors (**Fig. 1**). At low power, the expression of TTF-1 was

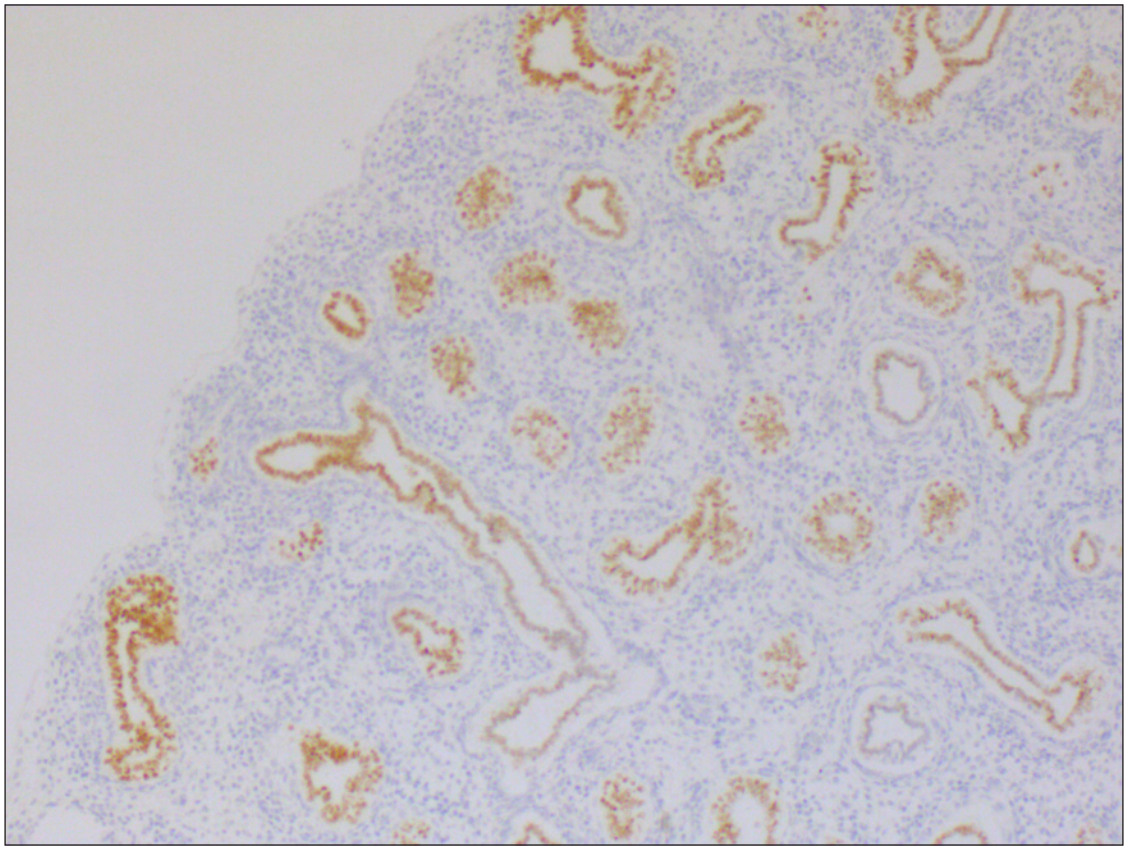

Figure 1 (ABS 83). TTF-1 marks nuclei of the stem/progenitor cells of the epithelial component (40X).

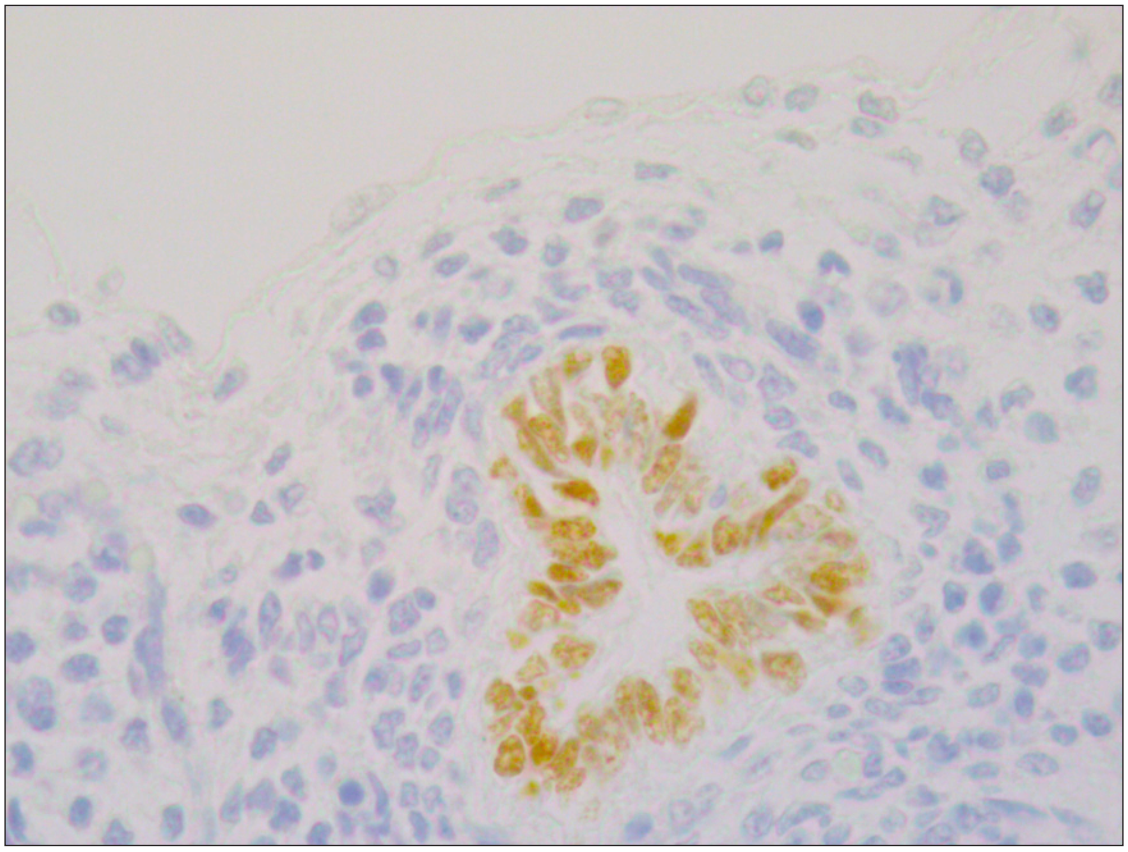

Figure 2 (ABS 83). Strong reactivity for TTF-1 of the stem cell niches of the sub-pleural zone (630X).

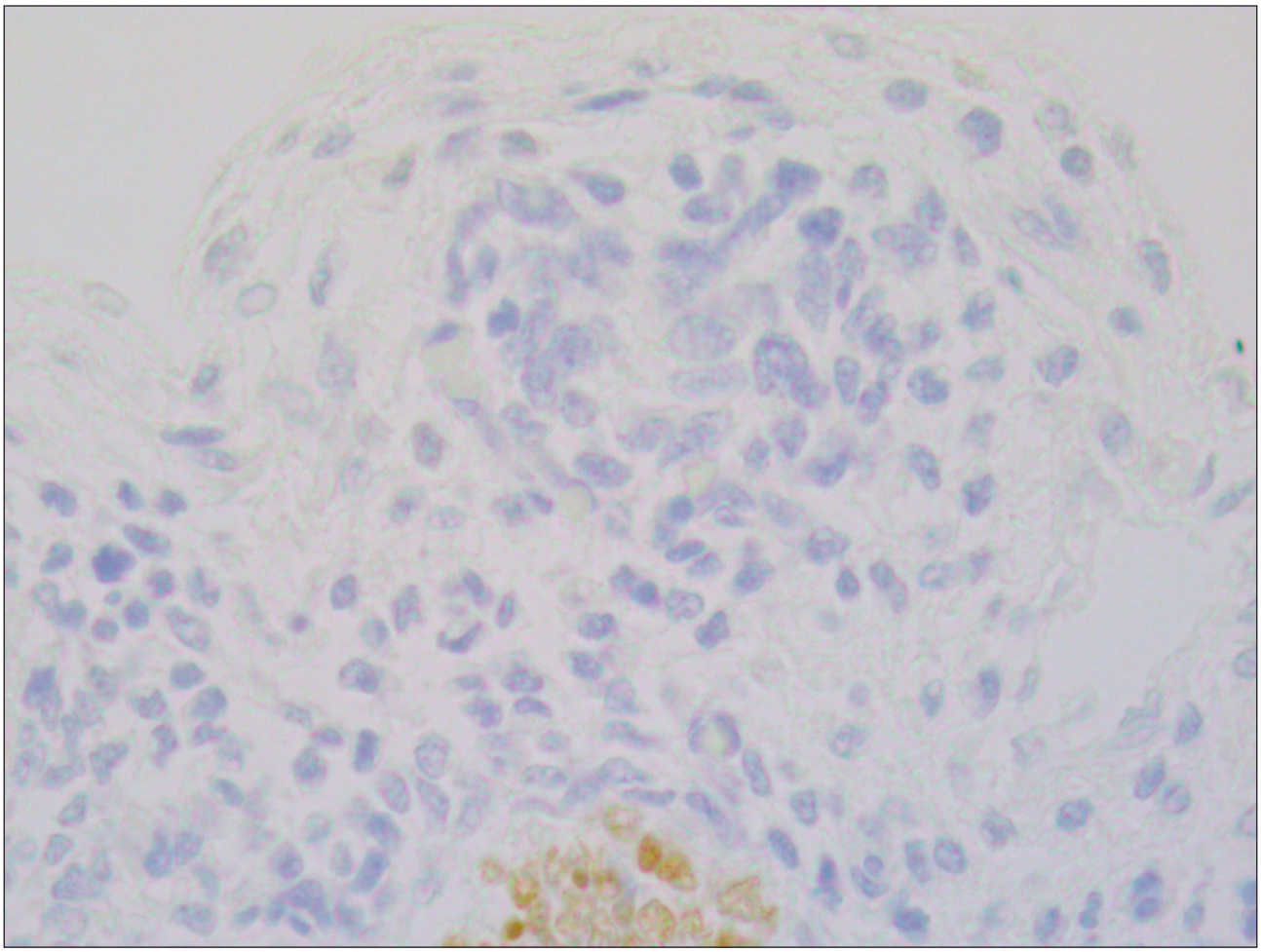

Figure 3 (ABS 83). Uneven reactivity for TTF-1 among different niches of the sub-pleural zone (630X).

uneven: immunostaining for this transcription factor was higher at the periphery of the lung, decreasing in the more proximal tracts of the developing bronchi. At high power, a strong reactivity for TTF-1 was detected in the epithelial progenitors of the tips taking part to the formation of the stem cell niches located in the sub-pleural zone (**Fig. 2**). When TTF-1 reactivity was compared among multiple niches, a strong variability was found: some niches were completely negative while other showed a focal reactivity (**Fig. 3**).

CONCLUSIONS

Our preliminary data confirm that TTF-1 plays a relevant role in human lung development [1]. Its preferential location in the distal lung structures confirms previous data [2, 3] on TTF-1 function in branching morphogenesis and in the induction of the mesenchymal precursors. The variability of TTF-1 reactivity among different niches represents a new tool for better understanding the stage of evolution and differentiation of human lung stem cell niches.

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ABS 84

BIOMARKERS OF AGGRESSION: METABOLOMICS

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Metabolomics is the quantitative study of a large number of low molecular weight metabolites that

are products of different metabolic pathways. The simultaneous analysis of patterns of metabolites produces a metabolic profile, which is the result of the interaction between gene expression, and environment. Each metabolic profile is, so far, peculiar of a kind of disease. The comparison between a pathological group and a control group, in fact, can be used for identifying metabolic variations that can discriminate different pathologies, such as those that are related with psychiatric disorder [1, 2]. Among them, the study of human aggression at a biological level by the use of biomarkers might be of particular interest. In fact, the hypothesis that a "deregulated" metabolic profile made up of several metabolites for many psychiatric disease has been already demonstrated by Rozen et al., 2005, Paige et al., 2007, Holmes et al., 2006; Kaddurah-Daouk, 2006; Kaddurah-Daouk et al., 2007 [3-7]. An increasing evidence of perturbations in a variety of metabolic pathways such as mitochondrial dysfunction, neurotransmitter deficiency, membrane composition loss, and immune functions impairment in psychiatric disease has been also noted [8-10]. Therefore, it is certainly possible to assume that metabolomics approach will provide a powerful tool to map in greater detail the perturbations related to human aggression and the possible relationship in response to therapy. A crucial step will be the decision of which analytical tool will need to be used. Gas and Liquid Chromatography coupled with Mass Spectroscopy (GC/LC-MS) can be used to obtain the largest number of metabolites, while the use of nuclear magnetic resonance (NMR) can be useful for metabolite structural determinations, and for multi-step longitudinal study. However an essential step will be avoiding possible confounding factors, and in order to do this an accurate age matching of patients and controls, a control of diet, gender, ethnic background and the use of drugs must be pondered carefully. Finally, blinded studies are needed to validate markers identified.

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ABS 85

SOX 2-IMMUNOSTAINING ALLOWS THE IDENTIFICATION AND LOCATION OF NEURAL STEM PROGENITORS IN THE DEVELOPING HUMAN HIPPOCAMPUS

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BACKGROUND/AIM

It has been shown that SOX2 is crucial for the self renewal and differentiation of neural stem cells (NSCs). Furthermore several studies have shown that SOX2 is expressed during development of neural tube and in proliferating central nervous system progenitors [1, 2]. SOX2, together with other transcription factors, are master regulator of pluripotency [3]. Studies on SOX2 expression have been mainly carried out on the rat brain. In light of the absence of studies on SOX2 expression in the fetal human brain, our attention was aimed at describing the immunoreactivity of the stem/precursor cells of the dentate gyrus in the early stages of human development.

METHODS

The expression of SOX2 was evaluated in samples from the hippocampus from three human fetuses, received from the Obstetric Division of the University of Cagliari, as voluntary termination of pregnancy (VTOP). All the fetuses included in this study had no congenital malformation. All procedures performed were approved by the Ethics Human Studies Committee of University Medical Centre of Cagliari (according to the instructions of the Declaration of Helsinki).

The fetuses, ranging from 17th to 21st week of gestation, have been completely sampled and histologically and immunohistochemically studied. Hippocampus samples were fixed in 10% formalin, routinely processed, and paraffin-embedded. For immunohistochemical analysis, the samples was treated with 10 min heat-induced epitope retrieval in buffer pH 9.00 (EnVision™ FLEX Target Retrieval Solution High pH, Code: K8004; Dako Denmark A/S, Glostrup, Denmark). Slides were then incubated for 20 min at room temperature with SOX2 (Santa Cruz Biotechnology) mouse monoclonal antibody 1:50 dilution.

RESULTS

Significant reactivity for SOX2 was detected in both 17th to 21st week of gestation in the hippocampal region. At 17 weeks, immunostaining for SOX2 was detected in the nuclei of hippocampal glioepitelium. Whereas the majority of granular cells were negative, some large precursor cells showed a strong reactivity for SOX2 (**Fig. 1**). At higher power, it was possible to identify a large number of voluminous neural stem/progenitor cells inside the granular layer of the dentate gyrus (**Fig. 2**). At 21 weeks, the number of SOX2-reactive neural progenitors was increased. Moreover, a strong immunostaining for SOX2 was detected in the subventricular neuroepithelium (**Fig. 3**). At higher power, SOX2-positive precursors showed the tendency to migrate out of the granular layer toward the subpial regions (**Fig. 4**).

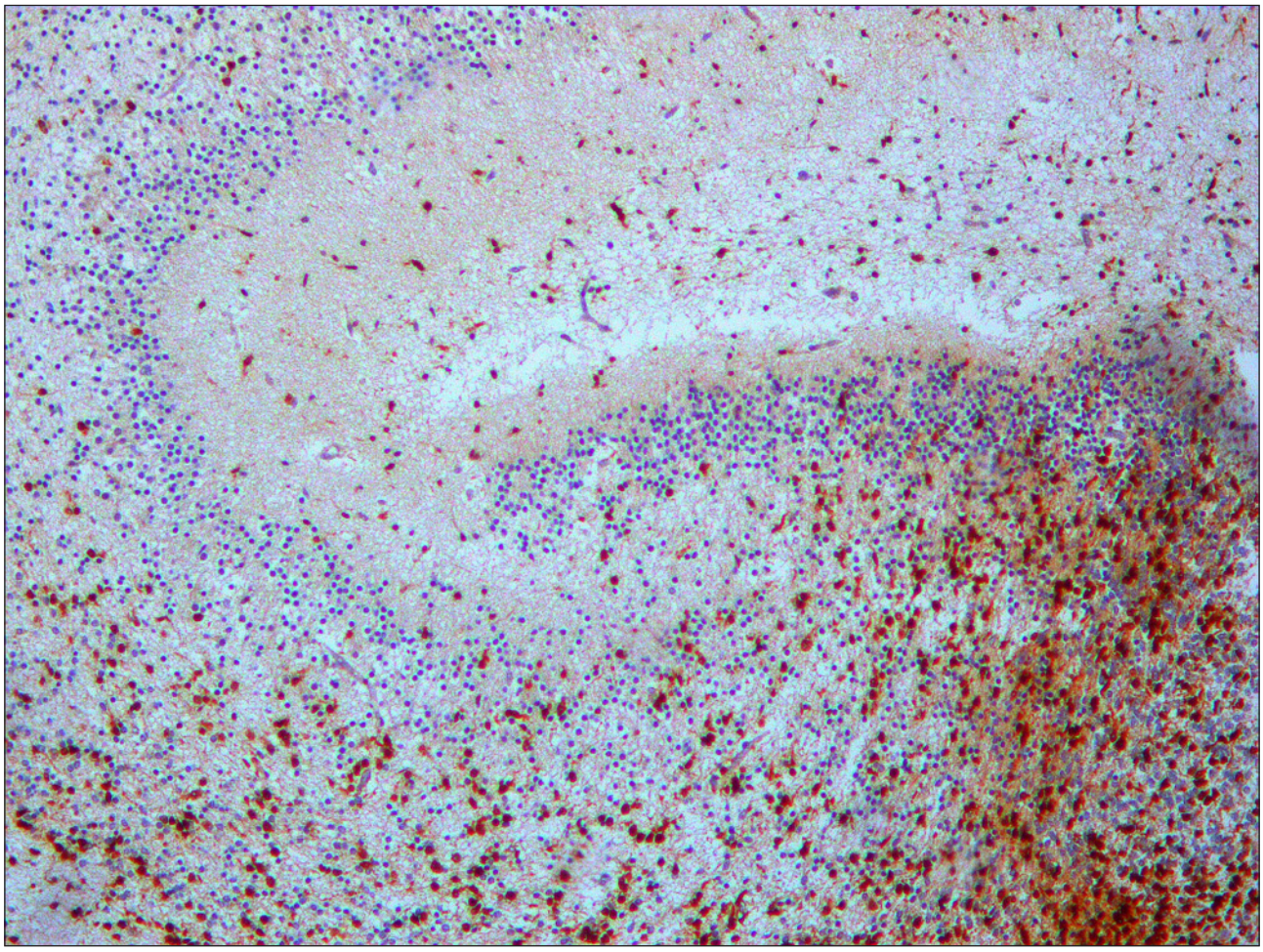

Figure 1 (ABS 85). 17 weeks: some large precursor cells in the hippocampal glioepitelium show a strong reactivity for SOX2.

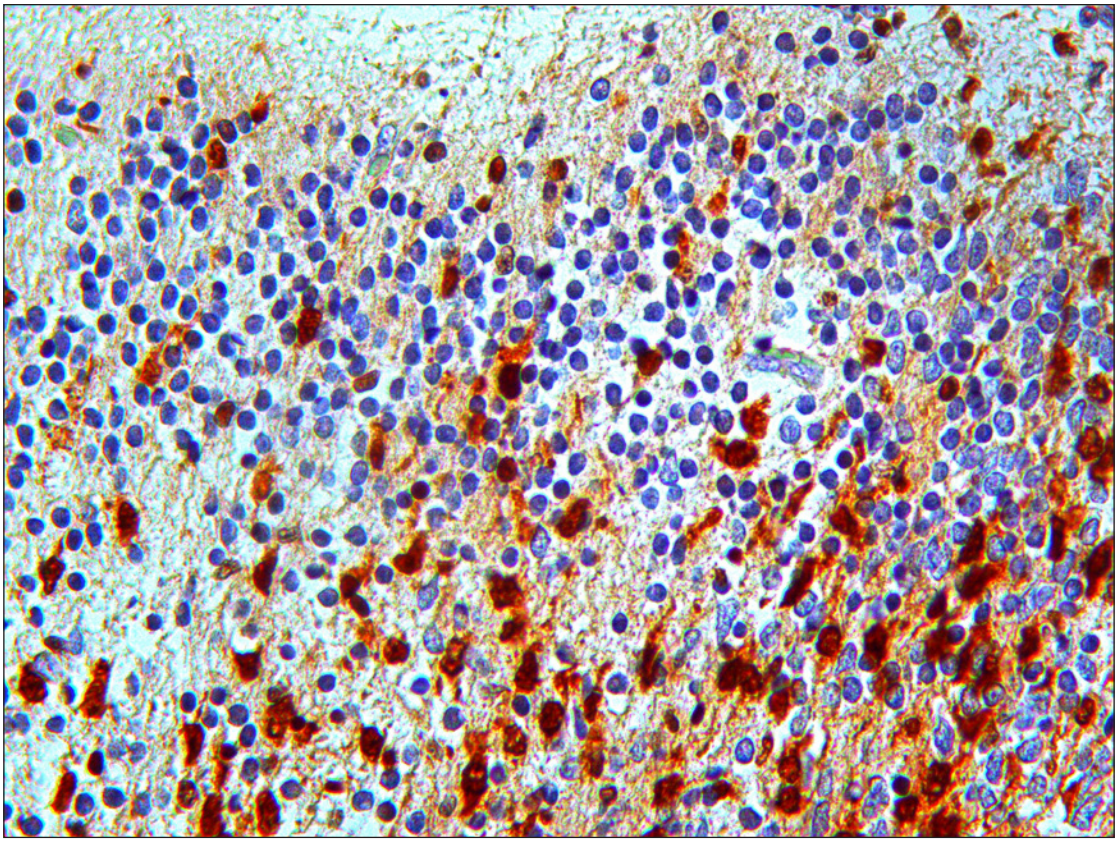

Figure 2 (ABS 85). 17 weeks: at higher power, it is possible to identify a large number of voluminous neural stem/progenitor cells inside the granular layer of the dentate gyrus.

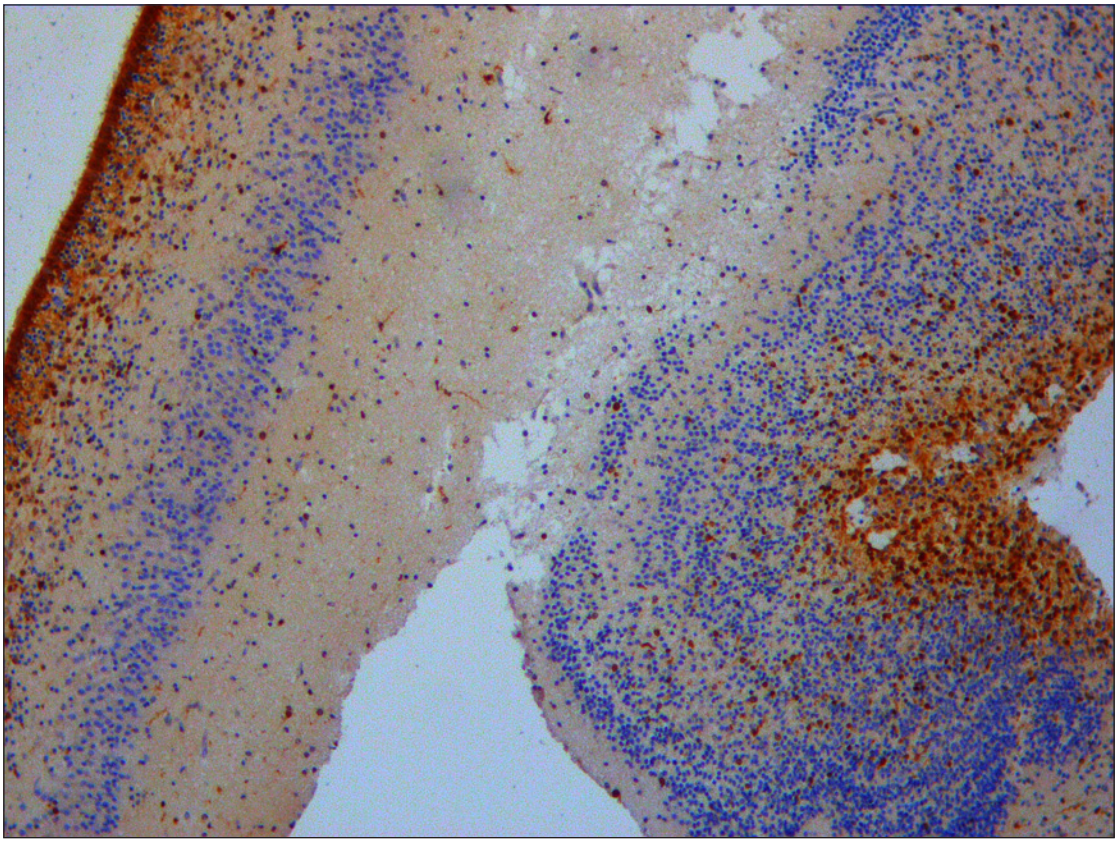

Figure 3 (ABS 85). 21 weeks: a strong immunostaining for SOX2 is detected in the subventricular neuroepithelium.

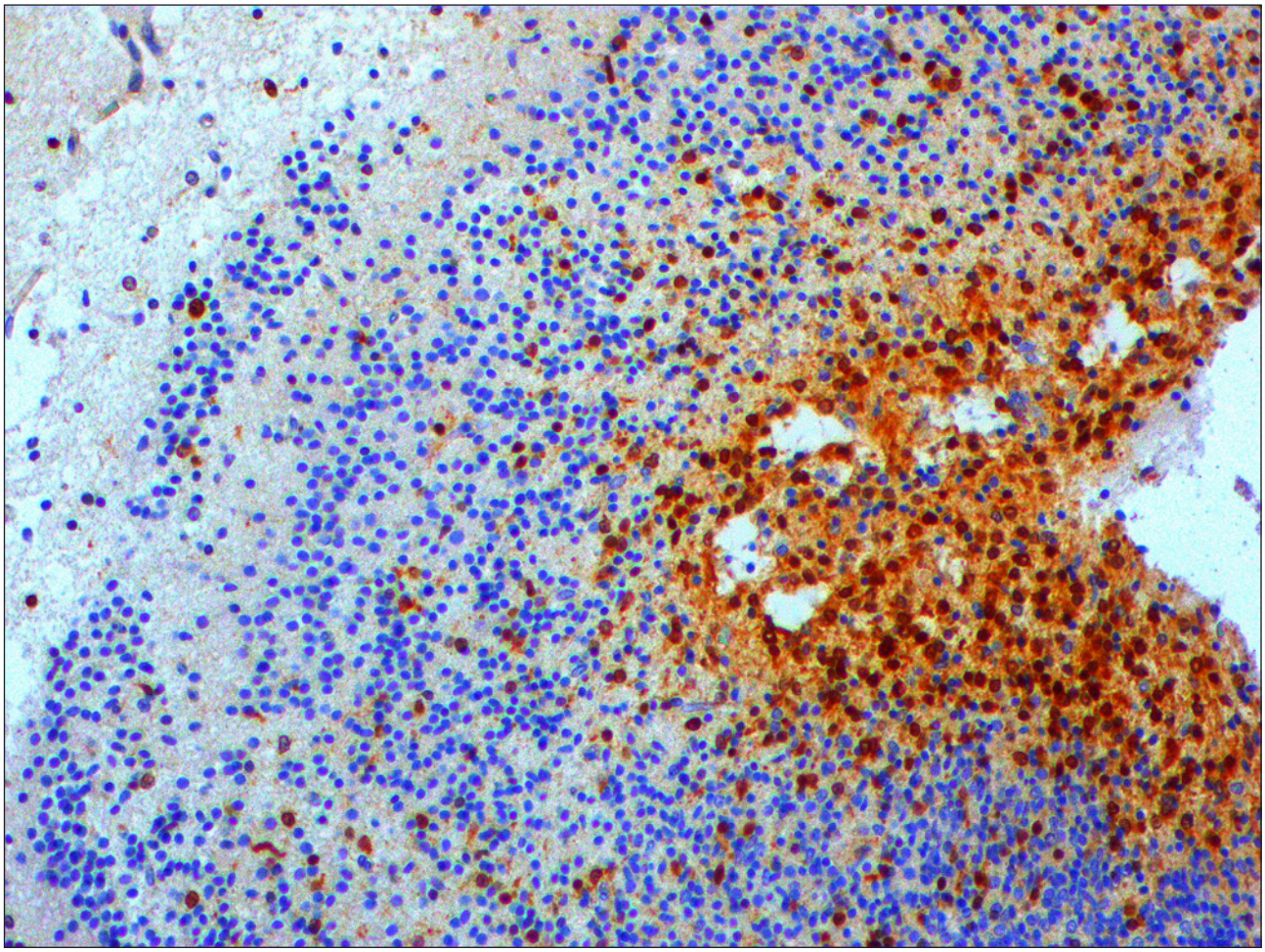

Figure 4 (ABS 85). 21 weeks: at higher power, SOX2-positive precursors show the tendency to migrate out of the granular layer toward the subpial regions.

CONCLUSIONS

Our preliminary study shows that neural stem/precursor cells are abundant in the dentate gyrus of the human brain in the early phases of development. Immunostaining for SOX2, a typical marker of stemness, appeared at a useful tool for a better comprehension of the multiple stages of differentiation of the multiple cell types involved in the maturation of the dentate gyrus. In particular, the vast majority of cells of the granular layer did not show any reactivity for SOX2. This finding probably indicates their differentiation into postmitotic neurons. On the othe hand, large neural progenitors, scattered in the granular layer, were strongly positive for the transcription factor, suggesting their stemness. Further studies are needed in order to better characterize the multiple cell types involved in the development of the human hippocampus and the role played by SOX2 at different gestational ages, till birth.

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ABS 86

STEM PROGENITOR CELLS IN THE LIVER

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4Pathology Department, ZOL, Genk, Belgium

BACKGROUND

Liver stem/progenitor cells are characterized by self-renewal ability and cellular plasticity.
They are able to give rise to multiple cellular populations and to differentiate both into hepatocyte and cholangiocyte lineages, or into extra-hepatic cell types (intestinal, pancreatic cells) and generate malignant cells.
The identification and the role of human liver stem/progenitors has been a challenge topic of the recent scientific literature.
The CD34, c-kit, CK7, CK19, alpha-fetoprotein, OV6, CD90, CD133, SOX9, SOX17 and CK8/18 were the major markers used in order to demonstrated the existence of liver stem/progenitor cells.
The niche is the stationary compartment where the liver stem/progenitors cells are localized.
The liver stem/progenitor cell niche is composed of numerous cell types, including portal myofibroblasts, hepatic stellate cells, endothelial cells, hepatocytes, cholangiocytes, Kupffer cells, Pit cells and immune cells.
Each of these cell types can interact and cross-talk with liver stem/progenitor cells, thereby influencing proliferation and differentiation within the niche itself.
The cellular and extracellular microenvironment controls the proliferation and differentiation of the stem/progenitor cells and the interaction with other cell types.
The composition of the surrounding signals supports and regulates the maintenance of the liver stem/progenitor cells.
Many and numerous studies claimed that the liver stem/progenitor cell niche is sited in portal tracts: some author focused their attention on the small cholangiocytes of the canals of Hering, wheras others attributed this ability to the periportal hepatocytes.

AIM

This work was aimed at identifying the morphological and immunohistochemical of hepatic stem progenitor cells features in the developing human liver.

MATERIALS AND METHODS

Human liver samples from 3 fetuses, at 11-12 weeks of gestational age, were routinely processed, H&E stained; then the immunohistochemical analysis of CK19, SOX9, c-kit, GATA2, CYP3A4, CYP3A5, CYP 3A7, CK7, and ISL1 was performed.

RESULTS

We have identified the vast majority of stem cells in the niche within the immature portal tracts, located in the mesenchyme, near the ductal plate.
Small undifferentiated cells with oval nuclei and scant cytoplasm, similar in shape to the rat oval cells, isolated or arranged in small groups, were observed in the immature portal tracts in H&E-stained sections (**Fig. 1**).
SOX9 (**Fig. 2**), CK19 (**Fig. 3**) and c-kit (**Fig. 4**) were expressed in the same small undifferentiated cells observed in the immature portal tracts.
SOX9, CK19 and c-kit were weakly expressed in the hepatoblasts and in the interemdiated hepatobiliary cells as well. GATA2, CYP3A4, CYP3A5, CYP3A7, CK7, and ISL1 reactions were positive in hepatoblasts and the more differentiated cells.
Hematopoietic stem/progenitor cells were negative for all markers considered in this study.

DISCUSSION

The human fetal liver represents the major source of stem/progenitor cells.
Our data support the hypothesis that the preferential site of the niche is located in the mesenchyme of the immature developing portal tracts.
They can be easily recognized as undifferentiated, with oval large nuclei and scant cytoplasm cells.
CK19, SOX9 and c-kit are the most interesting markers among the panel of immunohistochemical ones we evaluated in order to identify the hepatic stem/progenitor cells, since CK19, SOX9 and c-kit were positive in the undifferentiated cells morphologically detected.
The other markers of "stemness" evaluated in our study (GATA2, CYP3A4, CYP3A5, CYP3A7, CK7, and ISL1) did not underlined stem/progenitor cells, being highly expressed in the more differentiated cells as well.
This study represents the basis for identifying possible subtypes of hepatic stem progenitor cells and to characterize the stem cells themselves.
Another challenge is represented by the exact composition of the liver cell niche, mainly focused on the relationships existing among the so different cell types cooperating with stem cells for assuring liver regeneration.

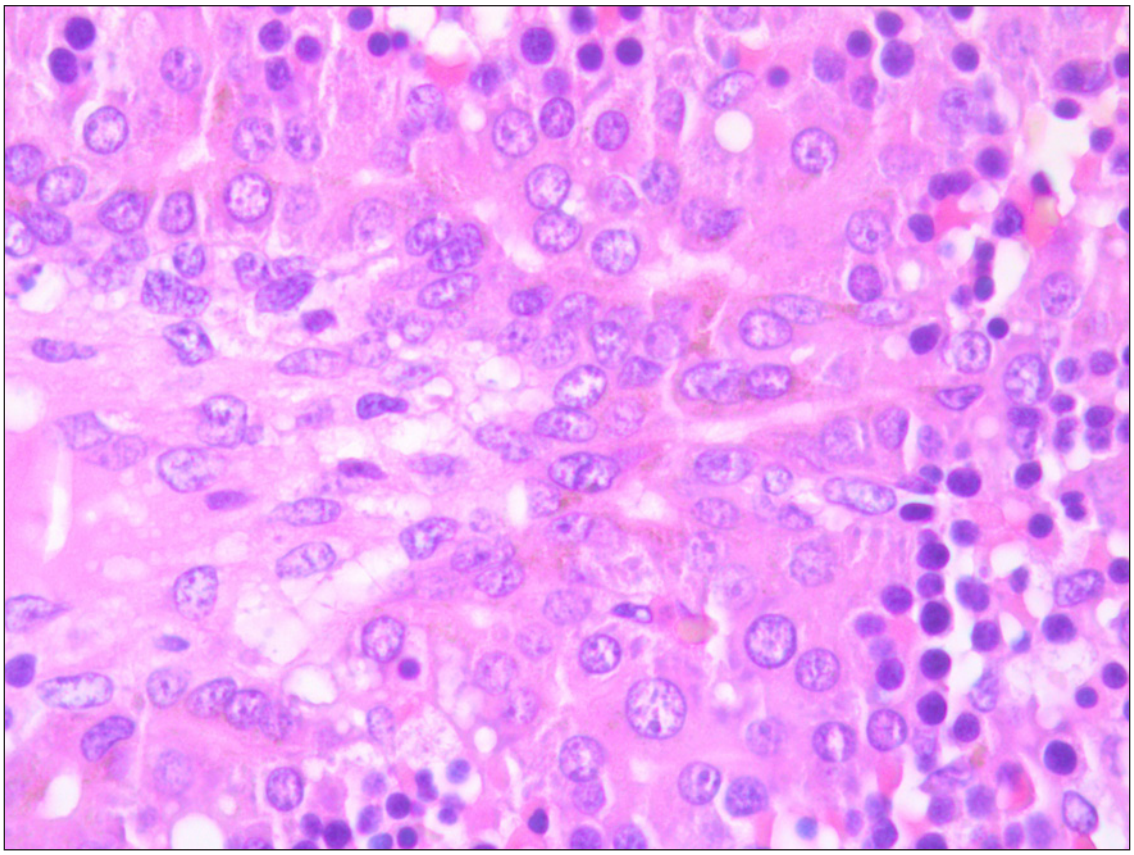

Figure 1 (ABS 86). Small undifferentiated cells observed in the immature portal tracts (H&E-stained section).

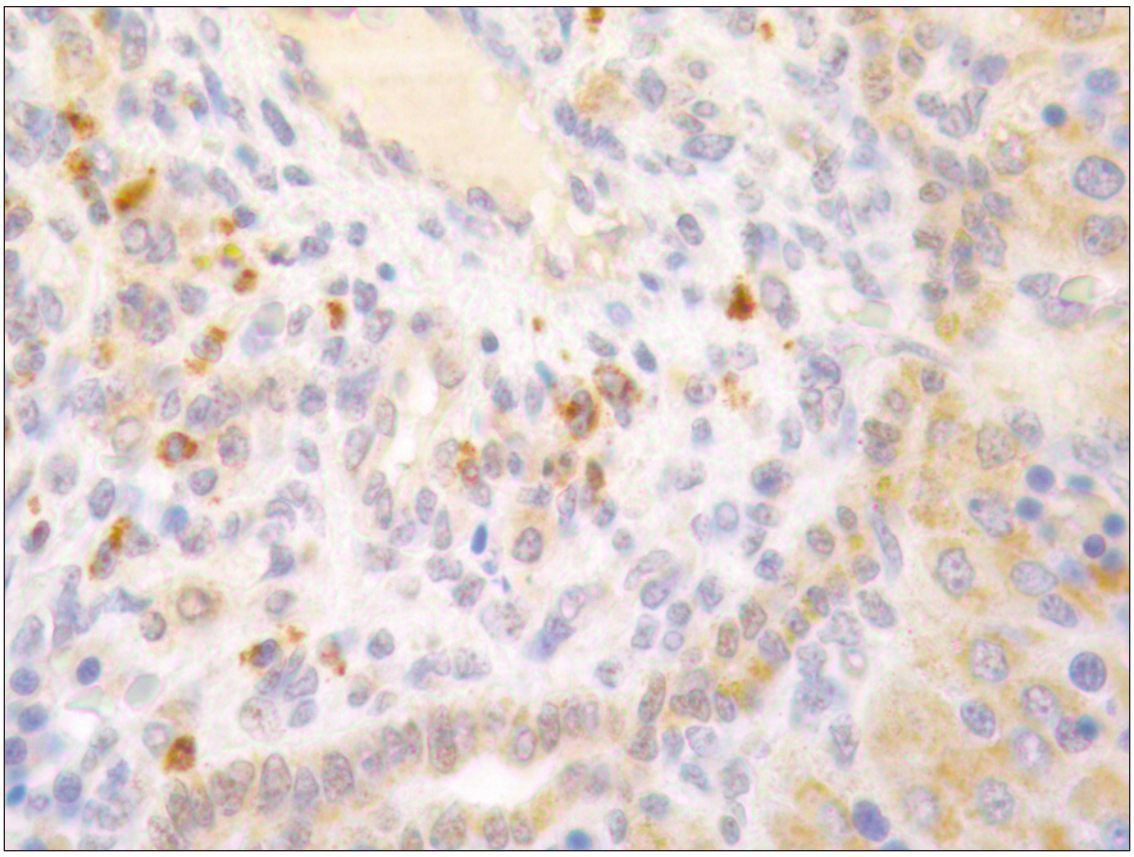

Figure 2 (ABS 86). SOX9 is expressed in the small undifferentiated cells observed in the immature portal tracts.

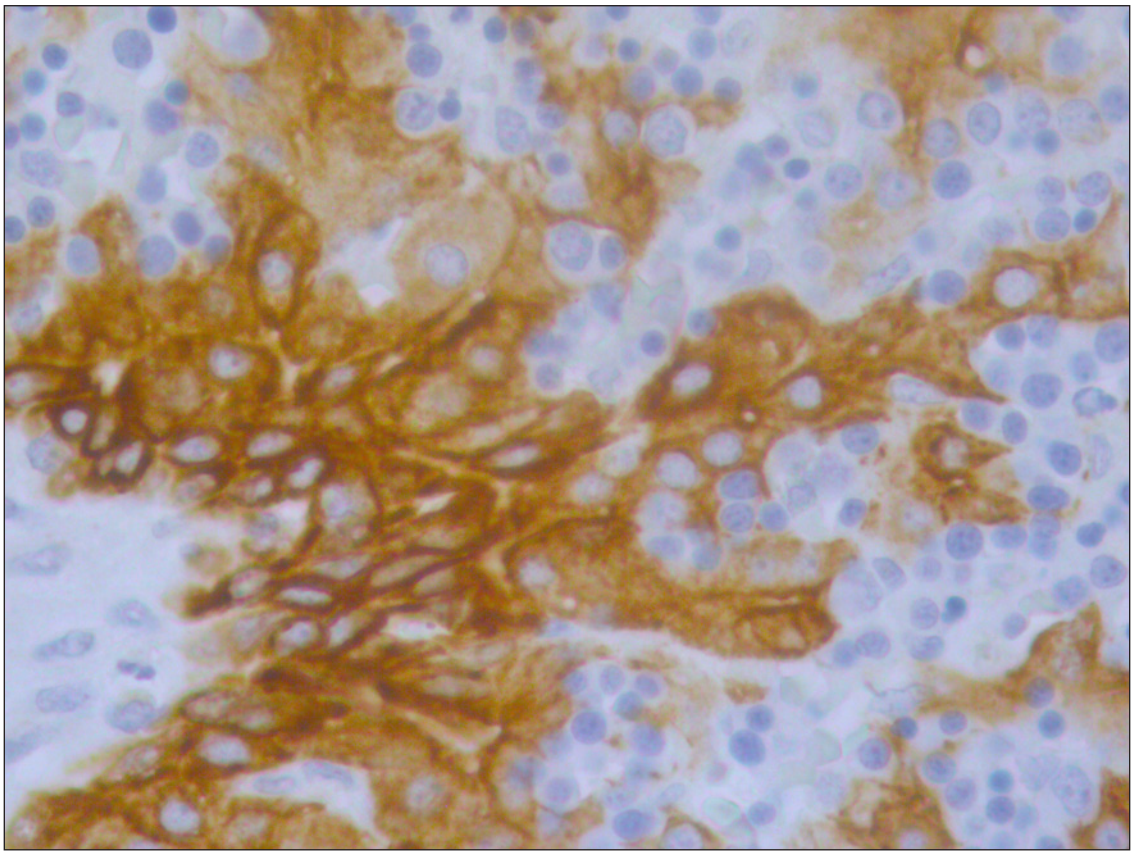

Figure 3 (ABS 86). CK19 is expressed in the small undifferentiated cells observed in the immature portal tracts.

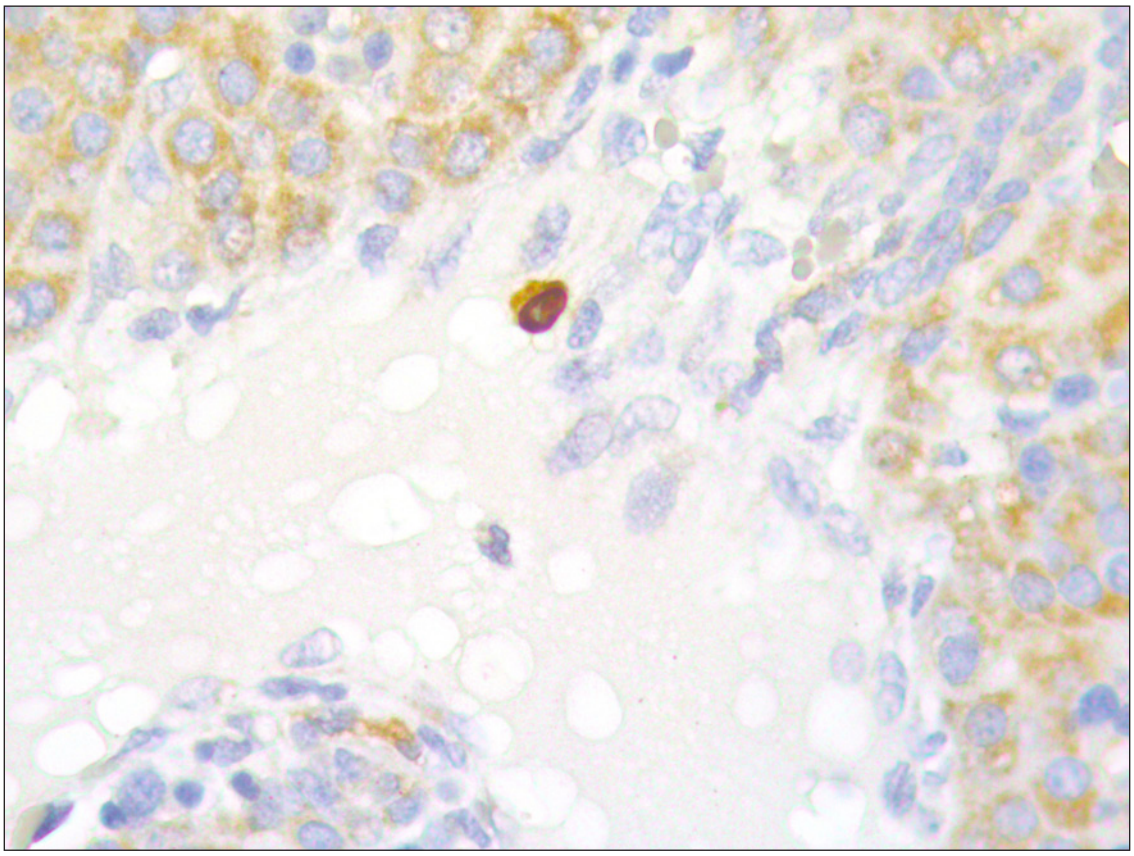

Figure 4 (ABS 86). c-kit is expressed in the small undifferentiated cells observed in the immature portal tracts.

ABS 87

SACROCOCCYGEAL TERATOMA AND THE SECRETS OF STEM/PROGENITOR CELLS

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INTRODUCTION

Sacrococcygeal teratoma is the most frequent neonatal congenital tumor. Sacrococcygeal teratoma can be benign or malignant although malignancy is rare [1]. There is a high risk of neonatal mortality, linked to complications such as rupture of teratoma at birth, fetal hydrops, fetal hydronephrosis or bleeding. The risk of mortality increases in solid and highly vascularized tumors [2, 3].

Teratomas are tumors composed of stem and progenitor cells that differentiate into organs and tissues of all three germ embrional layers.

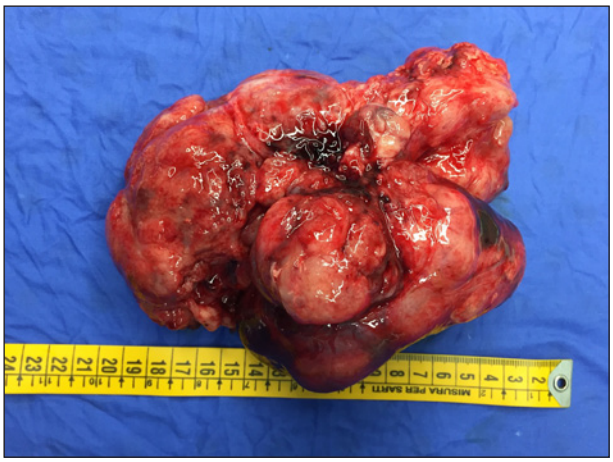

Figure 1 (ABS 87). Sacrococcygeal teratoma: the tumor weight was 708 grams and the maximum size was 18 x 12 x 9 cm.

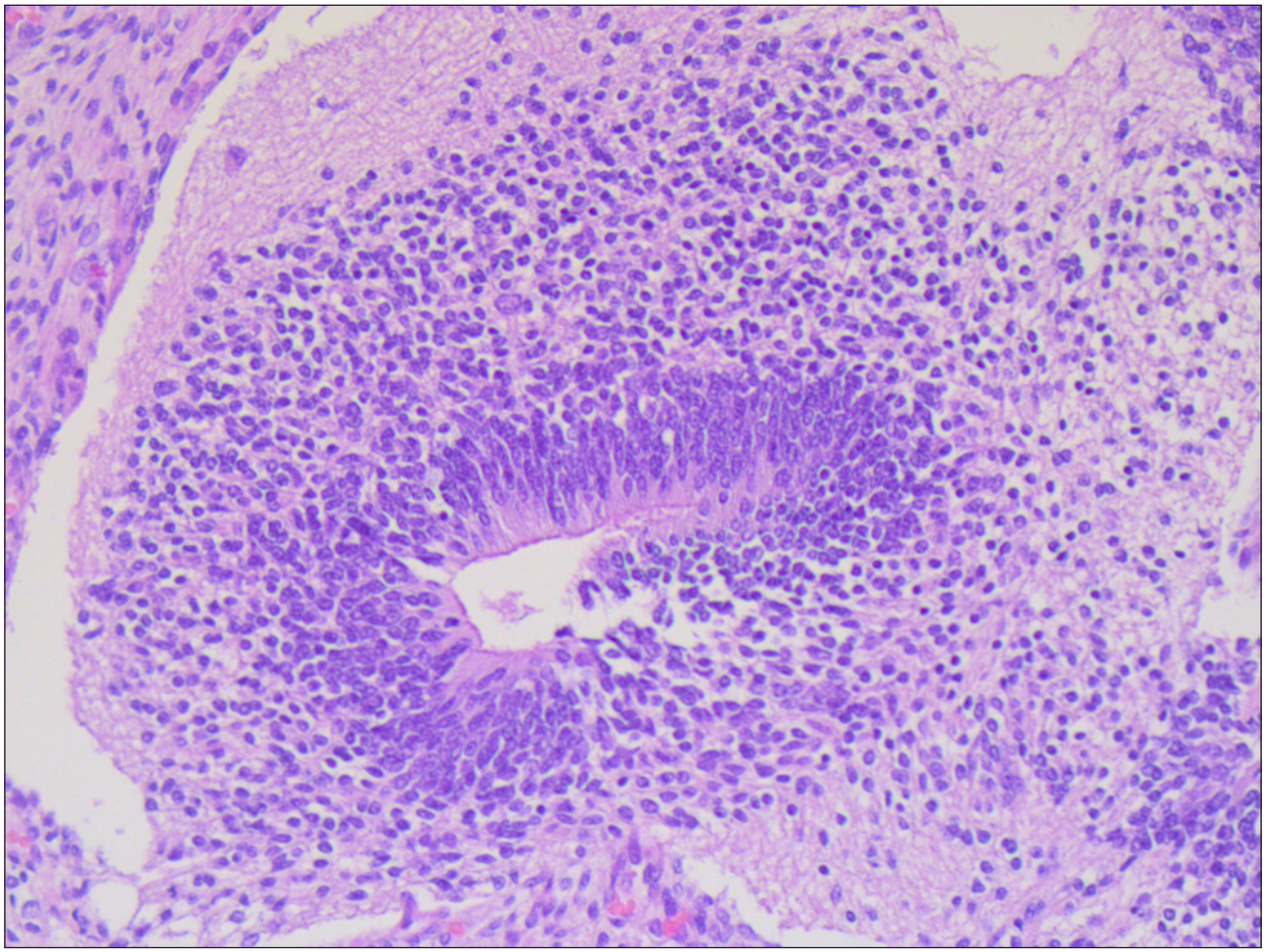

Figure 2 (ABS 87). At low power, teratoma tissue was formed by multiple organoid structures, including neural crest-like structures.

METHODS

We studied a case of sacrococcygeal teratoma in a preterm newborn of 31 weeks.

The tumor weight was 708 grams and the maximum size was 18 x 12 x 9 cm (**Fig. 1**). Multiple tumor samples were fixed in formalin, routinaly processed and embedded in paraffin. 5µ thick sections were stained by hematoxylin and eosin and observed at the microscope.

RESULTS

At low power, teratoma tissue was formed by multiple organoid structures, including neural crest-like structures (**Fig. 2**). In the centre of the tumor, solid areas formed by undifferentiated stem/progenitors cells were detected (**Fig. 3**). Island of respiratory epithelium with well-differentiated cilia were frequently identified (**Fig. 4**). An area of renal-like tissue (**Fig. 5**), containing one large glomerulus surrounded by multiple tubular structures, was also found.

CONCLUSIONS

The study of this case of sacrococcygeal teratoma allowed us to observe a large number of stem and progenitor cells and the various features of their progressive differentiation. In the teratoma stem cells undergo differentiation and never develop into mature tissue. Observation of a rudiment kidney-like structure similar to a pronephros leads us to consider the hypothesis that stem cells have the ability to differentiate just in a rudimentary organ. The organogenesis in fetus requires stem cells nonetheless a series of structures (expecially the stem cell niche) and interaction between each other. The development follows an order and a precise pattern that lacks in teratoma.

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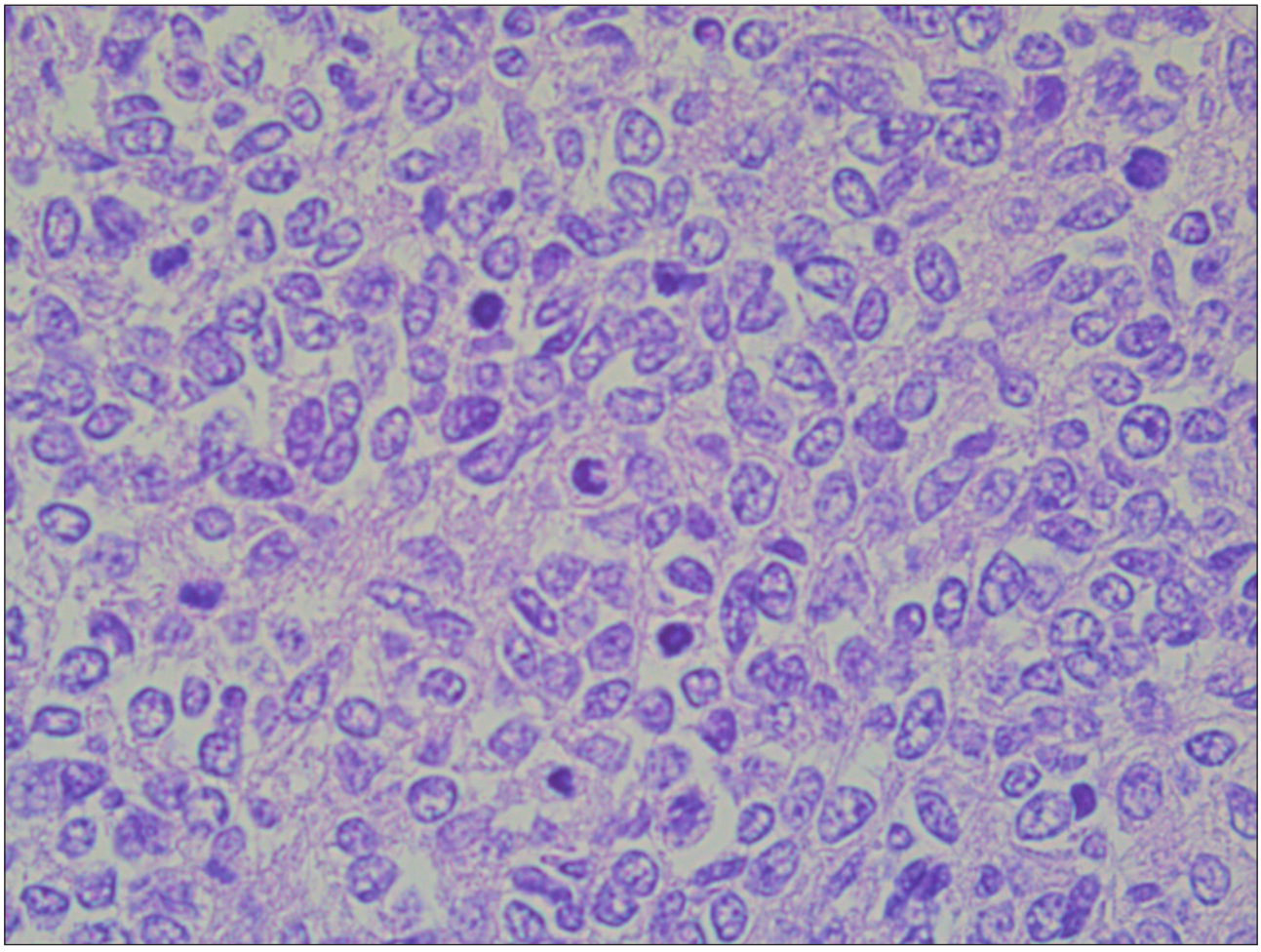

Figure 3 (ABS 87). In the centre of the tumor, solid areas formed by undifferentiated stem/progenitors cells were detected.

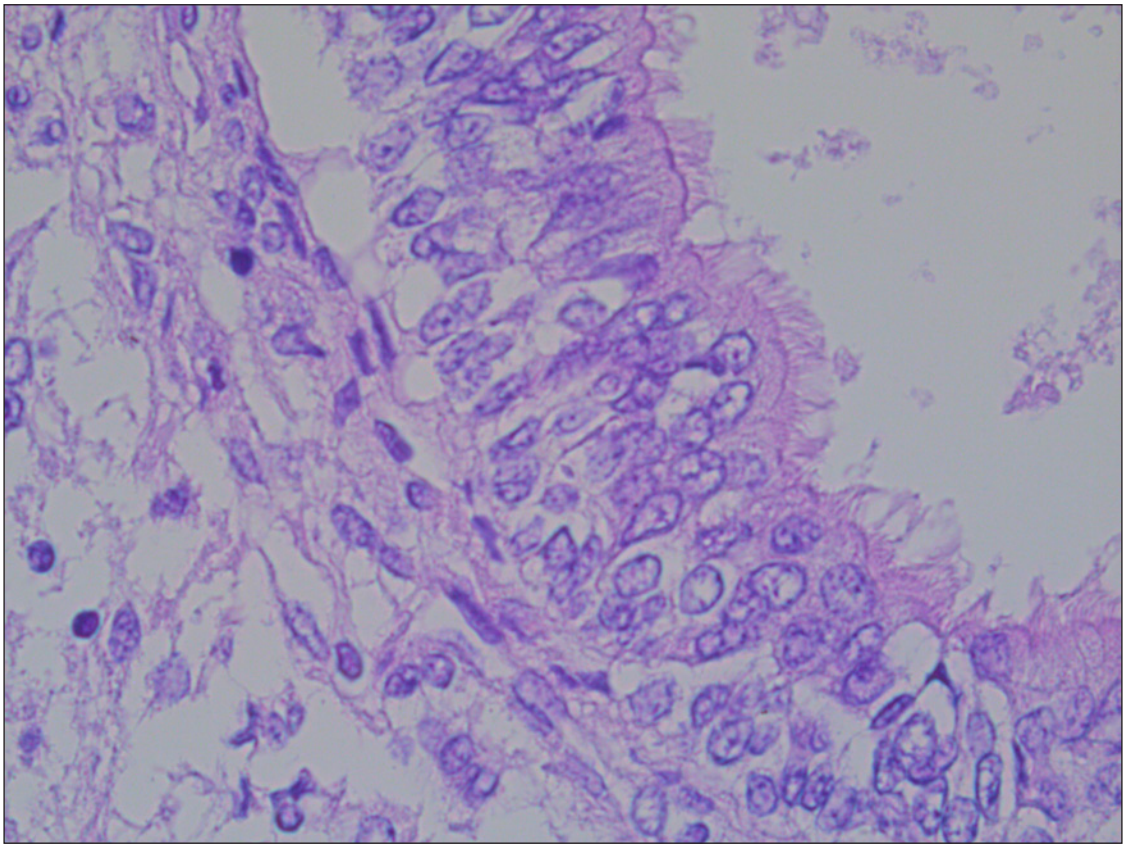

Figure 4 (ABS 87). Island of respiratory epithelium with well-differentiated cilia were frequently identified.

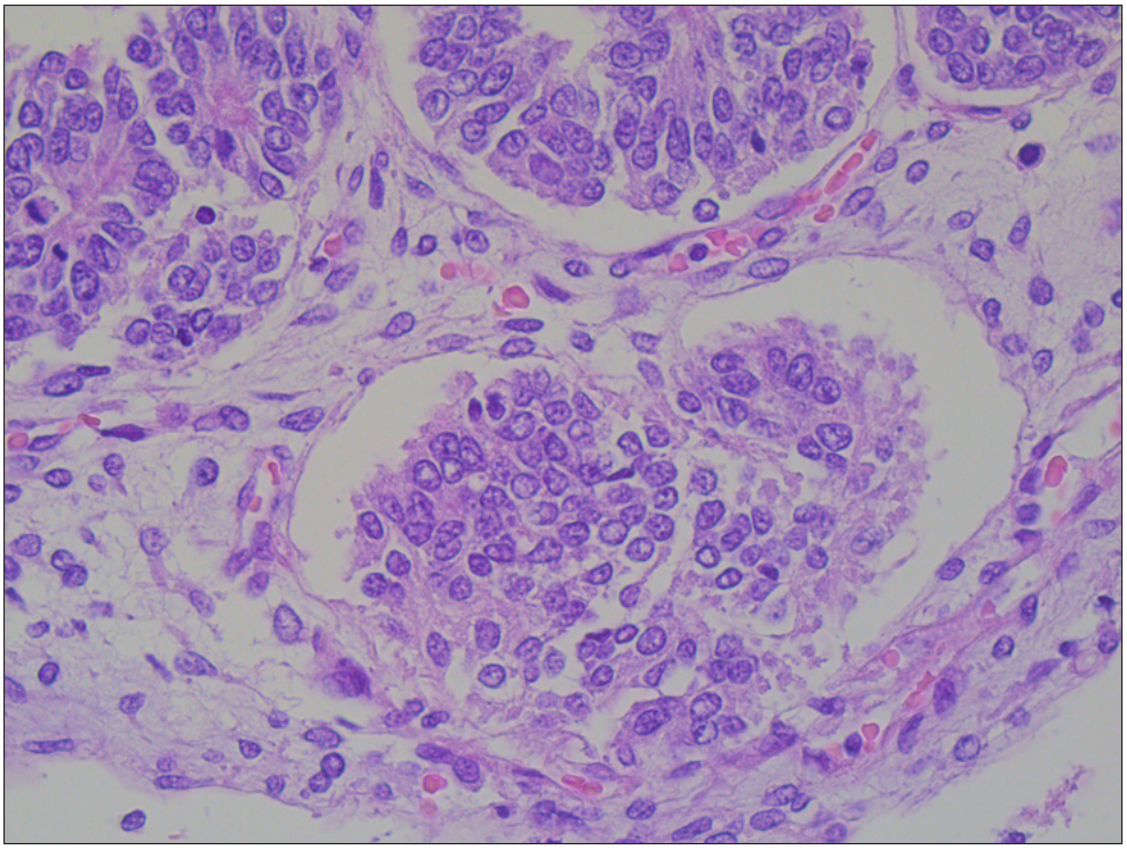

Figure 5 (ABS 87). An area of renal-like tissue, containing one large glomerulus surrounded by multiple tubular structures.

ABS 88

NUTRIMETABOLOMICS IN IUGR AND LGA NEWBORNS

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Nutrition plays a key role in the life of human being and can affect the quality of life in several ways. It is now a common knowledge that obesity and the risk to develop a metabolic syndrome are correlated with fetal malnutrition, both excessive and insufficient, which can permanently alter the fetal programming. It is also important how the nutrition in neonates could affects their life quality or could prevent the risk of chronic pathologies. According to the foetal hypothesis of Barker, which suggested the relationship between low birth weight and increased risk of developing diseases in adulthood, it assumes more value to understand how to treat these patients, to reach new qualitative and valid standard in the field of infant nutrition. Intrauterine growth retardation (IUGR) and large for gestational age (LGA) newborns seem so different, but they have something in common, because both at birth show a reduced carbohydrate tolerance with hypoglicemia. This condition expose these infants to an higher risk of developing metabolic disease such as type 2 diabetes and obesity in adulthood. There is a general agreement in recognizing breastfeeding as the best type of nutrition in newborns, but not every time the precious resource of human milk is available and so the formula must be used. For this reason, it is desirable learning more about the constitutive elements which are presents in both type of milk, the attempt should be to improve the formula milk, making it more similar to the highest neonatal feeding standard: breastfeeding. A scientific method helpful in this analysis is the metabolomics. Metabolomics is a new field of research in the panorama of "-omics" sciences, and it focus on the quantitative measurement over time of the metabolic response of a living system to pathophysiological stimuli and genetic modifications. The use of this approach allows to evaluate the effects which occur in the metabolism of the neonates and these are strictly related to the type of nutrition received in the first period of life. Metabolomics can be considered a valid, noninvasive instrument in the study of the components produced by metabolism even in fetal and neonatal life. The studies published to date seem represent a starting point of fundamental importance in assessing the metabolic correlations between IUGRs and LGAs. Above all, metabolomics appears to offer for these patients the possibility in the near future to personalized nutrition in order to prevent the onset of chronic diseases in adulthood.

ABS 89

FETAL PROGRAMMING AND RENAL DAMAGE: NEW PERSPECTIVES?

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Fetal programming concerns every altered *in utero* conditions that induce defects on organs development. Consequently, it increases the risk of diseases and organ failure in adulthood.
Several conditions have been involved. Particularly, it has been seen that a low birth weight (LBW) correlates with higher risk of developing renal failure (defined as altered blood pressure, albuminuria and eGFR levels reduction) over time compared to a referred normal birth weight (NBW). Additionally, it has been found a higher proportion of relapses in nephrotic sindrome (minimal change disease/ focal segmental glomerulosclerosis) and a worse clinical course of IgA nephropathy. An early reduction of fetal nephrons number appears to be correlated to LBW and may lead to a reduced adaptation in later life, probably due to a prolonged hyperfiltration of the remaining nephrons. A poor nephron endowment can be due to both environmental (maternal diet and smoking habits, maternal diseases) and epigenetic factors.
Furthermore, solitary functioning kidney (SFK), conditions either congenital and secondary (i.e. CAKUT disease), might increase the risk of chronic kidney diseases. In these conditions, nephrons number at birth seems to be implicated in the chronic kidney disease susceptibility, too. Nevertheless, this condition tends to be experienced if an acute event happens, so that it reflects a poor adaptation to external stimuli. Finally, people with fetal SFK onset seems to be at a higher risk of late-onset chronic kidney disease respect to those that develop a SFK condition after their childhood (i.e. unilateral renal donors). Thus, a postnatal follow-up is required even though it is actually determined by opinion-based recommendations rather than codified guidelines.

Renal fetal programming is yet a widely unknown field of research. Nevertheless, new efforts should be spent in increasing our knowledge on those mechanisms leading to renal impairment in adult age in order to make possible both prevention and early diagnosis, to retard or totally avoid the ESRD onset and its related consequences.

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ABS 90

THE PAEDIATRIC PARENT'S ASSOCIATION AND "THE CARE": THE THREE-YEAR PROGRAM OF "*ASSOCIAZIONE AMICI BAMBINI CARDIOPATICI SARDI*" 2015-2017

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The "*Associazione dei Bambini Cardiopatici Sardi*", called ABCS, was founded in 1993 by a group of parents of children with heart problems. The role of this association has always been to provide support to the family. It initially started at the Brotzu Hospital in Cagliari (at the Department of Paediatric Cardiology) and later in 2014 at the Department of Neonatal Pathology and Childcare, Section of Neonatal and Paediatric Cardiology, of the University Hospital (Azienda Ospedaliera Universitaria Cagliari) under the supervision of Dr. P. Neroni.

Based on the agreement signed in February 2014 between the ABCS and the AOU a new program of activities was presented to the AOU executive manager and approved by him, structured in three points.

1. It will be activated a social Secretariat, ruled by ABCS, with aims to:
   - establish a place that can be used as a reference point for parents of children with heart problems, which can help to complete the bureaucratic practices and specific needs related to the disease;
   - create a network of information always active and updated;
   - providing connections with other national associations to ensure the care of children with heart disease and their families during transfers abroad.
2. ABCS also will actively participate in the construction of a course of therapeutic education (Patient Education) organized by the Department of Puericulture (AOU). The course " therapeutic education of patiens" (ETP) will aim to:
   - to help patients for acquiring or maintaining the skills they need to better manage their life with a chronic disease;
   - to create a permanent organization for taking care of the patients;
   - to help them to understand the disease and the treatment, to better cooperate and to assume their responsibilities of the disease, with the ultimate aim to improve their quality of life.

   The experimental course, organized by Dr. Neroni and dr. Zonza will have the active participation of the ABCS members. Then, a course aimed directly at children with heart disease, will be prepared.
3. Last but not least a Support Group for Parents will be organized. The Support Group, in the view of the "Peer Tutoring", will allow us to share and support each other's experiences that will help to support of other parents in the same position.

The program will be implemented in collaboration with the Medical Humanities Project of AOU Cagliari coordinated by Dr. M. Zonza.

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ABS 91

CLINICAL RISK IN NEONATOLOGY: HOW TO PREVENT IT IN DAILY PRACTICE?

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Iatrogenic events (IEs) are a major problem because the forensic costs compromise the basis of the common healthcare, causing not only a biological damage to the patient, but also economic and image deterioration to the insurances, to the hospitals, the staff and the health system.
In 1999, it was found that the secondary deaths to medical errors were between 44,000 and 98,000 per year in the US. In 2002, the Consortium Cineas and Zurich Consulting estimated that about 8 million hospital admissions/year, approximately 14-50 thousand deaths were related to some medical error in Italy.
The neonates undergoing intensive care are particularly exposed to IEs, among all patients who afferent to the health care facilities. In fact, compared to the older age, the incidence of adverse events and medication errors, is greater for the frequent use of drugs that have not a pediatric and neonatal formulation and for the specific pathophysiological characteristics of the newborn, even due to a peculiar pharmacokinetic and pharmacodynamic profile in the neonatal period.
A prospective approach, preventive, continuous and non-punitive especially through the creation of a team dedicated to the careful management of the maintenance of the structure and medical equipment; to the careful control of internal and external emergencies; the computerization and robotization of pharmacological treatments; the use of incident reporting; the control, monitoring and prevention of hospital infections; the creation of itinerary and therapeutic/welfare protocols, are the key to reducing the IEs, improving the quality of care and patient safety.
Therefore, the IEs may decrease just through a Structured Error Handling and the development of a program, centered primarily on the promotion of a cultural change in the approach of the errors, improving and making safer the care system.

ABS 92

DRUG-INDUCED METABOLIC PROFILE CHANGES IN PRETERM INFANTS

F. Bazzini[1], M. Longini[1], M.L. Tataranno[1,2], S. Perrone[1], I. Sirgiovanni[5], S. Negro[1,2], M. Calderisi[6], A. Vivi[4], M. Tassini[4], M. Fumagalli[5], M.J.N.L. Benders[2,3], G. Buonocore[1]

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[3]Centre for the Developing Brain, King's College, London, UK
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[5]Foundation "IRCCS Ca' Granda Ospedale Maggiore Policlinico", Milan, Italy
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BACKGROUND
Clinical management of preterm infants (PREM) represents a challenge for physicians due to the immaturity of organs, tissues and metabolic pathways. Several drugs are used during PREM's hospitalization in NICUs according to local standardized protocols. Metabolomics is a unique metabolic "fingerprint". It refers to the complete set of small-molecule metabolites: metabolic intermediates, hormones and other signaling molecules, to be found within a biological sample.
OBJECTIVE
To test the hypothesis that drugs administered during the first 2 days after birth can modify the metabolic profiling of PREM.
DESIGN/METHODS
94 extremely PREM (GA < 28 wks) were prospectively enrolled in three tertiary European centers: 50 from Utrecht (mean GA 25.9), 11 from Siena (mean GA 25.3), 33 from Milan (mean GA 25.9). Drugs administered during the first 2 days of life were recorded. Urine samples were collected at 48 hours after birth and tested using proton nuclear magnetic resonance (1H-NMR) spectroscopy. NMR urine spectra were analyzed through Principal Components Analysis (PCA) on mean centered and Pareto chart scaled data.
RESULTS
35 different drugs were administered in the first 2 days of life, mainly antibiotics, vitamins

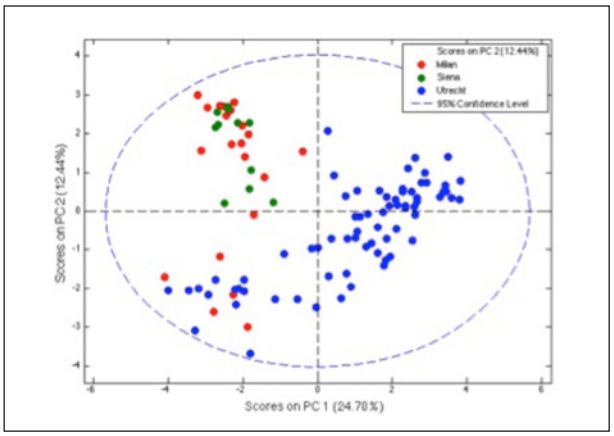

Figure 1 (ABS 92). PCA of urine metabolomics in newborns treated with drugs in the first two days of life.

and caffeine. Utrecht used different medications compared to Milan and Siena, who had 12 drugs in common, whereas Utrecht had only 4-5 drugs in common with the other hospitals. Newborns from Utrecht had a different metabolic profile compared to Siena and Milan, as shown by the scores of the second component (PC2) of the PCA analysis (**Fig. 1**).

CONCLUSIONS

The different therapeutic approaches among NICU determine specific and locally different metabolic profiles in PREM. Our data show that even small differences in drug therapy result in wide metabolic changes, suggesting that PREM have high differences in metabolic susceptibility to drugs. . More specific analyses are needed to discriminate metabolic differences occurring in relation to gestational age and severity of illnesses.

ABS 93

USEFULNESS OF MOLECULAR GENETICS IN THE DIFFERENTIAL DIAGNOSIS OF MELANOCYTIC SPITZOID LESION IN CHILDREN

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INTRODUCTION

In 1948, Sophie Spitz, coined the term 'childhood melanoma' for a group made up of melanocytic skin lesions developing in children, characterized by fusiform and/or epithelioid melanocytes. Later on, it was reported that these tumors may arise in adulthood, usually manifesting an indolent biological behavior. This finding led to the introduction of the term "Spitz nevus" to indicate their benign nature. The extreme rarity in of adverse outcome death of in patients with Spitz nevus arising before puberty, induced Dr. Spitz to believe that the onset after puberty was a determining risk factor for increased mortality. The same lesion might be considered benign when diagnosed in before puberty, and potentially malignant when diagnosed in adulthood. Tumors with spitzoid features showing an aggressive clinical behavior, similar to conventional melanomas, were called "spitzoid melanomas". The differential diagnosis between Spitz nevus and spitzoid melanoma if often challenging, due to overlapping morphological features. Some Authors proposed to replace the term Spitz nevus with the more suitable term of Spitz tumor, to be further classified as: 1) Spitz tumor without atypia, 2) Spitz tumor with atypia and 3) spitzoid melanoma [l-4]. Pursuing an effort to unravel the complexity of spiyzoid tumors, advances in the molecular characterization lead to the introduction of fluorescence in situ hybridization (FISH) (both conventional melanoma probe [RREB1 (6p25), MYB (6q23), CCND1 (11q13) and centromere 6] and 9p21 probe) and aCGH for risk assessment [5-6]. In particular, the 9p21 FISH probe may further support the diagnosis of lesions with Spitzoid morphology, and increase the sensitivity of the current melanoma FISH probe assay in identifying Spitzoid melanomas without affecting specificity.

CASE REPORT

A 10-year-old male presented with a recurrent papule 4 mm in size on the left cheek (**Fig. 1**). The lesion had

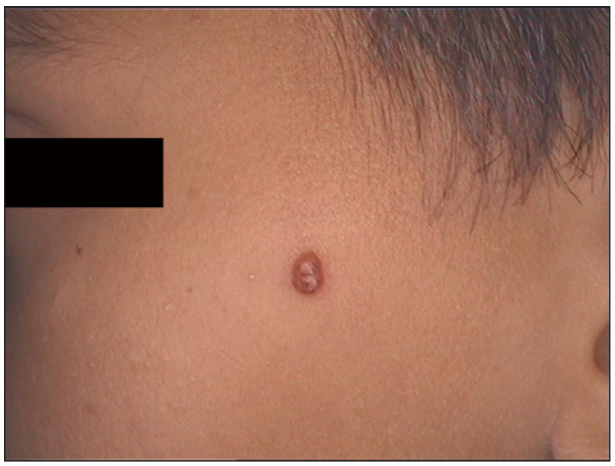

Figure 1 (ABS 93). Papular lesion presenting in the left cheek bone, of a 10-year-old child.

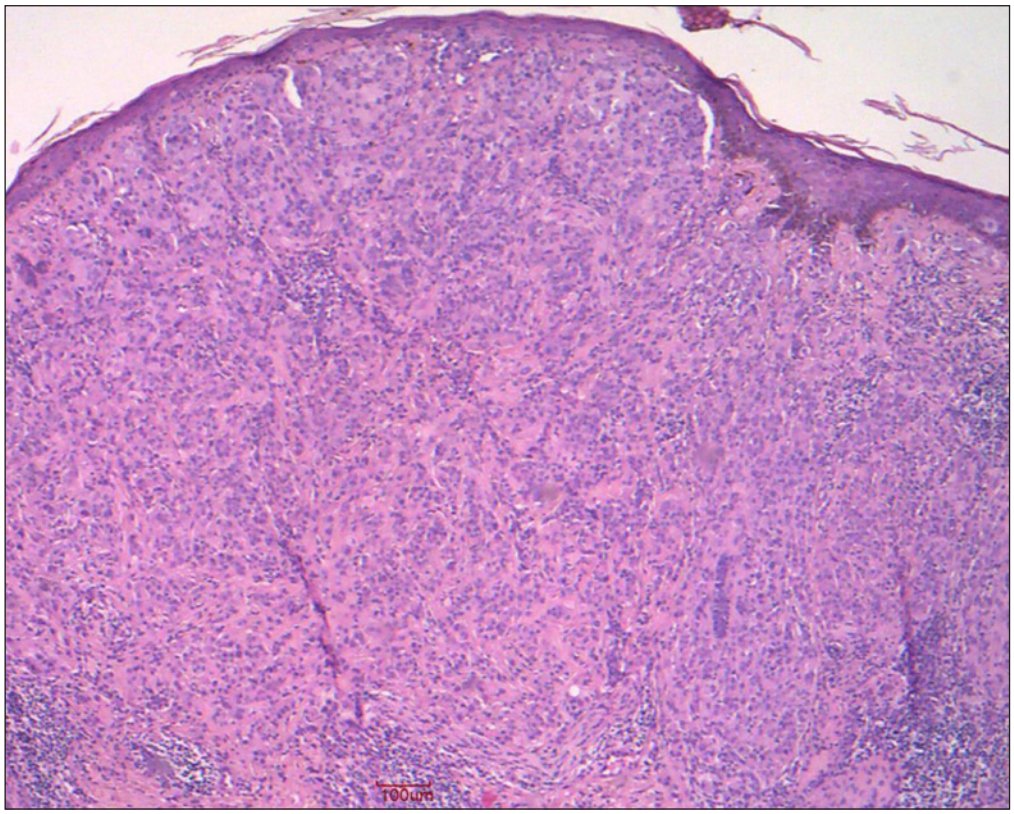

Figure 2 (ABS 93). Melanocytic predominantly dermal proliferation (scal-bar 100 μm), almost circumscribed, slightly asymmetrical, with a diameter of 4.0 mm and 1.5 mm in Breslow thickness, consisting of epithelioid melanocytes (scal-bar 10 μm).

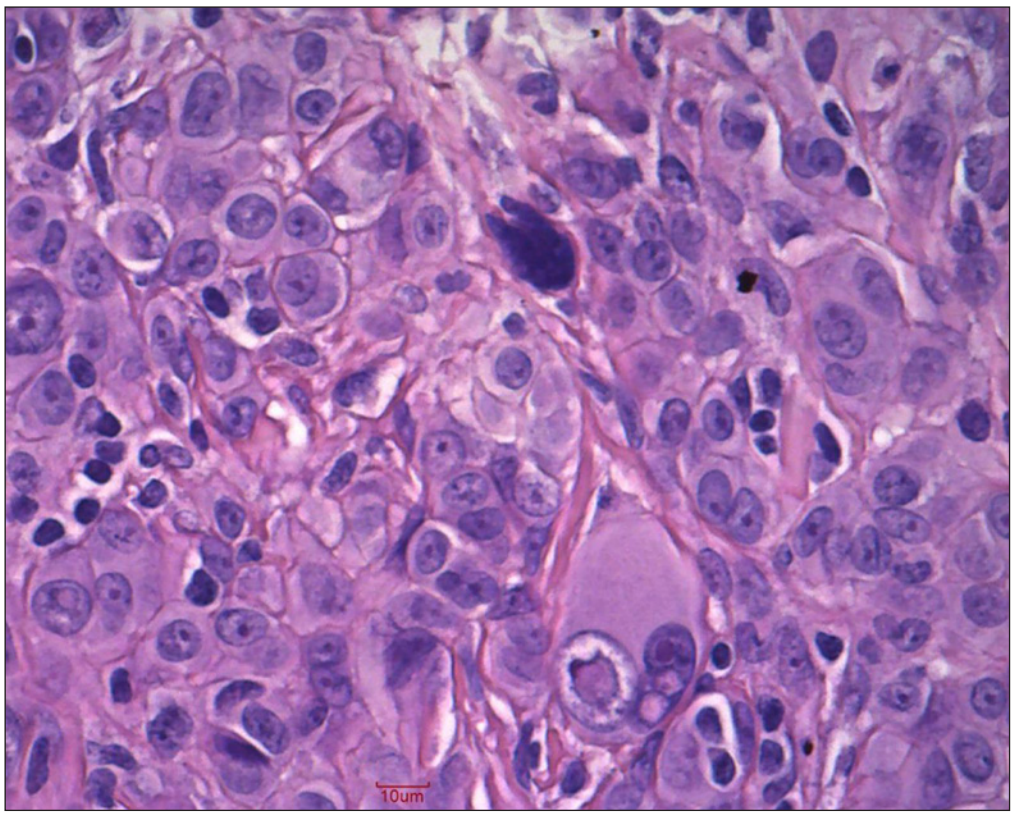

Figure 3 (ABS 93). Epithelioid melanocytes with large cytoplasm, large nucleus, sometimes iperchromic and with evident nucleolus (scal-bar 10 μm).

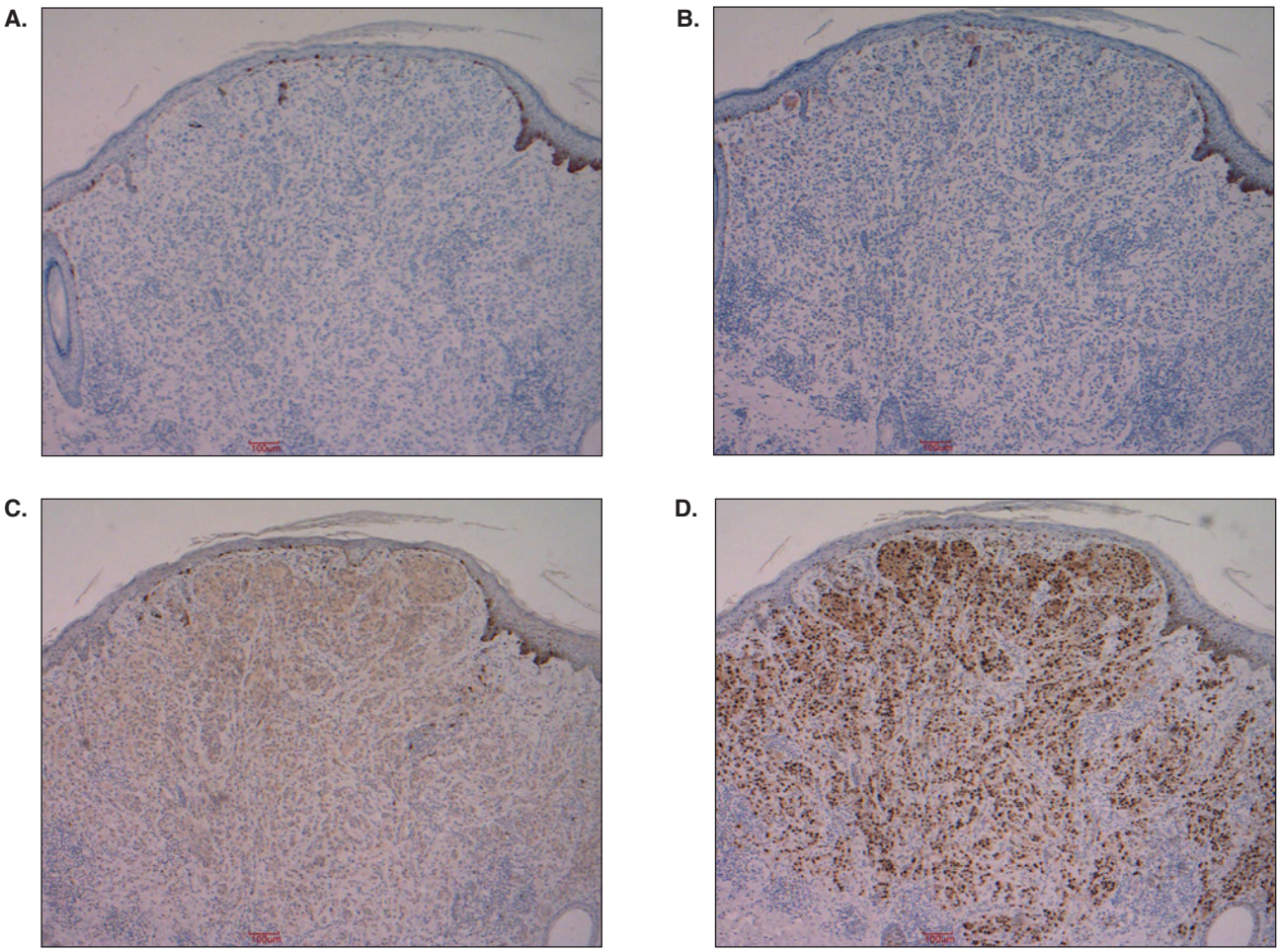

Figure 4 (ABS 93). Immunohistochemical investigations: **A**)HMB45, **B**) MelanA, **C**) p16 and **D**) ciclina D1 (scal-bar100μm).

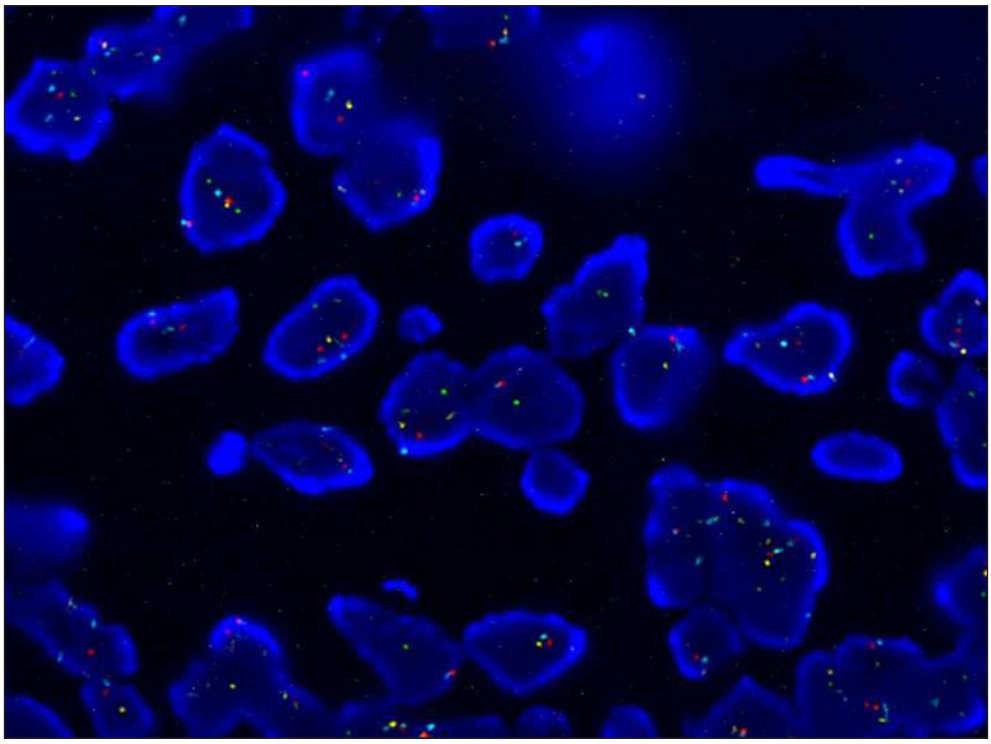

Figure 5 (ABS 93). FISH images with preservation (diploid copies) of the four probes, indicating a negative result.

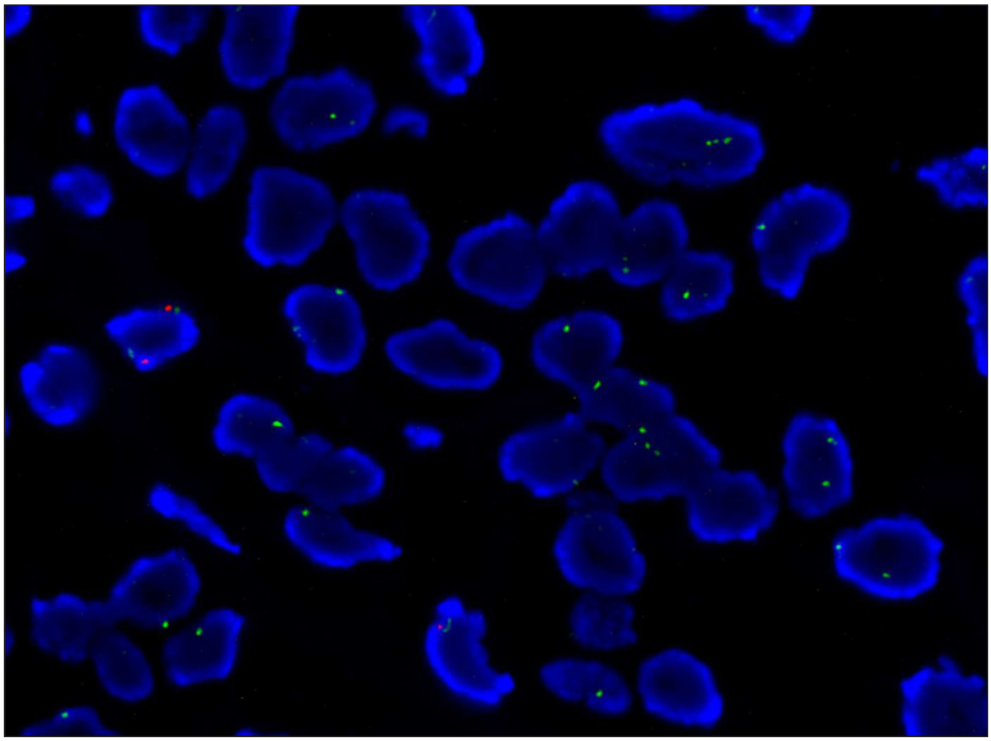

Figure 6 (ABS 93). A FISH analysis showing p16(9p21) deletion.

been previously excised (7 months before) without histopathological examination. Histopathologic examination showed predominantly dermal (**Fig. 2**) atypical melanocytic proliferation composed of spindle and epithelioid cells (spitzoid cytology) (**Fig. 3**). The lesion was asymmetrical and well circumscribed and extended to the reticular dermis up to 1.5 mm in thickness. There was no obvious maturation. Mitotic activity was 1-3 mitoses/ mm^2. There was a brisk intralesional lymphocytic infiltrate. Immunohistochemical analyses showed focal immunoreactivity for HMB-45 (**Fig. 4A**) and MelanA (**Fig. 4B**) in the superficial dermal and in the intraepidermal components. P16 was negative (**Fig. 4C**) while nuclear reactivity for Cyclin D1 was observed (**Fig. 4D**). Proliferation index (Ki67) was around 2-3%. The morpho-phenotypic findings were consistent with a diagnosis of recurrent atypical Spitz tumor, although a diagnosis of spitzoid melanoma could not be ruled out. FISH analysis for the detection of copy number of genes RREB (6p25), MYB (6q23), CCND1(11q13) and the centromere of chromosome6 (6p11.1-q11.1,), highlighted absence of chromosomal abnormalities. FISH analysis with LSI p16 (9p21) OS/CEP 9SG probe showed the presence of 9p21 homozygous deletion. The final diagnosis was "Spitzoid melanoma of childhood", or "Spitzoid melanoma, childhood type". This term has been introduced by in cases of a Spitzoid neoplasm in childhood to define tumors that are currently interpreted as clinically-indolent, low-grade melanomas with low metastatic potential.

We have described a difficult spitzoid tumor in pediatric age in which the final diagnosis resulted from the integration of morphological features with molecular genetic studies.

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ABS 94

JOB PROFILES IN THE NURSERY

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INTRODUCTION

Before we talk about professional profiles we should make a reflection on the concept of competence and more precisely from the reasoning which defines the historical conception of the trade to the concept of competence. The word competence has become the key word in the fields of human resources management in modern organizations. In literature, there is currently no model of shared responsibilities, each school of thought has its own solution. In language and professional training, there is often little clarity about what is meant by competence, but despite the differences you can identify some key features commonly attributed to the construct of competence.

ANALYSIS OF CONTEXT

I will introduce the concept of multi-professional and interdisciplinary, in a context such as that of neonatal care where there are many figures who revolve around the newborn. All actors in the process of care such as nurse, pediatric nurse and midwife, reflect what has just been said. First the physiological newborn to be serviced according to the best available evidences as quoted in all the codes of conduct currently in force. The keyword is the collaboration between the parts. By integrating the members of an organization, they have expressed their intention to contribute to the achievement of the established institutional order, that is the health of the newborn.

There is yet another aspect to consider, recent studies confirm that for 2,677 accidents in medicine and contributing factors for 5,610, 50% can be attributed to the lack of non-technical skills [1]. Communication barriers between the various professional hierarchies, the inability to recognize human fallibility, the lack of situational awareness, often combine with each other, generating what James Reason [2] defines "the trajectory of opportunity" which leads to the incident.

CONCLUSIONS

This new form of respect for the birth therefore requires, in addition to technical skills, scientific and social skills, the promotion of non tecnichal skills, which make it less intrusive and prescriptive the care relationship. Taking into account the health of the protagonists of the birth, their physiological, emotional, relational, of belonging and self-realization [3] needs.

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ABS 95

PAX2 AND PHENOTYPIC EXPRESSION

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BACKGROUND

PAX2 gene encodes a transcription factor that belongs to the paired-box family of homeotic genes and is widely expressed during the development of both ductal and mesenchymal components of the urogenital system. PAX2 mutations, arising de novo or inherited in an autosomal dominant fashion, have been associated with renal coloboma syndrome (RCS) (OMIM 120330). PAX2 mutations have also been reported in patients with renal hypodysplasia without eye anomalies, and recently in some FSGS families.

CASE REPORT

DC, male, 34 weeks with IUGR, born by CS from mother with gestosis. Birth weight 2,000 kg. APGAR score 7 and 8 at 1 and 5 minutes respectively. No respiratory distress. On day 2, his exams were unremarkable except of serum creatinine (1.5 mg/dl). Diuresis, BP and creatinine was monitored: serum creatinine remained high (1 mg/dl). Renal ultrasound scan revealed bilateral renal hypodysplasia. Eyes examination showed bilateral neural optic coloboma. The diagnosis was renal coloboma syndrome. Mother's history showed mild renal failure from 14 years old due to glomerulonephritis without ocular anomalies. Genetic analysis found c.239C>T (p.Pro80Leu) in both child and mother.

CONCLUSION

PAX2 variants may lead to an expanded phenotypic spectrum. Of note, most PAX2 mutations resulting in

truncated proteins lead to renal coloboma syndrome, more severe phenotype. In our patients renal coloboma syndrome was related with a missense mutation with a different phenotype expression in the mother. Moreover c.239C>T (p.Pro80Leu) mutation was observed firstly in one FSGS family and never in renal coloboma syndrome.

ABS 96

INFECTION WITH *HELICOBACTER PYLORI* IN CHILDREN

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The infection with *Helicobacter pylori* (*H. pylori*) is a very important pathogenetic link in children with gastroduodenal diseases. The role of *H. pylori* bacteria in the pathogenesis of digestive diseases is well established and currently known, but recent research argues the involvement of *H. pylori* in the occurrence of extradigestive diseases. The prevalence of infection differs from one geographical area to another, being in decline in developed countries and keeping stili high among the population of developing countries. It is known that there are specific bacteria that affect a particular organ, but also can cause systemic changes throughout the body. Chronic infection with *H. pylori* is associated with anemia, loss of weight and low stature. Also, *H. pylori* is involved in diabetic patients status and food allergies. Increasing the quality and accuracy of detection of infection with *H. pylori* causes a low rate of complications of there diseases by using constantly improved treatment strategies.

ABS 97

SYSTEMIC INFECTION BY *CANDIDA SPP.* RESISTANT TO ECHINOCANDINS

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BACKGROUND

The systemic fungal infection (SFI) is a primary cause of morbidity and mortality in VLBW infants.

CASE REPORT

We describe a case of SFI resistant to treatment with echinocandins. P.A. born at 30 wks GA from cesarean section. Weight 1,070 g. APGAR 7-8. Assisted in N-CPAP for 7 days, then breathes independently. MEF by 2nd day. Sudden deterioration of the clinical state in 21st day of life with need of mechanical ventilation, shock therapy, antibiotics and antifungal therapy with micafungin at dose of 5 mg/kg. We found blood culture positive for *C. albicans*, cerebrospinal fluid and urine cultures negative, high CRP, neutrophilia and thrombocytopenia. Cerebral ultrasound showed a cerebritis. Abdominal US and thoracic XR were normal. Because of the worsening of the clinical status and the persistent positivity of the second culture carried out after 7 days of therapy, micafungin was increased to 8 mg/kg. We found swollen knees from possible osteomyelitis and outbreak of chorioretinitis. We stopped micafungin and started double antifungal therapy with liposomal amphotericin B at dose of 5 mg/kg and fluconazole (better intraocular diffusibility) at dose of 12 mg/kg. After 1 week of double antifungal therapy clinical conditions and cerebral ultrasound improved fast. Cerebral MRI performed at 2 months was normal. Later slow resolution of the osteomyelitis and chorioretinitis. Liposomal amphotericin B and fluconazole were continued for 5 weeks, then only intravenous fluconazole for 2 weeks, and finally orally at the same dose continued at home for 6 months. At 6 months after discharge the clinical conditions are well.

CONCLUSIONS

We describe this case to show that 1) the SFI is not limited to a blood-borne sepsis, but often involves multiple organs despite adequate therapy; 2) we have to explore all organs and change recomended treatment with echinocandins, even if it is in contrasts with the in vitro sensitivity. That depends on the different diffusibility of the antifungals available and on the type of organ involved.

ABS 98

PREDICTIVE BIOMARKERS OF PRE-ECLAMPSIA

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Pre-eclampsia (PE) is one of the most human common pregnancy complication characterized by placental and maternal vascular dysfunction and represents one of the major causes of neonatal morbidity and mortality especially in low- and middle-income countries. It consists of a hypertensive multisystem disorder that affects 3-7% of women during the second half of the pregnancy. In 2014 the International Society for the Study of Hypertension in Pregnancy defined the PE as a de-novo hypertension present after 20 weeks of gestation coupled with proteinuria, other maternal organ dysfunction (renal insufficiency, liver involvement, neurological or haematological complications, uteroplacental dysfunction), or fetal growth restriction.

Thus, PE is considered a syndrome with multisystem involvement, so the ideal predictive test for this pathology should utilize a combination of many predictors derived from only maternal source, only fetal-placental source or both.

Although anticipatory treatments have been studied widely, nowadays it hasn't been already discovered an effective intervention to avoid PE to develop. The administration of aspirin before 16 weeks of gestation as preventative management of PE seems to reduce the inflammation and to improve trophoblast invasion reducing significantly the develop of the disease. However, the delivery still remain the more appropriate intervention if the fetus conditions and gestational age allowed it. PE can require delivery before 34 weeks (early forms), between 35 and 37 weeks (intermediate forms) or after 37 weeks (late forms). The early onset form is associated with abnormal placentation and fetal growth restriction, while the intermediate and late onset forms, the more recurrent forms, are often associated with maternal metabolic disorders.

Measurement in early pregnancy of a variety of biophysical and biochemical markers implicated in the pathophysiology of PE combined with clinical risk factors (such as familial history of PE, history of previous pregnancies complicated by PE or with chronic/gestational hypertension among the most relevant) has been proposed to predict the development of the syndrome mitigating an adverse outcome. Nowadays, no single biomarker can predict PE and many features complicate the prevention of PE pregnancies. Most are attributed to unknown etiology, time of onset, several presentations of the disease, duration of progression.

This presentation aims to evaluate the most promising biochemical markers in maternal blood and their related predictive value and grouping them on the basis of the pathogenic mechanism in which they are involved such as inflammation, hypoxia and oxidative stress and endothelial dysfunction.

ABS 99

THE NEW FIGO GUIDELINES FOR DIAGNOSIS AND MANAGEMENT OF GESTATIONAL DIABETES

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BACKGROUND/OBJECTIVES

Hyperglycemia is one of the most common medical conditions encountered during pregnancy; more women enter pregnancy with risk factors that make them vulnerable to hyperglycemia than ever before. Low and middle income countries account for 85% of the annual global deliveries and 80% of the global diabetes burden. Gestational Diabetes Mellitus (GDM) represents 84% of cases of hyperglycemia in pregnancy; given its association with higher incidence of maternal, fetal and neonatal morbidity as well as long term sequelae that can affect women and their offspring, international guidance on screening, diagnosing and providing care for women with GDM is of utmost importance.

METHODS

The International Federation of Gynecology and Obstetrics (FIGO) brought together international experts in GDM with the following aims: 1) To raise awareness of the links between hyperglycemia and poor maternal and fetal outcomes as well as future health risks to mother and offspring and demand a clearly defined global health agenda to tackle this issue, and 2) To create a consensus document which provides guidelines for testing, management and care of women with GDM and to disseminate these evidence based guidelines internationally.

RESULTS

A consensus document offering a pragmatic guidance on care for women with GDM was produced. The document received support from a large number of international groups. Suggestions are provided for various regional and resource settings based on their financial, human and infrastructure resources. Recommendations cover the following: prioritization of GDM as a public health issue, universal testing of pregnant women for GDM, criteria for diagnosis, and management of GDM including lifestyle and

pharmacological management throughout pregnancy, postpartum and after.

CONCLUSIONS

A document providing international guidance to healthcare professionals on care for women with GDM was long overdue. As pregnancy offers a window of opportunity to provide maternal care services to reduce traditional maternal and perinatal morbidity and mortality indicators, improve women's future health, as well as address intergenerational prevention of NCDs, healthcare workers must utilize this resource to improve diagnostics and care for women in their countries. Public health measures must work to increase awareness, access, affordability and acceptance of preconception counselling and antenatal and postnatal services for girls and women.

ABS 100

BRAIN MYSTERIES FROM THE WOMB TO THE ADULT: IS AGGRESSION CONGENITAL?

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Human aggression encompasses a wide range of behaviors and is related to many psychiatric disorders. Patients affected by bipolar disorder, psychosis, impulse control and personality disorders might present rates of aggression ranging from 26% to 84% [1]. Aggression appears to have a specific longitudinal developmental trajectory starting from toddlerhood to adolescence [2-5]. Environmental factors appear to modulate the liability to aggressive behavior determined by genetic factors [6]. Indeed, the heritability of aggressive behaviors is substantial (28% to 40% depending on the symptomatology assessed) [7]. Consistently, having a mother with early onset antisocial behavior was a potent predictor of high levels of physical aggression in children assessed longitudinally from birth to 42 months of age [4]. At the same time, the developmental trajectory to aggression appears to be modulated by environmental factors, such as low income, presence of mothers who smoked during pregnancy, mothers' coercive parenting behavior, and family dysfunction [4]. Of note, specific epigenetic signatures of peripheral white blood cells seem to correlate with the manifestation of physical aggression during childhood [8]. Taken together, these findings show that relevant antecedents for the manifestation of aggressive behavior later in life might exert an effect before birth, acting on and modulating the liability threshold determined genetically. This amount of evidence carries substantial clinical implications, as these environmental risk factors might be amenable to change, leading to effective preventive strategy and tailored treatment.

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ABS 101

SUPPORTING BREASTFEEDING IN HOSPITAL. NURSE LED EXPERIENCE AND NURSING STRATEGIES

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BACKGROUND

A growing number of studies carried out over the last 15-20 years have shown that, without a doubt, breastfed babies are healthier than formula fed babies. Numerous scientific studies have revealed the advantages and importance of mother's milk

for the health and growth of our babies. Besides its precious mix of nutrients, immune cells and stem cells, the recent and interesting discovery that highlights the important relationships between the components of maternal milk and intestinal microbiota microorganisms, shows us once more how precious and perfect this natural and vital element is.

Breastfeeding for 6 months (WHO recommends) is the best protection for every baby and continuing to breastfeed after weaning keeps up this protection. Other studies have noted that besides strengthening the bond between mother and child, women who breastfeed for long periods are more protected from breast and ovarian tumors and osteoporosis.

However, even when health workers are aware of this and are in favor of breastfeeding, they often do not know well how to promote it.

Although the basics about breastfeeding can be learnt from theoretical and practical lessons, supporting a mother in order to breastfeed successfully (empowerment) is based on an interpersonal relationship which requires sensitivity when listening and psychological support.

Health workers play a crucial role in helping with starting to breastfeed in hospital, because added to their medical role they can have an effect on a wide social spectrum and furthermore can influence programs and attitudes towards breastfeeding.

OBJECTIVES

The Nurse Led Clinic (started in 2011), was set up in order to offer post-demission assistance. The continued assistance is aimed particularly at supporting first-time mothers in breastfeeding (following the WHO/UNICEF guidelines).

The aims of the nurse-led neonatal clinic can be summed up in four fundamental points:

- support to exclusive breastfeeding;
- support to the mother-baby-father relationship;
- information: health education, pediatrics, auxological and growth check-ups, jaundice check-ups and "care" of the neonate;
- a moment of transition between hospital and pediatrician and/or family clinic.

METHODS

- Check-ups in Nurse Led Clinic within the first week of life and further check-ups within a month.
- Telephone interviews within a month of life and feeding assessment.

We use the "Latch Breastfeeding Assessment" chart in our research. The Latch score chart is an important evaluation tool.

The "Latch Breastfeeding Assessment Tool", is a chart for evaluating feeds and was created in 1994 by Jenson D, Wallance S, KelesayP.

Such a systemic method is able to supply individual data about the initial phases of breastfeeding.

The Latch Score is simple and fast for health workers to complete.

The cut-off indicated suggests that in all cases in which the score is less than 7, the mother-child couple could benefit from support and/or assistance from the health workers in order to help with successfully starting breastfeeding.

The advantage of the Latch chart is that it provides health workers with a clear and standardized report about each feed. In this way it is easy to identify which mother-child couple needs support and assistance both during the stay in hospital, after being discharged and the first few days post-partum.

RESULTS

Evaluation of feeds using the Latch Breastfeeding Assessment Tool and telephone interviews

All cases in question (200) (**Tab. 1**), were assessed using the Latch chart the day they were discharged from hospital and during the first check-up in the Nurse Led Clinic (4-6 days). In some cases a second check-up was arranged after 7-15 days, in all the other cases the mother was contacted by telephone 15-20 days after being discharged from hospital. This made it possible to obtain positive data in real time about breastfeeding. Moreover it provided us with interesting proof of the effectiveness of information and support offered by the Nurse Led Clinic.

Nurse Led Clinic, started in 2011, up to now has had 1,399 mother-baby couples. **Tab. 2**, indicates the ways of feeding at the first check-up in the clinic (4-6 days after being discharged from hospital).

Table 1 (ABS 101). Exclusively breatfeeding after hospital discharge (Latch table < 7, total: 200).

A.

Latch table < 7 on being discharged from hospital	Latch table < 7 and exclusively breastfeeding at first Nurse Led check-up (4-6 days)	Telephone interview and exclusively breastfeeding 15-30 days after hospital discharge
Total: 200	108 (54%)	131 (66%)

B.

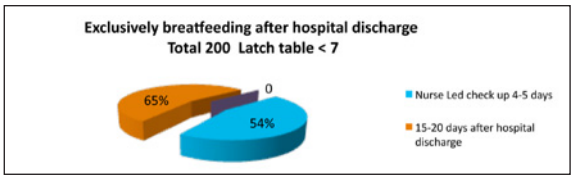

Table 2 (ABS 101). Ways of breastfeeding Nurse Led Clinic.

	2011	2012	2013	2014	2015 (6 months)
Exclusive breastfeeding	198 (68.5%)	209 (70%)	178 (58.9%)	201 (63.8%)	98 (50%)
Predominant breastfeeding	14 (2%)	18 (6%)	38 (12.5%)	61 (19%)	53 (27%)
Complementary breastfeeding	52 (18%)	63 (21%)	84 (27.8%)	47 (14.9%)	41 (20%)
Lactation formula	25 (9%)	7 (2.5%)	2 (0.6%)	6 (1.9%)	4 (2%)
Total babies	289	297	302	315	196

CONCLUSIONS

In recent years numerous WHO/UNICEF theoretical and practical courses on breastfeeding have been promoted for health workers. All the health workers who come into contact with expectant mothers should promote and support breastfeeding. However, often in hospitals the situation is very different. The personnel does not always have sufficient knowledge and practice and has little experience in providing adequate assistance to the mother-child couple.

Adequate assistance for each individual situation requires the health worker to be welcoming, compassionate and a good listener.

Breastfeeding support is a strong model for cultural and organized commitment in a modern health system. For this reason the experience of the Nurse Led Clinic is a positive strategy, a chance to improve and enrich the nursing assistance offered to mothers and children.

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ABS 102

DATA ON MATERNAL OBESITY CONCERNING OUTCOMES OF ASPHYCTIC TERM NEWBORNS UNDERGOING HYPOTHERMIA THERAPY

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HYPOTHESIS

The evaluation of the follow-up of asphyctic term newborns undergoing hypothermic therapy (HT) may allow us to assess not only the effects of hypothermia but also certain factors that are mainly associated with worse prognosis either *quod vitam* than *valetudinem*.

MATERIALS AND METHODS

Twelve asphyctic term newborns were included in the study. The degree of hypoxic-ischemia encephalopathy (HIE) was determined following the criteria of Sarnat (modified from Shankaran et al.) [1, 2]. All the newborns included in the study had a second grade of HIE whilst, those with severe or moderate to severe HIE as well as the cases with diabetic or with hypertensive mothers were excluded. The cases studied were selected taking into consideration the age of the mother, the status of maternal nutritional, the type of childbirth, and the type of the insult. All the HT criteria (Italian Society of Neonatology, 2013), which suggest performing blood gas analysis on arterial blood from the umbilical cord and the baby itself within 1 hour from childbirth, were satisfied.

RESULTS AND CONCLUSION

Seven newborns of twelve underwent HT for 72 hours without any complications; five of twelve had difficulties in being disconnected from the air ventilation. Among them, two of five had a delayed onset of sucking reflex, consequently they were fed with the gavage method for 8 days from the end of HT and the start of heating process. All twelve newborns were followed for 3 years. The five infants with problems of rehabilitation, although having the same parameters of the eight (without

complications) *id* gestational age, percentile of weight, length, high foetal stress blood gas analysis and HIE insult, they had a significant incidence of severe maternal obesity.

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ABS 103

NEONATAL PAIN MANAGEMENT

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Analgesia is a basic human right: in the 21st century it is not acceptable that patients should undergo pain without any treatment. And this should be mandatory when we deal with babies, the most fragile patients. Unfortunately, many procedures are still performed on them without an effective analgesia, even during clinical trials. This is what emerges from the analysis of scientific literature. Many attempts have been done to overcome babies' pain, and now we have good and effective drugs and treatments that allow us to say that every suffering in a baby patient is inacceptable and should be labeled not only as malpractice, but as abuse. This is why some years ago we started a program with the aim of finding new analgesic treatments and new tools to assess infants' pain level. The first step was to find a reliable pain treatment for newborns: we achieved to find a new non-pharmacological type of analgesia that we called "sensorial saturation" [1-3]. It is based on the evidence that multiple stimuli can antagonize the arrival of pain to the conscience, through a "gate-control" strategy. Sensorial saturation is a reinforcement of the well-known analgesic effect of oral sugar, which is only partially effective. It is composed of three steps, all aimed to attract the baby's attention: taste (giving oral sugar), touch (massage), talk (speaking to the baby). The marker that the union of these three "Ts" is working is the rhythmic sucking: when the baby begins sucking rhythmically, the puncture can be performed with a high rate of analgesic success.

The second step of our research was to find an easy tool to measure pain: available pain scales for acute pain are rarely used in clinical practice, because they are too complicated and oblige nurses to choice whether they assess pain or perform the procedure. We verified that the pain score given by the procedure-performing nurse, using two of the most used scales, much differs from the score given in a second time, when the same procedure is recorded via a video camera [4]. We also verified that more than thirty method exist to assess newborns' pain, and this is the sign that a golden method has not yet been achieved [5]. We analyzed more than sixty babies cries during a heelprick and saw that when pain is high cry becomes rhythmic and the first moan emitted is high-pitched [6]. On this basis we developed the ABC pain scale that we validated in both term and preterm babies [7, 8].

Recently we underlined [9] that the fundamental difference between scoring pain, that enable us to assess the level of pain, and detecting the absolute presence or absence of pain. In fact our main goal is the pain rapid detection to immediately stop it.

In conclusion, caregiver should avoid neonatal pain without any tolerance. Pain should be recognized and managed in everyday practice.

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ABS 104

STRATEGIES AND CHALLENGES FOR NEXT GENERATION METABOLOMIC ANALYSES

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Omics technologies are increasingly being applied to study complex biochemical and physiological states. Analysis of small-molecules or metabolites, metabolomics, has been widely used to characterize organismal phenotypes including identification of biomarkers associated with autism, infant birth weight, metabolic syndrome and cancer. Next-generation approaches integrating genomic, proteomic and metabolomic measurements have shown promise to aid researchers to better understand otherwise recalcitrant biochemical process and identify robust biological markers for disease diagnosis and treatment efficacy monitoring. Robust interpretation of experimental results measuring discreet biological domains remains a significant challenge in the face of complex biochemical regulation processes such as organismal versus tissue versus cellular metabolism, epigenetics, and protein post-translational modification. Integration of analyses carried out across multiple measurement or omic platforms is an emerging approach to help address these challenges. Key challenges remain for metabolomic researchers including large-scale studies data normalization, multivariate analysis, visualization and omics data integration.

Implementation of data normalization approaches including internal standard and quality control based methods maybe required to effectively remove analytical batch effects. Emerging methods incorporating replicated measurements to carry out LOESS or other non-linear based smoothing models have shown promise to deal with complex analytical modes of variance.

Omics integration methods are required to combine and analyze biological measurements carried out across multiple platforms within a biological context. Leading approaches for omic integration include biochemical pathway, network-based and empirical correlation-based methods.

Given the aforementioned challenges, advanced data analysis tools are required to carry out effective omic and specifically metabolomic data interpretation. Modern data analysis tools are necessary to allow researchers to implement analysis pipelines incorporating data normalization, integration, multivariate analysis and ultimate interpretation with in a biochemical context. An emerging approach termed network mapping shows promise to effectively integrate statistical, multivariate and functional domain knowledge to calculate richly connected biochemical networks which can highlight metabolic perturbations specific to researchers' areas of interest.

ABS 105

NEW PERSPECTIVES FOR THE STUDY OF SYSTEMIC SCLERODERMA

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INTRODUCTION

Systemic scleroderma (SS) is an autoimmune disease involving various organs and systems primarily affecting the skin. The prevalence of the disease in Italy is about 70,000 patients with approximately 300 new cases per year. Women are affected more than men by a ratio of about 3:1. The age of onset is generally comprised between 45 to 65 years however the causes are still unknown. Some hypotheses suggest a link with certain medications or exposure to some environmental toxicants (vinyl chloride, trichloroethylene, silicone). The disease has no hereditary factors although there is an association with certain genes (HLA A1, B8, DR1, DR3, DR5, DR11) [1]. A new approach to the study of these diseases can be given by metabolomics, which through the study of the metabolome may help for identification of the key metabolites characterising the disease. The metabolomics approach consists on the characterization and quantification of all the endogenous and exogenous metabolites aiming at capturing a certain number that can be intended as descriptors for a metabolic status. Indeed, this

concept is particularly interesting for the study of SS, which appears to be partially due to changes caused by the external environment.

AIM

The purpose of this study was to analyse by GC-MS urine samples collected from a court of patients affected by SS and compared with a control court matched with age and gender.

MATERIAL AND METHODS

In this study, 30 subjects affected by SS and 20 controls were enrolled. Urine samples containing an aliquot of sodium azide 1% were collected and stored (-80°C) at University of Florence, Italy. Afterwards, samples were analysed using an Agilent 5975C mass spectrometer interfaced to the GC 7820 equipped with a DB-5ms column (J & W), injector temperature at 230°C, detector temperature at 280 °C, helium carrier gas flow rate of 1 ml/min. The GC oven temperature program was 90°C initial temperature with 1 min hold time and ramping at 10°C/min to a final temperature of 270°C with 7 min hold time. 1 µL of the derivatized sample was injected in split (1:20) mode. After a solvent delay of 3 minutes mass spectra were acquired in full scan mode. Each acquired chromatogram was analysed by means of free software AMDIS (Automated Mass Spectral Deconvolution and Identification System) and NIST08 (National Institute of Standards and Technology's mass spectral database). A multivariate analysis was performed using metaboanalyst [3] by which a model PLS-DA was built using the classification of the two groups, pathological-controls.

RESULTS AND DISCUSSION

A preliminary investigation indicates 5 variables of importance (VIP) metabolites resulting principal discriminant between the two groups. Such compounds will be useful in the future for determining the most important pathways that are activated in SS. This preliminary analysis suggests that more research efforts should be devoted to identify a characteristic fingerprint for SS.

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ABS 106

SLEEP RESPIRATORY EVENTS AND CLINICAL PARAMETERS AND IN A GROUP OF OBESE CHILDREN

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INTRODUCTION

Obstructive sleep apnea in children is more frequently due to tonsil and adenoid hypertrophy. Children who are obese and have obstructive sleep apnea might continue to have breathing difficulties even after tonsils and adenoids removal.

POPULATION STUDY

The relationship between ambulatory clinical parameters and sleep respiratory events were assessed with a type III polysomnography in a group of obese children. A prospective respiratory sleep study was performed between 2013 and 2015 on 42 consecutive exogenous obese children.

METHODS

The tonsils and palate position were subjectively measured using a grading system. The Brouillette test was applied for scoring obstructive sleep apnea in children. An overnight limited-channel polysomnography was performed using a type III portable ambulatory device (SOMNOscreen™ PSN, SOMNOmedics GmbH, Randersacker, Germany). Correlation and linear regression analysis between sleep respiratory parameters (apnea-hypopnea index, respiratory disturbance index, mean SpO_2, oxygen desaturation index, % snoring and phase angle) and clinical parameters was performed. Statistical analysis was done using SPSS® Statistics 19.0 software for Windows®.

RESULTS

Statistical analysis showed that Friedman palate position correlated with respiratory disturbance index.

CONCLUSIONS

In conclusion, in our cohort of obese children, palate position was the best clinical parameter for predicting sleep respiratory disorders. Palate

position should be taken into account for the clinical evaluation of obese children.

ABS 107

ECHOCARDIOGRAPHIC ASSESSMENT OF DUCTAL SIZE COMPARED TO BODY SURFACE AREA FOR MANAGEMENT OF PATENT DUCTUS ARTERIOSUS IN PRETERM NEONATES

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INTRODUCTION

Patent ductus arteriosus (PDA) is the most common congenital cardiovascular malformation in preterm neonates. Incidence is about 28% in very low birth weight (VLBW) neonates and rises up to 60% in extremely low birth weight (ELBW) neonates less than 28 weeks of gestational age (GA). While ducuts arteriosus is important for fetal circulation, its persistence beyond transitional neonatal period is often associated with increased morbidity and mortality. Since there is no unanimous consensus on those infants who may benefit from intravenous ibuprofen, echocardiographic assessment of ductal diameter compared to allometric measures as body surface area (BSA) could be a potential predictor.

OBJECTIVES

The aim of this study is to evaluate an hemodynamically significant PDA after measuring the inner ductal diameter adjusted for BSA (according to Mosteller formula).

METHODS

Medical records of 37 ELBW (birth weight: 805 ± 135 grams; GA: 26 ± 2.2 weeks) and 22 VLBW (birth weight: 1,196 ± 140 grams; GA: 29 ± 1.78 weeks) neonates admitted from 1st January 2009 to 31th December 2014 to the Neonatal Intensive Care Unit of Di Venere Hospital, Bari, Italy, with diagnosis of PDA have been evaluate. The inner ductal diameter (cm) measured by echocardiography within 48 hours of life in left parasternal short axis view of great vessels, as reported in the medical record, has been retrospectively indexed for BSA (m^2) in each patient.

RESULTS

The average ratio between inner ductal caliber and BSA associated to a spontaneous closure was 1.6 cm/m^2 in ELBW (8%) as well as in VLBW (27%) neonates; the same ratio in those responsive to a single course of ibuprofen (55%) was 2.4 cm/m^2 and 2.2 cm/m^2, and a ratio of 2.6 cm/m^2 and 1.9 cm/m^2 was found in neonates who required a second course of ibuprofen in ELBW and VLBW respectively. In addition those neonates who had the ratio ranging from 2.6 to 3.2 cm/m^2, all in ELBW group, were unresponsive to ibuprofen and underwent surgical closure.

CONCLUSION

The ratio between inner ductal caliber and BSA greater than 1.6 cm/m^2 seems to highly correlate with hemodynamically significant PDA in both ELBW and VLBW. Differences in ductus arteriousus closure and ibuprofen effectiveness by birth weight populations have been observed. According to these results, in VLBW and ELBW neonates, echocardiographic measurement of inner ductal caliber and the calculated ratio with BSA would be a potential predictor of hemodynamically significant PDA that could benefit from pharmacological closure with ibuprofen treatment.

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ABS 108

METABOLIC PROFILE IN ADOLESCENTS AND YOUNG ADULTS BORN AT DIFFERENT GESTATIONAL AGE

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BACKGROUND AND AIMS

Prematurity is associated with an increased risk to develop metabolic syndrome in the adult age. Implicated factors still need to be elucidated. The aim was to investigate gestational age (GA)-related metabolic differences in a cohort of young adults born at term and preterm.

METHODS

Blood and urine samples were collected from 67 patients, 16-23 years (20.2 ± 3.4 yrs) subdivided in two groups: 49 born preterm with mean gestational age 30.5 ± 2.4 weeks and birth weight (BW) 1,398 ± 301 g; 18 born term with mean gestational age 38.5 ± 2.1 weeks and birth weight (BW) 3,440 ± 298 g. Perinatal data were collected retrospectively and a questionnaire including questions concerning lifestyle, current weight, body mass index, health, family structure, education and occupation was performed. Bone mineral density (BMD) was assessed using quantitative ultrasound (DBM Sonic BP, Igea, Italy). Amplitude Dependent-Speed of Sound (AD-SoS, m/s), Bone Trasmission Time (BTT, mS) and correspondent z-scores were calculated. Serum levels of calcium, HDL and LDL cholesterol, transaminases, creatinine were measured. Urine were analyzed using proton nuclear magnetic resonance (1H-NMR) spectroscopy and NMR urine spectra were analyzed through Principal Components Analysis (PCA) on mean centered and Pareto scaled data, plus classification analysis has been carried out by means of several classification techniques: PLSDA, Support Vector Machine, Random Forest, k-NN.

RESULTS

No statistically significant difference were found between the two groups regarding blood metabolic profile and BMD. In young adults born preterm, the univariate regression analysis showed a significant negative association between BTT z-score and each of the following variables: total lenght of hospital stay ($p = 0.021$, B coefficient -0.32), LDL blood levels ($p = 0.045$, B coefficient -0.01), and being small for gestational age (SGA; $p = 0.038$, B coefficient -0.71). The multivariable regression analysis adjusted for the above variables, showed that young adults born SGA have significantly lower values of BTT z-score ($p = 0.033$, B coefficient -0.77).

A different urine metabolic profile was found between young adults born term and preterm. The Random Forest algorithm gives the better classification (nearly 80% of corrected classification) and indicates that the following metabolites are responsible for the classification:

- Citrate ABX system CH3 Creatinine (3.13 ppm);
- CH2 Creatinine (4.28 ppm);
- Fumarate (6.8 ppm);
- Hippurate (7.6-7.8 ppm).

CONCLUSION

Prematurity is characterized by specific variations of metabolic profile and BMD. In adult life, SGA newborn have a high prevalence of BMD reduction. Urine metabolic profiles of young adults born preterm significantly differ from young adult born term. Plausibly, an adverse *in utero* environment may induce fetal re-programming leading to changes in growth patterns and body metabolism, with permanent alterations of the physiology and metabolism later in life.

ABS 109

DIFFERENCES AND SIMILARITIES AMONG STEM CELL NICHES IN MULTIPLE ORGANS OF THE HUMAN FETUS

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BACKGROUND/AIM

Stem/ progenitor cells are present in high percentages in the developing human organs. In previous studies from our group [1] we showed that stem/progenitors are arranged in complex structures defined stem cell niches [2].

The aim of this study was to compare the architecture and cell components of multiple stem cell niches detected in different organs of the same subject.

METHODS

Sample from lung, heart, liver, intestinal tract, pancreas, kidney, adrenal gland, prostate, esophagus, thyroid, pituitary and skeletal muscle were obtained from a 12 week-old human fetus. Specimen were formalin-fixed, paraffin-embedded and tissue sections were stained by HE.

RESULTS

Histological examination showed that stem cells niches can be identify in all examined organs.

Lung: subleuric stem/progenitor cells surrounding a glandular structure (**Fig. 1**).

Heart: subepicardic stem/progenitor cells adjacent to a vessel in differentiation stage (**Fig. 2**).

Liver: immature portal tract with central vein encircled by mesenchyme containing stem/ progenitor cells (**Fig. 3**).

Intestinal tract: intestinal niche with stem/progenitor cells and initial differentiation of intestinal crypts (**Fig. 4**).

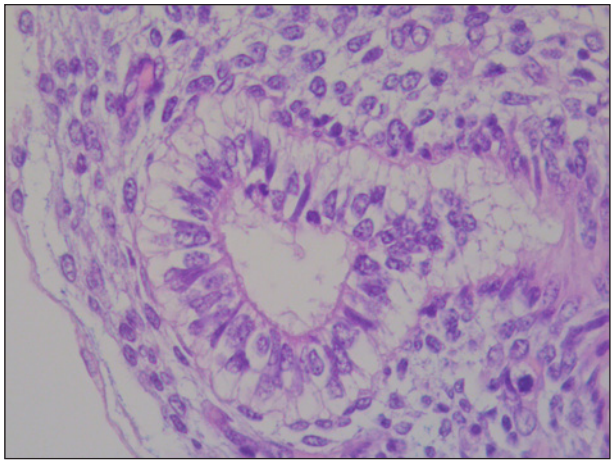

Figure 1 (ABS 109). Lung: subleuric stem/progenitor cells surrounding a glandular structure.

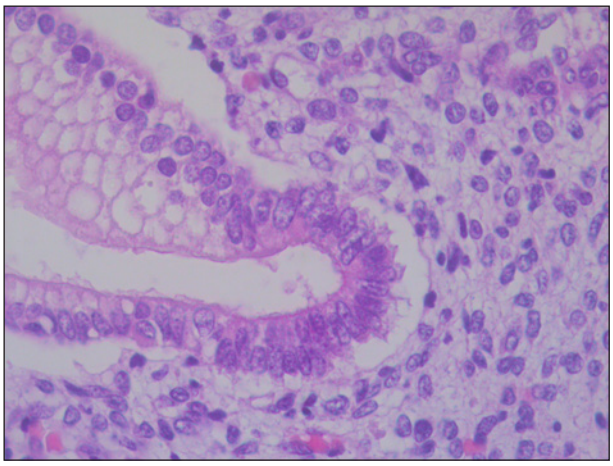

Figure 4 (ABS 109). Intestinal tract: intestinal niche with stem/progenitor cells and initial differentiation of intestinal crypts.

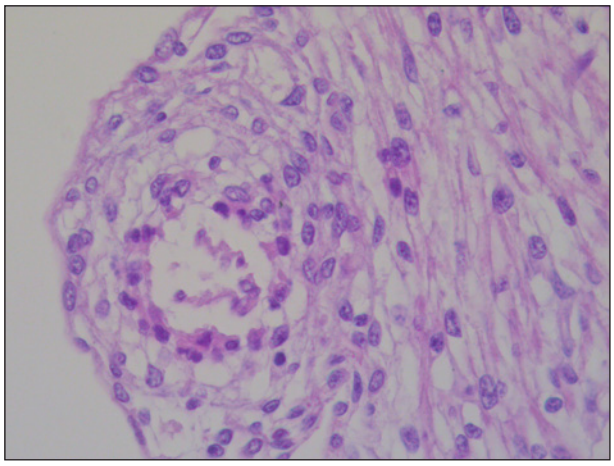

Figure 2 (ABS 109). Heart: subepicardic stem/progenitor cells adjacent to a vessel in differentiation stage.

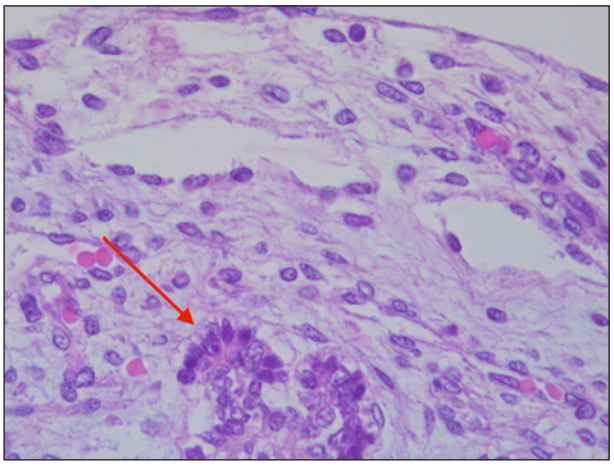

Figure 5 (ABS 109). Pancreas: mesenchyme with stem/progenitor cells and initial differentiation of pancreatic gland near a vein (arrow).

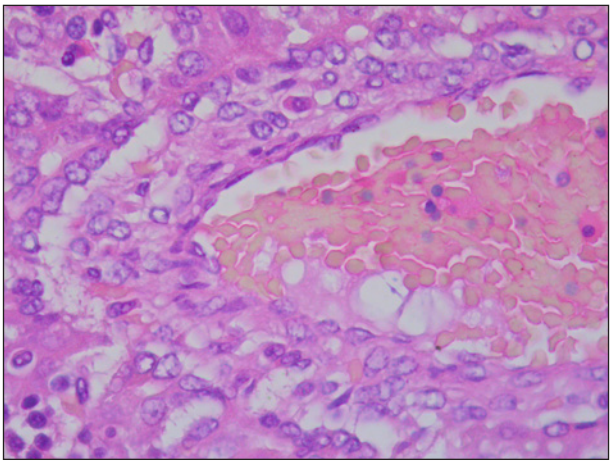

Figure 3 (ABS 109). Liver: immature portal tract with central vein encircled by mesenchyme containing stem/progenitor cells.

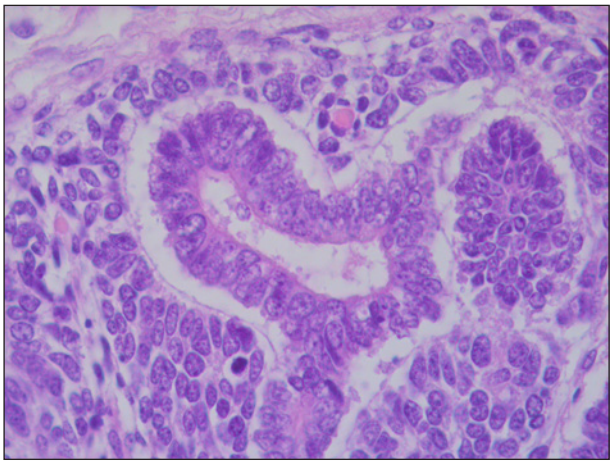

Figure 6 (ABS 109). Kidney: the niche composed by subcapsular stem/progenitor cells surrounding ureteric bud and vesicle.

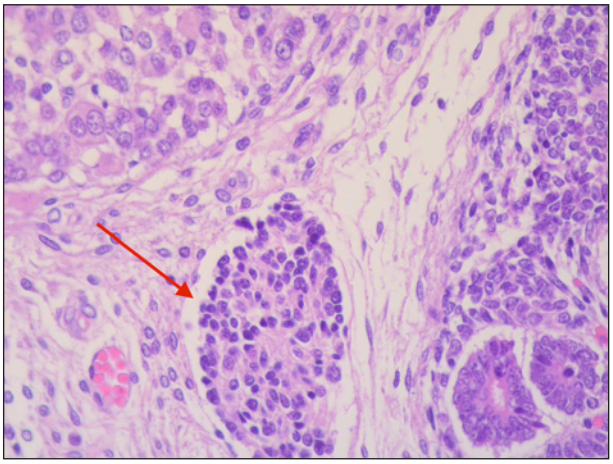

Figure 7 (ABS 109). Adrenal gland: the niche of stem/progenitor cells sited in between the kidney and adrenal gland (arrows).

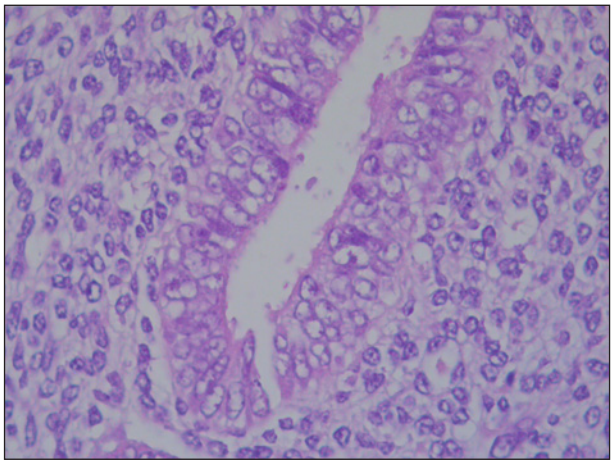

Figure 8 (ABS 109). Prostate: stem/progenitor cells close to the prostatic gland.

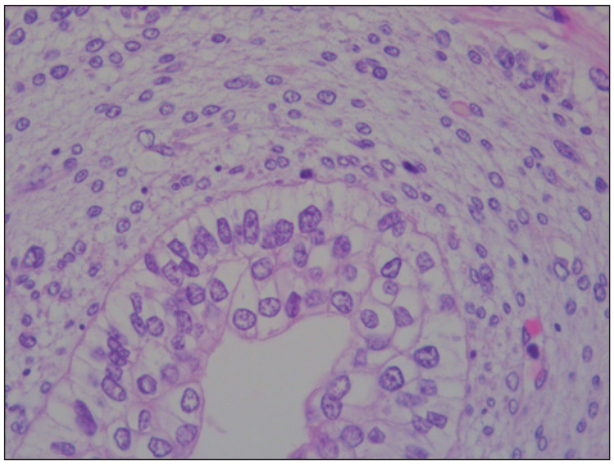

Figure 9 (ABS 109). Esophagus: stem/progenitor cells nearby the squamous epithelium.

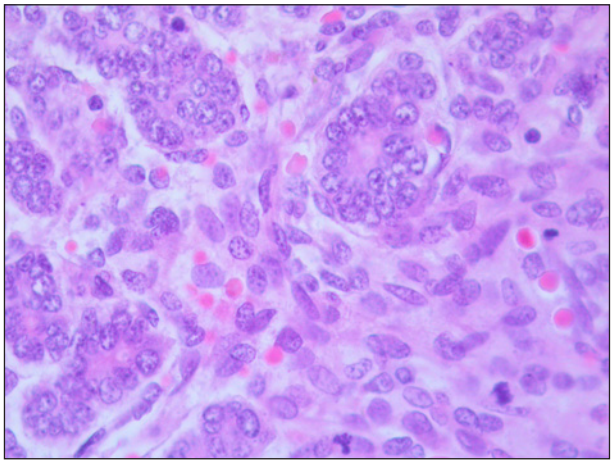

Figure 10 (ABS 109). Thyroid: mesenchyme with stem/progenitor cells surrounding thyroid follicles.

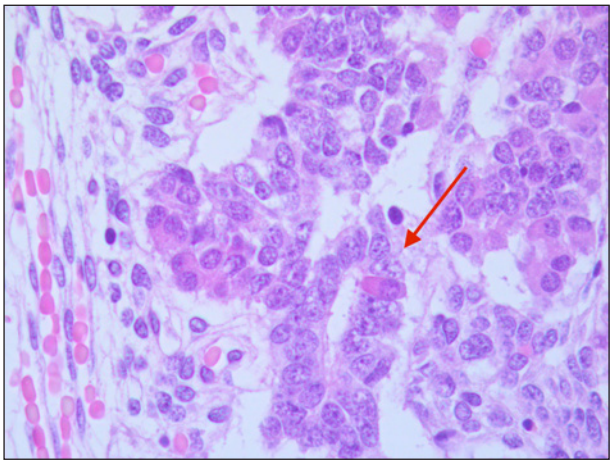

Figure 11 (ABS 109). Pituitary: this pictures show the initial transformation of stem/progenitor cells in glandular adenohypophysis (arrow).

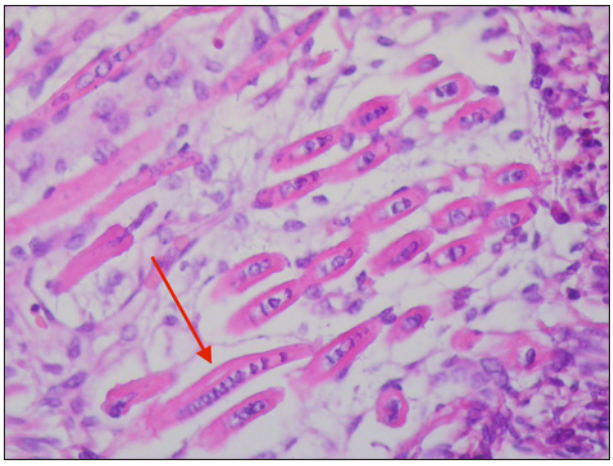

Figure 12 (ABS 109). Skeletal muscle: stem/ progenitor cells adjacent to immature muscular fibers with syncytial aspects (arrow).

Pancreas: mesenchyme with stem/ progenitor cells and initial differentiation of pancreatic gland near a vein (**Fig. 5**, arrow).
Kidney: the niche composed by subcapsular stem/ progenitor cells surrounding ureteric bud and vesicle (**Fig. 6**).
Adrenal gland: the niche of stem/progenitor cells sited in between the kidney and adrenal gland (**Fig. 7**, arrows).
Prostate: stem/progenitor cells close to the prostatic gland (**Fig. 8**).
Esophagus: stem/progenitor cells nearby the squamous epithelium (**Fig. 9**).
Thyroid: mesenchyme with stem/progenitor cells surrounding thyroid follicles (**Fig. 10**).
Pituitary: this pictures show the initial transformation of stem/progenitor cells in glandular adenohypophysis (**Fig. 11**, arrow).
Skeletal muscle: stem/ progenitor cells adjacent to immature muscular fibers with syncytial aspects (**Fig. 12**, arrow).

CONCLUSIONS

Our study of a 12 week-old human fetus, clearly shows that each organ of the developing human organism is characterized by peculiar and specific stem cell niches. Differences among niches regarded both the architecture and the cell components. Besides, structures in different differentiation stage can be easily recognized in all the examined organs as well as vessels and primitive mesenchyme, suggesting the developing organogenesis in the specific gestational age evaluated in our study. All niches and all stem/ progenitor cells are strictly and directly related with the developing structures in different differentiation stage, vessels and mesenchyme since organogenesis requires organization, order and specific feedbacks.

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ABS 110

POST-NATAL CATCH-UP GROWTH IN SARDINIAN INFANTS WITH INTRAUTERINE GROWTH RETARDATION

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INTRODUCTION

Recent studies have shown that infants with intrauterine growth retardation (IUGR) undergo catch-up growth during infancy.

AIM

The aim of this study was to evaluate the growth during the first year of life in a group of IUGR infants born in a single Maternal-Infant Unit in North Sardinia.

METHODS

A retrospective case-control study was performed on the clinical data of 24 infants, classified into two groups: twelve IUGR infants (group A) and 12 appropriate for gestational age control infants (group B). The auxological parameters of weight (W), height (H) and head circumference (HC) of group A were compared to those of group B infants at 5 time intervals from birth to the 12th month of age. The Student t-test was used for statistical analysis, considering significant a value of $p < 0.05$.

RESULTS

The significant difference present at birth between group A and group B infants for all the auxological parameters considered (W, mean 1,846 vs 3,170 g, $p < 0.0001$; HC, 30.09 vs 34.41 cm, $p < 0.0001$; H mean 43.36 vs 49.36 cm, $p < 0.0001$), showed a progressive, rapid and significant reduction during the first year of life, with final values of $p = 0.02$ for W (W, mean 7,861 vs 9,165 g), $p = 0.04$ for HC (HC, mean 43.47 vs 45.68) and p = ns for H (H, mean 72.58 vs 76.53 cm) at 12 months of life.

CONCLUSIONS

The majority of infants born IUGR in our series underwent a significant catch-up growth during the first 12 months of life. Further studies focused on improving our knowledge on the causes of IUGR will help to develop measures for prevention of the IUGR and for individualized treatments.

ABS 111

METABOLOMIC STUDY OF AMNIOTIC FLUID IN GESTATIONAL DIABETES

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INTRODUCTION

Gestational diabetes (GD) occurrence is increasing in pregnant women belonging to most ethnic groups, showing different rates depending on the different ethnicity. In particular, while Caucasians seem to be less affected by this condition, Asians are characterised by the highest incidence rate [1]. Notably, also other variables such as obesity may influence the predisposition toward this pathology. The reason why GD is of clinical relevance is its association with higher risks of adverse perinatal, pregnancy, and delivery outcomes; indeed, type II diabetes incidence increases more than seven-fold within 5-10 years after pregnancy for women which experienced GD [1]

MATERIALS AND METHODS

Study design and population

A preliminary retrospective cohort study was conducted on 14 pregnant women; 7 healthy, and 7 GD affected subjects. Amniotic fluid was collected from all pregnant women through amniocentesis at 15-16th week, hence written informed consent was obtained from each woman undergoing invasive procedures.

Sample preparation

AF samples were thawed at room temperature and vortex mixed to homogenize. 200 µL were transferred in Eppendorf tubes (1.5 mL) and treated with 400 µL of acetone for protein precipitation. The mixture was vortexed for 30 s and centrifuged (1,400 rpm for 10 min). 400 µL of supernatant were transferred in glass vials and evaporated to dryness overnight in an Eppendorf vacuum centrifuge. 60 µL of a 0.24 M solution of methoxylamine hydrochloride in pyridine was added to each vial, samples were vortex mixed and left to react for 17 h at room temperature. Then 80 µL of MSTFA (N-Methyl-N-trimethylsilyltrifuoroacetamide) were added and left to react for 1 h at room temperature. The derivatized samples were diluted with hexane (100 µL) just before GC-MS analysis.

GC-MS analysis

Samples were analyzed using a Agilent 5975C interfaced to the GC 7820 equipped with a DB-5ms column (J & W), injector temperature at 230°C, detector temperature at 280°C, helium carrier gas flow rate of 1 ml/min. The GC oven temperature program was 90°C initial temperature with 1 min hold time and ramping at 10°C/min to a final temperature of 270°C with 7 min hold time. 1 µL of the derivatized sample was injected in split (1:20) mode. After a solvent delay of 3 minutes mass spectra were acquired in full scan mode using 2.28 scans/s with a mass range of 50-700 Amu.

Data analysis

Each acquired chromatogram was analyzed by means of the free software AMDIS [2] that identified each peak by comparison of the relative mass spectra and the retention times with those stored in an in-house made library comprising 255 metabolites. Other metabolites were identified using NIST08 (National Institute of Standards and Technology's mass spectral database) and the Golm Metabolome Database (GMD) [3]. Through this approach 97 compounds were detected and quantified. AMDIS analysis produced an Excel data matrix to be treated for data analysis.

Statistical analysis

Data matrix was processed through multivariate analysis, exploiting MetaboAnalyst 3.0 (http://www.metaboanalyst.ca) PLS-DA strategy. Calculations highlighted some VIP metabolites as discriminant for the two different phenotypes: hippuric acid, stearic acid, glutamic acid, and palmitic acid. Such compounds were then used to determine which canonical biological pathways were influenced the most by the pathological occurrence.

RESULTS AND CONCLUSIONS

PLS-DA analysis showed significant changes in some important metabolites as hippuric acid, stearic acid, glutamic acid, and palmitic acid between the two groups under study. These metabolites can be defined as "hubs" of the differential network of the two groups. Finally, metabolomics proved to be a powerful tool also for the amniotic fluid characterization of several pathological conditions with a remarkable amount of information about the fetal and maternal health. Our preliminary GD metabolomics analysis of amniotic fluid highlights the contribution of the phenylalanine metabolism and fatty acid biosynthesis pathways.

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ABS 112

IMMUNOLOGY: FROM THEORY TO CLINICAL PRACTICE. IMMUNE-MODULATED RESPONSES FROM NEONATOLOGY TO ADOLESCENCE

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Immune system cells are generated from totipotent stem progenitors starting from the fifth week of pregnancy. In particular, T and B lymphocytes, the major actors of adaptive immune responses, develop in different organs during gestation: in the bone marrow between the eighth and the tenth week, in the thymus at eight weeks, in lymphonodes at eleven weeks, and in pharyngeal tonsils during the fourteenth week. By diffusion in those organs, the various components of the immune system spread and localize in different body districts.

At birth active immune response, which requests a direct involvement of newborn immune cells, is weak and unable to adequataly protect the baby from pathogen attacks. However, the newborn is protected by passive immune system mechanisms: mother's antibodies acquired during gestation through placental transfer and, at birth, by breast milk antibodies.

Maternal antibodies are not equally vertically transmitted. At birth, the newborns are able to responde to viruses and Gram+ bacteria insults, but can't get by Gram- pathogen infections. Unfortunately, Gram- bacteria are responsible of several pathologies characteristic of neonatal period (e.g. *B. pertussis*, *Salmonella spp.*, *Shigella spp.*, and *E. coli* infections).

Anyway, in the newborn passive immunity is an efficient support to the not yet well functional active immunity. The latter, infact, that reaches the higher grade of maturation during adult age, is characterized by a reduced ability to produce cytokines, a lower activity of complement, and a delayed and weaker T cell response.

All these elements contribute to make the neonatal period at higher risk of infections, characterized by both viral and bacterial infective processes with more serious progresses.

The immune system development completes during the first years of life as a consequence of interactions between the child and enviroment and antigenic stimulations. However, the diversity of antigenic exposure and B-T lymphocyte cooperation contribute to a complete maturation of immune system within the first year of life.

During this period, recurrent respiratory tract infections (RRTIs) commonly occur in healthy children due to an increased risk of exposure to infectious agents associated with immune system immaturity. The development of both innate and adaptive immunity is gradual, and it is not fully functional until 5-7 years of age. Compared to adults, younger children have lower concentrations in respiratory mucus of several proteins with antibacterial activity (e.g. defensins, lysozyme, IgA) as well as proteins that have immunomodulatory (e.g. cytokines) and protective functions.

The lower expression on epithelial cell membranes of toll-like receptors (TLRs), molecules involved in antigenic recognition, leads to less efficient recognition of pathogens and to a delayed and less effective induction of innate immune responses. Moreover, lymphocytes, macrophages, and dendritic cells activity is still inadequate in the first years of life, reaching adult efficiency only after repeated exposure to pathogens. During the first years of life the secretion of pro-inflammatory cytokines and chemokines and the systemic and mucosal antibodies production are low.

In young children, many attempts to improve immune function have been performed using alternative medicines (e.g., plant preparations), dietary supplements (e.g., vitamins C and D, zinc), and other preparations such as probiotics, bacterial lysates and pidotimod (PDT), a synthetic dipeptide molecule (3-L-pyroglutamyl-L-thiazolidine-4carboxilic acid). In only a few percentage of cases, scientific evidence of positive effects was reported. However, the results are encouraging for PDT, that seems to present immunomodulatory activity on both innate and adaptive responses. In fact, *in vitro* studies in both animal and human subjects have documented after treatment with PDT higher expression of TLR2, induction of dendritic cell maturation (accompanied by an increased release of pro-inflammatory molecules), HLA-DR expression upregulation, stimulation of T

lymphocyte proliferation and differentiation toward a Th1 phenotype, inhibition of thymocyte cell death and promotion of phagocytosis.
Moreover, *in vivo* studies have demonstrated that prophylactic use of PDT for a long period can be of benefit in children with RRTIs: the total number of new infectious episodes and the consequent use of drugs, including antibiotics is reduced.
In a previous study we reported that in subjects with Down syndrome PDT administration induces a stronger response to the influenza vaccine, suggesting a preferential activation of effector mechanisms and a potential beneficial effect of immunization by PDT [1].
Recently, we evaluated the immunomodulatory activity of PDT administered in association with standard antibiotic therapy in children hospitalized for community-acquired pneumonia (CAP). We observed that the percentage of dendritic cells expressing activation and co-stimulatory molecules were significantly higher in children receiving PDT plus antibiotics than in the controls. A significant increase in tumor necrosis factor-α and/or interleukin-12 secretion and expression of TLR2, followed by an increased release of proinflammatory cytokines by monocytes, was observed in PDT-treated children compared with controls. In the PDT-treated group, mRNA expression of antimicrobial peptides and genes involved in the inflammatory response were also increased in comparison with the controls.
These results demonstrate that PDT administered together with standard antibiotics is associated with a favorable persistent immunomodulatory effect in children with CAP [2].

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ABS 113

NEWS ON PEDIATRIC UROLOGY

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Pediatric urology is a pediatric specialty dedicated to the diagnosis and treatment of congenital and acquired genitourinary tract diseases. It is a specialty that is rapidly changing, thanks to the technological development that has been emerging in recent years. There have been important diagnostic and therapeutic news.
Congenital anomalies of the kidneys and urinary tract (CAKUT) include various entities of structural malformations that result from defects in their morphogenesis. Clinical research and genetic studies on the origins of CAKUT are quickly evolving, with significant growth of high-quality research.
Management goals of CAKUT include prevention of febrile urinary tract infections (UTI) in newborns and toddles and renal injury, while minimizing the morbidity of treatment and follow-up. Treatment options include observation with or without continuous antibiotic prophylaxis (CAP) and surgical correction. Now, randomized controlled studies show that children with normal urinary tracts or low-grade vesicoureteral reflux (VUR) do not benefit from prophylaxis.
All children with known mechanical or functional obstructions of the urinary tract are considered to have UTI. Functional obstruction often results from lower urinary tract dysfunction (LUTD) of either neurogenic or non-neurogenic origin and dilating VUR.
The role of bladder and bowel dysfunction (BBD) in children with UTI and the long-term risk of renal scarring, have shed new light on treatment strategies. Often it is BBD, rather than reflux, that causes UTI in children older than 2 years.
Pediatric urology has evolved in recent years, with a greater focus on bladder and renal function, minimally invasive treatment, evidence-based interventions, and guideline adherence.
Other topics in Pediatric urology include urinary incontinence in children with special needs and the use of robot-assisted laparoscopic surgery (RALS) in children, with advantages over conventional laparoscopic surgery.

ABS 114

PEDIATRIC TUBERCULOSIS IN NORTH SARDINIA, 2009-2014

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INTRODUCTION

Tuberculosis (TB) is a rare disease in the developed countries and in Italy (< 10 cases/100,000 inhabitants/year). In 2008, the Italian National TB Surveillance System has reported a gradual increase of pediatric TB cases.

AIM

Aim of the study was to evaluate demographic and clinical characteristics of pediatric TB in two Provinces of North Sardinia, Sassari (SS) and Olbia-Tempio (OT), from 2009 to 2014, in native and immigrant residents.

METHODS

The study population included both the symptomatic TB-infected children and the TB-exposed household contacts. Contacts were classified into positive and negative tuberculin skin test (TST). Data were collected retrospectively by consulting clinical records. The χ^2 test was used for statistical analysis.

RESULTS

In the period 2009-2014, total of 244 (M:F = 1:1) TB-exposed children were identified, among whom 217 (89%) were natives and 27 (11%) immigrants, with the majority (82%) aged 5 to 10 years. An equal number of cases was detected in each Province (n = 120 in SS vs n = 124 in OT, p = ns). However, considering the total number of residents, the incidence of pediatric TB in OT is double than that found in SS ($p < 0.05$). Among the 194 (75%) TST negative subjects, 60 (31%) underwent 3 months of isoniazid prophylaxis because were at high risk. Active disease involved 22/244 (9%) children. Pulmonary involvement with lymphoadenopathy was the most common form (73%), followed by pleuritis (14%), laterocervical lymphoadenopathy (9%) and disseminated TB (miliary and meningitis) in one case (4.5%). In our study population, one case presented with secondary infection by multidrug-resistance tuberculosis (MDR-TB).

CONCLUSIONS

Pediatric TB in North Sardinia represents a relevant health problem, where immigrants do not appear to affect significantly its global incidence. The emergence of MDR-TB cases is worrisome. As the household contacts showed less severe clinical forms, both early identification of TB-exposed children by a careful surveillance system and appropriate treatment protocols are necessary to prevent the most severe forms and to limit the spreading of TB infection.

ABS 115

SOX2, A MARKER FOR NEURAL STEM CELLS IN THE DEVELOPING HUMAN CEREBELLUM AT DIFFERENT GESTATIONAL AGES

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INTRODUCTION

The transcription factor SOX2 [SRY (sex-determining region Y)-box] is expressed during the development of mammalian central nervous system [1]. It is known as a marker for neural stem/progenitors cells. SOX2 belongs to the SOX family of transcription factors characterized by the presence of a homologous sequence known as hight mobility group (HMG) box, a DNA binding domain highly conserved among species.

The SOX proteins are expressed in different tissues during development. High levels of SOX2 are found in the developing nervous system. However, the expression of SOX2 persists also in adulthood where SOX2 is expressed in the neural stem cells of the subventricular zone and in the subgranular zone of the dentate gyrus of the hippocampus [2].

The function of SOX2 has been investigated using several transgenic mice models. Experimental studies demonstrated that a constitutive expression of SOX2 maintains the properties of stem cells and inhibits neuronal development. On the other hand, in these transgenic mice, the inactivation of SOX2-encoding genes resulted in an early exit of neural precursors from the cell cycle. This was associated with a loss of progenitor markers and the onset of early neuronal differentiation markers [3]. Additionally, SOX2 expression was lost during neuronal differentiation. These data suggest that SOX2 is involved in the mainteinance of the "stemness" identity of the neural stem cells. In humans, few data have been reported, at the best of our knowledge, regarding the expression of SOX2 in the developing nervous system and, in particular, in the human cerebellum.

AIM

This study was aimed to investigate the role of SOX2 during the development of the human cerebellum.

MATERIALS AND METHODS

To this end, we analyzed the immunoreactivity for SOX2 in human fetuses at different gestational ages. Cerebellum samples were obtained from 6 human fetuses, ranging from 11 to 38 weeks of gestation. The fetuses have been completely sampled and histologically and immunohistochemically studied. Samples were fixed in 10% buffered formalin, paraffin-embedded and immunostained for SOX2.

RESULTS

Our data show that, at 11 weeks of gestation, immunostaining for SOX2 is strongly detected in the nuclei of stem/precursor cells in the neuroepithelium of the IV ventricle, which is considered the source of primary precursors that give rise to many types of cerebellar neurons including basket cells, stellate cells and Purkinje cells (**Fig. 1**).

At 20 weeks, in the surface of the cerebellar cortex, SOX2 is expressed in the nuclei of precursor cells that are migrating from the subventricular neuroepithelium towards the pial surface (**Fig. 2**).

At 24 weeks, SOX2 continues to label the nuclei of migrating cells in the surface of the cerebellar cortex (**Fig. 3**).

Conversely, our observations show that at 30, 34 and 38 weeks of gestation, SOX2 is not expressed in the cerebellar cortex (**Figures 4-6**).

CONCLUSIONS

The interesting finding of our study is the different expression of SOX2 at the different gestational ages. Our data show a positive immunostaining for SOX2 in the cerebellum specimens at 11, 20 and 24 weeks of gestation and a negative immunostaining for SOX2 in the cerebellum specimens at 30, 34 and 38 weeks.

As known, SOX2 is one of the earliest transcription factors expressed in neural stem and precursors cells in the developing nervous system.

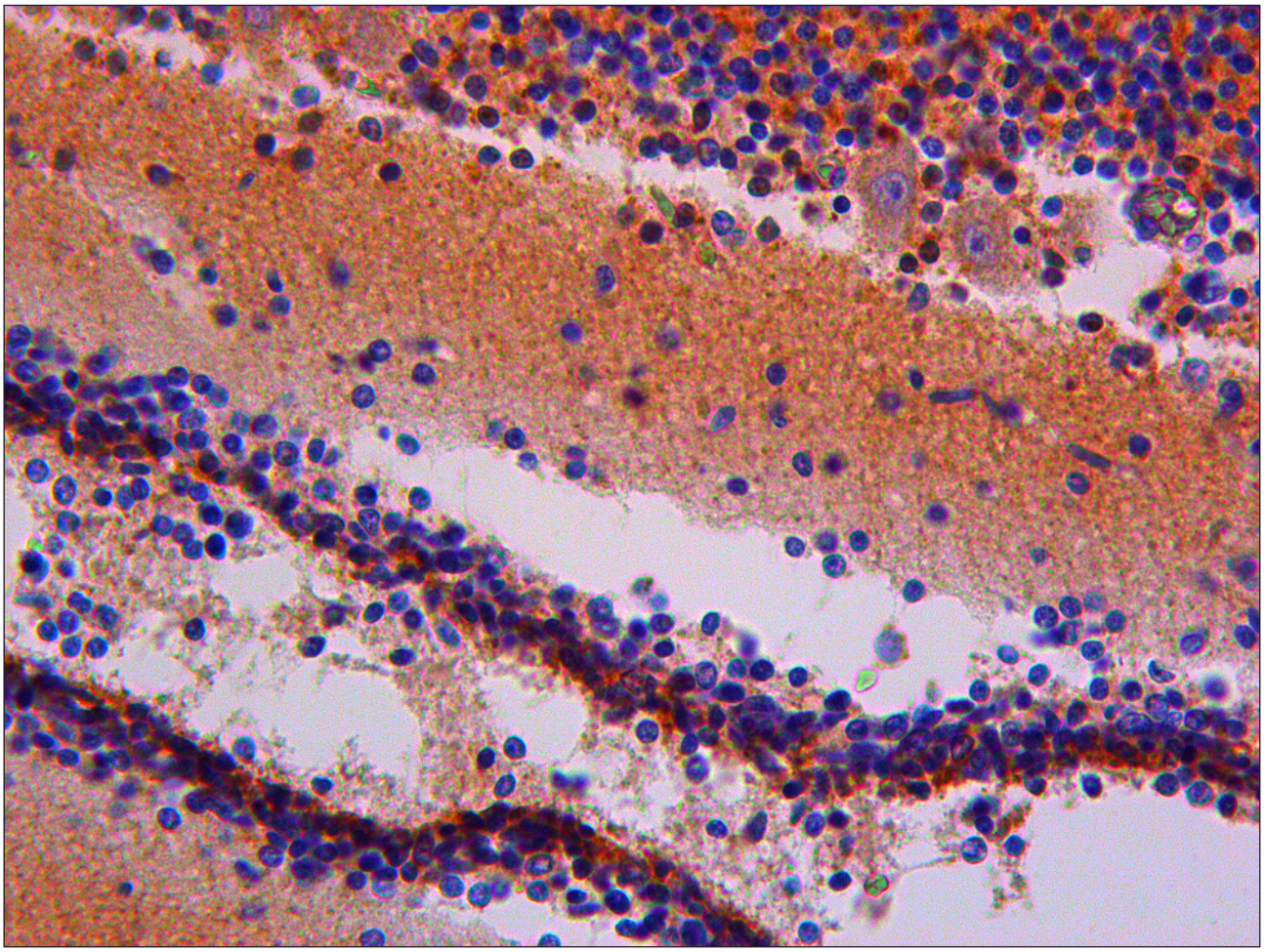

Figure 1 (ABS 115). 11 weeks: immunostaining is strongly detected in the nuclei of stem/precursor cells in the neuroepithelium of the IV ventricle.

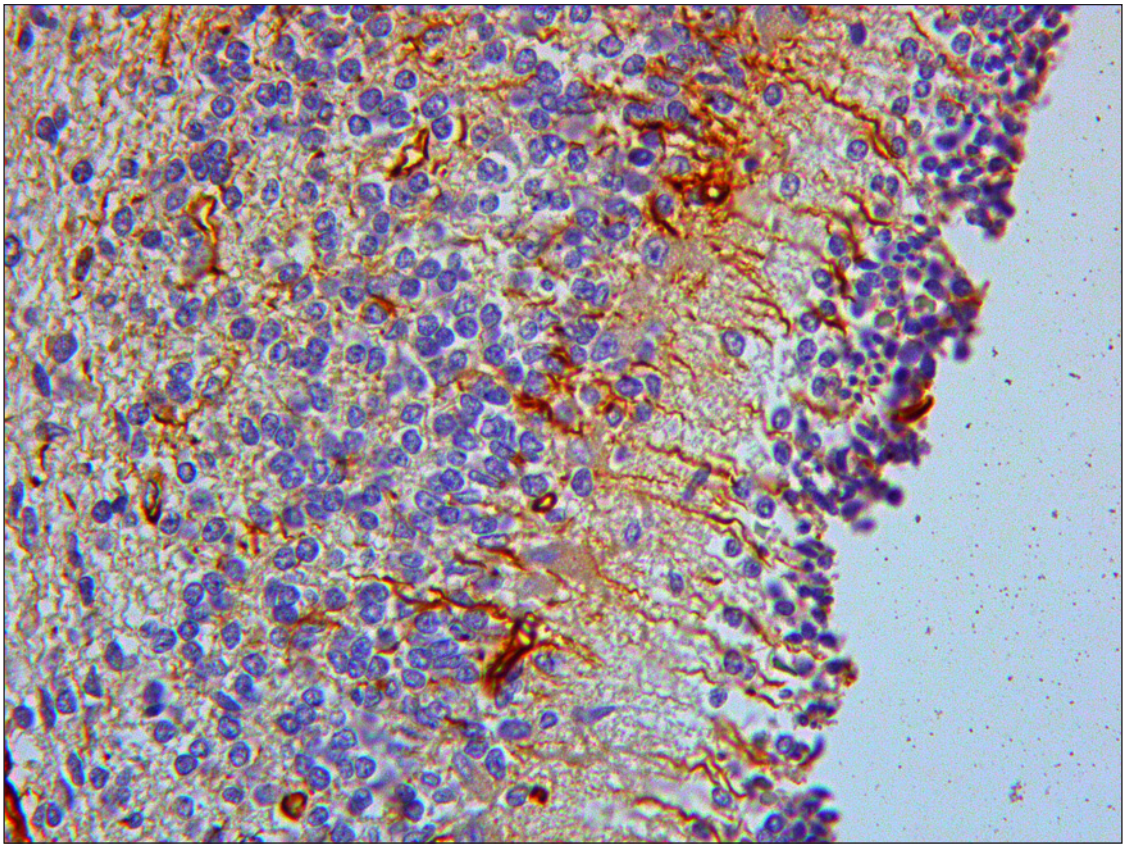

Figure 2 (ABS 115). 20 weeks: in the surface of the cerebellar cortex, SOX2 is expressed in the nuclei of precursor cells that are migrating from the subventricular neuroepithelium towards the pial surface.

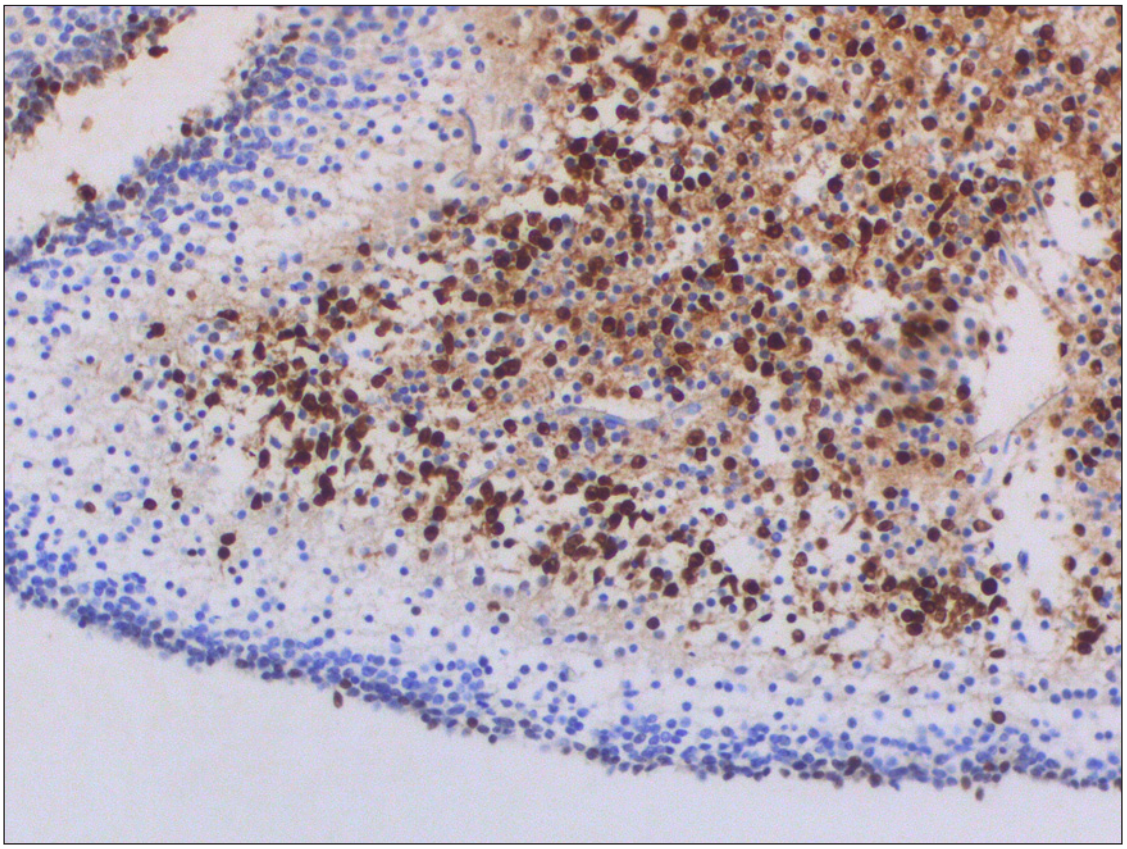

Figure 3 (ABS 115). 24 weeks: SOX2 continues to label the nuclei of migrating cells in the surface of the cerebellar cortex.

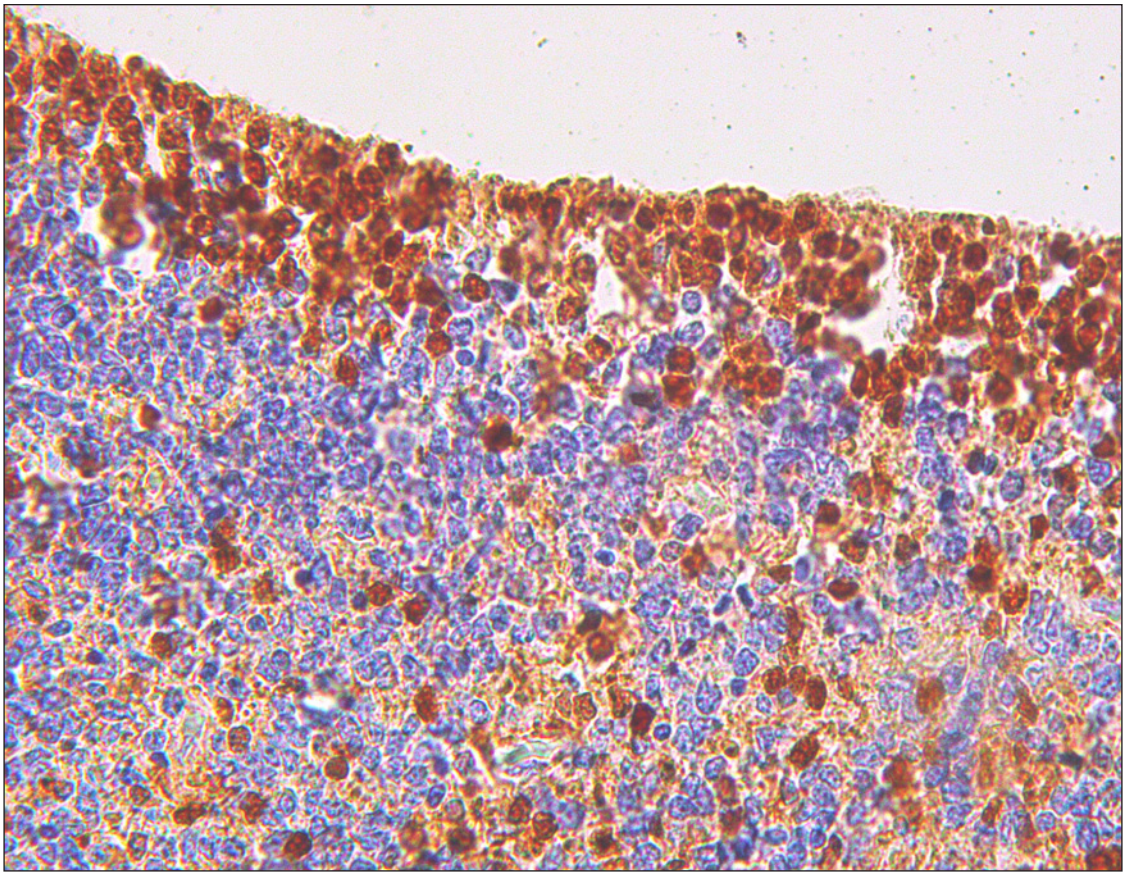

Figure 4 (ABS 115). 30 weeks: SOX2 is not expressed in the cerebellar cortex.

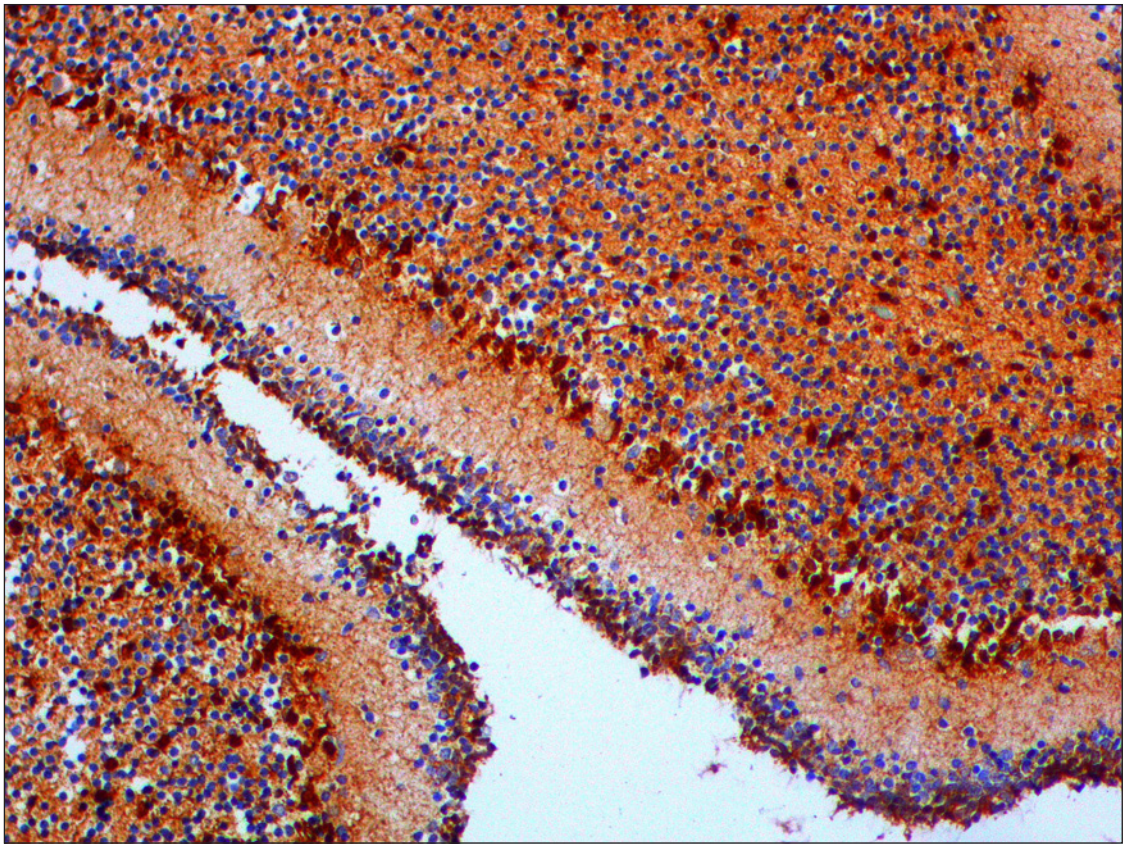

Figure 5 (ABS 115). 34 weeks: SOX2 is not expressed in the cerebellar cortex.

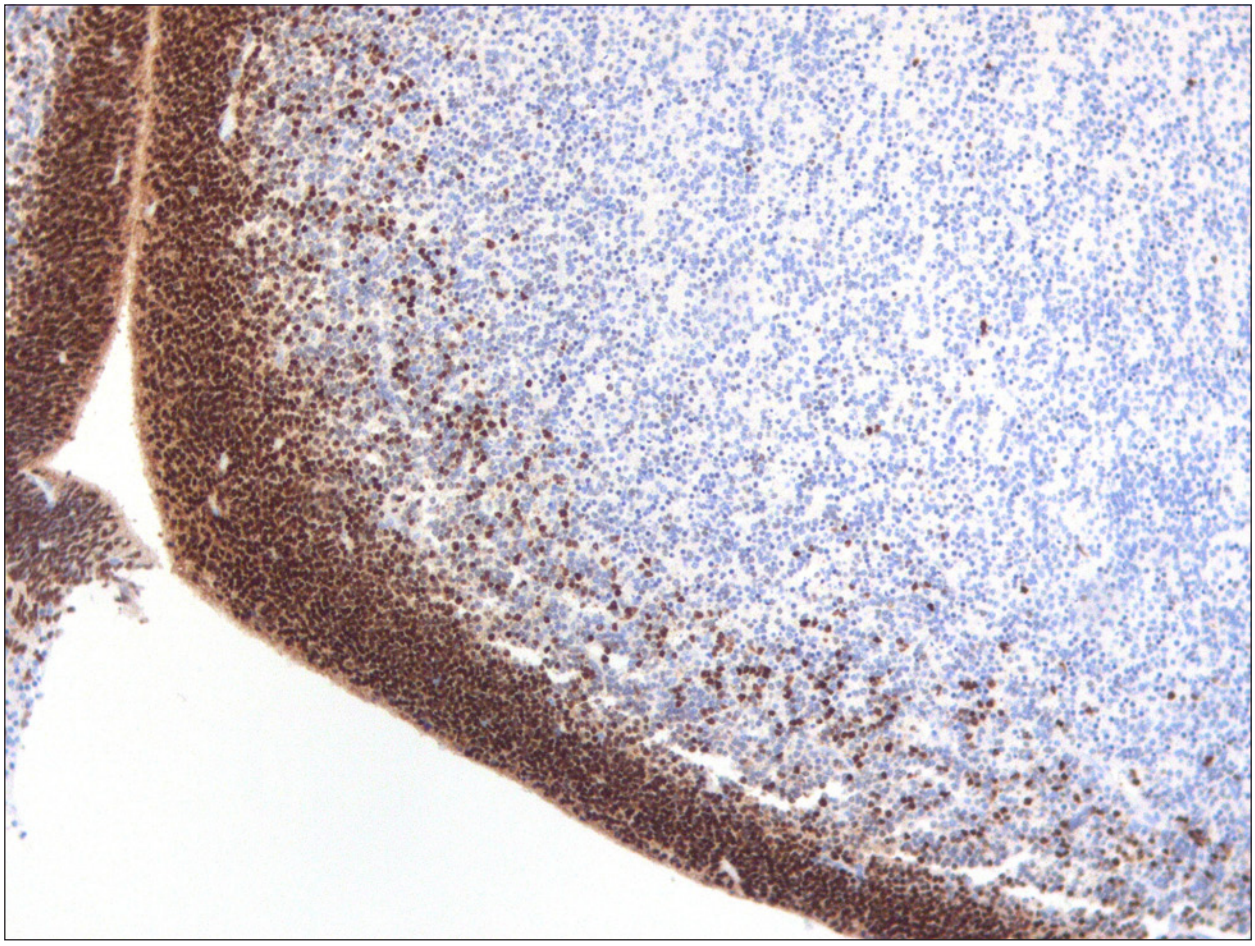

Figure 6 (ABS 115). 38 weeks: SOX2 is not expressed in the cerebellar cortex.

This study show a prevalent SOX2 expression in the nuclei of the ventricular neuroepithelium, that represents the primary source of progenitors in the developing human nervous system.

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ABS 116

NEC AND TRANSFUSIONS

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Necrotizing enterocolitis (NEC) is an inflammatory gastrointestinal (GI) disease and the most common surgical emergency in neonatal intensive care units (NICU), which occurs in 12% of very low birth weight (VLBW) infants with a mortality rate of 30%. Compared to those without NEC, the affected neonates have longer hospitalization (average 22 days, up to 60 days if surgical NEC) and higher operating cost (about 19%). Although NEC is inversely related to gestational age, it also involves about 12% of early term and full term infants, since all conditions potentially inducing hypoxia or hypoperfusion during antepartum (intrauterine growth restriction, maternal illicit drug use such as cocaine, hypertensive disease, infections) intrapartum (chorioamnionitis, sepsis, prolapsed cord or placental abruption) or postpartum (patent ductus arteriosus, indomethacin treatment, sepsis, polyicythemia, respiratory distress syndrome, mechanical ventilation, congenital heart diseases, umbelical catheters and exchange transfusion) period expose neonates to intestinal blood shunt from gut to heart and brain. Furthermore, after injury, reperfusion and risk of bacterial invasion may exacerbate the inflammatory response with

additional mucosal gut damage. In very preterm neonates these phenomena are enhanced by mucosal IgA deficit.

NEC is characterized by mucosal and/or transmural necrosis, and its clinical presentation, well staged with Bell's criteria, can be slowly insidious or rapidly evolving and worsening as to require respiratory and cardiovascular supports. It generally occurs in the first two weeks of life with aspecific or gastrointestinal signs with or without altered laboratory tests. Radiographic findings (intestinal dilation, intestinal pneumatosis, air in the portal venous system, pneumoperitoneum, ascites) are constant and evocative.

Pathogenesis of NEC is multifactorial with risk differences related to gestational age, with 90-95% of incidence before 36 weeks of postmestrual age. Independent risk factors such as prematurity or formula feeding combined with gut immaturity contribute to a major incidence of NEC in preterm neonates. The latter have disrupted tight junction integrity and reduced peristalsis with lasting contact among antigens and enterocytes that lead to sepsis. *Enterobacter spp.*, *Enterococcus spp.*, *Staphylococcus spp.* and *Clostridia spp.* proliferate at the expense of commensal bacteria species (*Bifidobacteria*), as occur during prolonged and early antimicrobial treatment.

Formula fed neonates develop more NEC, regardless of the rate of feeding advancement. Feeding intolerance due to gastric tube contaminations (frequently by *Klebsiella spp.* or *Enterobacteriacee spp.*, especially during H_2 blocker therapy) have been reported.

Recent clinical evidences show a not well explained but intriguing chronological association between packed red blood cell (PRBC) transfusions and NEC. It has been hypothesized that in VLBW infants transfusion may be responsible for NEC when administerd 24-72 hours before the onset.

Concomitant enteral feedings, storage conditions of blood products, peculiar gastrointestinal and immunological immaturity, which alters mesenteric oxygenation even in absence of alterated gastrointestinal perfusion, may independently act in "transfusion related" NEC (TR-NEC).

Studies designed to evaluate TR-NEC showed that 25-40% of all neonates with NEC received a transfusion within 48 hours before onset, and those with lower gestational age at birth or with a patent ductus arteriousus or under mechanical ventilation most prone to be affected.

TR-NEC occurs later at 3-5 weeks of life, with a more severe course than non transfusion related-NEC.

In contrast, some authors suggest a protective effect of PRBCs transfusions on "late onset" NEC by reducing tissutal hypoxia due to chronic anemia, as confirmed by lower hematocrit value in some VLBW with TR-NEC .

In infants with birth weight ≤ 1,000 grams exposure to transfusions was associated with NEC significantly less than in infants with birth weight of 1,001 to 1,500 grams, with a prevalence of onset at 30.4 ± 2.6 post-mestrual age, probably due to susceptibility of immature gastrointestinal tract to neovascularization, angiogenic factors and oxygen toxicity, similarly to what happens in retinopathy of prematury.

It has also been supposed that enteral feeding may contribute to pathogenesis of TR-NEC by increasing oxygen extraction in premature gut during digestion or probably by increasing infections risk due to gastrointestinal dysbiosis. It remains controversial whether withholding feedings around 4 hours before and after transfusion can be beneficial.

Anatomic and immunological gut immaturity, dismotility as well as impaired intestinal circulation and barrier integrity are included in TR-NEC pathogenesis, even though alterations in gastrointestinal perfusion is generally common in VLBW with NEC directly due to tissue ischemia or reduced splancnic perfusion after bacterial colonization or inflammatory mediators release. It has been reported tha mucosal disruption mainly occurs if a transfusion or enteral feedings coexist.

In addition, immune mechanisms similar to those seen in transfusion related acute lung inyury (TRALI) can be postulated to determine NEC. This particular condition refers to immunological vascular damage triggered by antibodies against cellular components and by cytokines in stored blood, which similarly could lead to vascular gut injury in presence of bacterial or nutritional antigens.

Using single unit of stored blood, preferred in VLBW to reduce the risk of infections, does not protect against inadeguate intestinal perfusion potentially induced by storage lesions of RBC altered in structure and function. Studies performed with the near infraed spectroscopy (NIRS) technology demonstrated that altered RBCs could cause an immune leukocyte mediated response causing the release of vasoconstrictors

mediators that enhance endothelial damage already affected by concomitant lack of intestinal autoregulation.

Adverse outcomes in term of morbidity and mortality may occur in neonates after receiving leukoreduced RBCs older than 14 days, because of rapid biochemical storage lesions (also influenced by anticoaugulant and additive solutions), which cause erytrocites hemolysis conseguently to lipidic membrane peroxidation with iron and hemoglobin release. This causes iron overload and oxidative reaction with alteration in immune function and exposure to infections.

Although TR-NEC pathogenesis remains unclear, strong evidences suggested a temporal association between NEC and transfusion, with higher prevalence in VLBW, lower birth weight and enteral fed. The predictable GI immaturity of preterm neonates and quality of blood products seems to act like the substrate of complex immune and proinflammatory mechanisms responsible for intestinal mucosal damage which occur at NEC onset. Knowledge of risk factors, breast feeding encouragement and prevention of anemia to avoid transfusions are required to prevent TR-NEC and improve clinical care.

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ABS 117

SNORING IN OBESE CHILDREN AND ITS CLINICAL SIGNIFICANCE IN AMBULATORY SETTING

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INTRODUCTION

Frequency of habitual snoring is significantly higher in obese than in normal-weight subjects. Obesity and adeno-tonsillar size are risk factors of snoring. Other factors, such as fat distribution and upper airway collapsibility, could explain the relationship between obesity, snoring and OSAS.

AIM

The aim of the study was to investigate clinical and instrumental significance of snoring in exogenous obese children referred to our department.

METHODS

A prospective respiratory sleep study was carried out from January 2014 to March 2015 in 36 consecutive exogenous obese children. Body mass index (BMI) and BMI Z score were calculated according to age and sex. Nasal patency, tonsil size, Friedman palate position scoring were also recorded.

An overnight limited-channel polysomnography was performed using a type III portable ambulatory device (SOMNOscreen™ PSG, SOMNOmedics GmbH, Randersacker, Germany). Statistical analysis was done using SPSS® Statistics 19.0 software for Windows®. The strength of the association between two variables (snoring versus respiratory variables or versus clinical scoring) was evaluated by calculating simple and partial correlation coefficients, adjusting for clinical scoring when appropriate.

RESULTS

Snoring, objectively measured by respiratory polysomnography, was associated with palate position and with oxygen desaturation index (ODI). The correlation between snoring and ODI completely disappeared when adjusting for palate position scoring.

CONCLUSION

Low palate position can be identified as an adjunctive factor that can contribute to making snoring and increased desaturation events possibly related to increased risk of upper airway collapsibility during sleep in obese children.

ABS 118

BIOMARKERS FOR BRONCHOPULMONARY DYSPLASIA (BPD)

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The pathogenesis of BPD is multifactorial, with complex interactions between genetic and environmental factors. In new BPD alveolarization is arrested in an immature stage due to factors such as inflammation and oxidant stress caused by chorioamnionitis, sepsis, hyperoxia, ventilation etc. Useful biomarkers must be reliable and have the capacity to detect BPD at an early stage so that early intervention can avoid or minimise the detrimental effects on the lung. In general biomarkers are a reflection of the pathogenetic processes involved in BPD and can be measured in different tissues and fluids obtained from the preterm infant (or its mother or placenta). The advantage of serum (including from cord blood) and urine is that is easy to obtain but it is influenced by inflammation and other processes in other parts of the body. Tracheal aspirates (TA) or bronchoalveolar lavage fluid (BALF) better reflects pulmonary changes but are more difficult to obtain especially with the avoidance of invasive ventilation nowadays. Classically, old BPD was characterised by severe injury to the lung with a severe inflammatory response, and biomarkers studied related to this (inflammatory cells in TA or BALF, or elastase, cytokines etc…). In new BPD, we found that early cytokine concentrations in BALF discriminate less good between later BPD or no BPD, than growth factors such as VEGF and TGF-alpha. This reflects the importance of developmental arrest of the alveoli. VEGF and other growth factors are known to play important roles in alveolar development. Factors associated with injury and remodelling have also been shown to be early markers of BPD (for ex. MMP-9 in TA). Recently, decreased endothelial progenitor cells in blood have been shown to be associated with later BPD. These stem cells are also known to be important for development and repair. As oxidative stress also plays a major role in BPD, as is especially known from animal studies, several markers of damage by reactive oxygen species have been studied for an association with BPD with contradictory results. Instead of studying all these pre-defined biomarkers new technologies allow us to apply more unbiased approaches with the use of "OMICS". Genomic and epigenomic studies found associations of single nucleotide polymorphisms (SNP's) and epigenetic changes with BPD but most data were not confirmed in different studies. The study of microRNA's seems promising. Transcriptomics and proteomics have found some changes between BPD and no BPD, but it is to early to state the value of these. Metabolomics on urine or TA's, measuring profiles of small metabolites, seem promising as well as the measurement of volatile organic compounds in exhaled breath or on TA's with mass spectrometry techniques or even with so called electronic noses which can be easily used bedside in the future.

In conclusion, biomarkers reflect the pathogenesis of BPD and could allow early risk detection and interventions in the future. New omics techniques are promising but have not been studied enough yet. Better data collection and biobanking in large cohorts is needed to make larger steps forward.

ABS 119

INCIDENCE OF *FUSOBACTERIUM NUCLEATUM* IN TONGUE BIOFILM OF MOTHERS AND NEWBORNS. A NEW WAY FOR THE OLFACTORY PERCEPTION?

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INTRODUCTION

The anaerobic Gram negative bacterium *F. nucleatum* so far has been considered a putative pathogen; it is frequently associated with different human affections, such as chronic periodontitis in vaginosis microbiota and in preterm births [1]. Commonly this bacterium is isolated in oral biofilm, e.g. dental plaque, even in healthy subjects. Starkenmann et al. [2] published an article that

remarks a possible role of *F. nucleatum*, and others anaerobic oral bacteria, in transforming cysteine-S-coniugates, contained in fruits and vegetables, in soluble thiols able to modulate the odor's perception [2]. In this context the role of anaerobic tongue biofilm could be interesting, i.e. the mouth acts as bioreactor transforming S-coniugates from foods to various sulfur compounds able to stimulate the olfactory system (**Fig. 1**).

AIM

The aim of this preliminary study was to investigate the presence of *F. nucleatum* in the tongue surface of the mothers and the respective newborns. In fact, the significant presence of this bacterium in healthy subjects in the first day of life could suggest an unexpected physiologic function for the putative pathogen *F. nucleatum*.

METHODS

Fifty newborns (preterms) and forty mothers were recruited from the Neonatal Intensive Care Unit, Puericulture Institute and Neonatal Section, AOU and University of Cagliari. The patients showed this age's profile: mothers from 22 to 48 years (mean 37), children from 1 to 48 months (mean 15). The mothers have given informed consent to take part in the microbiological analysis. For each subject we have analyzed: (i) the breath's air to reveal, by gas-chromatograph (OralChroma™), the presence of volatile sulfur compounds (VSC), (ii) a tongue swab to analyze the bioma extention and the presence of *F. nucleatum* in the tongue. Microbiological analysis was performed by real-time PCR using a protocol with SYBR Green fluorescent Dye. Primers for *F. nucleatum* were designed using a hypervariable region of *RRS* gene, extracted from the NCBI database (GenBank) with accession number AY692453. The amount of tongue bioma (exfoliated epithelial cells plus bacteria) was measured following the total DNA quantization by Qubit® photometer [3, 4].

RESULTS AND DISCUSSION

Breath analysis and VSC production

The VSC analysis has been performed by dedicate portable gas chromatograph OralChroma™. This analysis is useful to analyze the VSC produced by the mouth microflora [5]. The presence of VSC in the breath of newborns could suggest a mature tongue biofilm able to transform S-compounds. First of all, 15% of the mothers showed high levels of H_2S (> 112 parts per billion, ppb) indicating a clinical sign of oral halitosis, despite only 4% of their children showed clinical evidence of bad breath. **Fig. 2** shows the increase of different VSCs in the newborns in comparison with the age. The results suggest a progressive production of oral H_2S correlated with the months of life; this suggests a progressive enrichment, in the children, of anaerobe bacteria on the tongue biofilm, characterized by VSC production, despite the gastric gas $(CH_3)_2S$ being very low or negative. In the mothers we have observed a positive correlation ($p < 0.05$) only with their body mass index (data not shown).

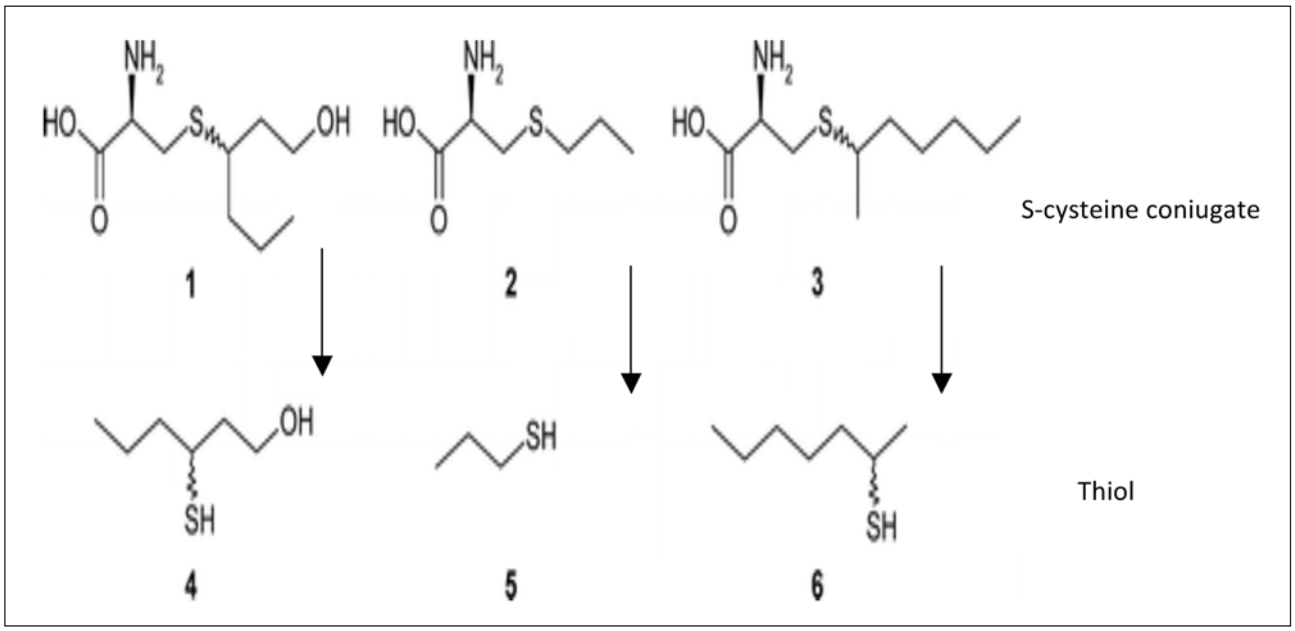

Figure 1 (ABS 119). Pathway of the enzymatic degradation of vegetables cysteine-S-coniugates by oral anaerobic bacteria. The correspective thiols are involved in the olfactory modulation for the fruit or vegetable.

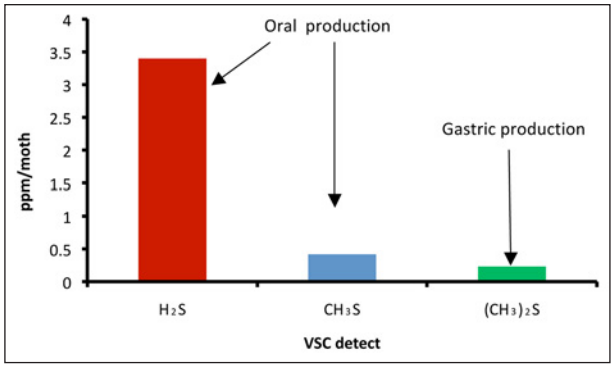

Figure 2 (ABS 119). VSC increase ppb/moth from bird evaluated in 50 newborns included in this study.

Tongue bioma and F. nucleatum analysis

The tongue bioma extension, calculated by the molecular procedures described above, showed that the amount of DNA in newborn's tongue was not correlated with the newborn age and the extension of mother bioma (data not shown): these results could be due to variability of the sampling. **Tab. 1** represents the association of *F. nucleatum* within the analyzed patient cohort. We can observe that 37 mother out of 40 (75%) resulted positive for this bacterium and none of these reported oral infectious diseases, in particular periodontitis; in addition, also a considerable number of newborns resulted positive for this anaerobic bacterium (34 out of 50 [68%]). *F. nucleatum* resulted associated in the mother and her newborn in 62.5% of the families, despite 7% of newborns resulting *F. nucleatum* positive with mother negative, indicating a possible extra-maternal way of transmission (**Tab. 1**). The molecular analysis showed that *F. nucleatum* is present in tongue biofilm in the first weeks of life, and after 24 months all newborns showed this bacterium in the oral cavity (**Fig. 3**).

CONCLUSIONS

The increase of the protease activity (H_2S production) with *F. nucleatum* presence, in comparison with the age, suggests a physiological role of this bacterium in human oral cavity already in the first days of life. Following the experimental hypothesis of Starkenmann and collaborators, this microorganism could be useful in the tongue biofilm for modulating the olfactory perception of vegetables. In this context, the mothers have a crucial position in this process, transmitting anaerobic bacteria by saliva to oral cavity of the newborn. This study is preliminary and other experiments are necessary to confirm this hypothesis i.e. following the mother salivary follow-up of the bacterium and the corresponding presence in tongue biofilm of the child.

Table 1 (ABS 119). Presence of *F. nucleatum* within the patient cohort.

	Mother	Newborn 1	Newborn 2	Newborn 3
1	+	-		
2	+	-		
3	+	-		
4	+	-		
5	+	-	-	
6	+	+		
7	+	+		
8	+	+		
9	+	+		
10	-	+		
11	+	+		
12		-		
13	+	+		
14	+	+		
15	+	+		
16	+	+		
17	+	+	+	
18	+	+		
19	+	+	+	+
20	+	+	-	
22	+	+		
23	+	+	+	
24	+	+	+	
25	+	-		
26	+	+		
27	+	+		
28	+	+	+	
29	+	+		
30	+	+		
31	+	-		
32	+	+		
33	+	+		
34	+	-	-	
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36	+	+		
37	+	+		
38	+	-		
39	+	-		
40	-	-		

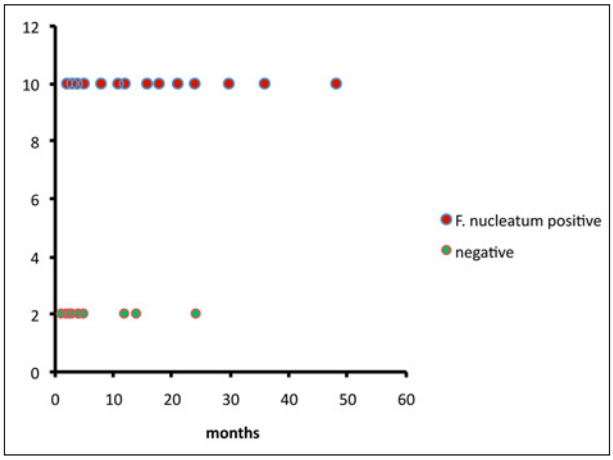

Figure 3 (ABS 119). Recurrence of *F. nucleatum* at different ages, evaluated in 50 newborns.

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ABS 120

PROCALCITONIN IN NEONATOLOGY

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Procalcitonin was described as a marker of sepsis in 1993 by Assicot et al., is the precursor of calcitonine and is normally produced by neuroendocrine C cells of the Tyroid gland. In the sepsis there is multiorgan massive production and release of procalcitonin. Procalcitonin levels may be increased in not infective inflammatory condition (surgery, trauma ,cardiogenic shock, cardio-pulmonary resuscitation, severe SIRS, severe renal or liver failure, first 72 hours after birth, rabdomyolisys). Many papers showed good sensibility and specificity for procalcitonin as biomarker of sepsis versus other markers (C-reattive protein and cytokine exspecially). Procalcitonin kinetic profile is more favourable agains C-reattive protein because of its short induction time (2-4 hours), its peak levels at 24-48 hours and its hemivita,of 24-36 hours. Cut-off for sepsis diagnosis is > 0.5 ng/ml but in first 72 hours after birth is more elaveted, necessitating adjiustments to the normal ranges. Several different assays and analyses are avaible to measure procalcitonine with different functional assay sensivity, assay time measurement and sample type. Because of its favourable kinetic procalcitonin may be used as early marker of sepsis, as early indicator of outcome in sepsis, as indicator for initiating or discontinuing antibiotics therapy (two consecutive Procalcitonin determinations > 0.5 ng/ml or inferior to 80% of procalcitonin peak), for monitoring appropriateness of antibiotic therapy. Procalcitonin in clinical use may be utilized for differentiating gram negative from gram positive infection and for differentiating bacterial sepsis from *Candida spp.* infections by a significantly higher value. The procalcitonin level is a potentially predictor of causative pathogens and a guide decision for treatment. The procalcitonin such as all the markers of sepsis must be considered in general context of physical examination and medical history. It is possible that contemporary use of procalciton and C-reactive protein for differented kinetics, for their sensibility and specificity related to non bacterial induced SIRS, for bacterial sepsis and candida infection, may be used in clinic for differentiating these situations and for guide decision making in therapy. Procalcitonine is a helpful biomarker for early diagnosis of severe sepsis in newborn but are necessary other studies to define optimal cut off in early onset infections and for the use in monitoring appropriateness and stop of antiobiotic therapy.

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