Abstracts

Selected Abstracts of the 10th International Workshop on Neonatology

THE LAST TEN YEARS, THE NEXT TEN YEARS IN NEONATOLOGY

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How to cite

ABS 1

UTILIZING MATERNAL FACTORS TO PREDICT ACUTE KIDNEY INJURY IN VERY LOW BIRTH WEIGHT INFANTS

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BACKGROUND

Despite the progress made over the last 20 years, acute kidney injury (AKI) remains associated with increased morbidity and mortality in very low birth weight (VLBW) infants. Although nephrotoxic medications, sepsis, hypotension and asphyxia are well known causes of AKI in the VLBW population, there is little information regarding the maternal factors that may be associated with the development of AKI in the VLBW infant. A model including both and maternal and neonatal factors for predicting AKI risk would allow for the opportunity for interventions prior to the development of AKI.

OBJECTIVE

We sought to determine which maternal factors are associated with the development of AKI among VLBW infants in a level IIIc neonatal intensive care unit (NICU) at the University of Virginia (UVA).

METHODS

We reviewed all infants admitted to the NICU of the UVA Hospital from April-October 2011 with birth weight ≤ 1,500 grams. Patients admitted > 2 days or died during hospitalization were excluded. AKI was defined according to KDIGO classification modified to include only serum creatinine (sCr). Maternal factors collected included maternal health and pregnancy history, medications, and general demographics. Statistical analysis was performed using X^2 for categorical variables and t-tests for continuous variables.

RESULTS

Of the 44 infants evaluated, 22.7% experienced AKI. Among patients experiencing AKI, mean maternal age was significantly increased compared to those who did not experience AKI (32.7 ± 7.6 vs 25.2 ± 4.7; p = 0.0002). Maternal aminoglycoside administration during pregnancy (p = 0.003) and the diagnosis of chorioamnionitis (p = 0.012) were found to be significantly correlated with AKI in VLBW infants. There was no association between AKI and maternal mode of delivery, history of diabetes, smoke exposure, or family history of renal disease).

CONCLUSIONS

In this retrospective study of VLBW infants, neonatal AKI occurs more frequently when a maternal history of advanced age, aminoglycoside therapy and chorioamnionitis are present. Refinement and validation of the model with the addition of patients are the next steps.

ABS 2

EFFECTS OF CESAREAN SECTION AND INFANT FEEDING ON LATER OBESITY RISK

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INTRODUCTION

Cesarean sections' (CS) number is increasing in many countries and a lot of concerns about short and long-term effects of this procedure, such as allergic diseases, are gradually emerging. Moreover, CS-born children show inadequate initial gastrointestinal colonization and aberrant mucosal immune function: in fact, vaginally delivered infants acquire a collection of bacterial communities similar to those in their own mother's vagina and skin, whereas CS-delivered infants acquire different and less diverse bacterial communities which may resemble the microbiota of the assisting personnel and the general delivery environment. Recent scientific advances point to an aberrant compositional development of the gut microbiota and low-grade inflammation as contributing factors to later metabolic risk, including obesity [1]. Another key driver for early weight gain and later obesity risk is the choice of infant feeding: feeding practice has a high potential role for long-term health

effects [2, 3]. It is already known that breastfeeding is protective against the risk of becoming obese; on the contrary, feeding formula with a higher protein content is associated with early weight gain, earlier adiposity rebound and higher body mass index (BMI) z-score at school-age.

AIM

To investigate whether delivery mode (vaginal or CS) may influence BMI z-score and obesity risk at 6 years of age.

MATERIALS AND METHODS

259 Italian children were recruited at birth (61 CS, 198 vaginal birth). Children were formula fed (n = 164, 43 CS and 121 vaginal birth) or exclusively breastfed (n = 95, 18 CS and 77 vaginal birth). Children were anthropometrically examined at the inclusion of the study (n = 259), at 12 months (n = 259), at 24 months (n = 259) and at 6 years of age (n = 39 CS, 9 BF and 30 FF; n = 126 vaginal birth, 44 BF and 82 FF). BMI was transformed to age and sex-specific z-scores according to the WHO growth standards [4]. Obesity was defined according to WHO criteria [4]. Analysis was performed using SPSS®-20.

RESULTS

In overall sample, mean [SD] BMI z-score was different between CS and vaginal birth group at 12 (0.654[0.980] vs 0.395[0.852], P = 0.046) and at 24 months (0.571[0.823] vs 0.320[0.836], P = 0.040). Obesity prevalence at 6 years of age was higher in CS group (17.9%) compared to vaginal birth group (6.4%) (P = 0.05). At 6 years of age BMI z-score wasn't different between BF and FF children both in the overall population (P = 0.665), nor in CS (P = 0.124) and vaginal birth (P = 0.781) children. No difference among BF and FF children occurred for obesity prevalence at 6 years in overall population (5.6% vs 10.7%, P = 0.391), in CS (0% vs 23.3%, P = 0.169) and in vaginal birth group (6.8% vs 6.1, P = 0.999). Including infant feeding choice in the model, a logistic regression analysis showed an independent association between delivery mode and obesity risk at 6 years of age (P = 0.046), with a risk to become obese of 3.05 (95% CI 1.02 to 9.12) for children deliverd by CS.

CONCLUSIONS

The results of the present study show that CS may be considered as a risk factor for increased later obesity. This association seems to be independent of the feeding choice. Further studies, especially on microbiome and metabolomics, are needed to understand the underlying mechanisms. The finding of an association between birth by CS and childhood obesity is important and points out the need to avoid, in absence of real medical indications, this type of delivery.

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ABS 3

A PROPOSAL FOR MULTICENTER STUDY ON FAMILY-CENTERED CARE IN NICU, PARENTS' SATISFACTION AND EXPERIENCE

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BACKGROUND AND AIM

The quality of family-centered care (FCC) in Neonatal Intensive Care Unit (NICU) is often assessed through Parental Satisfaction (PS). At the moment, FCC in NICU is not expressed homogeneously across Europe.

The objective of the study is to evaluate the quality of FCC by a validated PS instrument and to explore its relationship with the level of FCC expressed by professionals and by the organization of the NICU.

METHODS

A multicenter, observational study will be performed. In particular, a survey across all the Italian NICUs will investigate NICUs' organization, using the "FCC in the NICU: A Self-Assessment Inventory" from the FCC Institute. Then, a cross-sectional design will assess the satisfaction of, at least, 400 parents of discharged newborns, using the "EMPATHICN" questionnaire, developed by Latour et al. (2012) and the professional attitude of 400 healthcare providers with the "FCCQR" questionnaire by Bruce et al. (1997), revised for neonatology setting.

Ethical approval for the study has been submitted to the Bambino Gesù Children's Hospital Ethical Board. Written informed consent forms will be collected among all the participating parents and healthcare providers.

RESULTS

Descriptive statistical analysis will be performed on the results from the samples (NICU, parents and healthcare providers) and items normality will be tested through asymmetry and kurtosis index.

CONCLUSIONS

A reliable description of PS and the identification of variables associated with it is expected. The results of the study will provide guidance for organization and training relevant to the FCC interventions, to increase the quality of care and the PS. In addition, the results provide evidence based outcomes for benchmarking the NICU clinical practices.

ABS 4

DORSAL PENILE GLANS EPIDERMOID CYST: A CASE REPORT IN A CHILD

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INTRODUCTION

Penile epidermoid cysts are rare, frequently presenting as a congenital lesion located ventrally along the median raphe [1-3]. We report the first case of dorsal epidermoid cyst of the glans penis in a pediatric patient, successfully treated with surgical excision, with a very satisfactory cosmetic and functional result.

CASE REPORT

A 9 year old boy was referred to our Center because of phimosis and an asymptomatic, whitish, quickly growing soft mass of the dorsal aspect of the penis, measuring approximately 5 millimeters, closely resembling a smegmatic cyst. Following gentle retraction of the foreskin, no smegmatic inclusion cyst was found. Surprisingly, the mass appeared to be included in the glans, deforming its dorsal profile (**Fig. 1**).

An ultrasound scan (US) with color doppler was promptly performed revealing an homogeneous, hypoechoic mass measuring 1.5 cm, without vascular signals nor urethral involvement (**Fig. 2**). Ultrasound was repeated after one month, showing no significant differences.

An elective surgical excision was therefore planned along with a preoperative urethrocystoscopy, which documented a normal penile urethra, without sign of extrinsic compression. The urethra was catheterized to prevent injuries during dissection. Surgical excision of the mass was completed with an haemostatic loop at the base of the penile shaft. The cyst appeared to be full of a cheesy content and was completely excised (**Fig. 3**). A foreskin dorsal plasty completed the procedure to facilitate complete retraction of the prepuce over the glans (**Fig. 4**).

Histologic examination revealed a fully excised epidermoid cyst, lined with stratified squamous epithelium and laminated keratin along with surrounding granulomatous aspects, identified by histiocytic infiltration. Three months postoperatively the child showed no dysuria or discomfort. The foreskin retracts with ease showing an excellent local cosmetic result of the glans. No signs of recurrence were detected.

DISCUSSION

Congenital epidermoid cyst of the penis is a very rare clinical entity [4]. Glans localization is even rarer, only 2 cases having been described so far worldwide [1, 5]. To our knowledge, this case is the first report of an epidermoid cyst involving the dorsal aspect of the glans penis in a child.

When located ventrally, the cyst may be secondary to circumcision or other surgical procedures [6]. More frequently, the primary etiology remains unclear, and may be related to possible abnormal closure of the median raphe or a folding defect of ectodermal tissue surrounding the urethral plate,
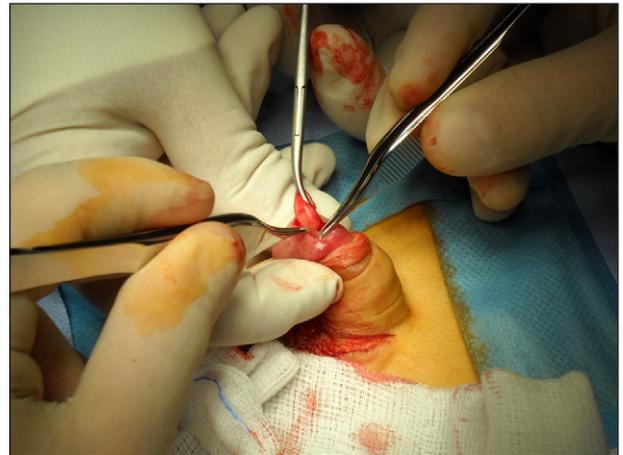
Figure 1. Preoperative aspect of dorsal penile glans lesions following gentle foreskin retraction.
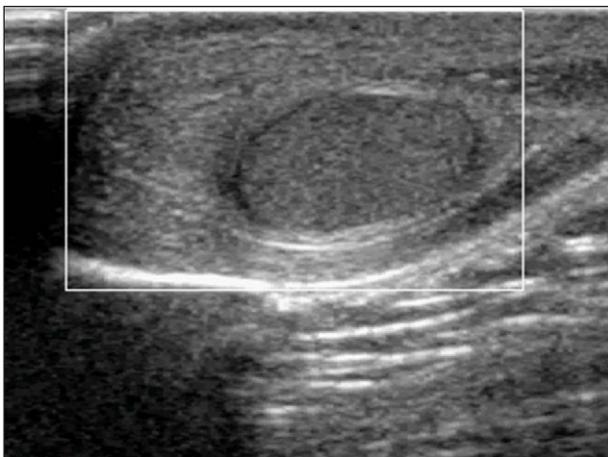
Figure 2. Ultrasonographic appearance of the glanular lesion.

Figure 3. Intraoperative view of cyst excision.

Figure 4. Immediate postoperative aspect of glans reconstruction and foreskin dorsal plasty.

which may cause epidermoid inclusions. Cysts localized along the median raphe may, therefore, present different and heterogeneous surrounding epithelium, such as epidermoid, urothelial, or both [2, 3]. Other Authors suggest that penile epidermoid cyst may represent a monolayer germ cell teratoma, as proposed for intratesticular epidermoid cysts [7]. Clinical presentation is usually as a painless swelling of the penile shaft, slowly growing. Young adults generally complain sexual discomfort, whereas parents usually report the incidental finding of an asymptomatic mass in children [8]. Almost all reports describe a single epidermoid cyst. Differential diagnosis include other cystic lesions as parameatal urethral cysts or pylosebaceous cyst, vascular, neurogenic, infectious lesions, or diphallia [4]. When multiple lesions are present, other dermatological affections such as juvenile xantho-granuloma, fibroma, pearly penile papules, macules or nevi should be considered in the differential diagnosis [4, 9, 10].

A voiding cystourethrogram (VCUG) and eventually a preoperative urethrocystoscopy under general anesthesia may help in differentiating epidermoid cysts from other urethral anomalies such as urethral diverticulum or fistula [3, 4, 8].

Whenever an intrapelvic extension of the lesion is suspected, further diagnostic imaging is mandatory (i.e. pelvic US and/or MRI) [7].

Surgical indications include a symptomatic (pain, sexual discomfort, infection, urethral obstruction) or an enlarging cyst. Frequently, simply cosmetic reasons may warrant surgical excision [4]. Although theoretically possible, there is no evidence suggesting malignant transformation in penile epidermoid cysts [8]. In the reported case, surgical treatment was considered and prompted because of enlargement of the lesion, as well as some concern about urethral obstruction and possible histology due to unusual localization of the lesion. Surgical complete excision represent the first choice treatment. Aspiration and drainage and/or incomplete excision are burdened with high recurrence risk [9]. Patients should be followed up regularly for at least 1 year. No further investigations are required unless based on clinical suspicion.

CONCLUSION

Glans malformations are generally benign and amenable to complete excision without further treatments, as for epidermoid cysts. On the other hand, definite histological diagnosis is usually achievable only with surgical excision. Therefore, as a minority of glans lesions are linked with malignant transformation [4], clinicians should document and promptly treat these affections in order to confirm clinical diagnosis and eventually decrease parental anxiety.

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ABS 5

A NUMERICAL PREDICTION OF THE ITALIAN SCHOLASTIC POPULATION IN 2020

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We realized a numerical prediction of the Italian scholastic population in 2020 based on the ISTAT data. In five years, number of students globally will drop 1% (i.e. -61,389 students), but not equally everywhere. Scholastic population will increase in some regions in the following order: Emilia Romagna (+5.62%, i.e +23,566 students), Lombardy (+3.41, i.e. +34,368 students), Lazio (+2.73%), Tuscany (+2.50%), Umbria (+2.44%), Piedmont and Aosta Valley (+1.22%), Veneto (+ 0.62%). In Friuli Venezia Giulia the number of pupils will be stable. Only two northern areas will have a negative trend: Trentino - South Tyrol (-2.35%) and Liguria (-2.17%).

Southern regions and islands will have a general negative trend: Basilicata (-10.12%), Molise (-8.96%), Apulia (-7.94%, i.e. -35,939 students), Campania (-7.21%, i.e. -51,087 students), Calabria (- 6.31%), Sicily (-5.91%) and Sardinia (-3.41%), Abruzzo (-1.83%).

About Emilia Romagna, the increase of scholastic poulation will be distributed as follows: Reggio Emilia (+ 7.40%), Parma (+ 7.07%), Ravenna (+ 6.25%), Modena (+ 6.11%), Forlì-Cesena (+ 5.97%), Ferrara (+ 5.34%), Bologna (+ 5.14%), Rimini (+ 3.23%), Piacenza (+ 1.93%). About Lombardy, the increase of scholastic population will be distributed as follows: the strongest growth is expected in Mantua (+ 5.1%, i.e. +2,062 students), followed by Milan (+ 4.75%), Lodi (+ 4.5%); Brescia (+3.83%); Monza and Brianza (+ 3.35%); Pavia (+3.22); Bergamo (+2.64); Varese (2.46%), Como and Cremona (+2%), Lecco (+1.31%). The only negative trend in Lombardy will be in Sondrio (-4.65%, i.e. -880 students).

ABS 6

RESPIRATORY TRACT INFECTIONS (RTI) IN PEDIATRIC POPULATION

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OBJECTIVES

Respiratory tract infection (RTI) is a pervasive public health issue and a great burden to both families and society in general [1]. Upper and lower respiratory tract infections are commonly encountered in the emergency department [2]. The aim of this study was to evaluate the prevalence of respiratory symptoms as motive for emergency room visits by pediatric patients, describing the major clinical syndromes.

METHODS

We performed a retrospective, transversal, observational study in the pediatric emergency department at the University Hospital of Messina. Study population included children aged 1 month - 16 years who presented at our emergency room with respiratory symptoms, over a 5-year period (from September 2008 to December 2013).

RESULTS

A total of 1,041 children aged 1 month - 16 years were included in this retrospective investigation and diagnosed with RTI at our hospital. There was an increase in the number of visits in winter months (December, January, and February). The group aged 1 month - 1 year was most affected by respiratory problems. During the first 4 months of life, male patients represented the larger number of pediatric emergency room visits for respiratory problems. Bronchospasm emerged as the most frequent (36%) respiratory problem, followed by laryngospasm and bronchitis (18%, both). The incidence of pneumonia increased from 9 months of age onwards.

CONCLUSIONS

Studying the epidemiological profile of pediatric patients presenting at an emergency room with respiratory symptoms, we observed a high prevalence of access to pediatric emergency room for upper and lower RTIs. This study suggests to encourage preventive measures against RTIs and emphasizes the importance of an adequate therapeutic approach, in order to combat growing antibiotic resistance in pediatric patients [3].

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ABS 7

FETAL-NEONATAL H[1]NMR NUTRIMETABOLOMICS IN THE FIRST WEEK OF LIFE

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INTRODUCTION

It is known that nutritional imbalances and metabolic disorders that occur during the critical period of fetal development can have both short-term or long-term repercussions on the child's health.
Several studies have been taken into consideration, until now all of them have addressed the existing relation between low and high birth weight and the bigger risk for these babies to develop chronic pathologies such as diabetes mellitus type 2, arterial hypertension, dyslipidemia and metabolic disorders.

PURPOSE OF THE STUDY

Through a metabolomics holistic approach we have analyzed several urinary metabolic profiles of neonates born with intrauterine growth retardation (IUGR), adequate for gestational age (AGA) and large for gestational age (LGA), in order to identify the predictive biomarkers of the metabolic profile.

MATERIALS AND METHODS

We took into consideration a cross-section of 116 newborns from whom urine have been collected on the 1st, 3rd, and 7th day. A metabolomic analysis has been carried out in order to allow the characterization of the metabolomic signatures of the three classes of newborns.

RESULTS

Our preliminary data show more metabolic analogies between IUGR and LGA, rather than

AGA, in particular some alterations in metabolites of Krebs Cycle such as citrate, betaine and glycine.

CONCLUSIONS

Once again, metabolomics turns out to be a promising tool for the study of the newborn metabolic basal state, in order to take account of gestational nutrition and its possible complications in an advanced age.

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ABS 8

VARIABILITY IN LANGERHANS ISLETS NUMBER AT BIRTH: MARKER OF SUSCEPTIBILITY TO DEVELOP DIABETES LATER IN LIFE?

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INTRODUCTION

The development of the human pancreas is a complex process, that requires the fusion between two buds emerging from the endoderm and necessitates molecular interactions between the pancreatic endoderm and the adjacent notochord [1]. Recent studies have been carried out on the multiple factors that play a role in the epigenetic modulation of pancreas development, including intrauterine growth restriction and perinatal asphyxia [2] (**Fig. 1**). Perinatal programming is fundamental in determining the health or the disease status in adulthood, since the predisposition to many pathologies may start in the first stages of life. The aim of the present work was to study pancreatic histology in fetuses and in preterms of different gestational age, in order to correlate the degree of pancreas development and in particular of Langerhans islet burden with gestational age [3].

PATIENTS AND METHODS

20 pancreas samples, 10 from fetuses and 10 from neonates were obtained at autopsy. Gestational age ranged from 12 up to 36 weeks. Pancreas samples were formalin-fixed, paraffin-embedded and routinely processed. 4 μ-thick sections were stained with H&E (Hematoxylin and Eosin) and examined by two pathologists (G.L.; S.N.). In each pancreas the number of Langerhans islets was evaluated, by counting the number of islet cells in 5 consecutive histological fields at 200x.

RESULTS

Islet number increased with increasing of gestational age. Pancreatic samples under 20 weeks of gestation didn't show any organized Langerhans islet, endocrine cells forming small clusters distributed throughout pancreatic parenchyma, indistinguishable at H&E from other surrounding cell types (**Fig. 2**). At 21 weeks the presence of islets was observed. From the 26th week, a higher islet number was observed. A subject at the 26th week presented an average number of 3 islets, with a maximum of 5 islets observed (**Fig. 3**). A subject at the same gestational age presented a number an average islet number of 2, with a maximum of 4 islets observed. Moreover, two twin subjects of 27 weeks, did not show a marked interindividual variability in Langerhans islets burden (**Fig. 4**). A strong variability in islet number emerged among subjects with the same gestational age. From the 28th week, the average number observed were 5 islets. A peak in islets development was observed in a subject at the 28th week, with a maximum number of 8 islets.

CONCLUSIONS

Our data show that Langerhans islets are distinguishable in human pancreas starting from the 20th gestational week. Islets burden is strictly influenced by gestational age, as demonstrated by the increasing number of islets that reach an adult conformation at 26th week. A strong variability in islets number emerged in subjects with the same gestation age. In this study, the incomplete pancreatogenesis was associated with various epigenetic intrauterine injuries, including asphyxia and multiple organ failure, that resulted in a reduction of quantity and quality of the endocrine compartment. Further studies are needed in order to better understand relevance of fetal programming and Langerhans islet burden at birth on the predisposition to develop type I diabetes in childhood or type II diabetes in adulthood.
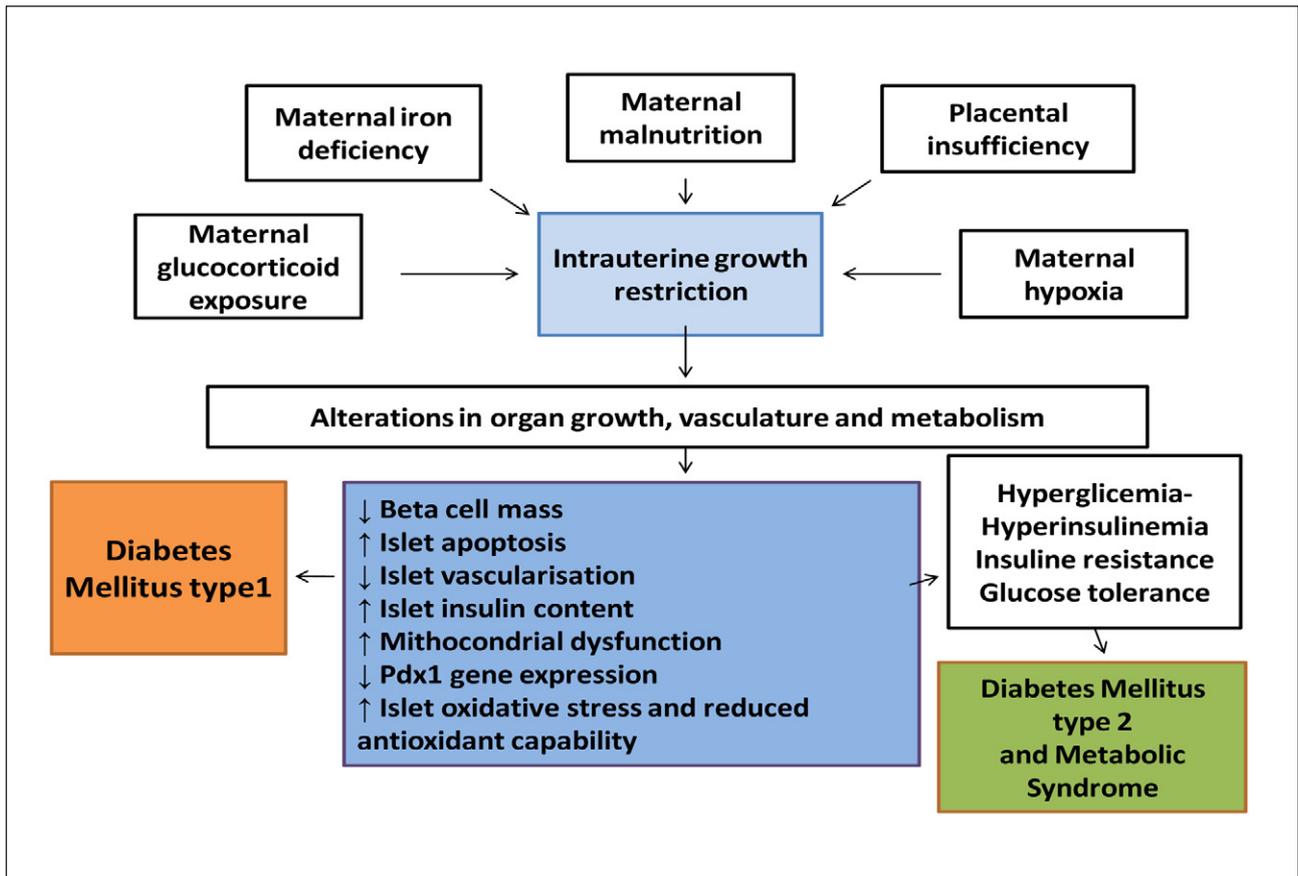
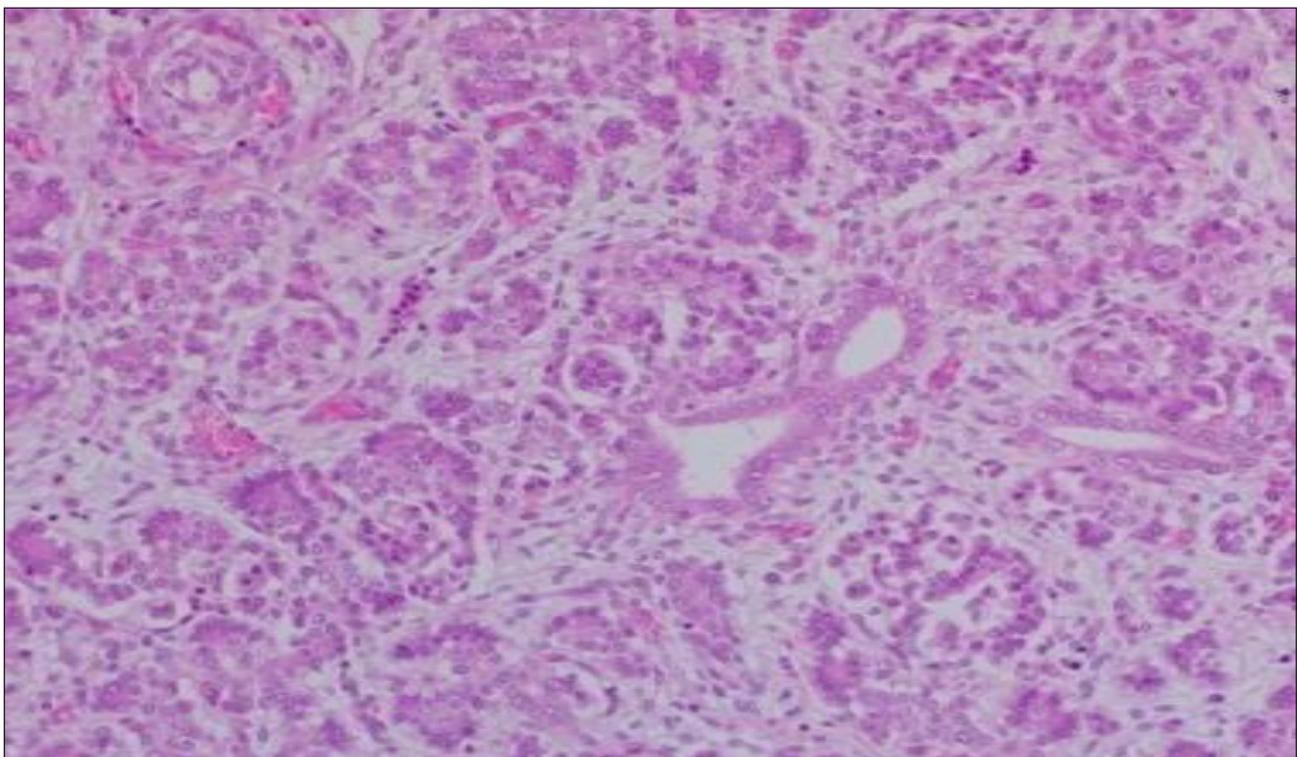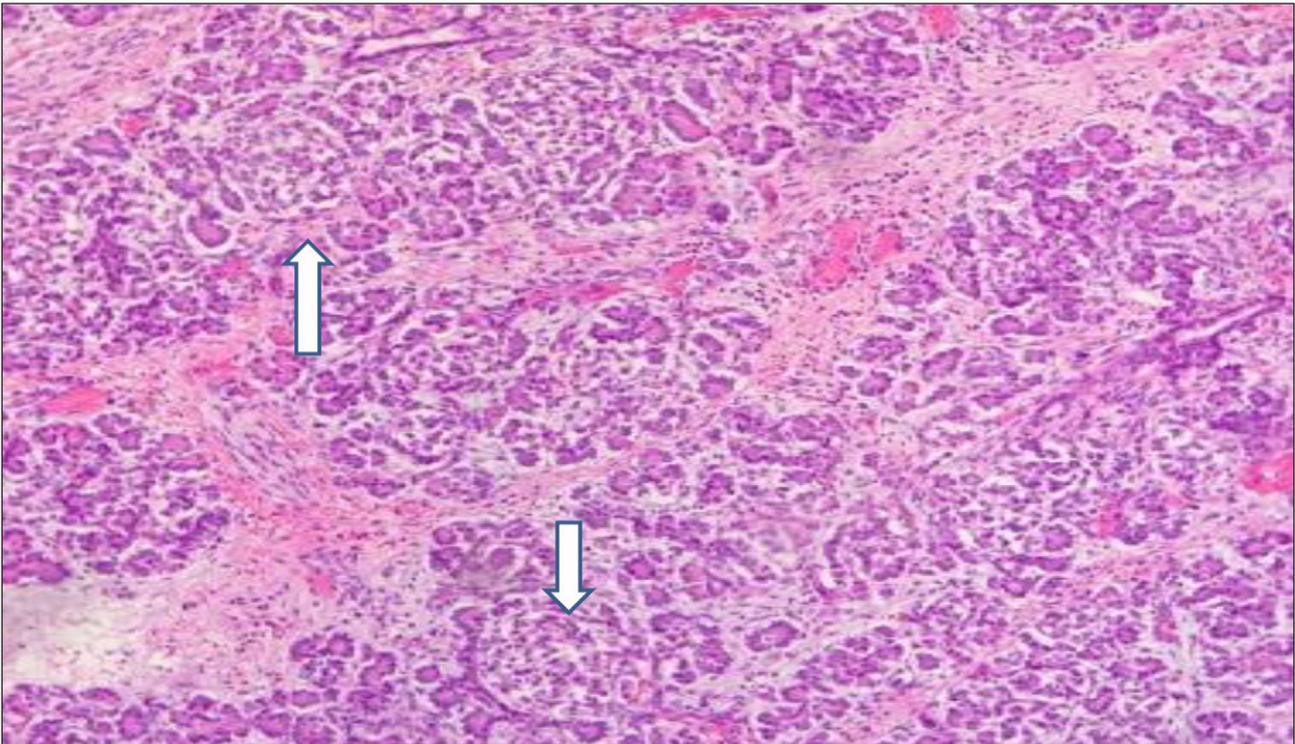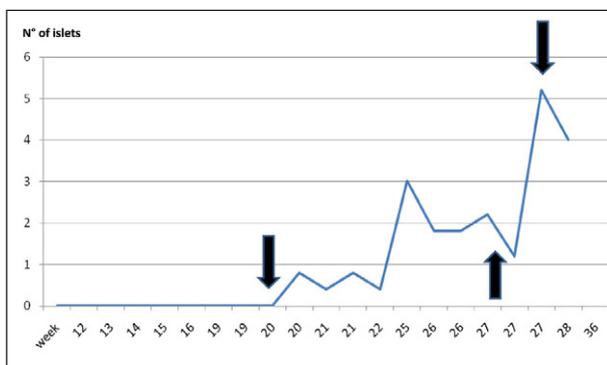**Figure 1.** Epigenetic mechanisms involved in diabetes mellitus risk.

Figure 2. Human pancreas of subjects at 19 weeks of gestation: no organized Langerhans islet is evident. Hematoxylin and Eosin (200x).

Figure 3. Human pancreas of subjects at 26 weeks of gestation: organized islets are shown (arrows). Hematoxylin and Eosin (200x).

Figure 4. Interindividual variability in Langerhans islets burden during gestational weeks. Langerhans islets were evaluated by counting the number of islet cells in 5 consecutive histological fields at 200x.

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ABS 9

TRAFFIKING OF THYMOSIN BETA-4 IN THE CELLULAR COMPARTMENTS OF HEPG2 CELLS AT DIFFERENT CONDITIONS

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INTRODUCTION

Due to its actin-sequestering properties, thymosin beta-4 (Tβ4) is considered to play a significant role in cellular metabolism. Several physiological properties of Tβ4 have been reported [1], however many questions concerning its cellular function remain to be ascertained. To better understand the role of this small peptide we have analyzed by means of transmission immunoelectron microscopy the ultrastructural localization of Tβ4 in HepG2 cells, which are a suitable *in vitro*

model system for the study of polarized human hepatocytes.

MATERIALS AND METHODS

Samples of HepG2 cell culture were fixed in a mixture of 3% formaldehyde and 0.1% glutaraldehyde in 0.1 M cacodylate buffer and processed for standard electron microscopic techniques. The samples were dehydrated in a cold graded methanol series and embedded in LR gold resin. Ultrathin sections were labeled with rabbit antibodies to Tβ4, followed by gold-labeled goat anti-rabbit, stained with uranyl acetate and bismuth subnitrate, observed and photographed in a JEOL 100S transmission electron microscope (TEM).

RESULTS

High-resolution electron microscopy showed that Tβ4 may have different localization, in the cellular compartments of HepG2 cells at different conditions. HepG2 cells growing in complete medium where characterized by a strong Tβ4 reactivity in the cytoplasm and in the perinuclear region, being strictly associated to the endoplasmic reticulum, whereas the nucleolus resulted labeled. In HepG2 cells growing for 48 h in the absence of fetal bovine serum evident Tβ4 immunostaining was observed in both cytoplasm and nucleus. In the nuclear compartment, Tβ4 was uniformly distributed in the nucleoplasm and only few gold particles decorated occasionally the nucleolus.

CONCLUSION

The above immunoelectron microscopic results confirm and extend previous observations at light microscopic level [2], highlighting the ability of Tβ4 to change subcellular distribution in both cytoplasmic and nuclear compartments according to different environments. The meaning of Tβ4 localization in the nucleolus is not at the best of our knowledge clarified yet. It could account for the interaction of Tβ4 with nucleolar actin being Tβ4 the most important actin monomer sequestering molecule in human cells. According with this hypothesis, Tβ4 could contribute, together with other nucleolar actin binding proteins [3] to modulate the transcription activity of RNA polymerases.

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ABS 10

THYROID CARCINOMA IN SARDINIAN CHILDREN AND ADOLESCENTS IS OFTEN ASSOCIATED WITH AUTOIMMUNE THYROIDITIS

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INTRODUCTION

Thyroid cancer is generally considered uncommon in childhood and adolescence and radiations are generally considered the most important risk factors associated with this type of tumor [1, 2]. Probably for this reason, unlike other cancer types in children, differentiated thyroid cancer (DTC) has not been extensively and prospectively studied in non-exposed pediatric patients [3]. On the other hand, thyroid cancer is the most common endocrine malignancy in pediatric patients [4]. For these reasons, the aim of this study was to elucidate the clinical and pathological findings in thyroid carcinomas diagnosed in Sardinian children and adolescents in our Institute.

METHODS AND PATIENTS

A total of 41 consecutive patients aged 11-20 years, including 33 females and 9 males, underwent surgery for thyroid cancer between January 2001 and December 2011. The medical history did not evidence any risk factor, including external radiation. No family history of thyroid cancer was present. All patients underwent total thyroidectomy coupled with lymphadenectomy in the cases with ultrasonographic evidence of enlarged lymph nodes. Molecular analysis of B-RAF mutations was performed using a direct PCR sequencing method.

RESULTS

At macroscopy, 8 cases showed extrathyroid extension. At histology, all cases were follicular-derived differentiated carcinomas, including 36 papillary thyroid carcinomas (PTCs), 2 follicular carcinomas (FTCs), and 3 well differentiated carcinomas Not Otherwise Specifed (NOS). Regarding the subtype of PTCs, 6 were aggressive variants, including 4 tall cell and 2 diffuse sclerosis variants. In 21 out of 36 PTCs (58.3%), histological signs of lymphocytic thyroiditis were detected in

the peritumoral thyroid tissue. Cervical lymph node metastases were present, at presentation, in 15 out of 42 subjects (36.6%); no distant metastasis was found. Whereas the two FTCs and the three NOS did not show any mutation in the BRAF gene, 6 out of 30 cases of PTCs examined (20%) carried the V600E BRAF mutation.

DISCUSSION

Our study shows that thyroid cancer in Sardinian children and adolescents shows some peculiar features: 1. follicular-derived differentiated thyroid cancer represents 100% of our cohort, in the absence of any case of medullary carcinoma; 2. PTC shows an incidence (87.8%) higher than that reported in other pediatric populations; 3. PTC is associated in the majority of cases (58.3%) with lymphocitic thyroiditis; 4. the V600E BRAF mutation was present in 20% of the PTCs examined. This finding, together with the absence of any exposure to radiation in our patients, indicates that other factors should be considered in the pathogenesis of PTC in Sardinian children and adolescents. Among these, autoimmune thyroiditis might represent the prevalent predisposing factor.

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ABS 11

PROLONGING NEPHROGENESIS IN PRETERM INFANTS: A NEW APPROACH FOR THE PREVENTION OF RENAL DISEASE IN ADULT-HOOD?

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INTRODUCTION

Chronic kidney disease (CKD) represents a global public health issue with different features to take into account in different parts of the world, causing a dramatical increase in the prevalence of end-stage renal disease (ESRD). Kidney transplantation does not represent the definitive therapy for end-stage renal disease, and when the complex kidney structure is disrupted by ESRD, traditional stem cell-based approach has been demonstrated to be unable to regenerate the damaged organ. The aim of this study is the introduction of a new potential approach for the prevention of CKD, based on the utilization of a previously unrecognized physiological regenerative source: the huge amount of stem cells that are present in the kidney of preterm babies, giving rise to the so called blue strip [1].

MATERIALS AND METHODS

Kidney samples were obtained at autopsy from 63 consecutive fetuses and newborns ranging from 20 up to 41 weeks of gestation. Tissue samples were formalin-fixed, routinely processed, and paraffin-embedded. Histological sections were stained with H&E. The width of the sub-capsular blue strip was measured by a method based on combination of color and morphological observation.

RESULTS

Kidneys in preterm newborns showed relevant structural differences as compared to at term newborns. Preterm kidneys were characterized by the presence of a huge amount of stem/progenitor cells in close proximity of the renal capsule, appearing as densely-packed small cells with scant cytoplasm, giving rise to a blue-appearing strip in H&E-stained kidney sections (**Fig. 1**). In at term newborns, the stem cell compartment was scanty or absent (**Fig. 2**).

CONCLUSION

After birth, nephrogenesis may go on only for some weeks, and then stops [2]. As a consequence, in a preterm born at 26 weeks, nephrogenesis will stop around the 32nd postconceptional week, linking prematurity with oligonephronia and with higher susceptibility of preterms to develop kidney disease later in life. Our data show that the presence of a high number of active endogenous stem cells in the preterm kidney represents a unique opportunity for starting regenerative medicine in the perinatal period. The main obstacle for the actual utilization of this regenerative potential is the abrupt interruption of their nephrogenic activity few weeks after birth, irrespectively of the gestational age at birth. Understanding how to switch on stem/progenitors of the blue strip, maintaining their activity in the generation of new nephrons till the 36th postconceptional week will represent
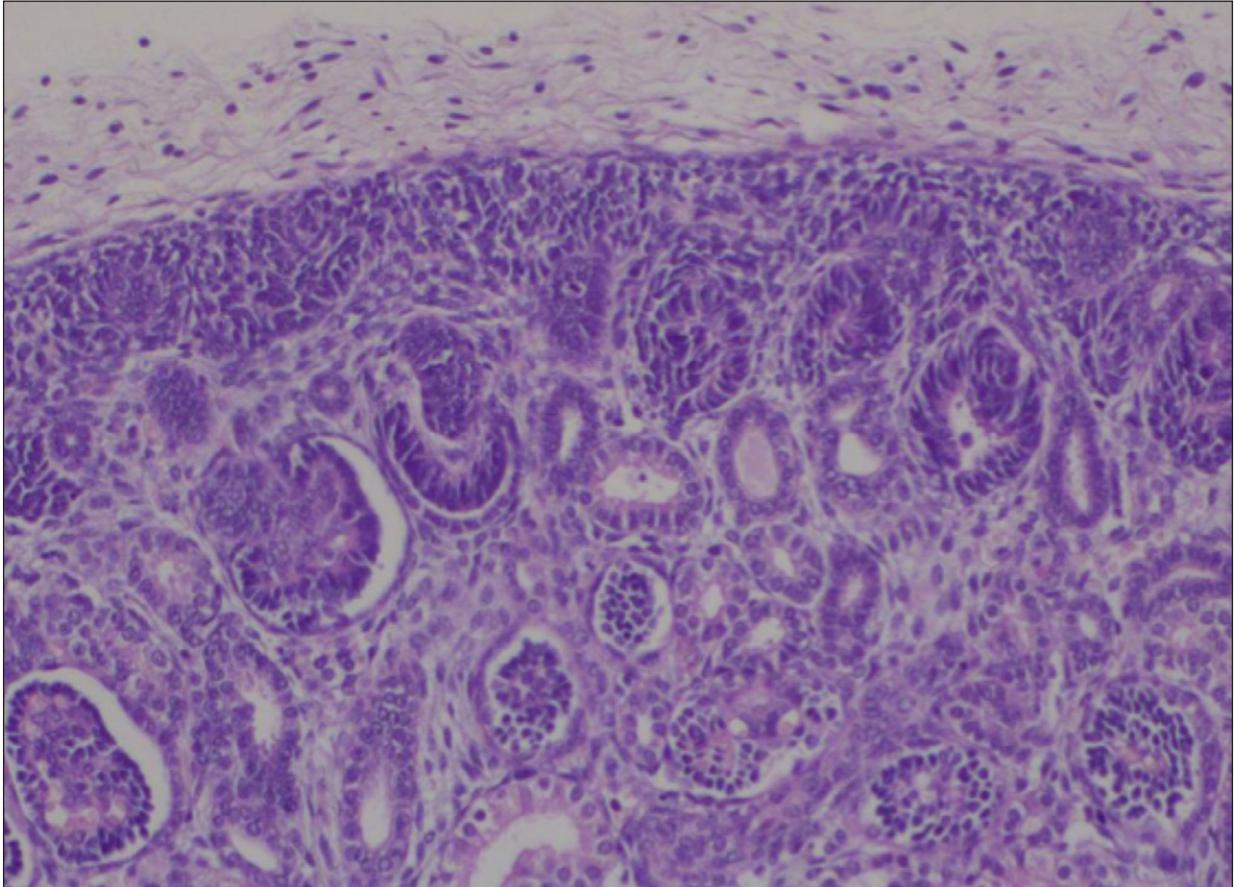
Figure 1. Autoptic kidney at 16 week of gestation (H&E): presence of Blue Strip in the sub-capsular region.
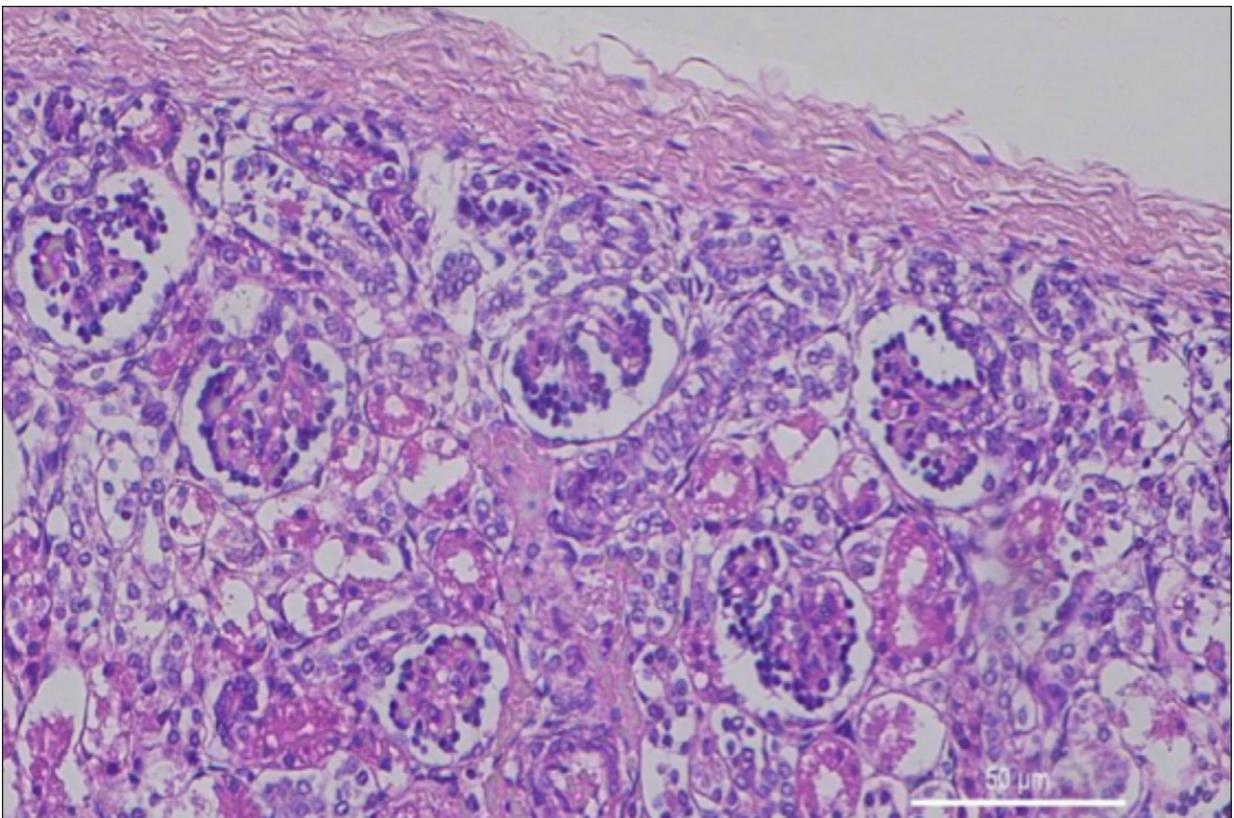
Figure 2. Autoptic kidney at 39 week of gestation (H&E): absence of Blue Strip in the sub-capsular region.

the new challenge for the prevention of CKD in adulthood. This new approach should be defined as "physiological renal regenerative medicine" [3].

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ABS 12

HUMAN ADRENAL GLAND DEVELOPMENT: MORPHOGENESIS AND MOLECULAR MECHANISMS

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INTRODUCTION

The fetal developing adrenal glands are characterized by marked differences regarding the general architecture and the cell types as compared to mature adult adrenal glands. The adrenal gland, a major hormone secreting organ, is composed of two distinct zones: the cortex, derived from the intermediate mesoderm, is organized into three concentric areas, zona glomerulosa, zona fasciculata and zona reticularis, which produce different steroid hormones; the medulla, derived from the neural crest of neuroectoderm lineage, produces catecholamines [1]. Human adrenal gland development begins at around the fourth week of gestation and continues into adult life. The development of human adrenal gland describes five landmarks: 1) condensation of the celomic epithelium (3-4th week of gestation); 2) proliferation and migration of celomic epithelial cells (8-10th week); 3) morphological differentiation of fetal adrenal cortical cells into two distinct zones: Fetal Zone (FZ) and Definitive Zone (DZ) (8-10th week); 4) decline and disappearance of the fetal zone (3rd postnatal month); 5) establishment and stabilization of the adult zonal pattern (10-20 years of age) [2] (**Fig. 1**). Between FZ and DZ, a thin region named Transitional Zone (TZ) containing both DZ and FZ cells is present, characterized by DZ cells forming cords that invade the FZ [3-4] (**Fig. 2**). During gestation, the adrenal medulla is absent but small clusters of medullary cells are observed scattered throughout the cortex (**Fig. 3**). During the 1st postnatal week, after the involution of the FZ, these cells appear to be concentrated around the central vein and form a rudimentary medulla. By the fourth postnatal week medullary cells cluster in the centre of the gland giving rise to the medulla in its adult-like in appearance [4] (**Fig. 4**).

MATHERIALS AND METHODS

Adrenal glands of 20 human fetuses and newborns, ranging from 11 up to 36 weeks of gestation, were 10% formalin-fixed, routinely-processed and paraffin-embedded.

Adrenal samples were stained with H&E and immunostained with antibodies against S100 protein, Chromogranin A, NCAM (CD56), and WT1.

RESULTS

At histology, adrenal development during gestation was characterized by a progressive increase in thickness of the DZ, appearing as a "blue strip" in the subcapsular area, paralleled by a deacrease in thickness of the FZ. Moreover, in the TZ, the invasion of DZ cells increased during gestation. Medullary cells, scattered and mainly localized in the cortex in fetuses, progressively aggregated in small clusters and localized to the inner adrenal zones. By immunohistochemistry, it was possible to better identify the different adrenal cell types: chromogranina A and S100 marked medullary cells, CD 56 (NCAM) marked DZ and medullary cells (**Fig. 5**), and WT1 specifically immunostained FZ cells.

DISCUSSION

Morphogenesis of the human adrenal gland, according with our preliminary data, appears as a complex process characterized by: invasion of the FZ by cells originating from the DZ (blue strip), with a process that appears very similar to invasion occurring in neoplasia; migration of neural crest-derived cells from the capsule towards the central zones. Immunohistochemistry allows to identify the multiple cell types participating to adrenal development. Finally, our preliminary morphological and immunohistochemical data evidence a previously unreported interindividual
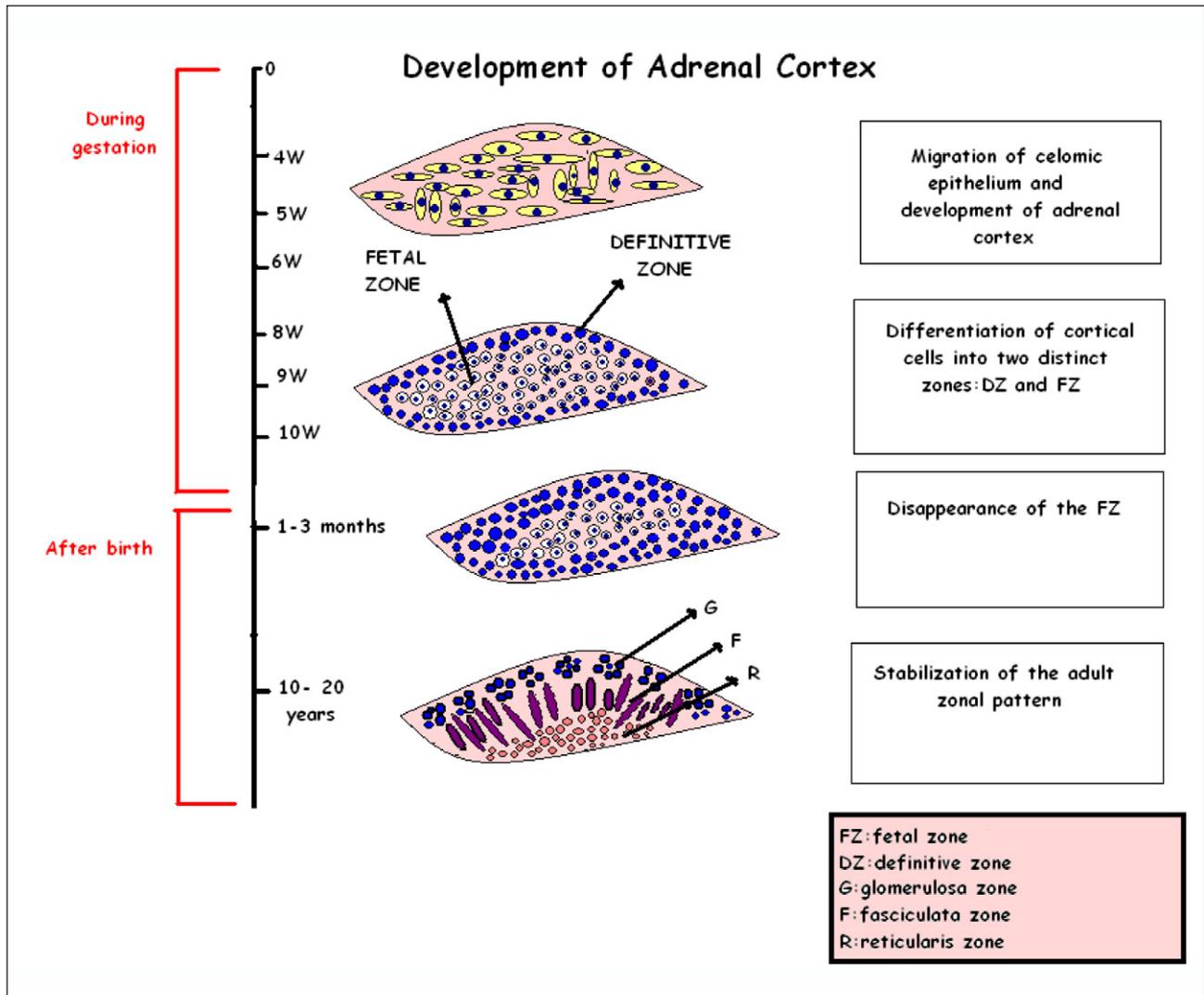
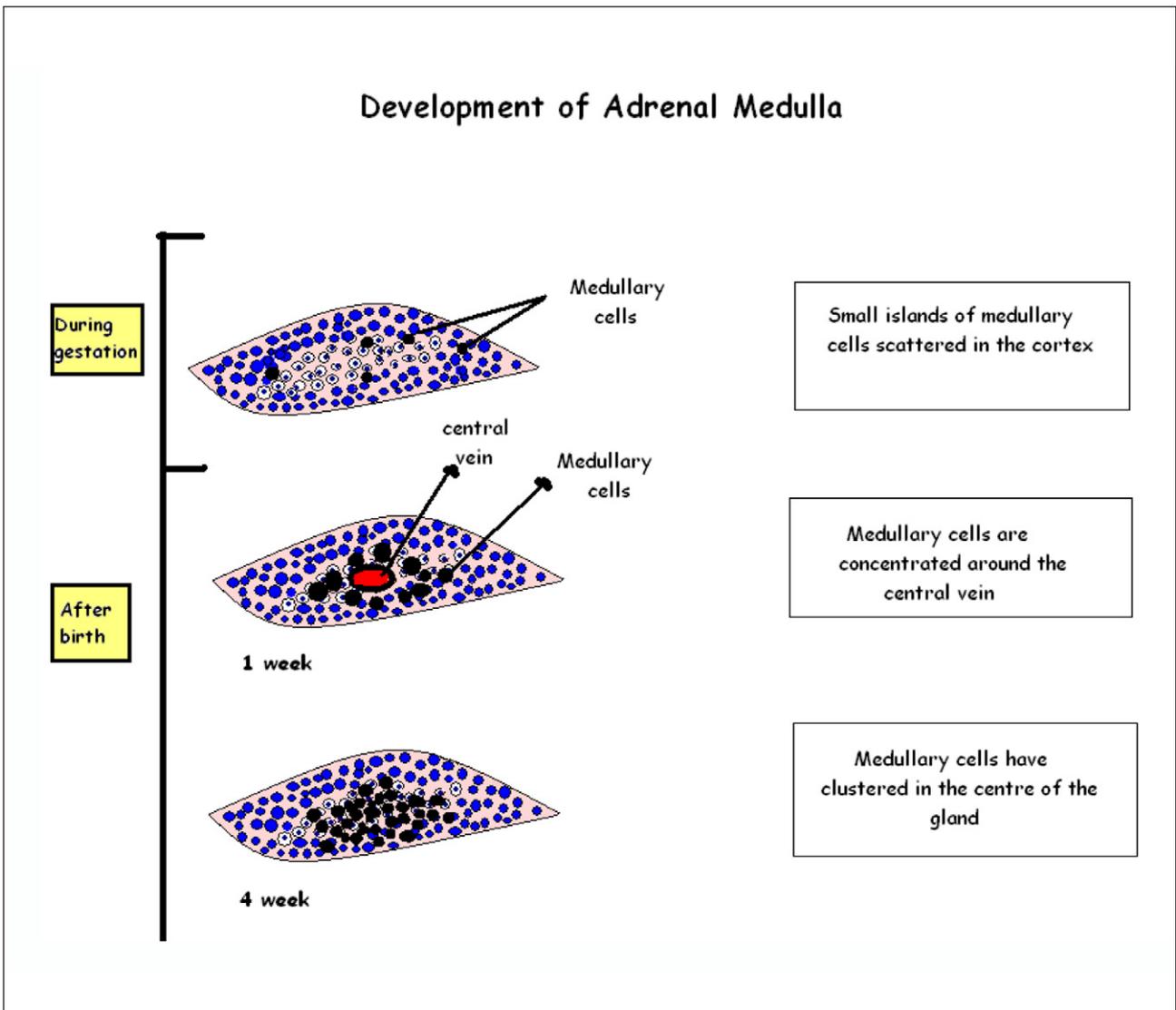**Figure 1.** The development of adrenal cortex during gestation and after birth. Human adrenal development can be represented as a continuum beginning at around the fourth week of gestation and continuing into adult life.
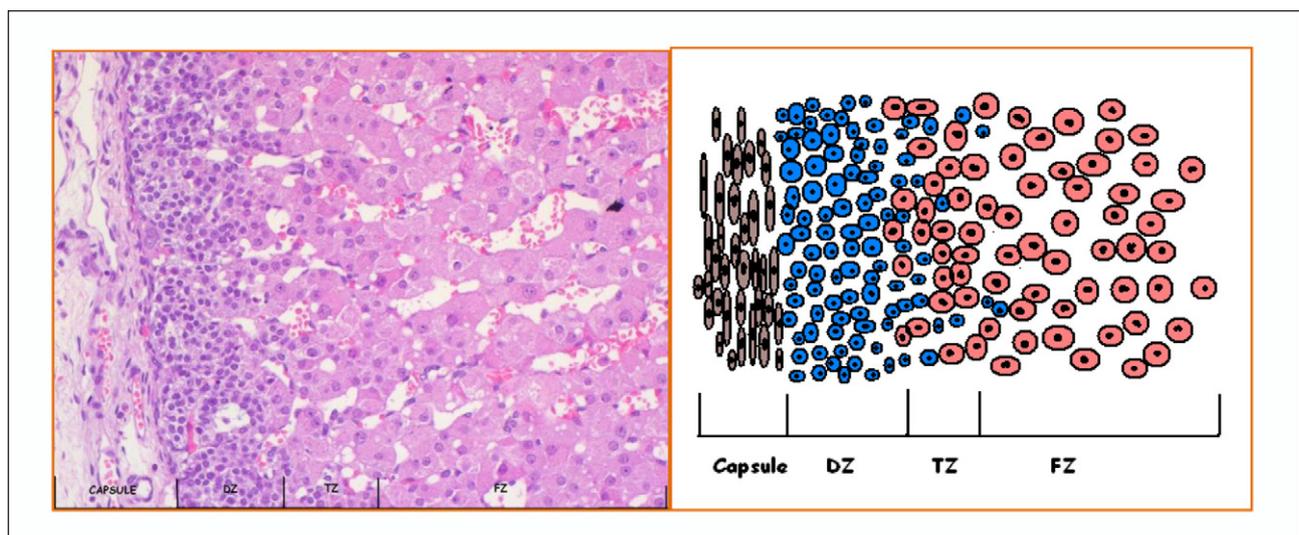
**Figure 2.** Between Fetal Zone (FZ) and Definitive Zone (DZ), a thin region named Transitional Zone (TZ), containing both DZ and FZ cells is present, characterized by DZ cells forming cords that invade the FZ.
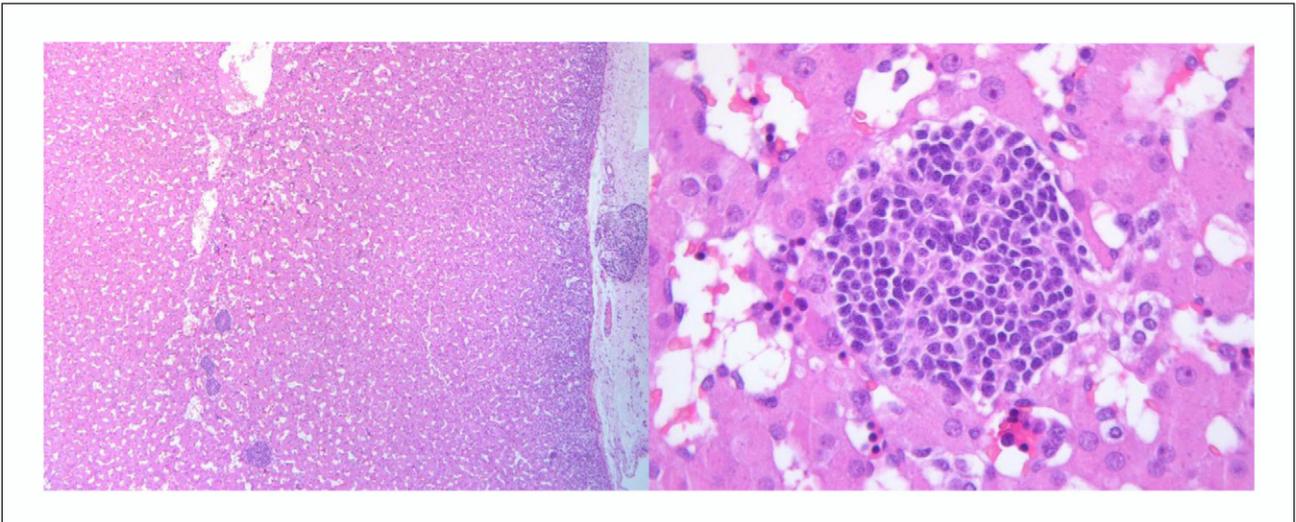
Figure 3. During gestation small cluster of cromaffin cells are scattered in the body of the cortex (fetus of 12 weeks and 6 days).

Figure 4. Development of Adrenal Medulla during gestation and after birth.
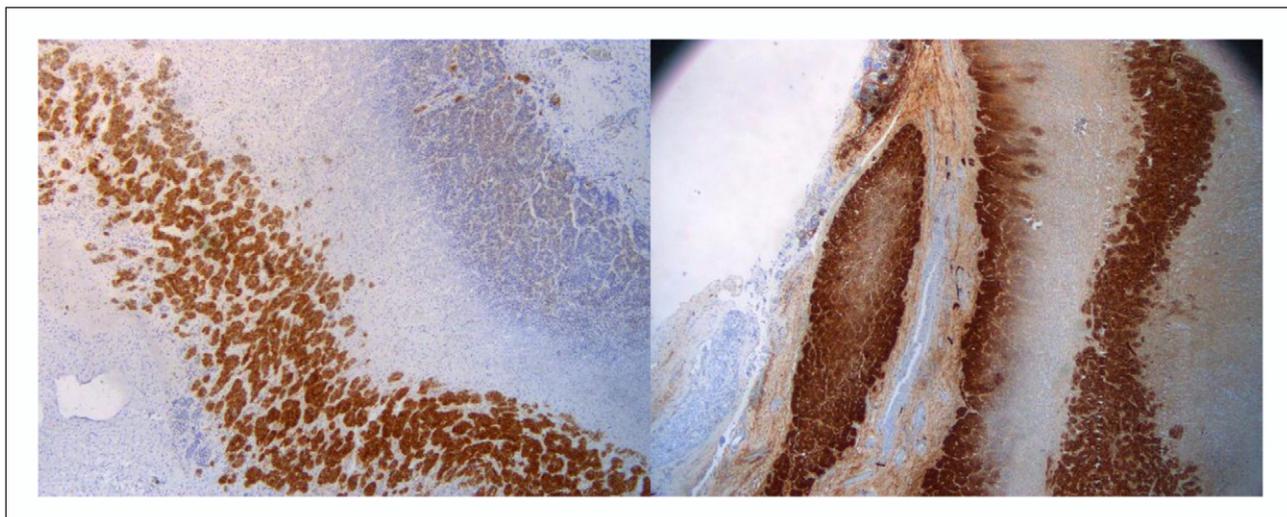
Figure 5. S100 marks the medulla, weather CD 56 (NCAM) marks the medulla and the Definitive Zone (DZ) in a fetus of 23 weeks.

variability in human adrenal gland development, suggesting the possibility of a fetal programming of adrenal gland disease later in adulthood.

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ABS 13

BIOMETRICS APPLICATION FOR NEWBORNS SAFE IDENTIFICATION IN NEONATAL INTENSIVE CARE UNITS

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Biometrics researchers have been recently showed interest in solving the problems of univocal identification of newborns, in order to avoid situations like loss of informations or, especially, cradle swapping [1]. The latter is, nowadays, one of the most concerning issues affecting the hospital's neonatal unit. Moreover, identification of newborns is one of the main tasks of the medical team in the newborn section. Several approaches dealing with biometric identification in medical field have been proposed, up to now; in this paper we propose a novel smartphone application that, basing on some ear physiological features, allows a fast and reliable recognition of the newborn previously enrolled. Although further tests are needed, the obtained results are already reassuring enough to deeply support the idea that this ear-based application can become the solution to the cradle swapping problem.

INTRODUCTION

Nowadays, most of the childbirths happen in hospitals and clinics rather than at home, in order to increase the safety of the event and also to promptly face unexpected complications that may happen. On the other side, in crowded neonatal sections, the use of labelled bracelets may carry to a newborn swap, due to operators' occasional distraction. Identification and labelling operated by immediately taking a photo of the newborn is not reliable enough, due to the fast, continuous and deep changes starting soon after delivery and, in case of natural delivery, related to the physiological stress of the labour and delivery processes which may significantly alter some traits

like the shape of the head. In this paper we propose a novel application, ear-based, for assessing the newborn identity. If compared to other biometric traits, face for example, the ear presents several advantages: first of all, the smaller surface and the quite simple structure allow faster processing as well less complex recognition strategies [2]; ears are relatively static in size and structure in the very first years, when possible changes are not so quick to invalidate recognition along a period of few days. But we must remark that, at present, the best accuracy in ear biometrics is achieved in controlled conditions (pose and illumination), conditions that can nevertheless easily occurring in the case of newborns in a delivery room. No expensive equipment is needed, since the acquisition phase simply requires a smartphone camera-provided, and a person with a sufficient experience in taking photos (which is very easy to find). Last but not least, a comparison only requires a new photo and to match them through a biometric algorithm.

Recently biometrics researches have confirmed the high uniqueness of the ear; this characteristic is mostly confined to the external ear "flap", technically defined as "pinna", with its morphological components. Furthermore, the remaining structure also significantly varies across different individuals and this leads to the conclusion that ear can be considered suitable in solving identification problems, even more in environments like neonatal wards, where the number of subjects to be enrolled consists of few dozens.

The strongest impulse to this research came from the pioneering study by Iannarelli [3] that have demonstrated that the ear provides a good discriminating power. His anthropometric recognition technique relies on a series of 12 measurements, corresponding to different segments identified by the margins of the three-dimensional ear structures, besides sex and race. The main limit of this procedure is just the accuracy required to identify the central point, which affects the following measures and therefore the whole recognition procedure. An important assessment of this study is that ear variations are very limited

along time. Growth is proportional from birth to the first 4 months, then only the lobe elongates from 4 months to 8 years. A further growth in size, until about the age of 20, does not alter the shape, which undergoes a further elongation only after 60/70. In the years subsequent to the Iannarelli studies, other researchers have explored the field, even if not intensively as for other biometrics. As a consequence, even different approaches to ear structures acquisition have been exploited, both in 2D and 3D.

As in any biometric system, recognition is only the last step in a process starting from acquisition, continuing with detection of the required trait, e.g., face or ear, and then taking to feature extraction and template matching. Each step presents different issues according to the acquisition modality especially in the comparison between 2D vs. 3D. The 3D imaging provides crucial enhancements when dealing with illumination and pose variations, but has as drawbacks to be much more expensive and computationally demanding. Since the application we propose is aimed to run on mobile platforms, with limited hardware resources, we strongly preferred 2D images.

METHOD

During the enrolment phase, four images for each left ear for a population of 24 newborns have been acquired. The process pipeline used in our work can be split up into four general steps, as shown in **Fig. 1** and explained in the following.

The image is first resized so that height and width are no greater than 800 pixels. Then, Viola-Jones algorithm [4] is exploited to identify one or more candidate regions of interest (ROIs) possibly containing the ear. Starting from the original position and rotating the image of 10 and 20 degrees clockwise and counter-clockwise, we look for a positive response from the detector. For each image rotation, a growing threshold is then iteratively applied to finally possibly select a single region of sufficient size for each image. After the ear is detected, we used an approach based on the Active Shape Model, namely STASM [5], that, as results of a training phase, in which some landmarks are manually selected,

Photo Shoot → Localization → Normalization → Feature extraction → Identification → Identity
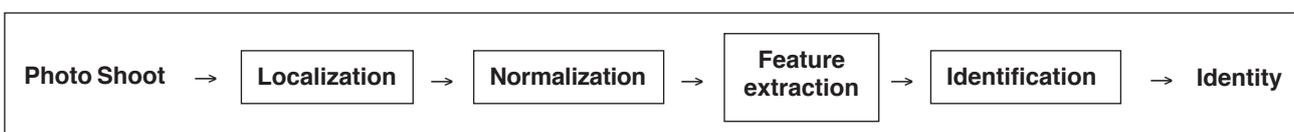
Figure 1. Phases involved in the ear recognition system.

automatically segments and crops the quasi-elliptical shape related to the ear.

In the normalization phase, the image is carried to a normalized format to facilitate the recognition process. As regard the orientation, the centre of the found ellipse is used as pivot for the rotation of the image, performed in such a way that the major axis of the ellipse is parallel to the y axis. Size normalization is achieved choosing an image size of 90 x 144, with a height/width ratio of 1.6, which is a good approximation of the average size for the ears of newborns; for colour normalization, we applied greyscale conversion, histogram equalization to improve contrast, and median filter for noise reduction.

The feature vector is computed exploiting the multiscale linear binary pattern LBP [6] after dividing the image in 32×32 square regions. After this, in order to reduce the dimension of the feature vector, we applied four different techniques and compared the recognition performance obtained by Euclidean distance between probe and gallery vectors: Principal Component Analysis (PCA) [7], Linear Discriminant Analysis (LDA) [8], Neighbourhood Preserving Embedding (NPE) [9], and Orthogonal Locality Preserve Projections (OLPP) [10].

RESULTS

We tested the recognition process using the before mentioned reduction techniques, and with a varying number of images in the gallery of each subject. This element should positively influence recognition, since having more images to compare can help 60 catching some variations and improve both verification and 40 identification. In practice, when a probe is submitted, it is compared with all images in the gallery (pertaining to the 20 supposed subject if in verification mode). We used the Euclidean metric among templates for matching, measuring performance by setting a false acceptance rate threshold at 10^{-3}, which is an acceptable value in this context; we calculated NPE, OLPP, PCA and LDA measuring their performances calculating Genuine Acceptance Rate (GAR) and False Rejection Rate (FRR), and finally, we further computed the equal Error Rate (ERR).

In **Tab. 1** we reports the results obtained at FAR = 10^{-3} in the 4 cases in which the gallery for each subject includes 1, 2, 3 and 4 images, and for the 4 algorithms for dimensionality reduction applied to LBP feature vectors.

As expected, increasing the number of images in the gallery from 1 to 4 for each subject positively affects performance of all four methods, in both verification and identification operations. The differences among dimensionality reduction techniques are often negligible, and this can be assumed to depend on the characteristics of multiscale LBP.

DISCUSSION

We presented in this paper a preliminary study on the possibility to assure newborns' identity by means of ear characterization with images acquired

Table 1. Results obtained at FAR = 10^{-3} in the 4 cases in which the gallery for each subject includes 1, 2, 3 and 4 images, and for the 4 algorithms for dimensionality reduction applied to LBP feature vectors.

$GAR^{1img}_{NPE} = 0.8;$	$GAR^{2imgs}_{NPE} = 1;$	$GAR^{3imgs}_{NPE} = 0.8;$	$GAR^{4imgs}_{NPE} = 1$
$FRR^{1img}_{NPE} = 0.2;$	$FRR^{2imgs}_{NPE} = 0.00;$	$FRR^{3imgs}_{NPE} = 0.2;$	$FRR^{4imgs}_{NPE} = 0.00$
$EER^{1img}_{NPE} = 0.08;$	$EER^{2imgs}_{NPE} = 0.07;$	$EER^{3imgs}_{NPE} = 0.07;$	$EER^{4imgs}_{NPE} = 0.06$
$GAR^{1img}_{OLPP} = 0.83;$	$GAR^{2imgs}_{OLPP} = 0.92;$	$GAR^{3imgs}_{OLPP} = 0.87;$	$GAR^{4imgs}_{OLPP} = 1$
$FRR^{1img}_{OLPP} = 0.17;$	$FRR^{2imgs}_{OLPP} = 0.08;$	$FRR^{3imgs}_{OLPP} = 0.13;$	$FRR^{4imgs}_{OLPP} = 0.00$
$EER^{1img}_{OLPP} = 0.09;$	$EER^{2imgs}_{OLPP} = 0.08;$	$EER^{3imgs}_{OLPP} = 0.08;$	$EER^{4imgs}_{OLPP} = 0.08$
$GAR^{1img}_{PCA} = 0.83;$	$GAR^{2imgs}_{PCA} = 0.92;$	$GAR^{3imgs}_{PCA} = 0.87;$	$GAR^{4imgs}_{PCA} = 1$
$FRR^{1img}_{PCA} = 0.17;$	$FRR^{2imgs}_{PCA} = 0.08;$	$FRR^{3imgs}_{PCA} = 0.13;$	$FRR^{4imgs}_{PCA} = 0.00$
$EER^{1img}_{PCA} = 0.09;$	$EER^{2imgs}_{PCA} = 0.08;$	$EER^{3imgs}_{PCA} = 0.08;$	$EER^{4imgs}_{PCA} = 0.07$
$GAR^{1img}_{LDA} = 0.63;$	$GAR^{2imgs}_{LDA} = 1;$	$GAR^{3imgs}_{LDA} = 0.93;$	$GAR^{4imgs}_{LDA} = 1$
$FRR^{1img}_{LDA} = 0.37;$	$FRR^{2imgs}_{LDA} = 0.00;$	$FRR^{3imgs}_{LDA} = 0.07;$	$FRR^{4imgs}_{LDA} = 0.00$
$EER^{1img}_{LDA} = 0.11;$	$EER^{2imgs}_{LDA} = 0.11;$	$EER^{3imgs}_{LDA} = 0.10;$	$EER^{4imgs}_{LDA} = 0.07$

by smartphone. Mobile phones, despite the low resolution obtaining in the images, are important devices because they are ubiquitous, always available, easy to handle, and do not require an additional expense from the interested institution (hospital) since each operator can ultimately use its personal one. The obtained results are quite promising. Future work will regard testing on larger dataset, and performing extensive comparison with state-of-the art methods.

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ABS 14

METABOLOMICS IN NEONATAL HYPOXIC-ISCHEMIC ENCEPHALOPATHY (HIE): A PROPOSAL FOR A EUROPEAN MULTICENTER STUDY

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BACKGROUND

Hypoxic-ischemic encephalopathy (HIE) (incidence between 0.5‰ and 2‰ live births) can lead to significant morbidity and mortality. With standard supportive care, approximately 30% of such infants will die, and approximately 30% to 40% of survivors will have permanent neurodevelopmental disabilities. Accumulated evidence from RCTs shows the benefits of therapeutic hypothermia (TH) in reducing rate of death and disability in survivors. A major issue in HIE is the difficulty to establish early and reliable prognostic criteria. The first question addressed by the parents to the Neonatologist in the first few hours of the infant's life relates to the prognosis both in terms of survival and of long-term neurological outcome. The integration of clinical and instrumental data (EEG and MRI) is proven useful: in particular MRI imaging pattern is highly predictive of death and neuromotor outcome in infants with HIE treated with TH. Metabolomics is the study of the intermediates and products of specific cellular processes. This research is usually done in biological fluids: urine may be collected non-invasively, which is important in newborn infants. Significant changes of the metabolome have been found in animals and preterm infants after perinatal brain injury.

EUROPEAN MULTICENTER STUDY PROPOSAL

Our aim will be to study some urinary metabolites involved in HIE-induced brain damage. Urinary concentration of these metabolites at various times (i.e. at birth, 48 and 72 hrs, 1st week, 1 month of age) could reflect the extent of brain damage and therefore predict the neurodevelopmental outcome of the child. The integration of these data with clinical and instrumental findings could help to establish a reliable prognostic judgment, and allow a more complete communication with the family.

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ABS 15

LONGITUDINAL EVALUATION OF MYOCARDIAL FUNCTION IN INFANTS WITH HYPOXIC-ISCHEMIC ENCEPHALOPATHY (HIE): PRELIMINARY DATA

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BACKGROUND

Perinatal hypoxic-ischaemia is an important cause of mortality and morbidity in newborn infants, with a prevalence of 1-2/1,000 live births.

Multiorgan failure (MOF) can follow perinatal asphyxia. In particular, the hypoxic ischaemic insult can lead to a cardiac disregulation (reported incidence varies from 29% to 78%) which can compromise the blood supply to other organs leading to a multi-organ dysfunction [1, 2]. Longitudinal data on myocardial function in infants with hypoxic-ischemic encephalopathy (HIE) are scarce.

AIM

Aim of this study is to assess longitudinally myocardial function in infants with various grades of HIE, by means of laboratory and ECHO techniques.

PATIENTS AND METHODS

Newborns with gestational age ≥ 35 weeks and perinatal asphyxia were recruited. Infants were diagnosed with perinatal asphyxia if they met all the following criteria: (1) Apgar score ≤ 5 at 10 min; (2) severe metabolic acidosis (pH ≤ 7 or BE ≤ -12 mmol/l) documented by a blood gas analysis performed within 60 minutes from birth; (3) clinical evidence of encephalopathy confirmed by amplitude-integrated electroencephalogram (aEEG) recorded for at least 30 minutes. Patients were divided into two groups according to the severity of HIE: moderate-severe HIE requiring hypothermia (group 1) and mild HIE non requiring hypothermia (group 2).

Serum T troponin (cTnT) was evaluated in all infants at 6, 12, 24 hours after birth and then at 4 and 7 days. Conventional ECHO and Tissue Doppler Imaging were performed in both groups within 6 hours of life, at 36 hours and at 7 days.

All the patients underwent a long-term ECHO follow up (at 1 and 6 months of life in group 1, and at 12 months of life in both groups).

RESULTS

From January 1st 2013 to January 31st 2014, 9 infants were recruited (5 in group 1 and 4 in group 2) and completed the 12-months follow-up.

Mean ± SDS cTnT serum concentrations were higher in group 1 compared to group 2 at 6 (0.58 ± 0.76 mcg/l vs. 0.13 ± 0.02), 12 (0.38 ± 0.29 mcg/l vs. 0.13 ± 0.02 mcg/l) and 24 hours (0.42 ± 0.31 mcg/l vs. 0.09 ± 0.03), as well as at 4 days of life (0.55 ± 0.87 mcg/l vs. 0.17 ± 0.2). No difference between groups was documented at 7 days of life (0.11 ± 0.06 mcg/l vs 0.12 ± 0.07).

TDI systolic S' wave was significantly lower in group 1 compared to group 2 for both left and right ventricle at 6 hours (3.32 ± 0.6 cm/sec vs. 4.53 ± 0.88, and 4.53 ± 1.2 vs. 5.96 ± 0.95, respectively) and also at 36 hours (3.38 ± 0.88 cm/s vs. 4.21 ± 0.55, and 5.42 ± 1.6 vs. 6.3 ± 0.94, respectively). No difference was documented at 7 days.

At 12 months of life, no difference was documented between groups in TDI values. Infants in group 1 showed a significant improvement of systolic S' wave over time, achieving normal age values for both left and right ventricle at 12 months (9 cm/sec and 13.6, respectively). Both left and right systolic S' wave values at 12 months were significantly higher compared to values detected before (p = 0.002 and 0.005, respectively) and immediately after hypothermia (p = 0.001 and 0.04, respectively).

CONCLUSIONS

Preliminary results of the present study show that, in infants with moderate-severe HIE, cTnT values in the first 24 hours of life are markedly altered, while in infants with mild HIE these values are only slightly above normal age values. Similarly, both left and right ventricular systolic function are altered before and during hypothermic treatment in infants with HIE requiring hypothermia. However, the 12-months follow-up shows that myocardial function significantly improves over time, regardless the severity of HIE.

In conclusion, early laboratory and ECHO evaluation of myocardial function in infants with HIE appear to reflect accurately the severity of the disease in the first days of life, and thus could represent a valid diagnostic tool in early evaluation of MOF.

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ABS 16

CHANGES IN PODOCYTE NUMBER DURING INTRAUTERINE DEVELOPMENT

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INTRODUCTION

In recent years, many authors clearly demonstrated that total nephron number is highly variable in humans at birth, glomerular number and kidney size in neonates being associated to birth weight [1]. Nephron number at birth has a relevant clinical importance [2] with serious implications for long-term renal health [3]. The podocyte depletion hypothesis has emerged as an important concept in kidney pathology. Consequently, the estimation of podocyte number is often a significant component of studies of progressive renal diseases. This study was aimed at verifying if podocyte number of the human developing kidney changes significantly in different periods of intrauterine life.

PATIENTS AND METHODS

Sixty-two subjects with gestational age ranging from 20 up to 41 weeks were examined. Postnatal age ranged from 1 h to 3 months. Only subjects with no evidence of kidney pathology were enrolled into the study. Subjects died for renal reasons were excluded. Both IUGR (IntraUterine Growth Restriction) and non-IUGR subjects were included in the study. Kidney samples were formalin-fixed and embedded in paraffin, sectioned with microtome at 5 μm. Kidney sections were stained with hematoxylin and eosin and digitally scanned at 400X magnification. According to gestational age, subjects were organized in three groups: fetuses (gestational age ≤ 24 weeks, n = 5), preterms (gestational age ≥ 25 and ≤ 36 weeks, n = 39), at term (gestational age ≥ 37 weeks, n = 18). For each subject we selected 10 glomeruli along 3 straight lines extending from the renal capsule towards the deepest zone of the cortex. Each straight line was chosen in order to hit at least 3 glomerular profiles. Podocyte number was estimated by marking with black signs nuclei of podocytes in each glomerular section. Dark sign were discriminated and counted with a specific algorithm based on segmentation techniques, developed with Matlab® software.

Volumetric podocyte number of each glomerulus was performed using Weibel-Gomez method [4].

RESULTS

It was observed an average podocyte number of 1,908 ± 645 in fetuses, 1,394 ± 498 in preterm infants and 1,126 ± 256 in at term infants. Two-way ANOVA showed no interaction effect between gestational age and sex in podocyte number. There was not significant main effect of sex. There was a statistically significant main effect ($F (2, 56) = 5.82, P = 0.0051$) of gestational age (**Fig. 1**). Bonferroni posttest indicated that podocyte number was significantly lower in at term group than in fetuses ($t (21) = 3.07, P < 0.001$). There were not significant differences between preterm infants and nearby groups (fetuses and at term newborns).

DISCUSSION

This study outlines for the first time a decreasing trend in podocyte number during the gestation (**Fig. 2**). Podocyte number was significantly higher in fetuses compared with at term group. This suggests that podocytes undergo a cellular selection in order to form what will be the mature glomerulus. A marked intervariability at the same gestational age was also observed (**Fig. 3**). A variation in podocyte number in the newborn could be correlated to several events occurring during the intrauterine life. A deficient podocyte number might represent a predisposing factor to develop podocytopaties later in life.

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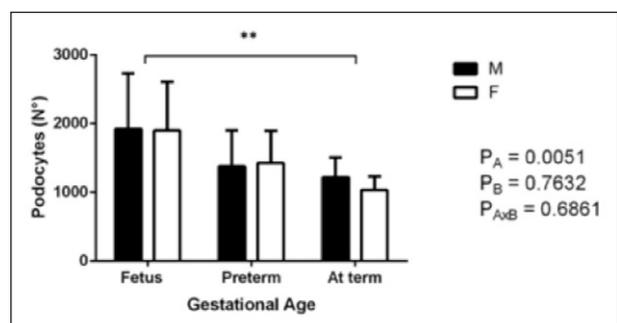
Figure 1. Decreased podocyte number in fetuses, preterm and at term subjects. Total podocyte number between fetuses, preterm and a term in male (black bars) and female (white bars). Bars represent mean ± SD. P_A, gestational age; P_B, sex; P_{AxB}, interaction.
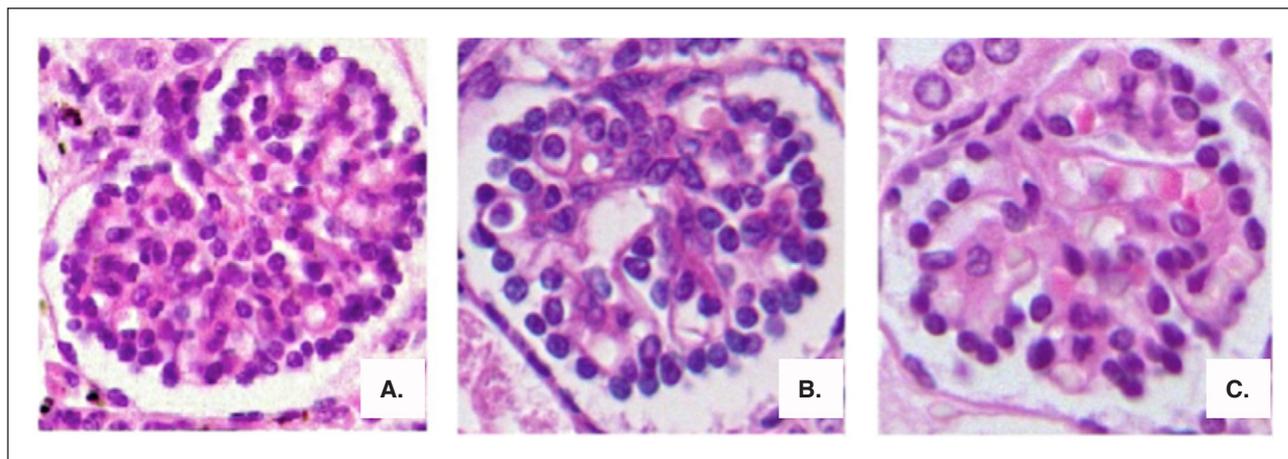
Figure 2. Glomeruli of subjects at (**A**) 23rd (fetus), (**B**) 36th (preterm) and (**C**) 41th (at term) week. Podocyte number significantly decreased with increasing gestational age.
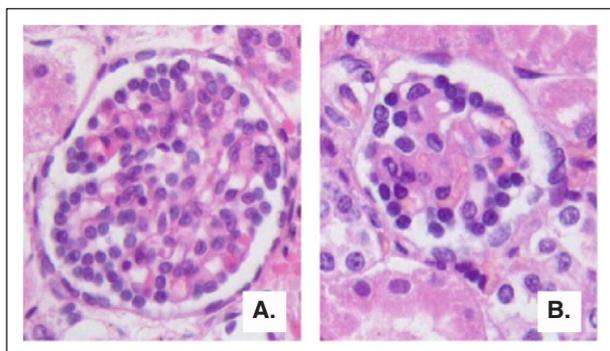
Figure 3. Intervaribility in podocyte number at the same gestational age. Glomeruli of two subjects at 40th week who exhibited respectively 1860 (**A**), and 700 (**B**) podocytes in the whole glomerulus.

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ABS 17

EFFICACY OF RECOMBINANT HUMAN ERYTHROPOIETIN IN ANEMIC PREGNANT WOMEN WITH HETEROZYGOUS BETA-THALASSEMIA

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OBJECTIVE

The aim of this study was to determine the response to recombinant human erythropoietin (rhEPO) in anemic pregnant women with heterozygous beta-thalassemia.

METHODS

A prospective study including 30 consecutive pregnant women with anemia and heterozygous hemoglobinopathy was performed. 10,000 U of rhEPO was administered alternated days with intravenous iron sucrose and parenteral folic acid.

RESULTS

In 23 patients, a good response to therapy was observed (mean Hb increase 1.6 ± 0.5 g/dl). In 7 patients, resistance to rhEPO was noted (mean Hb increase 0.5 ± 0.5 g/dl). Transferrin saturation and ferritin levels were normal at the end of the study. There was an increase of the percentage of hypochromic red cells, indicating functional iron deficiency after rhEPO administration despite supplemental iron. Reticulocytes increased significantly only in responder patients. The mean gestational age at the start of therapy was 29 weeks of gestation and at the end 33 weeks. The mean duration of a complete therapy was 3.5 weeks (range 2-4.5 weeks).

CONCLUSION

RhEPO stimulates both HbF synthesis and erythropoiesis in pregnant patients with heterozygous beta-thalassemia and anemia. Since it is known that high HbF levels ameliorate thalassemia symptoms in non pregnant patients, use of rhEPO for the treatment of severe anemia in thalassaemic patients during pregnancy might be further evaluated.

ABS 18

WT1 EXPRESSION IN ONTOGENESIS OF HUMAN FETUS

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INTRODUCTION

Wilms' Tumor 1 gene (WT1) encodes for a zinc finger transcription factor involved in the onset of Wilms' tumor and in the normal development of different tissues, including the urogenital system [1]. On the basis of the expression of this transcription factor in the urogenital system, as well as in other organs such as heart, spleen and adrenal glands [2, 3], the purpose of this study was to analyze the immunoreactivity of the WT1 protein in different tissues and organs in a human fetus of 12 weeks of gestation and to assess its possible role in human ontogeny.

MATERIALS AND METHODS

A human fetus of 12 weeks of gestation has 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 sections were obtained from each paraffin block; after dewaxing and rehydrating, one of these was stained with hematossilin-eosin, the other pre-treated for immunohistochemical analysis, then incubated for 20 minutes with anti Wilms' Tumor (WT1) mouse monoclonal antibody.

RESULTS

WT1 immunoreactivity was mainly cytoplasmic and only in few cases nuclear. WT1 was strongly expressed in the cytoplasm of endotelial cells, subepidermal mesenchyme, striated scheletric (**Fig. 1**) and cardiac muscle cells (with higher positivity in atrial walls than in ventricular ones), CNS (**Fig. 2**) and PNS axons, retinal neuroepithelium, cellular elements of sclera, brown adipose tissue, cells of adrenal glands "fetal zone" with ascending gradient from external to internal side, "blue strip" elements forming renal tubules, interstitial renal cells, very weakly in splenic cells, and focally in major salivary glands. Viscera of gastrointestinal
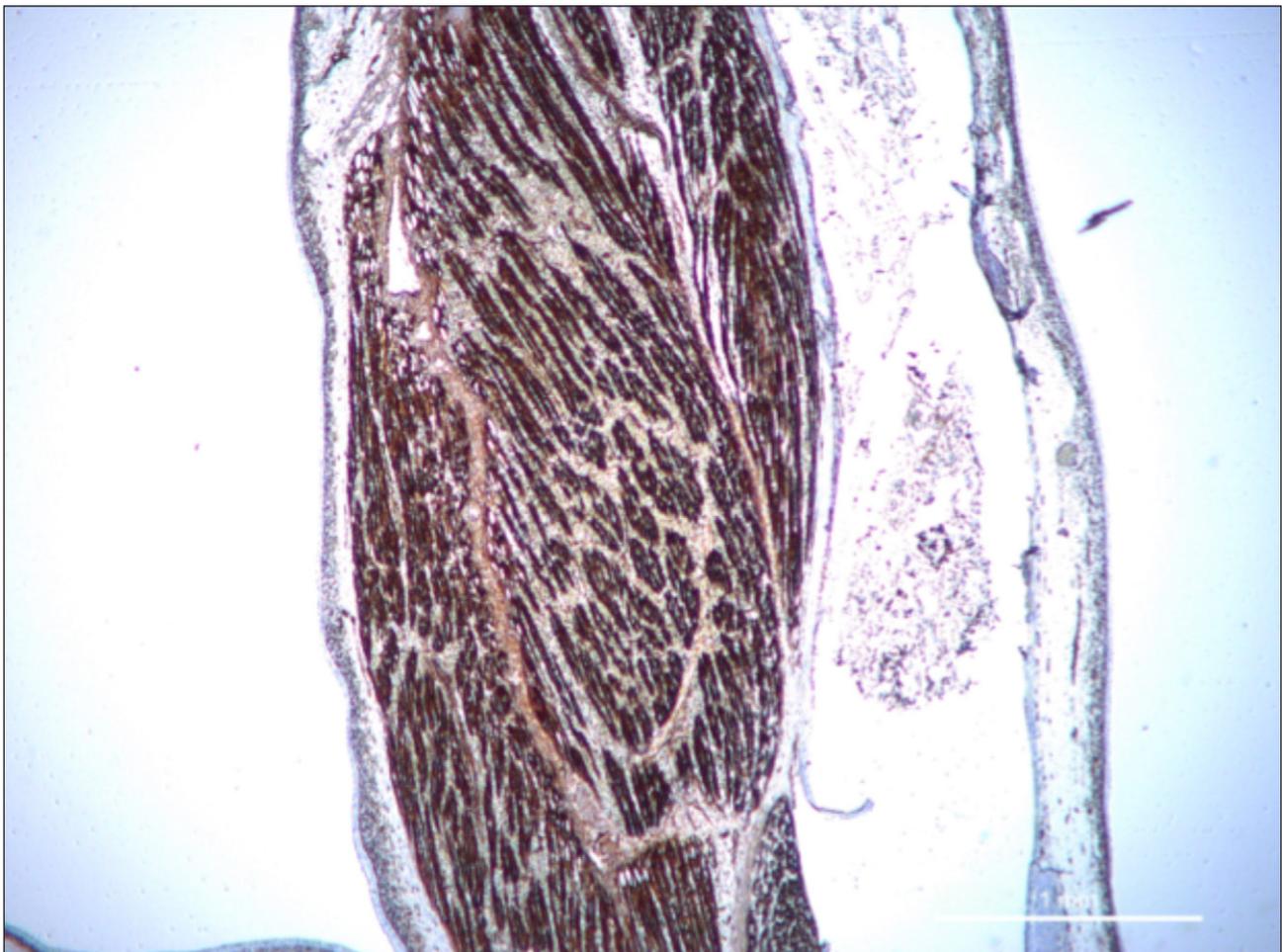
Figure 1. WT1 immunostaining in fetal arts. Highest levels of WT1 immunoreactivity were observed in developing skeletal muscles (2.5X HPF).
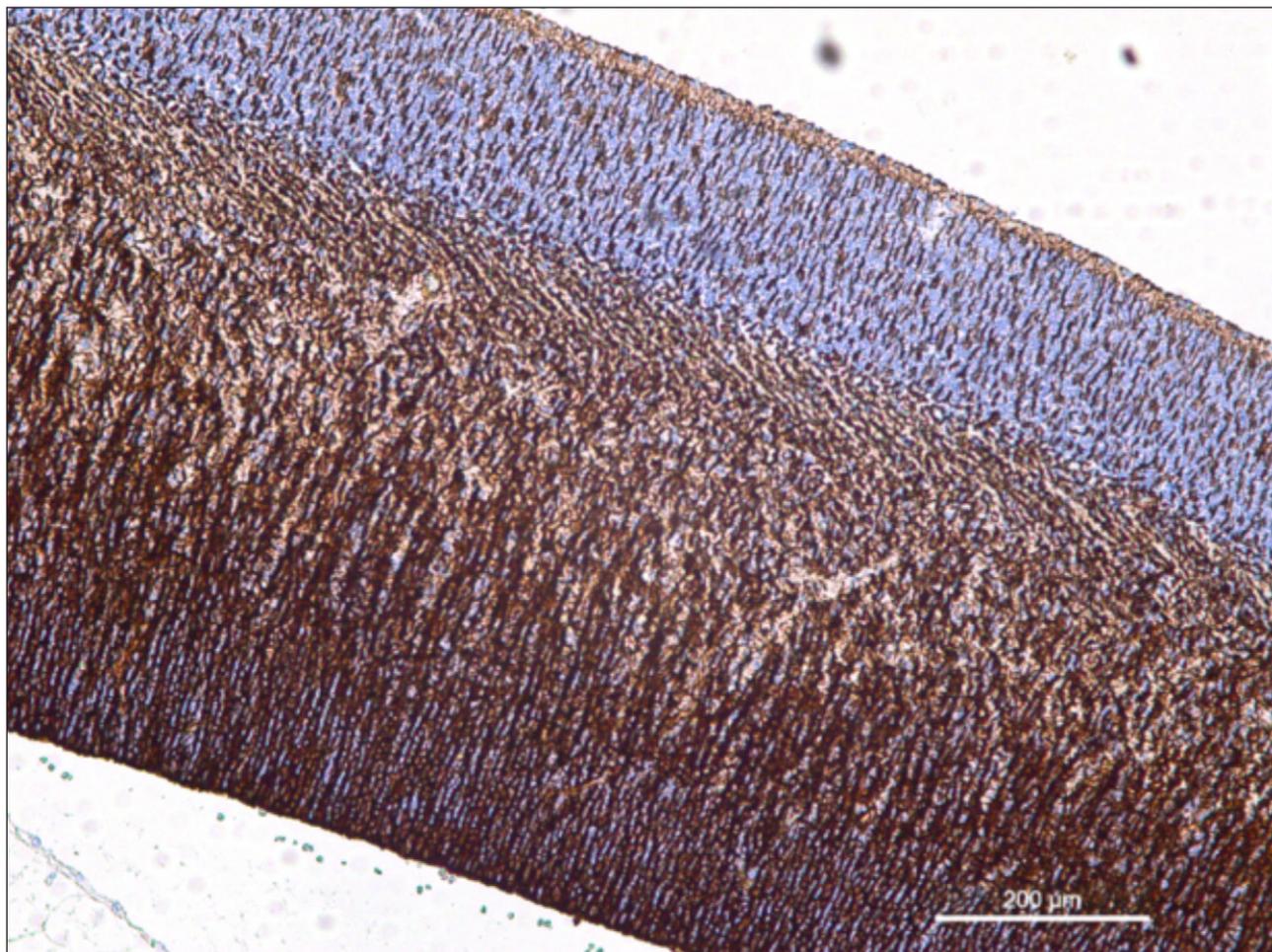
Figure 2. WT1 immunostaining in fetal brain. WT1 was detected in the axons extending from the cortex towards the white matter (10X HPF).

(GI) tract showed cytoplasmic positivity in spindle cells of the superficial half of submucosa and in the myoenteric plexus. Nuclear positivity has been detected strongly in perisplenium, in podocytes that showed membrane "reinforcement", and weakly in the epithelium of the Bowman's capsule. No reactivity was found in placenta, superficial (epidermis, GI tract, endometrium, renal tubules, urotehlium) and glandular (salivary glands, thyroid, pancreas, liver, ovary) epithelia, undifferentiated cells of renal "blue strip" and mesangium, lung, thymus, adrenal "blue strip", smooth muscle tissue, glial cells, olygodendrocytes and Schwann cells, crystalline lens, cartilage and blood cells.

DISCUSSION

Our study first shows that WT1 is involved in a more vast series of cells types during human development than previously reported. Immunostaining for WT1 was characterized by a predominant cytoplasmic positivity in several tissues and organs, which suggests an involvement of this protein in the processing of mRNA and in the regulation of translation of this messenger, in addition to the already known role of transcription factor in the nucleus [4]. Future studies are needed to detect differences in the expression of WT1 in various organs at different gestational ages of the human embryo and fetus in order to better evaluate the fundamental role of WT1 in cell proliferation and differentiation during intrauterine development, as well as in cancer insurgence and progression.

ACKNOWLEDGEMENT

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ABS 19

BRAIN HYPOTHERMIA IN PERINATAL ARTERIAL ISCHEMIC STROKE (PAIS)

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BACKGROUND

Perinatal arterial ischemic stroke (PAIS) is the most frequent form of cerebral infarction in children, as well as major source of neurological sequelae. Animal studies of focal cerebral ischemia and RCT in adult patients with ischemic cerebro-vascular accidents suggest a potential benefit of therapeutic hypothermia (TH) in PAIS. Systematic reviews of animal studies show that TH improves outcome and reduces infarction area by 44% (95% CI: 40-47%). A recent study in human newborns suggests that TH may decrease occurrence of seizures in infants with HIE and focal infarction.

PATIENTS AND METHODS

From November 2009 to November 2013, TH has been offered to 7 patients with stroke. TH was started within 6 hours of life in 4 patients (57%); at 12 hours in 1 pt (14%) for appearance of abnormal videoEEG, and between day of life 2 and 3 in 2 patients (29%) for MRI evidence of stroke. TH was mantained by a cooling device servo-controlled at $33 \pm 0.5°C$ for 72 hours.

RESULTS

During the follow-up all patients were examined every 3 months until 2 years of life. VideoEEG was performed in all patients at 1 month of age and repeated only if pathologic. Cognitive performance was tested with Griffith's Test at 6 months of age in all subjects; in 3 at 12 and 24 months. All subjects had a regular height-weight growth at 6 months; in 3 cases microcrania appeared as possible consequence of atrophic brain damage (Griffith score at 6 months < 100 in 2 cases). The 5 patients (71%) who had normal videoEEG at 1 month, also achieved a good motor-behavioral assessment at 6 months (Griffith score > 100). Despite severely abnormal MRI, 3 children had an adequate outcome (Griffith score > 100).

CONCLUSIONS

TH is likely to be effective in conditions other than neonatal hypoxic-ischemic encephalopathy. These findings may suggest a possible neuroprotective effects of TH in PAIS.

ABS 20

FAVORABLE OUTCOME AFTER SEVERE HEMOSTATIC COMPLICATIONS IN NEONATES RECEIVING THERAPEUTIC HYPOTHERMIA (TH) FOR PERINATAL ASPHYXIA

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INTRODUCTION

Although therapeutic hypothermia (TH) could exacerbate thrombotic and hemorrhagic events secondary to perinatal asphyxia, there is no evidence that asphyxiated infants treated with TH present a higher incidence of severe hemostatic complications than controls. Recent literature, however, have focused the attention of neonatologists on unfavorable outcome after severe coagulopathy in two asphyxiated neonates treated with TH.

CASE DESCRIPTION

This experience prompted us to review coagulation studies and clinical manifestations of coagulopathy among the 47 consecutive asphyxiated neonates we admitted for TH in the last 4 years. 17 (36%) showed abnormal hemostasis and platelet dysfunction, while 9 (52%) developed thrombotic and/or hemorrhagic complications. Specifically, there were the following 6 cases of hemorrhage: pulmonary (1), gastrointestinal (2), subdural (1), peri-intraventricular (2). Moreover, there were the following 6 cases of vascular thrombosis: renal vein (1), inferior vena cava (1), iliac vein (1), cerebral sinus trasversus (1), carotid artery (1), iliac artery (1). Coagulation studies before and during the 3 days of TH did not differ significantly between the symptomatic and asymptomatic infants. However, the symptomatic group showed lower Apgar score at 5', pH, and BE. Thrombotic and hemorrhagic

events were treated either with enoxaparin (150 IU/kg, subQ, bds, for 3 months or until resolution of symptoms), or with repeated ATIII and fresh frozen plasma transfusions. Only two patients with asymptomatic coagulopathy died (one secondary to renal insufficiency and the other following late onset sepsis).

CONCLUSIONS

Although severe hemostatic complications may occur in asphyxiated infants after TH, prompt medical treatment allows for successful management and favorable outcome.

ABS 21

VARIATIONS IN CARDIOMYOCYTE DENSITY IN DIFFERENT ZONES OF THE LEFT VENTRICULAR WALL CHARACTERIZE THE DEVELOPING HUMAN HEART

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INTRODUCTION

Cardiomyocyte burden at birth could represent a central feature in fetal programming of adult cardiac pathology. Excess cardiomyocyte attrition during a critical prenatal or perinatal growth could be at the basis of cardiac hypertrophy, representing the linkage between myocardial fetal growth restriction and cardiovascular morbidity and mortality in adulthood [1]. The aim of the present study was to evaluate any interindividual differences in human cardiomyocyte burden during fetal development. Furthermore the cardiomyocite density was assessed in subepicardial, intramyocardial and subendocardial zone, in order to observe variations related to different zones.

PATIENTS AND METHODS

Thirty-three subjects with gestational age ranging from 12 up to 41 weeks were examined. Portions of the heart were formalin-fixed and embedded in paraffin. Sections were stained with H&E and digitally scanned at 400X magnification. According to gestational age, subjects were subdivided into three groups: fetuses (gestational age ≤ 24 weeks, n = 7), preterms (gestational age ≥ 25 and ≤ 36 weeks, n = 12), at term (gestational age ≥ 37 weeks, n = 14). Cardiomyocyte burden was estimated by performing 4 random acquisitions per zone (subepicardial, intramyocardial and subendocardial). Cells were

discriminated and counted with a specific algorithm based on segmentation techniques, developed with Matlab® software.

RESULTS

In the subepicardial zone it was observed an average cell density of 5,215 ± 932, 5,120 ± 613, 4,835 ± 558 cells per mm^2 in fetuses, preterms and at term respectively. In the intramyocardial zone it was observed an average density of 4,657 ± 417, 4,795 ± 467, 4,330 ± 592 cells per mm^2 in fetuses, preterms and at term respectively. In the subendocardial zone it was observed an average density of 5,119 ± 749, 5,349 ± 533, 5,138 ± 652 cells per mm^2 in fetuses, preterms and at term respectively. One-way ANOVA showed no main effect of gestational age in cardiomyocyte density. There was a statistically significant main effect ($F (2, 96) = 9.55, P = 0.0002$) of zone (**Fig. 1**) in cardiomyocyte burden. Bonferroni posttest indicated that cardiomyocyte density was significantly higher in subendocardial zone than in intramyocardial zone ($t (64) = 4.26, P < 0.0001$). Thereby subepicardial zone exhibited an higher density then intramyocardial zone ($t (64) = 2.99, P < 0.05$). There were not significant differences between subendocardial and subepicardial zone.

DISCUSSION

Our preliminary data show the same cardiomyocyte density during intrauterine life. The constant density leads to an increase of the total number of cells with the fetal development, due to the growth in heart tissue volume. An increased number of cardiomyocyte supports the hypothesis of the study carried out by Mayhew et al. on the ventricular myocardium of human fetuses and newborns [2]. Our study outlines the presence of marked differences in cardiomyocyte density in different heart zones.
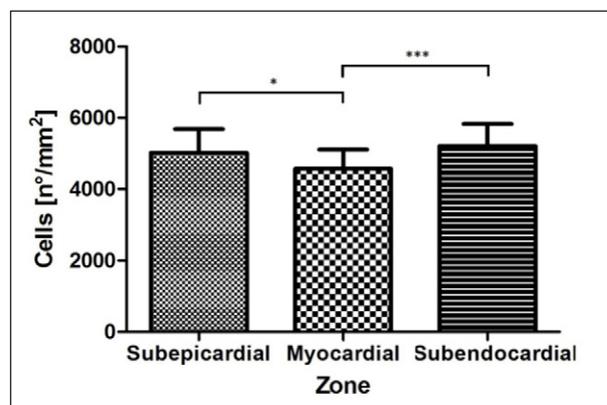
Figure 1. Cardiomyocyte density in subepicardial, intramyocardial and subendocardial zone. Bars represent mean ± SD.

Subepicardial and subendocardial zones exhibit a significant cardiomyocyte proliferation then intramyocardial zone. An interindividual variability is observed between groups. This variability is evident also in twin subjects. A variation in cell density could be correlated to epigenetic factors. Further studies are needed to evaluate the influence of epigenetic factor in cardiomyocyte burden.

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ABS 22

RENAL SHEAR WAVES VELOCITY CORRELATES WITH ESTIMATED GLOMERULAR FILTRATION RATE IN CHILDREN WITH CHRONIC KIDNEY DISEASE

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BACKGROUND

Acoustic radiation force impulse (ARFI) is a recently established ultrasound (US)-based diagnostic technique that allows physicians to obtain a measure of the elastic properties of an organ. Applications of the ARFI technique regard other parenchimatous tissues, including breast masses and thyroid nodules. Shear waves velocity (SWV) proves accurate in the grading of liver fibrosis in patients with chronic hepatitis or cirrhosis; on the contrary, these results when dealing with kidneys are today controversial. The aim of the present study was assessing the existing relationship between SWV values and eGFR (estimated glomerular filtration rate).

MATERIALS AND METHODS

A total of 29 patients were included in our study. There were 18 primary and 11 secondary (PUVs) VUR; 13 monolateral (7 right) and 16 bilateral VUR. The renal function of each patient was assessed measuring by standard methods the laboratory parameters (serum Scr, cysC, BUN, urinary protein and creatinine ratio). The eGFR was calculated by means of Schwartz's multivariate or univariate formulas for children with CKD. All ultrasounds were performed by a single qualified technician with 15 years of experience in abdominal US using a convex probe (frequency 4 MHz) on an S-2000 system (Siemens, Erlanger, Germany). All statistical analyses were performed employing the software R, version 3.0.0.

RESULTS

The mean SWV was negatively correlated, even adjusted for age and sex, with eGFR calculated both with univariate – Scr based ($eGFR_{cr}$) and cysC based ($eGFR_{cy}$) – and multivariate – Scr, BUN and cysC based ($eGFR_{ncc}$) – equations. The correlation was significantly different from zero for both $eGFR_{ncc}$ and $eGFR_{cy}$ and approached significance for the $eGFR_{cr}$ (**Tab. 1**).

Table 1. Main results of the study.

SWV (m/s) versus	Mean (n = 27)			
	r	p	r_{par}	p
$eGFR_{ncc}$	-0.409	0.034	-0.478	0.016
$eGFR_{cr}$	-0.365	0.062	-0.320	0.118
$eGFR_{cy}$	-0.654	< 0.001	-0.661	< 0.001

CONCLUSIONS

In conclusion, the results of this study showed a close correlation between mean SWV values of the two kidneys combined and $eGFR_{cy}$. In particular, there was a higher mean SWV value shown in kidneys with lower $eGFR_{cy}$. Therefore, this technique needs standardization and validation.

ABS 23

A POSSIBLE NEW APPROACH FOR INFORMATION TO PARENTS ON THE USE OF OFF-LABEL DRUGS IN NEONATOLOGY AND PEDIATRICS

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INTRODUCTION

Normative commitments made in recent years by the Food and Drug Administration and the European Medicines Agency with the aim of promoting the involvement of children in drug testing are well known. Despite improvements in the process of dynamic regulation of off-label drugs, for pediatricians and neonatologists it remains the duty of the information to parents (essential in terms of the expression of a valid consent to therapy) on the use of these drugs in young patients. Complying with this obligation, with respect to any off-label therapeutic administration, is often onerous for physicians in clinical practice and care.

MATERIALS AND METHODS

The poster will be presented under the auspices of the Italian Societies of Pediatrics and Neonatology with aim to propose a possible new approach for information on the use of off-label drugs. The aim will be to share information with the parents about the therapeutic project. This proposal was structured by us with the aim of offering parents a general informative: 1. what are the drugs off-label and on-label; 2. why they are used; 3. what are the most off-label drugs administered; 4. which data are available about their safety and effectiveness.

CONCLUSIONS

Through this content we want to transmit to parents the idea that off-label therapy is not an arbitrary choice of the individual physician, but is related to the therapeutic needs in a context of inadequate scientific support and/or regulatory compliance to drug delivery in infancy. In addition, it also want to ensure that the therapeutic choice of each center, despite its legitimate autonomy, is essentially shared by the scientific community.

ABS 24

NEONATAL HYPOGLYCEMIA

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BACKGROUND

Metabolic emergencies need to be recognized early. The prompt initiation of an appropriate treatment, even without having an exact diagnosis, determines the overall long-term outcome. Patients frequently are admitted in the neonatal section in an emergency situation [1].

DIFFERENTIAL DIAGNOSIS

As shown in **Tab. 1**, hypoglycemia, which represents a common metabolic emergency, may be caused by disorders of glucose homeostasis (glycogen storage disorders, disorders of gluconeogenesis, galactosemia and congenital hyperinsulinism), by disorders of inappropriate ketone synthesis (fatty acid β-oxidation defects or inappropriate ketone utilization or by disorders of amino acid metabolism (MSUD, tyrosinaemia type 1). Congenital hyperinsulinism, which is characterized by an uncontrolled excessive insulin secretion for the prevailing glucose levels, is the most frequent cause of severe and persistent hypoglycemia in neonatal period and early infancy. Patients present with recurrent episodes of profound hypoglycemia requiring rapid and intensive treatment with dextrose infusions and intravenous glucagon to prevent neurological sequelae [2]. The prevalence of the disease is approximately 1 in 50,000 live births.

TREATMENT

Congenital hyperinsulinism is a heterogeneous disease and may be classified into two major subgroups: "channelopathies" and "metabolopathies". Loss-of-function mutations in the genes encoding the SUR1 and Kir6.2 subunits of the ATP-sensitive potassium channel, account for more than 50% of cases and are associated with two histological aspects: a diffuse form, which is inherited either as autosomal recessive or dominant trait, and a focal form, which results from the combination of a paternally inherited germinal mutation and a somatic loss of heterozygosity of the maternal allele in a restricted group of beta cells. Mutations in other genes (i.e. *GCK*, *GLUD1*, *HADH*, *HNF1A*, *HNF4alfa*, *SLC16A1*, *UCP2*, *INSR*) have been associated with the "metabolic" forms of congenital hyperinsulinism [2]. Chronic medical treatment to prevent recurrence of hypoglycemia may include drugs, such as diazoxide or octreotide, often in association with frequent carbohydrate-enriched feeding. Diffuse congenital hyperinsulinism is first approached with conservative treatment, but when medical measures are ineffective, near-total pancreatectomy is required, whereas focal hyperinsulinism is an elective indication for partial pancreasectomy [3, 4]. ¹⁸F-DOPA PET imaging is useful to differentiate between the two forms, and precisely localize focal lesions in the pancreas [3, 4].

Table 1. Differential diagnosis of neonatal hypoglycemia (adapted from: Grünewald et al, 2014 [1]).

Disease	CHI	HI-HA	GALT	Adrenal insufficiency	FAO defects	HMG lyase	GSD 1	F 1,6 DP	MITO	HT-1	MSUD
Hypoglycemia	+	+	+	+	+	+	+	+	+	+	+
Ketones during hypoglycemia	↓↓	↓↓			↓↓	↓↓			↑	↑	↑
Lactate					n - ↑	n - ↑	↑	↑	↑↑		n - ↑
ASAT-ALAT			↑	n - ↑	↑	n - ↑	↑	n - ↑	n - ↑	↑	
CK					n - ↑				n - ↑		
Uric acid					↑	n - ↑	↑	n - ↑	n - ↑	↓	n - ↑
FFA	↓	↓		↓	↑	↑			↑		
Triglycerides							↑				
Direct bilirubin				↑							
Ammonia		↑			n - ↑	n - ↑			n - ↑		n - ↑
Coagulation			↓		n - ↓		n - ↓		n - ↓	↓	
WBC						n - ↓	↓ (1b)				
Glucagon response	+	+	-	+	-	-	-	-	-		
High glucose demand	↑↑	↑↑									
Abnormal organic acids		±			+	+	±	±	+	+	+
Abnormal amino acids			±			±			±	+	+
Abnormal acylcarnitines					+	+					

CHI: Congenital Hyperinsulinism; HI-HA: Hyperinsulinism Hyperammonemia syndrome; GALT: Galactosemia; FAO: Fatty Acid Oxidation defects; HMG: Hydroxymethylglutaryl-CoA deficiency; GSD 1: Glycogen Storage Disease type 1; MITO: Mitochondrial disorders; HT-1: Hereditary Tyrosinemia type 1; MSUD: Maple Syrup Urine disease.

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ABS 25

METABOLOMICS APPROACH FOR THE FUNCTIONAL EVALUATION OF A POPULATION OF KIDS BORN VERY PRETERM: PRELIMINARY RESULTS WITH GC-MS

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BACKGROUND

Children born preterm are at risk to develop renal damage by nephrotoxic action of drugs used in

Neonatal Intensive Care Units (aminoglycosides, NSAIDs) and following to anoxic damage by asphyxia, sepsis and hypotension.

Children born preterm are at risk of disease at adult age, such as cardio-renal syndrome. Our previous study showed that children born preterm had reduced renal size if birth weight was below 1,000 grams (extremely low birth weight) and had increased excretion of alpha1-microglobulin if postnatally treated with aminoglycosides. With usual renal markers, these children did not show a decline of renal function [1].

To study the variations of metabolites in biological fluids, the Magnetic Resonance Imaging and Fluid Chromatography are associated with Mass Spectrometry. Application of this method allows to obtain information on and quantification of thousand of metabolites. Modern metabolomics approach is a powerful tool to investigate the functionality in living being; its position as the final downstream product of gene expression enables the provision of a high-resolution multifactor phenotypic signature of causes, manifestations, and pathways of disease. Previously, the metabolomic approach was able to find a different metabolic profile between newborns with intrauterin growth retardation and controls [2]. In this study we show the preliminary results of the urinary metabolomic characterization of very preterms in childhood age.

SUBJECTS AND METHODS

We enrolled in the study 15 children (mean age 11 years) born preterm (gestational age: 29 ± 3 weeks; birth weight range 550-1,540 grams) and 15 controls (mean age 12 years) born at term. The pooled preterm children were the same of our previous study (5 years before).

At admission, they were all in good condition. In particular, they did not have signs of infection. A sample of second morning urine was collected, centrifuged, and stored at -80°C until analysis. Urinary samples were analyzed using an Agilent 5975C interfaced to the GC 7820. We performed both univariate and multivariate statistical analysis (R v3.0.1 statistical software).

RESULTS

GC-MS metabolomics was able to detect the urinary metabolic differences between the two groups of children. The metabolic differential profile is referring to the following molecules: Valine, Azelaic acid, Oxalic acid, Lysine, Glycerol, Galactopyranoside, 3-Aminoisobutyric acid, 3-Methylhistidine, Ornithine, Sedoheptulose, Glycine, 2,3-Dihydro-3,5-bis(3-methoxyphenyl)-1H-inden-1-one, Serine, N-(4-hydroxybenzoyl)glycine, Histidine, Threitol, Glutamine, Sucrose, D-Arabinose, 3-(3-Hydroxyphenyl)-3-hydroxypropionic acid, Ribonic acid, Tartaric acid, N-Acetylglucosamine.

CONCLUSIONS

By metabolomic approach we were able to differentiate urinary metabolic profile between preterm and at term in childhood age. Among different hypothesis, epigenetic variations of organs development and their metabolic pathways, as the consequence of preterm birth and related events, could be take into consideration. Following this step, it is necessary to interpret the intricated preliminary results, enroll more subjects and confirm the results.

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ABS 26

LIPOMA OF THE CORPUS CALLOSUS: A CLINIC CASE

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BACKGROUND

Lipomas of the corpus callosum (CC) are rare congenital brain malformations resulting in an abnormal differentiation of residual primitive meningeal tissue [1-2].

The sonographic (US) aspects of the CC lipoma consist in a nodular or curvilinear hyperechoic mass localized in the middle of the interhemisferic fissure [2]. The echogenicity of the neoplasm is very similar to that of the parietal bone. The margins are generally smooth but can also be irregular and may be extended toward the frontal lobes or the choroid plexus. The CC lipoma corresponds to a sharply delineated hypodensity with calcifications in the context on CT, while it appears hyperintense on both T1 and T2 MRI.

Two morphological forms of CC lipoma have been described [2]:

- tubulo-nodular form: appearing round, usually anterior, located in the genu and the trunk of the CC. It can reach the skull and may be associated with other kind of brain malformations;
- curvilinear form: consisting in a thin and elongated mass, sometimes replacing the CC splenium or extending into the choroid plexus.

One out of 2 of all fetal CC lipomas are linked with other brain malformations interesting more frequently the CC, which may result partially or totally absent or dysmorphic. Less frequently besides the CC lipoma, agenesis of septum pellucidum, bifid nose, spinal myelomeningocele, encephalocele, hypoplasia of the vermis and cortical malformations could be found.

The neoplasm can be unexpectedly discovered in healthy children or symptoms may include: seizure, delayed psychomotor and impaired intellectual development. Other associations are between CC lipomas and prenatal CMV infection and Goldenhar syndrome. Clinical markers of lipoma include subcutaneous lipomas, alopecia and facial dysmorphisms.

CASE PRESENTATION

We report the case of a female infant, born at term by cesarean section (for breech presentation), admitted to hospital for enlargement of the right occipital horn of the lateral ventricle (colpocephaly) detected by prenatal ultrasounds. The clinical and physical examinations were normal. The ultrasounds as well as the RMI scans confirmed the initial diagnosis, however, besides the ventricular enlargement a CC lipoma was observed. The mass was localized in the posterior trunk and in the splenium of the CC (**Fig. 1** and **Fig. 2**). It appeared curvilinear and markedly echodense with sharply delineated edges.

Other investigations conducted (routine blood tests, TORCH, CMV viral research, abdomen US, echocardiography, and ABR) were normal.

The EEG performed at seven weeks of age showed a diffuse theta and delta activity along with slow background activity, especially on the right side. The neurological exam revealed a stereotyped and stiff motor pattern.

Currently, the baby is five months old, she does not present motor abnormalities. Serial ultrasonography scans (performed every two months) show a stable US image.

DISCUSSION

Lipomas of the corpus callosum are rare congenital brain malformations, usually benign. They are often associated with other brain malformations
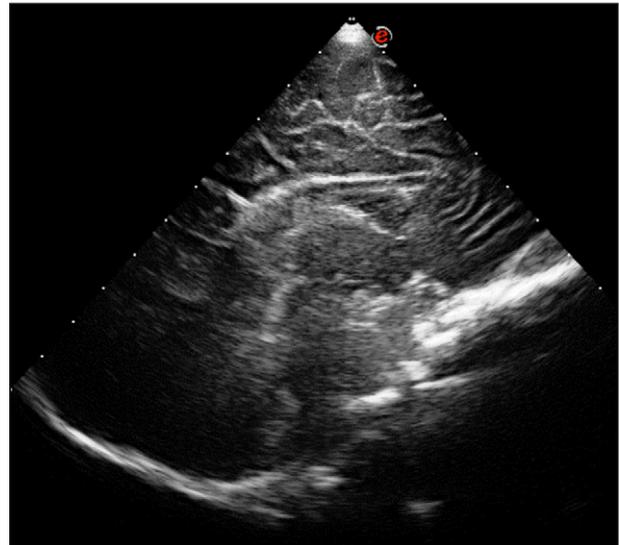
Figure 1. Sagittal US scan, curvilinear mass in the posterior trunk and in the splenium.

Figure 2. Frontal US scan, round echodensity replacing the splenium.

and, therefore, a careful radiologic investigation is necessary to evaluate more comorbid conditions [1]. Owing to CC lipomas are often asymptomatic, generally there is no need for specific treatment. Anticonvulsivant therapy should be considered in case of seizures because of the risk of epilepsy.

Since the neoplasm is non-malignant, non-progressive, hypervascular and deeply localized into the brain, surgical removal is generally not indicated.

In our case, the diagnosis was incidentally made during investigations following a report of prenatal enlargement of the right occipital horn of the lateral

ventricle. Colpocephaly may be present in the splenium of the corpus callosum disorders (agenesis or atrophy or lipoma) [1-3].

Currently, since the patient is asymptomatic, no medical/surgical therapy is required.

However, it is suggested to closely follow this kind of patients to identifie early signs or symptoms of the mass growth.

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ABS 27

CEREBRAL OXYGENATION, SUPERIOR VENA CAVA FLOW, SEVERE IVH AND MORTALITY IN 60 VERY LOW BIRTH WEIGHT INFANTS

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BACKGROUND

Hypotension and brain hypoperfusion during the immediate postnatal period have been reported to be related to cerebral damage in sick preterm infants, considering the extreme brain vulnerability and the impairment of cerebral autoregulation of these patients [1, 2].

Despite the perinatal care improvement, the search for valid tools to evaluate hypoperfusive states and regional cerebral tissue oxygenation (rSO_2) in these patients is still a great challenge for neonatologists [3].

A good marker of upper body systemic perfusion is Doppler echocardiographic measurement of blood flow in the superior vena cava (SVCf) [4, 5]. Cerebral rSO_2 is an indirect way of measuring cerebral blood flow (CBF) and a non invasive method to assess cerebral oxygenation is near-infrared spectroscopy (NIRS) [6-8]. It measures rSO_2 allowing to calculate Cerebral Fractional Oxygen Extraction (CFOE) thus discriminating between cerebral hypoxia and ischemic hypoxia [9, 10].

MATERIALS AND METHODS

In this study we enrolled 60 VLBW (GA 27.9 ± 2.4 weeks) in the first 48 hours of life to investigate whether continuous monitoring of cerebral rSO_2, CFOE and echocardiographic measurement of SVCf in VLBW in the immediate postnatal period are helpful to identify preterm infants at higher risk of adverse outcomes (death or intraventricular hemorrhage [IVH]).

RESULTS

The 8 patients who later died (6, 75%, also had high grade IVH) and the 52 patients who survived (only 1, 1.9%, had high grade IVH) were different in terms of number of rSO_2 values < 40% in the first 48 hours (OR for death: 1.01, p = 0.016) and number of SVCf values < 40 ml/kg/min (OR for death: 4.22, p = 0.019 (**Fig. 1**, **Tab. 1**). In patients who died, CFOE was higher at baseline and further increased from 12-24 hours, in patients who survived it tended to decrease over time (p < 0.001). At multivariate analyses, these associations retained significance.

CONCLUSION

Continuous monitoring of rSO_2, CFOE and SVCf could lead to a better tailoring of the treatment to each of these newborns, to identify those whose outcome may be improved by early interventions.

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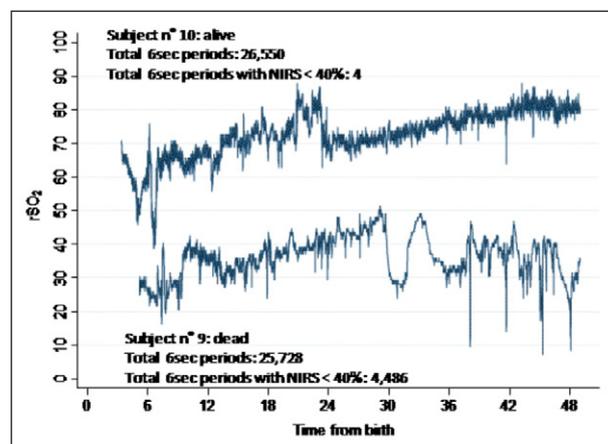
Figure 1. rSO_2 values in two patients (the first survived and the second died).

Table 1. Description of several summary measures of rSO_2 and SCVf over time, according to life status at discharge.

		0-6 hours		6-12 hours		12-24 hours		24-48 hours		
		Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	p^a
NIRS: Registration time		3.3 (1.6)	3.2 (2.0)	5.8 (1.8)	5.2 (2.1)	12.0 (1.8)	12.0 (12.0)	24.0 (23.0)	24.0 (24.0)	0.766
No of 6 sec. period with NIRS ≤ 40% / No of 6 sec. period recorded		145/143,245	2045/24,482	150/336,365	370/49,244	1057/664,845	1,866/97,578	658/259,853	10,526/34,615	
Periods of 6 sec. with NIRS ≤ 40%[b]		3.1 (8.1)	255.6 (633.7)	2.9 (7.8)	52.9 (103.8)	20.7 (49.3)	266.6 (650.6)	14.0 (50.1)	1503.7 (3,702.6)	0.016
Subject with at least one NIRS period ≤ 40% yes/tot (n, %)[c]		5/36 (13.9)	2/6 (33.3)	9/47 (19.1)	3/8 (37.5)	9/52 (17.31)	4/7 (57.1)	19/51 (37.5)	4/7 (57.1)	0.043
Time (hours) spent with NIRS:	≤ 40%	0.02 (0.06)	0.1 (0.2)	0.0 (0.0)	0.4 (1.0)	0.01 (0.03)	0.0 (0.0)	0.0 (0.0)	0.1 (0.4)	0.005
	≤ 50%	0.14 (0.4)	0.2 (0.3)	0.1 (0.4)	0.9 (2.0)	0.09 (0.42)	0.0 (0.3)	0.2 (0.2)	3.3 (2.5)	0.067
	≤ 55%	0.28 (0.58)	0.3 (0.4)	0.3 (0.9)	1.0 (2.1)	0.27 (1.00)	0.2 (0.5)	0.5 (0.4)	10.0 (4.6)	0.113
% of Time spent with NIRS:	≤ 40%	0.7 (2.4)	12.0 (29.0)	0.1 (0.2)	6.4 (16.0)	0.1 (0.3)	0.0 (0.1)	0.1 (0.1)	0.43 (1.5)	0.007
	≤ 50%	6.0 (18.0)	16.0 (35.0)	1.8 (6.0)	27.0 (46.0)	0.8 (3.5)	0.4 (2.3)	0.7 (0.6)	14.0 (10.0)	0.089
	≤ 55%	12.0 (26.0)	17.0 (35.0)	6.3 (16.0)	30.0 (45.0)	2.3 (8.5)	1.6 (4.4)	2.0 (1.7)	43.0 (19.0)	0.107
SCVf (ml/kg/min)		51.0 (17.4)	43.2 (14.8)	56.3 (20.8)	50.4 (17.8)	68.7 (21.6)	45.8 (10.7)	63.8 (20.4)	53.3 (17.2)	0.024
Subject with SCVf ≤ 40; yes/tot (n, %)[d]		12/38 (31.6)	4/7 (57.1)	5/38 (13.2)	2/5 (40.0)	2/37 (5.4)	2/5 (40.0)	2/36 (5.6)	1/5 (20.0)	0.019

Data are means and (SD), if not otherwise specified; [a]p values refer to comparison between the alive and dead newborns and are derived from logistic or linear regression models for repeated measures; [b]OR for dead: 8.0% every ten episode of NIRS ≤ 40% (95% CI: 1.5-15.0); [c]OR for dead: 3.3 (95% CI: 1.1-0.1); [d]OR for dead: 4.2 (95% CI: 1.3-14.0).

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ABS 28

A SARDINIAN HOSPICE BY THE SEA, FOUNDED IN CAGLIARI 135 YEARS AGO

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In 1879, 135 years ago, a committee was established to create in Cagliari (Sardinia, Italy) a hospice by the sea to take care of scrofolous children ("*Ospizio marino sardo per gli scrofolosi in Cagliari*"). The committee included many doctors. The president was A. Fara-Puggioni (lawyer, provincial director of Cagliari, Knight of the Crown of Italy); vice-president was I. Desogus, while L. Serra was the treasurer and prof. A. Falconi was the secretary. The councilors were six: prof. J. Flat-Borme, dr. G. Basso-Arnoux, G. Mereu, dr. R. Aresu, E. Timon, E. Cao. The members were assigned to this committee by Nicolò Ferracciù (**Fig. 1**), who was Minister of the Navy during the third Depretis government (19 December 1878 - 14 July 1879).

Ferracciù was born in Calangianus (the current province of Olbia-Tempio, in Sardinia) on May 19, 1815 (he died in Rome, March 1892). The committee asked the Minister the possibility to use the military hospital of Cagliari, located at the foot of the promontory of Sant'Elia, close to the Poetto beach (**Fig. 2**).

In this way, in the summer of 1879, the first Sardinian hospice by the sea was created, hosting 50 children from the provinces of Cagliari and Iglesias. It was a real hospital in front of the sea. Exposure to sunlight favored the transformation from the inactive to the active form of vitamin D, essential for bone formation. At the same time children, suffering from tuberculosis or rickets,
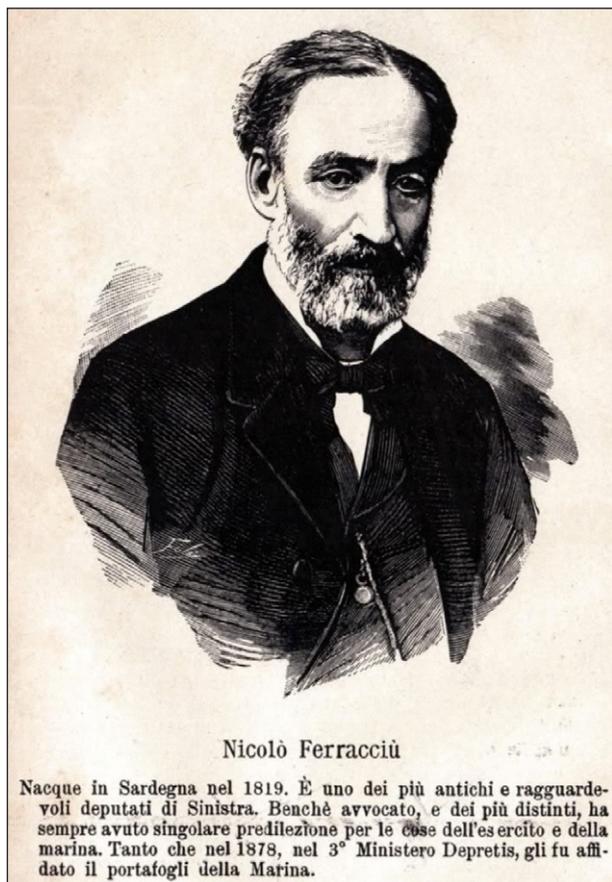
Figure 1. Nicolò Ferracciù (by Luigi Stefanoni, *Storia d'Italia contemporanea*. Roma: ed. E. Pierino; 1885).

had benefits for the respiratory system and in some cases a real rehabilitation.

The therapeutic efficacy of care provided in the hospice is demonstrated in **Tab. 1**: more than a half (53.88%) healed and one third (34.58%) had a relevant improvement.

Other hospices by the sea were established in other parts of Sardinia after this first one.

At the time of the introduction in Italy of the first health care reform (which came into force in 1888, signed by Francesco Crispi), 19 Italian hospices by the sea were present.

Table 1. Outcomes of care hospice marine Cagliari 1879-1885.

Outcomes at discharge	Number of children	%
Healed	215	53.88
Substantial improvement	138	34.58
Average improvement	32	8.02
Stationary conditions	13	3.25
Deceased	1	0.25
Total	399	100

Figure 2. Cagliari: the Poetto beach (1930).

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ABS 29

CHANGES IN PODOCYTE NUMBER AND GLOMERULAR VOLUME IN DOWN SYNDROME

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INTRODUCTION

Recent studies on the preterm neonatal kidney showed the existence of a decreasing trend in podocyte number during gestational age [1]. Our previously study showed that glomerular area values is greater in kidneys of children with Down syndrome (DS) compared to those with normal karyotype [2]. The aim of our study was to analyze morphological aspects of nephrogenesis such as podocyte number and density and glomerular volume in fetuses with chromosome 21 trisomy.

PATIENTS AND METHODS

5 fetuses from therapeutic abortions for chromosome 21 trisomy (Down Syndrome, DS) and 5 age-matched controls with normal karyotype (NK) from pregnancies interrupted for maternal reasons have been selected. Gestational age ranged from 13 to 20 weeks. Macroscopic examination of all the fetuses did not show any congenital malformation. Tissue samples were formalin-fixed and paraffin-embedded to obtain 5 μm-thick histological sections that were H&E-stained. Histological sections of the kidneys were digitally scanned at 400X magnification. For each subject podocyte number and density was estimated in five glomeruli chosen along at least two straight lines extending from the renal capsule towards the deepest zone of the cortex. Glomerular volume was assessed using Weibel-Gomez method [3]. Podocyte number and density were performed through a specific algorithm developed with Matlab® software.

RESULTS

Statistical analysis showed significant differences in glomerular volume between DS fetal subjects and normal subjects (t(48) = 2.507, p = 0.0156) (**Fig. 1**). Relevant histopathological features of DS fetal kidneys were glomerular changes consisting in glomerular hypertrophy and fusion, producing larger structures due to confluence of two or three glomerular tufts (**Fig. 2**). There were no statistical differences in podocyte density between the two groups. Podocyte number was significantly higher in DS fetuses than normal subjects (t(48) = 2.317, p = 0.0248) (**Fig. 3**).

CONCLUSIONS

Our preliminary study underlines significant differences in morphological aspects of nephrogenesis between DS fetuses and normal karyotype fetuses. A range of renal diseases have been described in patients with DS, increased
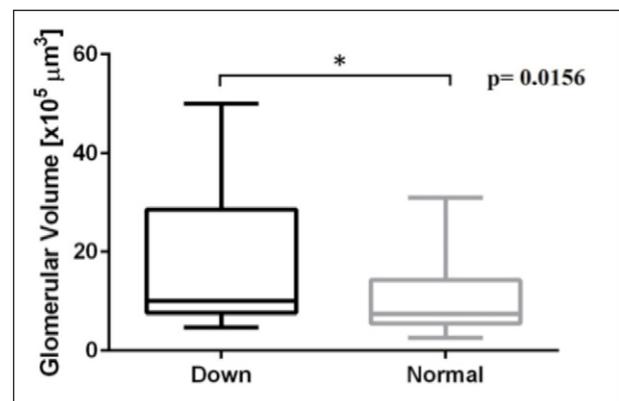

Figure 1. Differences in glomerular volume between DS fetuses and normal karyotype fetuses. Bars represent mean ± SD.
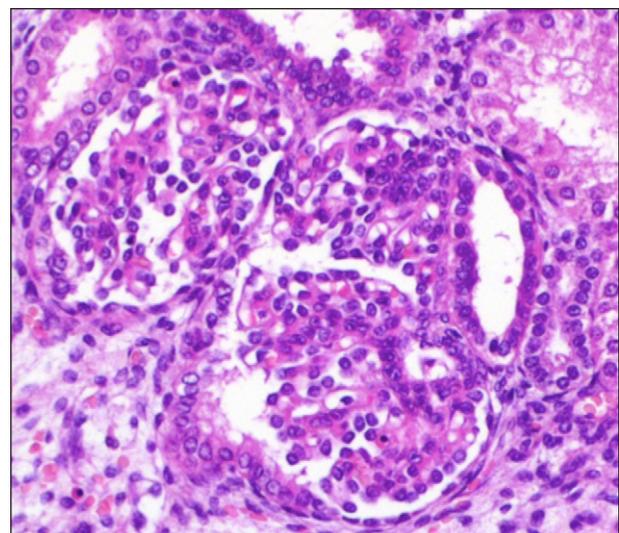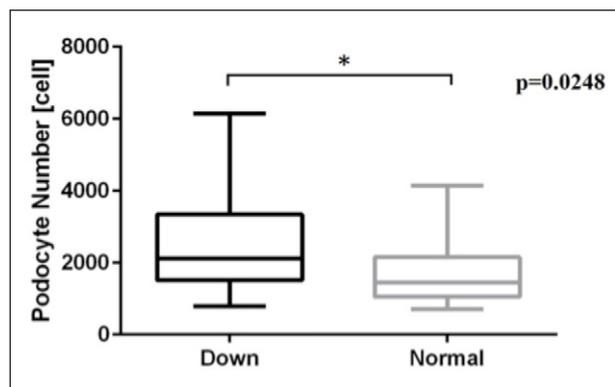

Figure 2. Confluence of glomerular tufts in a DS fetus.

Figure 3. Differences in podocyte number between DS fetuses and normal karyotype fetuses. Bars represent mean ± SD.

survival being associated with growing number of these patients presenting with chronic renal failure. The primary causes are still understood. Further studies on larger series are needed to validate the differences here reported.

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ABS 30

GIORGIO MAGGIONI, MASTER OF PEDIATRICS

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Giorgio Maggioni (**Fig. 1**) was born in Ruta (Camogli, Italy), on the 5th of July, 1918; he was raised in a refugee family coming from Belluno, by reason of the First World War. He graduated with honors in Padua (31st of May, 1941), obtaining the Lussana Prize, thanks to his best results as a student. He took part in the 2nd World War as a medical officer in Greece and Albania (1942-43).

From December 1943, he was one of the assistant professor of Gino Frontali, Director of the Paediatric Dept in the University of Rome. In 1947 Maggioni obtained the Child Health Diplome (CHD) in the University of London. In 1950 he started his carreer as a professor of Clinical Paediatrics, then, in 1954, in Children's Health (Puericultura). He carried out many investigation programs in the field of anemia, metabolic defects and nutrition.

From 1970, Maggioni spent seven years in Sardinia, first as Director of the Puericulture Institute in the University of Sassari, improving the neonatal care levels, then as director of the Paediatric Clinics in 1975-77.

He moved to Ancona, where, in a short time (1977-1979), he was able to create the University School of Paediatrics, working as Director of the Hospital Paediatric Departments, and having as a close coworker prof. Giuseppe Caramia, chief of the "Salesi Children's Hospital".

Professor Maggioni was able to gather and bring together all the Paediatricians in a great family.

In 1979 professor Giorgio Maggioni come back to the Sapienza University in Rome where he succeed in financing and organizing the new Puericultura University Service, obtaining a new building with many Laboratory and investigation units, especially in the field of Rh and AB0 immunological problems. He was Director during 10 years (1979-1988), improving his own Academic impact.

Considering his love and interest for books, history and the historical development of Paediatrics, he was continuously at work, even if he suffered, during the years, some important retinal damages.

Figure 1. Maggioni with his Colleagues Cataldi, Paladini, Gregorio and Fasani.

Professor Maggioni was an excellent investigator; he performed many important research programs, reaching, in 2010, the "Master" of Paediatrics Prize of the Italian Society of Paediatrics.

He was honorary President of the History of Paediatrics Study Group in the Italian Society of Paediatrics, from its foundation (Turin, 1998), until spring 2014 when, struggling to survive the cancer, he suddenly died the 1st of May 2014.

At the end of each of our meeting, Giorgio Maggioni greet us with his "Arrivederci!"

"Arrivederci Master, we still need your words and your supporting exemple!"

ABS 31

REGULATION OF THE HOSPICE BY THE SEA OF VIAREGGIO UNDER THE BORBOUN-PARMA DINASTY

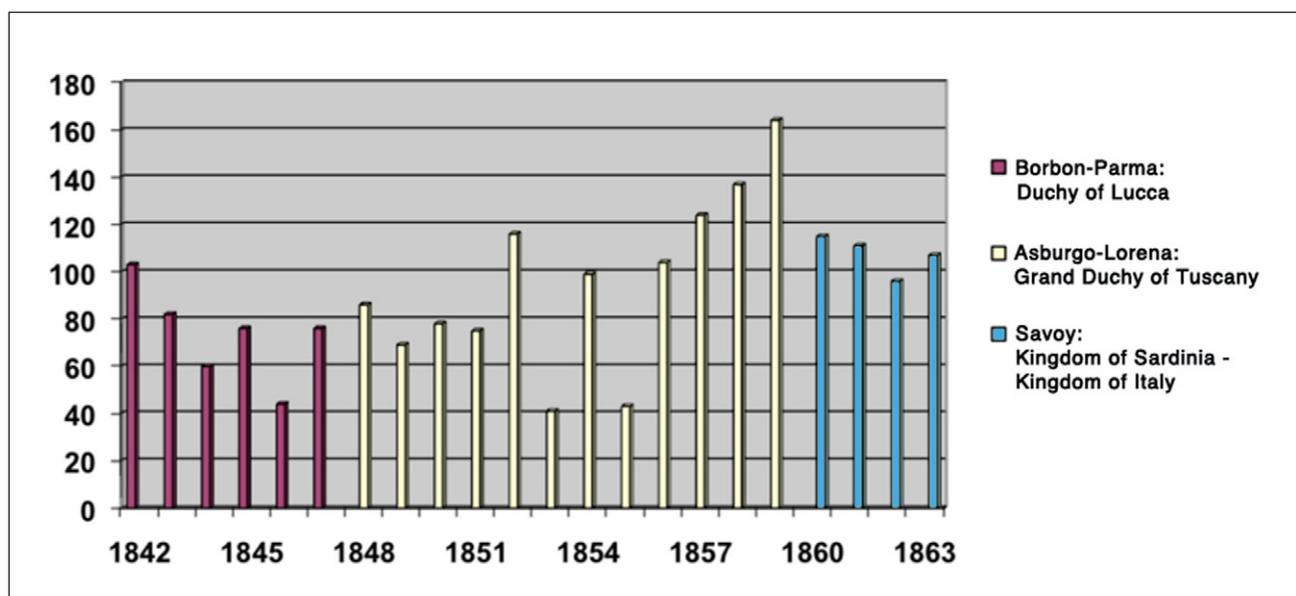I. Farnetani[1], F. Farnetani[2]

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The aim of the regulation of the hospice by the sea of Viareggio (**Tab. 1**) was to ensure the safety and the respect for each individual, while guaranteeing administrative transparency, a precise expression of the will of the government. The regulation was published on the 30th June 1842, signed by A.G. Di Grazia (Protocol No. 845, 1842) and is composed of 16 articles. We report some of them below.

- Male children were monitored by one or more male attendants, while females children were monitored by women.
- On the beach, children were cared for by attendants.
- Monitoring had to be careful. Negligence was judged and punished as a serious crime.
- Depending on the season, there were two swim schedules, one in the morning and one in the afternoon.
- The duration of the children's stay was 20 days, to allow rotation between the children who needed care.
- The attendants had to take care even of the religious aspect, accompanying the children, dressed in an appropriate manner, to the Sunday Mass.
- The kids could go out only in group, accompanied by attendants and dressed appropriately.
- Entrance of outsiders into the hospice was prohibited, except for the staff members.
- The meals were provided at noon and at eighteen. The children had to served by attendants.
- The food had to be purchased on a daily basis, to guarantee quality and quantity.
- The meals had to be prepared with an appropriate menu, reported daily in the logs.

Table 1. Number of children accommodated at the hospice by the sea of Viareggio from 1842 to 1863, divided by year of attendance and reigning dynasty (modified by I. Farnetani).

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ABS 32

MILK'S COLOURS AND LATCH SCORE: A TRANSCULTURAL EXPERIENCE IN NURSE LED CLINIC

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BACKGROUND

The act of breastfeeding has always played a key role in the society of all time. Today, western women undoubtedly recognize its importance, thanks to the numerous awareness campaigns, prenatal courses, information through the mass media. However, we must consider that to become mothers, as well as a biological phenomenon, is also the result of a cultural intervention. It is not easy to live and preserve traditions in a changing society, that is increasingly multiethnic, where one has to deal daily with other cultures.

Despite the cultural differences, our experience, dedicated to mothers who start breastfeeding in the hospital, helped us to understand that there are different ways to grow a baby. Every woman has a particular way of offering the breast to her baby, according to her own cultural roots.

The success of breastfeeding is achievable only by providing information to the woman-mother, providing also practical, psychological and emotional support.

METHODS

The research was conducted at the Maternal Neonatal Department and Nurse Led Clinic, University Hospital of Monserrato (Cagliari).

The study involved a group of 40 mothers from Tunisia, Morocco, Pakistan, Bangladesh, China, the Philippines, Latin America and a group of 80 Italian mothers.

The instrument used in our study is the "Latch Breastfeeding Assessment", drafted in 1994 by Jensen, Wallance and Kelsay [2]. This systematic method is able to provide individual data on the early stages of breastfeeding. Latch Score is a simple tool, quick to understand and use for operators who wish to observe the feeding.

The instrument, in fact, uses a list of essential questions with only 5 parameters: (L) = Latch attack breast; (A) = Audible swallowing; (T) = Type of nipple; (C) = Confort of the nipple and breast; (H) = Hold position of mother-child and correct attack on the breast.

For each of these parameters, it is assigned a score ranging from 0 to 2: 0 = mother and newborn require full assistance by the operator and/or the medical staff; 1 = mother and newborn require minimal assistance; 2 = mother and newborn do not need any kind of assistance.

After the observation of the feed, it will be assigned a score ranging from a minimum of 0 (feeding with numerous problems) to a maximum of 10 (feeding with no problem).

According to the cut-off indicated by the literature, a score below 7 indicates that the mother-child couple might be eligible for support and/or encouragement, to initiate and promote the success of breastfeeding.

The Latch board has the advantage of making available a clear and standardized report on each feed; in this way it is easy to identify mother-infant couples in need of some support both during hospitalization and after discharge from the hospital, in the early and sensitive days postpartum. The study was structured in three stages: at 24 hours after birth; at discharge (48-72 hours after birth); in Nurse Led 4-6 days after discharge from the hospital.

RESULTS

In our study, the migrant women have a mean age of 27 years (a minimum of 22 to a maximum of 36 years), while Italian women have an average age of 35 years (a minimum of 26 to a maximum of 42 years). Most of these women are primiparae. Regarding the migrants, almost none has attended a prenatal course; as regards the Italian women, 74% of them participated to a prenatal course.

In the first sample of 40 foreign mothers, it appears that only 20% would need encouragement to start the exclusive breastfeeding in the days following hospital discharge, compared with 35% in the second sample of 80 Italian mothers.

CONCLUSIONS

The experience of the Nurse Led shows that "Latch Breastfeeding Assessment" is an appropriate tool to provide competent care to support breastfeeding mothers.

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ABS 33

EPICARDIAL FAT THICKNESS, AN EMERGING CARDIOMETABOLIC RISK FACTOR, IS INCREASED IN YOUNG ADULTS BORN PRETERM

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INTRODUCTION

Intrauterine growth retardation and prematurity at birth constitute risk factors not only for neurological, nephrological, and ophthalmological sequelae, but also for future cardiovascular adverse events. In fact, some previous reports have highlighted that preterm born subjects may develop early atherosclerosis and coronary artery disease, hyperlipidemia, high blood pressure, type 2 diabetes, cardio-renal syndrome, QT tract prolongation at basal ECG, interatrial septal aneurysms [1-7].

Epicardial fat thickness (EFT), the large visceral fat storage located between epicardium and pericardium as well as around the large coronary arteries, is an emerging cardiometabolic risk factor [8].

This study aimed at measuring EFT in a group of young subjects who have been born preterm with an extremely low birth weight (< 1,000 kg) in comparison with born at term controls. A possible relationship with patients' characteristics, such as echocardiographic measures, gestational age, birth weight, and duration of stay in Neonatal Intensive Care Unit has been investigated as well.

METHODS

Sixty subjects were enrolled: thirty were high grade ex-preterm subjects (10 males [M] and 20 females [F], aged 17-28, mean 20.1 ± 2.5 years). They were compared with 30 healthy, age-matched born at term subjects (C, 10 M and 20 F). The carachteristics of the subjects in the study are summarized in **Tab. 1**. EFT was assessed non-invasively and easily by transthoracic echocardiograpy (see **Fig. 1**).

RESULTS

EFT was significantly higher (p < 0.001) in former preterm individuals than in controls. The above stated measure was correlated with birth weight (r = -0.47, p = 0.0009), as well as with gestational age (r = -0.39, p = 0.03) and cardiac left ventricular mass (r = 0.51, p = 0.004). When excluding the influence of body mass index, intrauterine growth restriction – expressed as birth weight – remains the only determinant of EFT, irrespective of gestational age.

CONCLUSIONS

EFT is significantly increased in former preterm subjects compared to controls and is associated with an increase in their cardiac left ventricular mass as well [9]. Since the latter is known to be associated with a higher incidence of clinical events attributable to cardiovascular disease, EFT appears to be an easy-to-measure tool for early detection of subclinical target organ damage able to predict the probable development of future adverse cardiovascular events in these subjects [10].

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Table 1. Characteristics of the subjects in the study.

	Ex ELBW (n = 30)	Control group (n = 30)	p
Age (years)	20.3 ± 2.4	20.8 ± 1.6	ns
Birthweight (g)	832.4 ± 116.5	3,138.1 ± 759.7	< 0.0001
Gestational age (weeks)	27.7 ± 2.1	39.3 ± 1.5	< 0.0001
Height (cm)	161.3 ± 4.2	167.5 ± 2.9	ns
Weight (kg)	51.8 ± 4.7	56.4 ± 3.2	ns
BMI (kg/m²)	19.7 ± 1.4	20.4 ± 0.9	ns
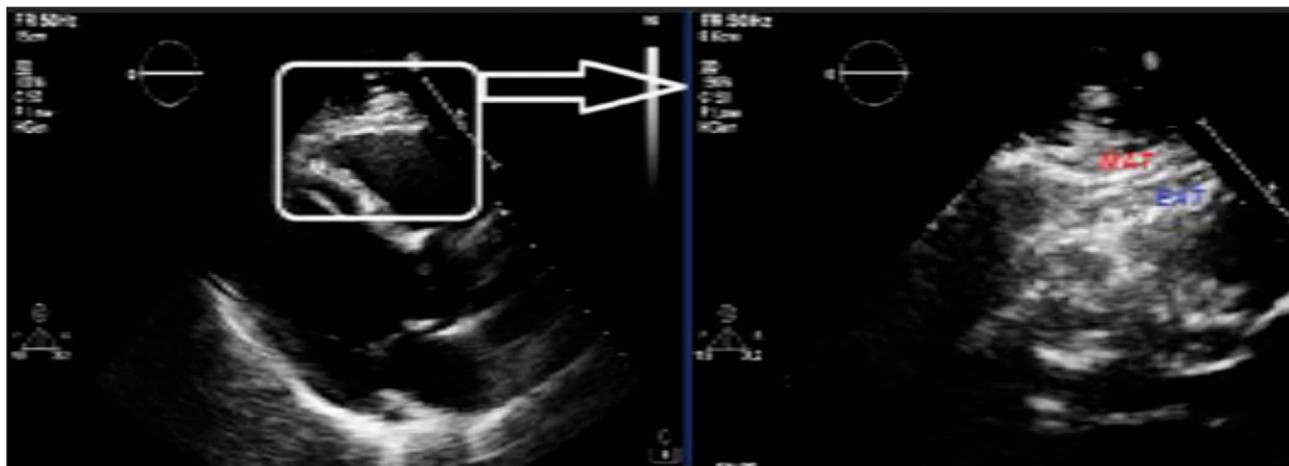
Figure 1. Measurement of epicardial fat thickness by echocardiography.

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ABS 34

A METABOLOMICS ANALYSIS OF CONGENITAL DIAPHRAGMATIC HERNIA: SURVIVORS VS NON SURVIVORS. PRELIMINARY RESULTS

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INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a fetal defect that develops during the formation of the diaphragm. In a CDH the diaphragm does not totally form, and an aperture rests between the chest and the abdominal cavities. The severity of this disorder ranges from a small to a large opening in the diaphragm and based on how large is the hole: the intestines, spleen, liver and/or stomach may move up into the chest cavity. It was estimated that 1 in every 2,200 births is affected by CDH with no predominance between the sexes. In addition, almost 25% of babies with CDH will have another birth defect. The exact cause is not known yet; infact the pathogenesis is still unclear. Two ideas exist both supported by animal experimental data. One describes CDH originating as a hole in the diaphragm, which causes the pulmonary sequelae. The second theory suggests CDH results from an abnormal mesenchymal plate. Despite recent remarkable advances in perioperative neonatal care and surgical procedures, CDH remains a condition with a significantly high mortality rate: 31-58% [1-5].

The aim of this study was to evaluate by GC/MS approach the presence of a urine metabolic profile characteristic of a population of infants died from CDH compared to infants who had survived from a preliminary cohort of CDH infants enrolled in the International Multicentre VICI Trial coordinated by Sophia Children's Hospital – Rotterdam [6].

MATERIALS AND METHOD

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 analyze 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 1h at room temperature. The derivatized samples were diluted with hexane (600 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

The chromatogram of the pool sample was used to build a dedicated library of urinary metabolites: some chromatographic peaks were identified through comparison of retention times and mass spectra with those obtained from authentic samples. Other metabolites were identified using the database NIST08 (National Institute of Standards and Technology's mass spectral database). In this way 100 target compounds were identified giving rise to a dedicated library to be used for automated analysis of samples with the free software AMDIS (Automated Mass Spectral Deconvolution and Identification System).

STATISTICAL ANALYSIS

We performed both univariate and multivariate statistical analysis. By univariate analysis the differences in mean metabolite concentrations (logarithm base 10 transformed) between CHD survivors and CHD un-survivor were accessed in R v3.0.1 statistical software [15] using independent sample t-test, adjusting for unequal variance and controlling for the false discovery rate. By multivariate analysis the principal components analysis (PCA) was carried out in R v3.0.1 and used to overview the data variance structure in an unsupervised manner.

A multivariate discriminant model about the significant differences between the samples of CHD-survivors compared to the sample of CHD-un-survivors population reveals the following metabolites: Inositol, Gluconic acid, Uric acid, Lactic acid, Glucose, Mannitol, Unknown monosaccharide 1, Citric acid, Phosphate.

O-PLS-DA model represents a multifactorial metabolism: survivors population are clustered all together due to a contribution of higher Phosphate, Gluconic acid d-lactone, Unknown 1, Unknown acid 1, Gluconic acid, Glucaric acid, Lactose and lower Uric acid and Inositol.

In the same way un-survivors are separated into two clusters: 55,48 and 35,27 with higher Gluconic acid d-lactone, Unknown 1, Unknown monosaccharide 1, Gluconic acid and Inositol and a lower levels of Lactic acid, Glycine, Glucose, Mannitol and Uric acid. This profile between survivors and un-survivors justifies the complex metabolic profile related to the random effects in both population.

Gluconic acid and its cyclic derivative, Gluconic acid d-lactone, are produced by oxidation of Glucose as well as Glucuronic acid, that is also formed by oxidation of Inositol.

CONCLUSIONS

Multifactorial models with higher number of samples are requested for a proper definition of the survivor to CDH diseases but our preliminary study demonstrate the ability of the GCMS-based urinary metabolomics to be sensitive to define the profile of the CDH-survivor phenotype.

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ABS 35

BERNARDINO RAMAZZINI AND OCCUPATIONAL EXPOSURE IN NEONATOLOGY IN THE SEVENTEENTH CENTURY

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This year marks the third centenary of the death of Bernardino Ramazzini.

A whole study day will be organized by the National Academy of Sciences, Letters and Arts of Modena, (http://www.accademiasla-mo.it/), which counts among its founders Ramazzini himself. Ramazzini, was born in Carpi (MO) in 1633. He taught first at the University of Modena; later on, he moved to Padua where he died on November 5, 1714.

Ramazzini is known as the founder of occupational medicine. In particular, in his book *The diseases of the workers* (**Fig. 1** and **Fig. 2**), he tracks and describes the occupational diseases of some categories of workers, introducing the concept of risk assessment. Of particular interest to neonatologists are the chapters on diseases of midwives and nurses.

We have summarized in some points the most important elements that affect perinatal medicine and neonatology, which are described with the sequence reported by Ramazzini.

- In Italy, women gave birth seated on a birthing chair, while in France, Germany and other European countries, pregnant women during delivery were lying down in their beds.
- In general, wet nurses suffered most from: wasting, hysterical attacks, pustules and itch, pain in the head, dizziness, shortness of breath and weakness of vision.
- Related to breastfeeding were especially breast engorgement, mastitis, sore nipples, and irritation caused by the contact "skin to skin" with the baby.
- Breastfeeding was prolonged for several years; usually it was not suspended because of problems for the baby, but because of mother consumption.
- Until the first decades of the twentieth century, the nurses lived at home with the baby family; turbulence was created, mainly because of the attentions of the male members of the families.
- Interestingly, donkey milk was used, following the advice of Hippocrates, as an alternative to cow's milk, richer in nutrients.
- The most common skin disease in the newborn was the "cradle cap" which is actually seborrheic dermatitis. For its treatment, drugs were used
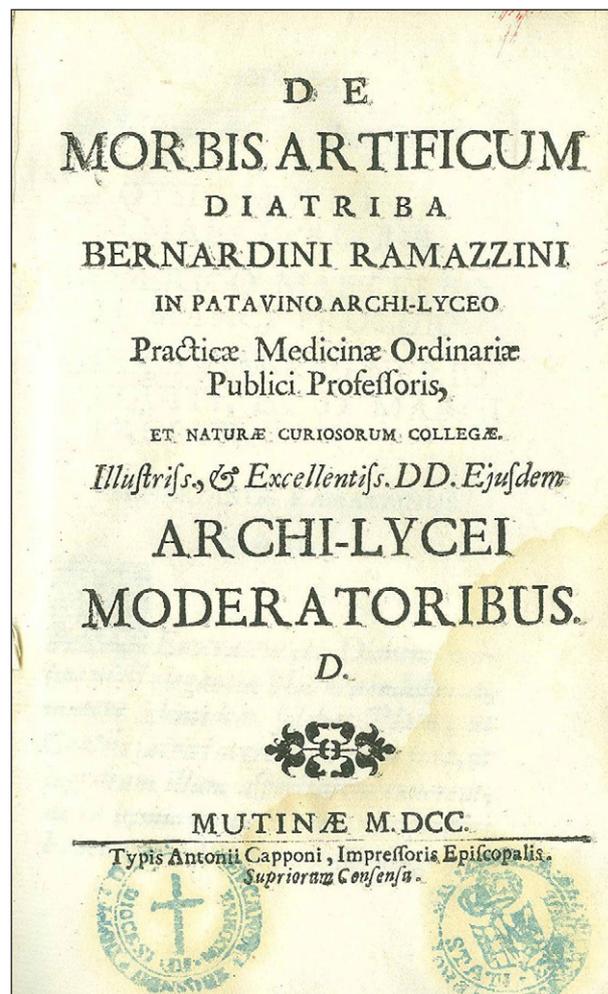
Figure 1. Frontispiece of Bernardino Ramazzini, *De morbis artificum*, published in 1700, kept in the library of National Academy of Sciences, Letters and Arts of Modena.
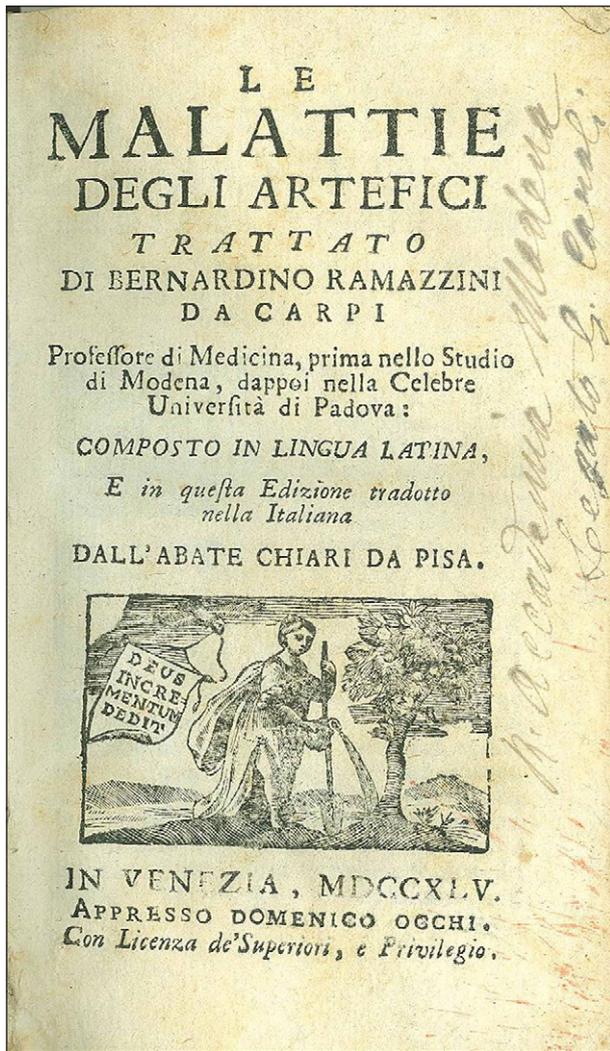
Figure 2. Frontispiece of Bernardino Ramazzini, *Le malattie degli artefici*, published in 1743, kept in the library of National Academy of Sciences, Letters and Arts of Modena.

against scabies and daily baths were preferred to systemic therapy.

- In the breastfed baby, three meals daily were recommended as opposed to the habit of keeping longer the baby to the breast. The Ramazzini advice was based on the observation of calves, that were fed only three times a day. This indication was therefore based on the observation of an animal model.
- In England and in Germany early weaning was implemented with a paste prepared from cow's milk, egg yolk and sugar.

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ABS 36

MARKED INTERINDIVIDUAL VARIABILITY IN SURFACTANT A AND B PROTEIN EXPRESSION IN THE FETAL AND NEWBORN HUMAN LUNG

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BACKGROUND

Pulmonary surfactant is a lipoprotein complex composed of lipids (90%) and proteins (10%), secreted by the alveolar type II pneumocytes. Surfactant proteins include surfactant protein A (SPA), B (SPB), C (SPC), and D (SPD). On the basis of these data, it seemed of some interest to better analyze, by immunohistochemistry, SPA and SPB expression during human development, in order to reach a better knowledge on the timing of expression of these surfactant proteins in bronchial cells and in alveolar cells.

PATIENTS AND METHODS

Immunohistochemistry for SPA and SPB was performed in autoptic lung specimens from 38 fetuses and newborns, ranging from 14 up to 41 weeks of gestation. In all subjects, SPA and SPB immunostaining was evaluated in brochial, bronchiolar and alveolar epithelial cells.

RESULTS

The first expression of surfactant proteins was detected in a 14 week-old fetus in the developing bronchial structures (**Fig. 1**). Immunostaining for SPA and SPB in the alvelolar epithelium was first detected in a 21 week-old fetus (**Fig. 2**). A marked inrindividual variability in surfactant protein expression was also observed: in the five fetuses of 21 weeks analyzed, alveolar reactivity was observed in 3 out of 5 lung samples (**Fig. 3**),
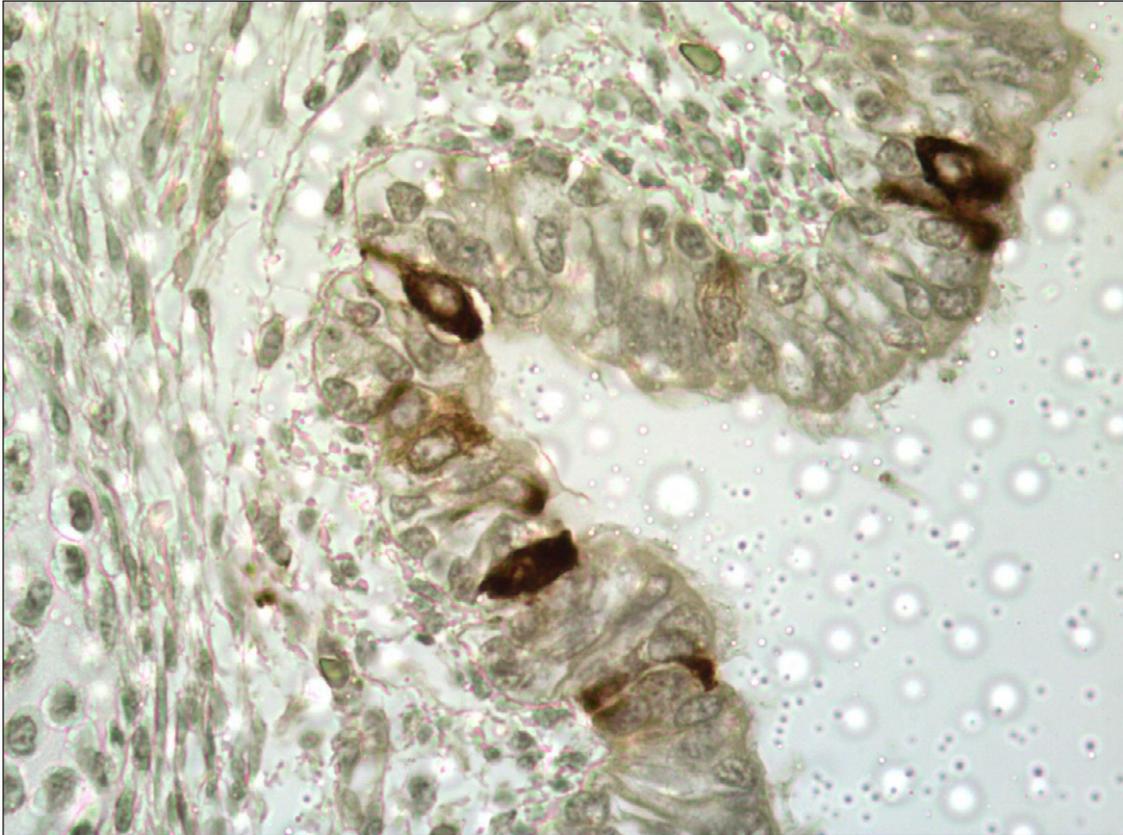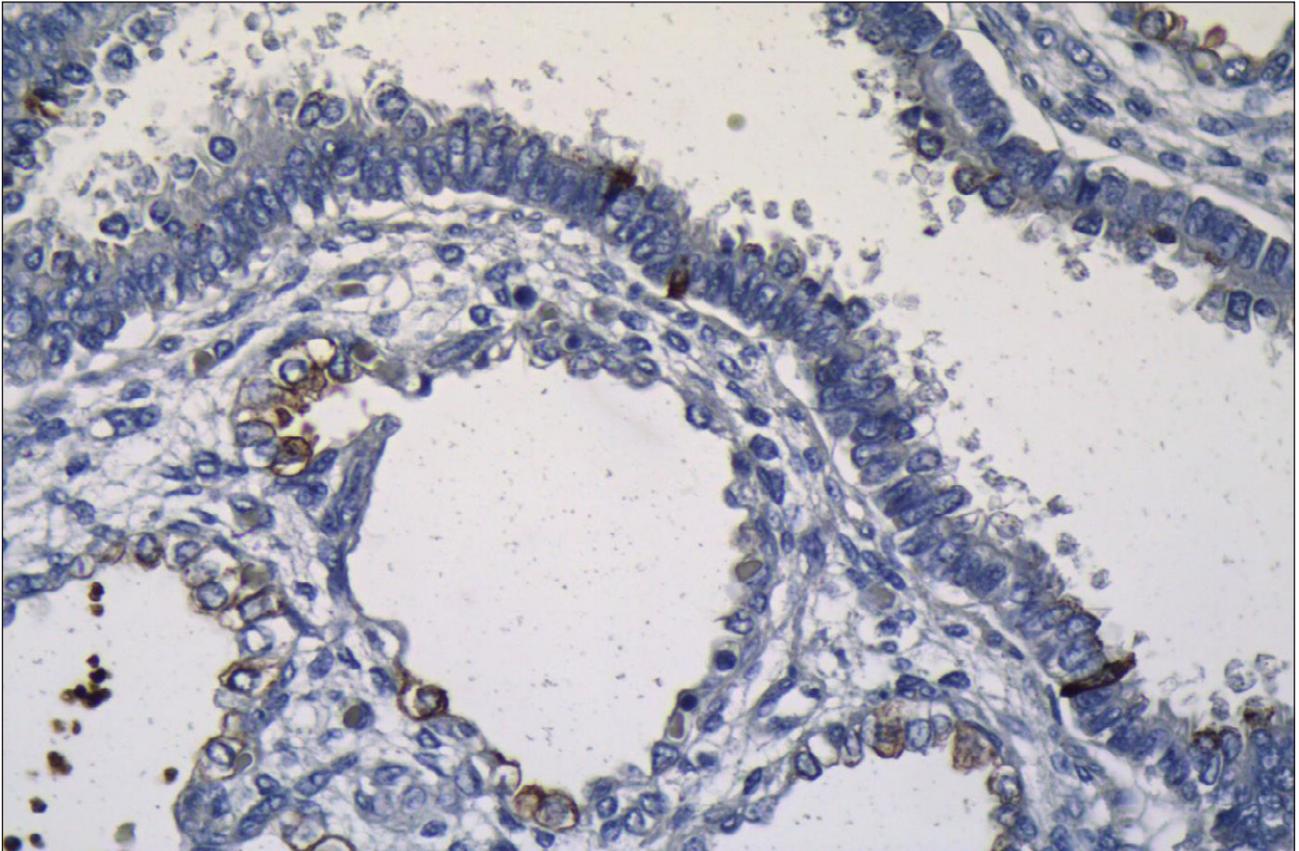
Figure 1. Developing bronchial structures, 14 week-old fetus.
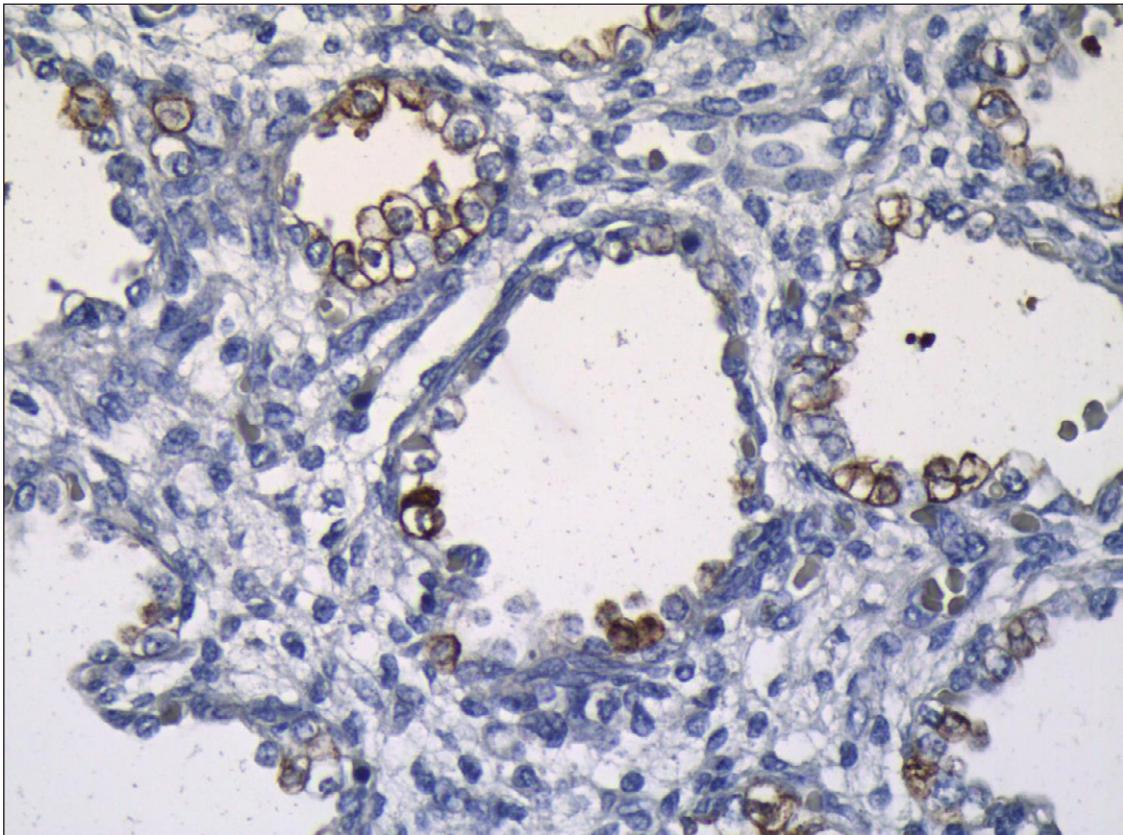
Figure 2. Alvelolar epithelium, 21 week-old fetus.
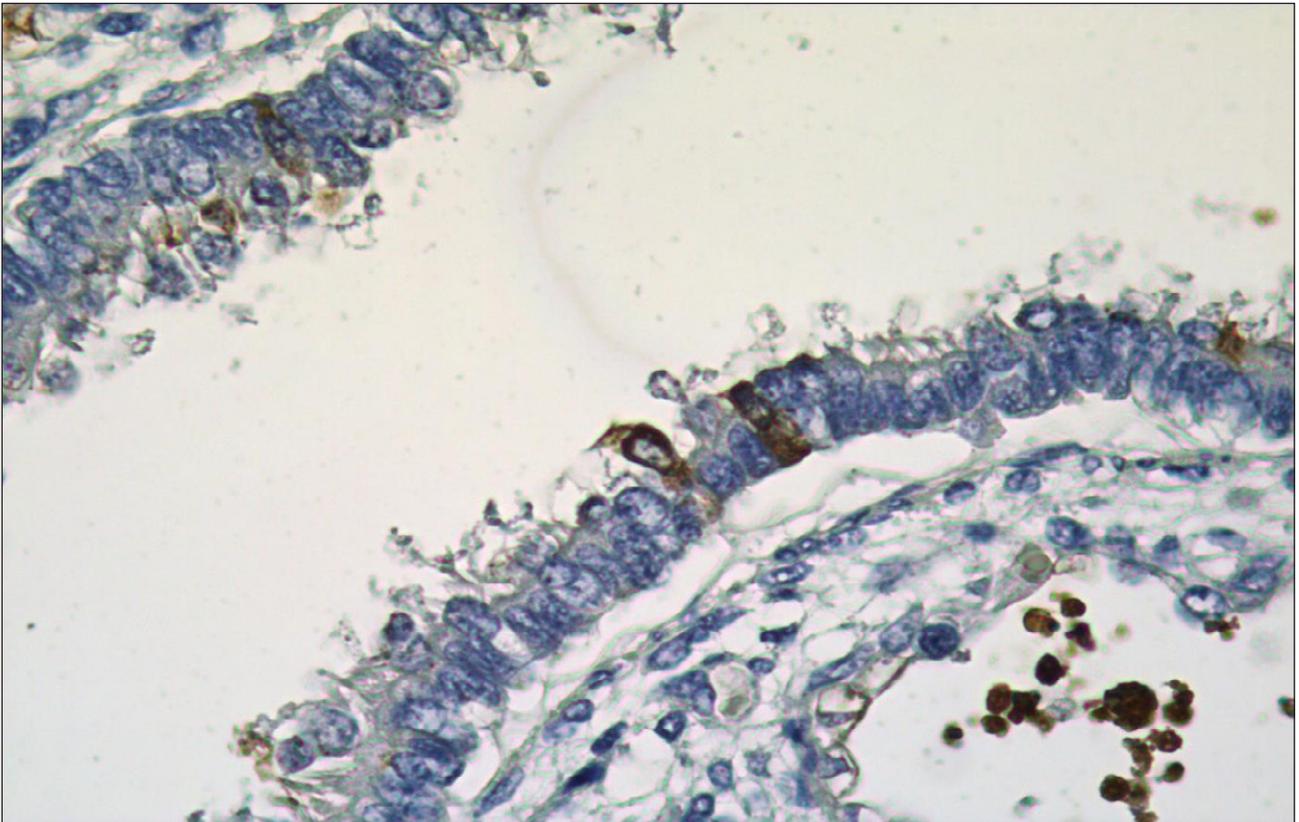
A.

B.

Figure 3. A, B. Alveolar epithelium of bronchial cells, 21 week-old fetus.

whereas bronchial cell reactivity was restricted to 2 out of 5 cases. Significant differences in surfactant expression were also found in newborns: the highest expression of alveolar surfactant proteins (grade 3) in a 33 week-old newborn, contrasted with the absence of any reactivity in a 34 week-old neonate.

CONCLUSIONS

Our data clearly show a previously unreported variability in surfactant protein expression in bronchial and alveolar pulmonary epithelium in the early phases of the human lung development during the intrauterine life. In this study, the degree of surfactant expression did not correlate with gestational age nor with the gender of the neonates. Further studies are needed in order to shed light on the epigenetic factors that may influence surfactant expression during human lung development, determining the ability of the neonate to adapt to the extrauterine life.

ABS 37

TRANSFUSION RELATED ACUTE LUNG INJURY IN NEONATES

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INTRODUCTION

Transfusion-related-acute-lung-injury (TRALI) in newborns is life-threatening and potentially fatal, which largely remains under-diagnosed and under-reported.

CASE REPORTS

The first case considers a newborn male with gestational age (GA) 28 weeks born with caesarean section, due to placental abruption and PROM. It was admitted in our NICU for prematurity and RDS and was transfused with FFP and packed red blood cells (PRBCs). One hour after the transfusion, under administrated O_2, it exhibited rapidly severe deteriorating respiratory failure and as a result it was intubated and ventilated with high frequency oscillated ventilation (HFOV). The chest radiography was typical of pulmonary edema in the absence of evidence of volume overload or cardiac dysfunction. The second case concerns a newborn female with GA 39 weeks. During the investigation of possible inborn errors of metabolism it exhibited disseminated intravascular coagulation (DIC) and it was transfused several times with FFP, PRBCs, PLT and cryoprecipitate. Four hours after the last blood component transfusion, the newborn exhibited abrupt deterioration of lung function and fresh lung infiltrations in the chest radiograph. The neonate admitted in our NICU was initially under conventional mechanical ventilation and, eventually, under HFOV. Despite of supportive therapy, both neonates died in 2 and 4 days, respectively, after the presence of TRALI.

CONCLUSIONS

The cases presented above indicate the importance of TRALI after transfusion in high-risk infants. There is a need of recording these cases, in order to base the transfusion of blood products on strict medical criteria and increase the clinical suspicion in early diagnosis of TRALI complication.

ABS 38

SEIZURES-LIKE WERE THE ALARMING SYMPTOMS IN AN AFRICAN CHILD WITH LIFE-THREATENING CONDITION FROM SEVERE UPPER RESPIRATORY TRACT OBSTRUCTION

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BACKGROUND

Obstructive sleep apneas (OSA) are characterized by episodes of complete or partial upper airway obstruction during sleep. In childhood, it is frequently due to tonsillar and adenoid hypertrophy. Symptoms and signs associated with sleep-related breathing disorders during sleep are odd sleeping position, snoring or snorts, gasping or labored breathing, witnessed apneas, sweating and enuresis; in the day are behavioral problems, poor concentration, excessive tiredness, failure to thrive, morning headaches, mouth breathing adenoidal facies, nasal speech and Harrison sulcus.

Type III sleep testing devices are covered when used to aid the diagnosis of OSA in patients who have clinical signs and symptoms indicative of OSA. Many centres (our own included) undertake cardiorespiratory sleep studies with effort bands (thorax and abdomen), airflow measures, heart rate and SpO$_2$ monitoring.

CLINICAL CASE

We report herein the case of a child hospitalized for suspected seizures, with severe life-threatening upper respiratory obstruction from tonsillar and adenoid hypertrophy who needed persistent intubation before surgical removal.

African boy, 3.5 year-old, with perinatal medical history unremarkable. Due to worsening of the nocturnal respiratory difficulties, snoring, drooling, revulsion of the eyes and tremors in all four limbs, he was conducted to the Hospital. Clinical history initially agreed for seizures. For this reason he performed urgent neurological evaluation. The EEG pattern did not agree for seizures, as initial suspected. Because the worsening of respiratory distress during sleep, with subcostal and jugular retractions, apneas, worsening of desaturations, the patient was transferred to the pediatric intensive care unit. Endoscopy examination of the airways showed marked edema of the nasal mucosa, turbinate and adenoids hypertrophy, tonsils slightly hypertrophic not tight. At the end, he was intubated. The day after intubation, an elective extubation, was followed by the position of a sleep study device (SOMNOscreenTM PSG, SOMNOmedics GmbH, Randersacker, Germany). Measurements were interrupted after 1/2 hour because appearance of prolonged apnea and severe desaturations (**Fig. 1**).

The day after, to rule out masses, a Magnetic Resonance Imaging of the neck showed hypertrophy of adenoids, tonsillars and linphonodes. Brain MRI was negative for recent ischemic lesions. Adenotonsillectomy was performed the subsequent day.

Vitelli et al. [1] reported a case of a 3-year-old child with nocturnal seizures and atypical OSA, paradoxical breathing, desaturations and tonic-dystonic posture. Following a cardiorespiratory polysomnography the patient was initially misdiagnosed as having severe OSA syndrome. On the contrary, the presenting signs during sleep of our patient agreed initially for seizures and finally diagnosed as severe OSA syndrome requiring intubation and followed by urgent surgical removal.
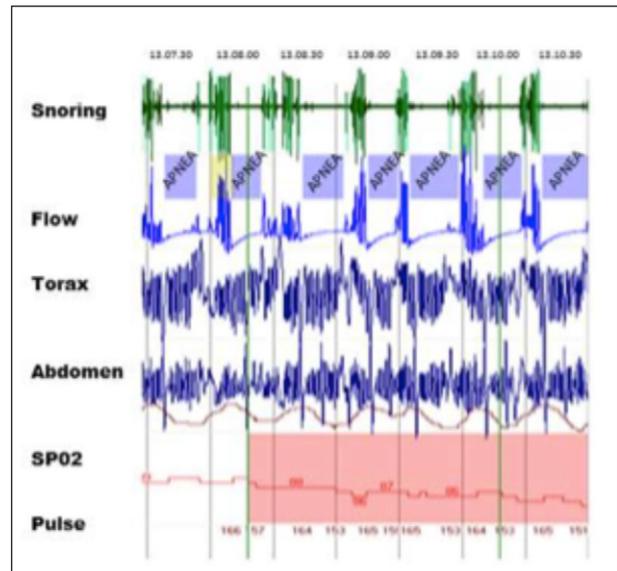
Figure 1. Prolonged apnea and severe desaturation during sleep study.

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ABS 39

DIAGNOSIS OF TUBEROUS SCLEROSIS COMPLEX IN THE NEONATAL PERIOD: A CASE REPORT

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INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant inherited disorder characterized by the formation of widespread hamartromas, especially in Central Nervous System, skin, kidney and eye [1]. It has an estimated incidence of approximately 1:6,000 [2-3]. About 85% of cases show mutations in 2 tumor suppressor genes, such as TSC1 and TSC2, with an high number of *de novo* mutations [4]. The diagnosis is based on the presence of 2 major or 1 major and 2 minor criteria according to the Tuberous Sclerosis Consensus Conference (June, 1998) [5]. It is possible to diagnose TSC

antenatally with fetal ultrasound and and MRI, showing cardiac and brain lesions [5]. The classic triad of symptoms of TSC, present only in 29% of cases, consists of seizures, mental retardation and adenoma sebaceum (angiofibromas) [6]. The diagnosis of TSC is difficult in neonatal life, because many features can be only apparent in this period [7]; most patient are not diagnosed until they reach adult age [8].

CASE REPORT

A female neonate was born at 38 weeks of gestation by spontaneous vaginal delivery. Her birth weight was 2,490 g (7th percentile), with a lenght of 47 cm (18th) and an head circumference of 30.7 cm (1st). Her fetal history was negative, while family history was positive for mental retardation in her older brother. The baby was admitted to our department at the 3rd day of life for systolic murmur (grade 1/6) at centrum cordis. The Doppler echocardiography displayed the presence of two hyperechoic formations, of which the greater protruded into the valvular aortic lumen without obstructing the left outflow tract and the second was located in the wall of the right ventricle. In suspicion of rhabdomyolysis, further investigations were performed. The cerebral ultrasound showed asymmetry of the lateral ventricles with prevalence of the left one and highlighted a nodular mass in the body of the caudate nucleus with an hyperechoic center and an hypoechoic perinodular rib, in which was observed vascular signal. The brain MRI revealed, along the medium cells of the lateral ventricles, multiple subependymal nodules characterized by hypersignal in T1-weighted sequences and hypointensity in T2 sequences. It also detected the presence of multiple areas of modest gyral swelling, more evident in both parietal lobes, in the right frontal lobe and in the left temporal lobe, with associated alteration of the cortical-subcortical signal, compatible with "tubers". Electroencephalographic examination, abdomen ultrasound and eye examination were normal. Moreover, dermatological examination showed a verrucous nevus in the abdominal region. Neurological consultation, based on the results of imaging studies, directed the diagnosis toward tuberous sclerosis. The subsequent genetic analysis demonstrated the absence of mutations in the TSC1 gene, and found an heterozygous mutation in the TSC2 gene (mutation A614D), which involved the replacement of aminoacid alanine with aspartic acid.

The child was discharged in good general conditions and a multicentric follow-up based on periodic neurological, cardiologic, genetic and dermatological checks was planned. Actually, there's not a modification in the echocardiographic examination, while electroencephalography performed 6 months after discharge showed the presence of sharp and slow anomalies in the right regions, not associated with the occurrence of convulsive manifestations. Therefore, at the moment it was not necessary to start any anti-seizure therapy.

CONCLUSIONS

Morbidity and mortality of TSC are related to the development of seizures [1], of subependymal giant cell tumor (SGCT) [9], renal failure and lymphangioleiomyomatosis [8]; these frameworks can be minimized through early diagnosis, long-term follow-up and early treatment [10]. In our case, a common cardiologic problem at birth, a systolic murmur, with no other symptoms, allowed us to make an early diagnosis, which is normally rare in the neonatal period [7].

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ABS 40

SAVE BREAST-MILK FROM POLLUTION

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INTRODUCTION

Breastfeeding has been recognized and promoted by public health officials as the most beneficial source of nourishment, growth, and development during infancy [1] providing a range of benefits for growth, immunity and development, and a significant decreasing risk for several acute and chronic diseases [1, 2].

However, in women both in industrially established and in developing nations, human breast-milk is not pure. In fact, several studies have identified, with advanced analytic methods, pollution residues in human milk [3]. Pollutants have been intentionally or inadvertently produced and introduced into the environment. Thus, chemicals tend to bio accumulate in long-lived species at the top of the food-chain, including in human milk.

CHEMICALS IN BREAST MILK

Levels of chemical residues in breast milk may be influenced by several factors, such as chemical peculiarity, individual (e.g., maternal age, number of pregnancies), demographic (place of residence), and lifestyle features (smoking, diet, occupation, household chemical use), as well as lactation related factors (e.g., previous lactation, duration of breastfeeding, volume and fat content milk) [4, 5].

Thus, through breastfeeding, a mother may transfer potentially toxic chemicals to the suckling infant, determining negative and/or irreversible effects in a developing subject, promoting the onset of several diseases [6].

CONCLUSIONS

Although scientific evidence indicates that the advantages of breast-feeding outweigh any risks from contaminants [1], we wish to underline that chemical contamination of human breast-milk requires higher attention and consideration. In fact, it is important to identify communities of women with major sources of human exposure, to limit the presence of pollutants in the food supply and to modify their critical short-and long-term effects in children. Consequently, safe breastfeeding could be ensured and encouraged [7].

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ABS 41

THE ROLE OF EPIGENETIC FACTORS ON DIABETES MELLITUS DEVELOPMENT

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INTRODUCTION

The pathogenesis of type 1 (T1DM) and type 2 diabetes mellitus (T2DM) has been related to different genetic and environmental factors characterized by the common capability to modify glucose homeostatic system. In T1DM the most relevant etiologic mechanism is autoimmune attack of hyper-reactive T-lymphocytes against islet beta cells causing cell loss, insulin secretion reduction and acute metabolic decompensation [1]. In T2DM insulin resistance is the principal factor involved [2]. In recent years, "the accelerator hypothesis" is emerging as a new unifying hypothesis, attributing to insulin resistance the basis for both T1DM and T2DM [3].

In this new perspective a precise distinction between the two types of diabetes could not exist and the pathogenesis of both diabetes might substantially overlap [4].

THE ROLE OF EPIGENETIC FACTORS ON PANCREAS DEVELOPMENT

There are several factors "accelerating" beta cell destruction, leading to beta cell failure. In

particular our study analyzes epigenetic factors that can act on pancreatic ontogenesis during gestation and, above all, on beta cell burden at birth, influencing childhood and adult life. Many maternal pathologies can predispose to fetal beta cell injury. Elevated blood glucose concentrations in women during gestation (related both to maternal diabetes and gestational diabetes) may be toxic for fetal beta cells, compromising cellular survival and function. Fetal hyperinsulinism in a mother affected by gestational diabetes mellitus is a risk factor for development of obesity and abnormal glucose metabolism in childhood and adulthood [5]. Even maternal obesity is associated with alterations in blood glucose homeostasis with consequences on pancreas functioning. Synthetic glucocorticoids exposure of pregnant mothers reduces birth weight of the offspring and determine hyperglycemia and hyperinsulinemia [6].

Maternal diet plays a key role. Low-protein intake can alter development of offspring endocrine pancreas, resulting in reduction of pancreatic islet cell size and proliferation, decreased pancreatic beta cell mass, and increase in islet cell apoptosis in fetal life. Maternal hyperlipidemia can amplify insulin resistance and modifying glucose blood level. Ethanol exposure during pregnancy produces oxidative stress in multiple tissues, including endocrine compartment. Viral infections (Echovirus 6, Rubella) may induce beta cell autoimmunity *in utero* increasing T1DM risk development [7].

Intrauterine growth restriction or fetal malnutrition (both IUGR and LGA), predispose individuals to an amplified risk of pancreatic damage in adult life, but it also increases nutrient availability in utero through gestational diabetes or maternal obesity, increasing the risk multiple of diseases such as obesity, impaired glucose tolerance, and metabolic syndrome.

Intrauterine hypoxia can alter DNA methylation of select genes in several tissues including the pancreas, that is susceptible to oxidative stress caused by its reduced antioxidant ability [8] (**Fig. 1**).

CONCLUSIONS

Further studies are needed to identify environmental factors predisposing to develop
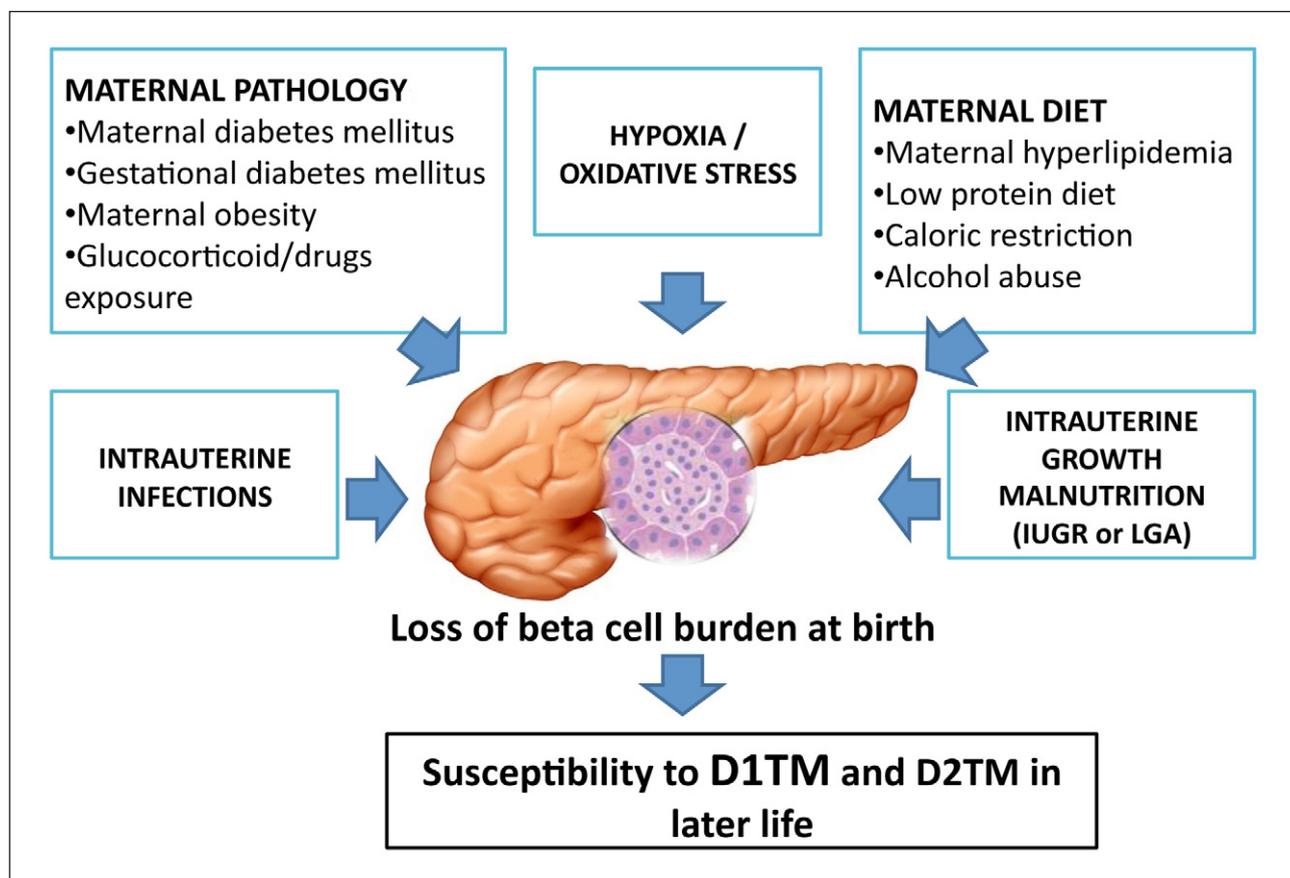
Figure 1. The role of epigenetic factors on pancreas ontogenesis and development.

mellitus diabetes. Studying the relevance of beta cell burden damage at birth can be fundamental to better understand mellitus diabetes pathogenetic mechanisms and to identify new preventing and therapeutic strategies.

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ABS 42

WT1 EXPRESSION IN THE HUMAN MESO-NEPHROS

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INTRODUCTION

The Wilms' Tumor 1 (WT1) protein is a zinc finger transcription factor (TF) encoded by the human gene WT1 and it is involved in the onset of Wilms' tumor, the most common primary renal tumor in childhood [1] and in development of several tissues, including early developing kidney [2].

AIM

On this basis, the purpose of this study was to analyze the immunoreactivity of the WT1 protein in the mesonephros of a human embryo in the 7th week of gestation and to assess its possible role in human development.

MATERIALS AND METHODS

A human embryo of 7 weeks of gestation has 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 sections were obtained from each paraffin block; after dewaxing and rehydrating, one of these was stained with hematossilin-eosin, the other pre-treated for immunohistochemical analysis, then incubated for 20 minutes with anti-Wilms' Tumor (WT1) mouse monoclonal antibody.

RESULTS

Immunostaining for WT1 was mainly located in the developing glomeruli, associated with a weaker immunostaining in the nephrogenic zone located under the renal capsule (**Fig. 1**). Tubular epithelial cells were completely negative. WT1 was strongly expressed in the nuclei of both podocyte precursors and endothelial cells, and along the developing basal membranes (**Fig. 2**). Staining for WT1 was also detected in the mesenchimal cells surrounding the developing glomeruli and the tubular structures. No immunostaining has been detected in the cytoplasm of podocyte precursors.

DISCUSSION

Our data suggest that WT1 is a very early marker of nephrogenesis and confirm previous reports from our group on a preferential WT1 expression in stem/progenitors cells of the developing human metanephros and that the protein plays a major role during kidney development [3]. Further studies are mandatory in order to better analyze the role of WT1 in podocyte development in the different phases of kidney development, as well as during the insurgence of podocytopathies in childhood and in adult subjects.

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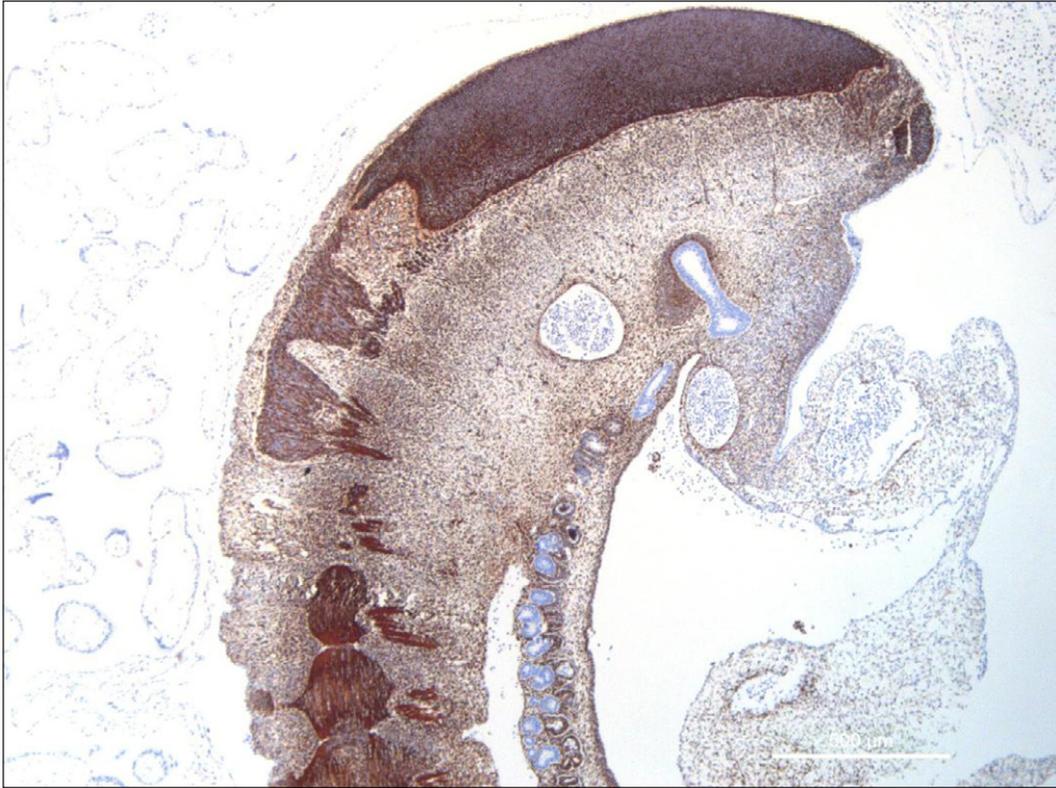
Figure 1. WT1 immunostaining in mesonephros. WT1 immunoreactivity is detected in the developing glomeruli, associated with a weaker immunostaining in the nephrogenic zone located under the renal capsule and in mesenchimal cells surrounding the developing glomeruli and tubular structures.
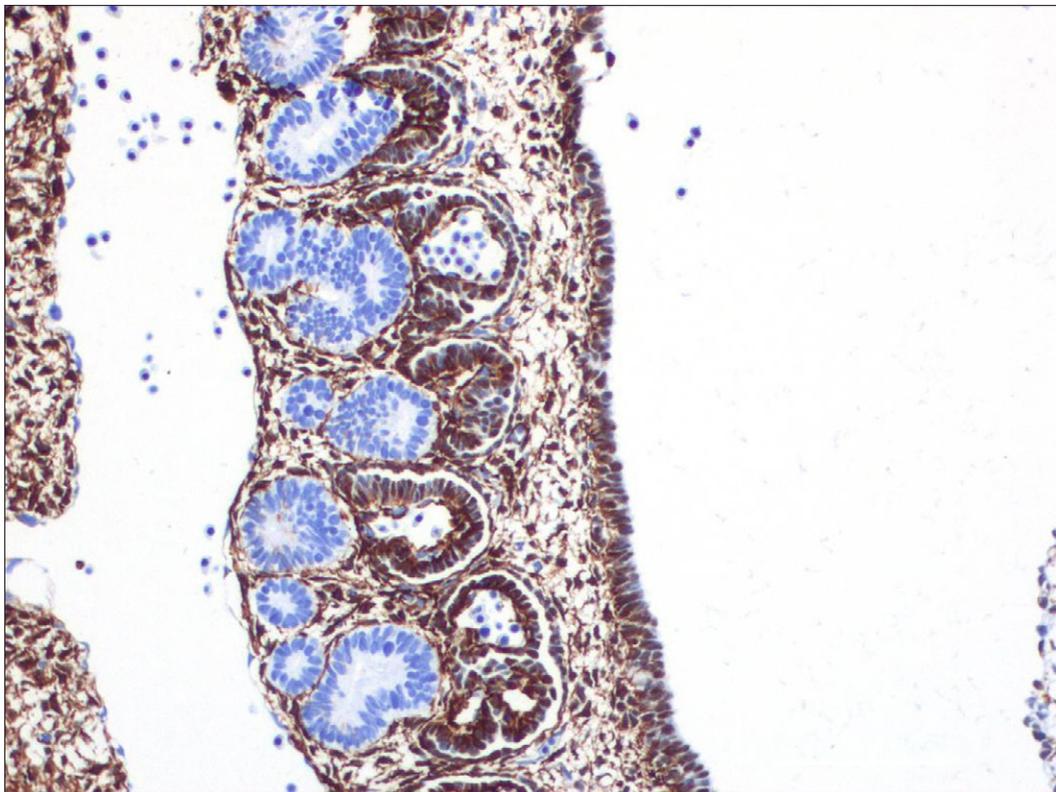
Figure 2. WT1 immunostaining in mesonephros. WT1 is strongly expressed in the nuclei of both podocyte precursors and endothelial cells, and along the developing basal membranes. Tubular epithelial cells are completely negative.

ABS 43

SINUSOIDAL CELL APOPTOSIS: A NEW MARKER OF PERINATAL SEPSIS?

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INTRODUCTION

Sepsis can be defined as a generalized inflammatory response of the organism and often manifests itself as a systemic inflammatory response syndrome (SIRS) [1]. The progression of SIRS usually leads to multiple organ dysfunction culminating in multiple organ failure (MOF) [2]. The endothelium is a major target of sepsis-induced events and endothelial cell damage accounts for much of the pathology of septic-shock [1]. Endothelial cells have mechanisms that recognize structural patterns of bacterial pathogens and subsequently initiate the expression of inflammation mediators [3].

In sepsis and septic shock a series of immunological events alters endothelial function in the macrocirculation and microcirculation. Endothelial cytoplasmic swelling, deformation and apoptosis with detachment from the vasculature occur and endothelial cells (EC) appear in the circulation [4]. The disorders of the normal function of the endothelium include derangement of the vascular tone, increase of endothelium permeability, activation of the endothelial cells, production of various regulators and disorders of coagulation [5]. The infant mortality rate of sepsis is related to the number of organs simultaneously inefficient.

CASE PRESENTATION

The aim of this study would show liver cell histological changes in a case of a fatal neonatal sepsis in a 2 day-old newborn.

At macroscopic examination, no congenital malformations were observed. Multiple samples from the liver were obtained. All the tissue samples 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.

RESULTS

At the authopsy, the most interesting feature was the presence of pathological changes in sinusoidal cells, characterized by endothelial detachment and exfoliation and apoptosis of Kupffer cells [6]. We can see these cells into the vessel lumen showing an elongated form with eosinophilic cytoplasm, that have lost their nucleus (**Fig. 1** and **Fig. 2**). Near the Kupffer cells, into the vessel lumen, we
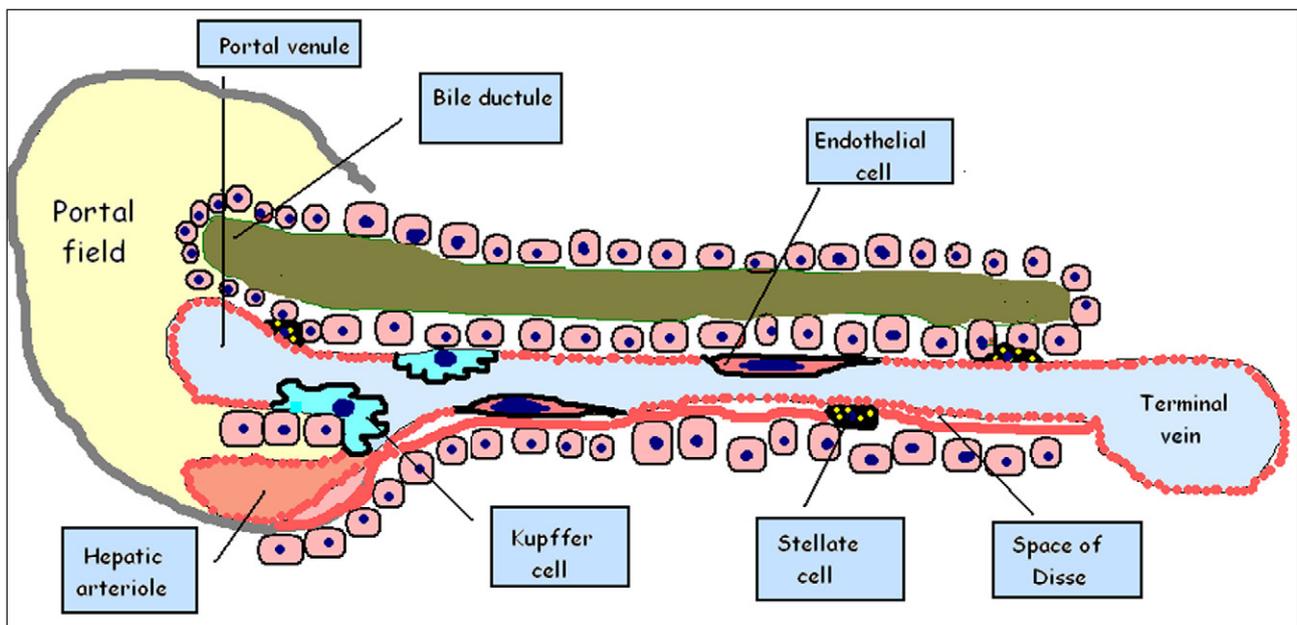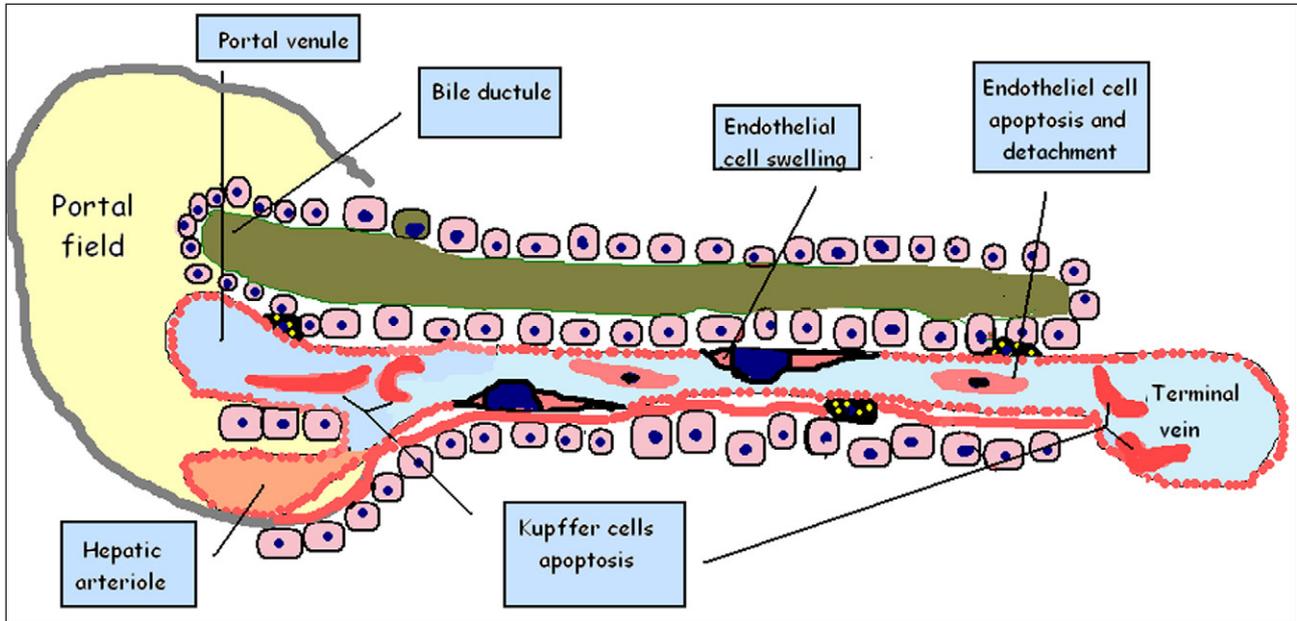

Figure 1. Normal sinusoidal cells: endothelial cells and Kupffer cells are supported on the basal membrane of sinusoidal capillary.

can observe also endothelial cells detached from the basement membrane. Endothelial changes are mainly represented in sinusoidal capillary and in lobular vein and are characterized by swelling, detachment and apoptosis (**Fig. 3** and **Fig. 4**).

CONCLUSIONS

Our data are the first report of sinusoidal severe changes in neonatal sepsis, represented by Kupffer and endothelial cells apoptosis. Endothelial changes are present during MOF that

Figure 2. Sinusoidal cells during sepsis: endothelial changes are characterized by swelling, detachment and apoptosis. Kupffer cells begin elongated with eosinophil cytoplasm.
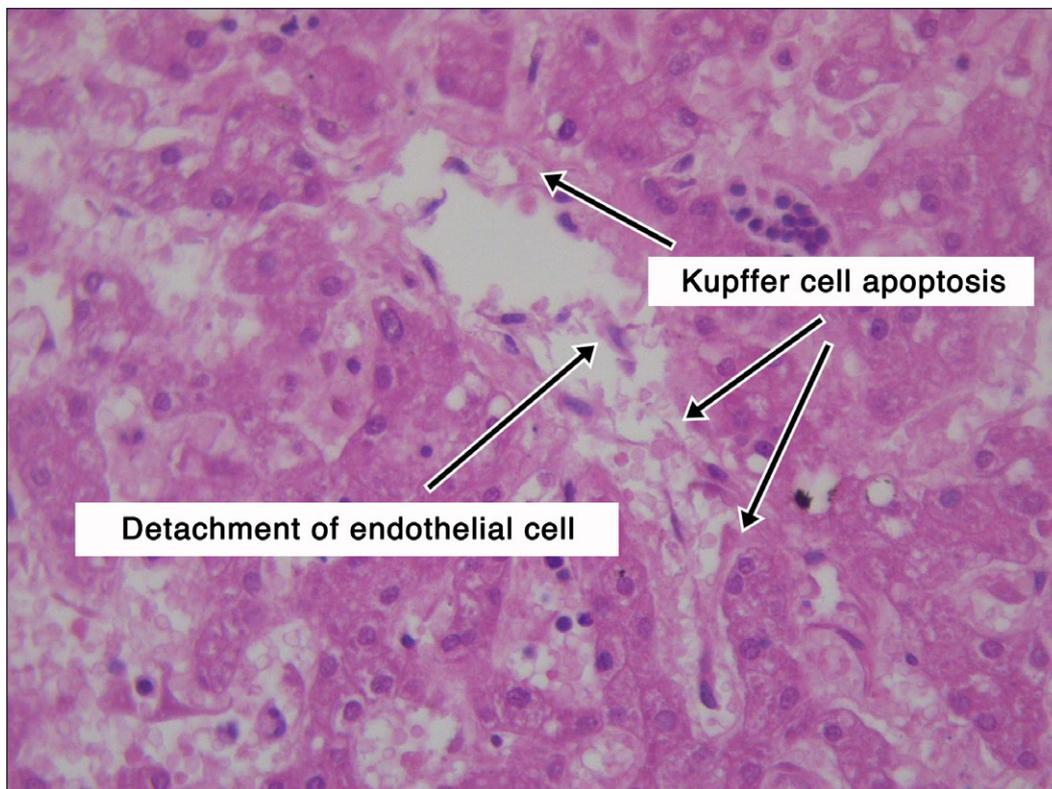
Figure 3. Sinusoidal cell apoptosis during sepsis.
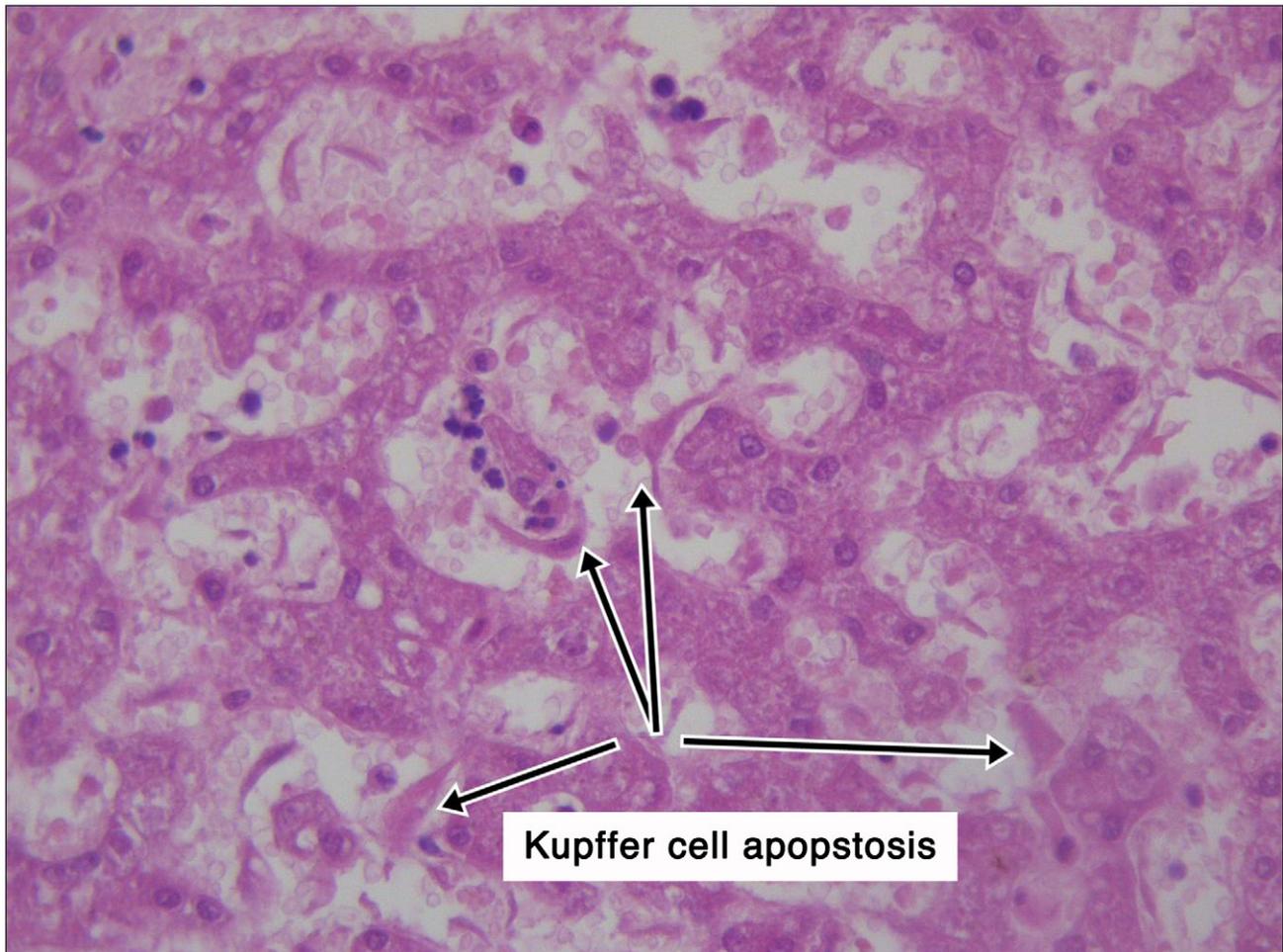
Figure 4. Kupffer cells apoptosis in sinusoidal capillary during sepsis.

represents a common but complex problem in critically ill patients in NICUs, a major cause of morbidity and mortality in newborns and a severe complicance of neonatal sepsis.

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ABS 44

WNT1 EXPRESSION IN THE HUMAN EMBRYO LIVER AT 7 WEEK OF GESTATION: SIMILARITIES WITH HEPATOCELLULAR CARCINOMA

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INTRODUCTION

The WNT1 protein is a member of WNT signaling proteins family, via the transcription co-activator β-catenin, that controls key developmental gene expression programs. WNT1 signaling participates in several events during embryogenesis and is

also involved in the homeostasis of adult tissues [1]. Mutations in the WNT pathway are often linked to human birth defects cancer and other deseases [2]. Some of the target genes such as c-Myc, cyclin D1, c-Jun, Fra-1 and u-PAR influence the promotion, progression, invasion and metastasis of tumor. Aberrant activation of the Wnt/β-catenin signaling is reported in a significant subset of hepatocellular cancers (HCC) [3]. Studies conducted on animals have highlighted the role of WNT signaling in liver cell differentiation [4]. Reserches showed that WNT1 plays an important role as a signaling molecule in central nervous system development [5] but there are not evidences about the role of WNT1 during early phases of liver development.

AIM

The purpose of this study was to analyze the WNT1 immunoreactivity in the liver of a 7 weeks human embryo, and to assess its possible role in human ontogeny and the relationship with HCC in adults.

MATERIALS AND METHODS

A human embryo of 7 weeks of gestation has been completely sampled, histologically and immunohistochemically studied and subsequently compared with the HCC of adult liver. 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 hematossilin-eosin, the other pre-treated for immunohistochemical analysis, then incubated for 20 minutes with anti-WNT1 rabbit polyclonal antibody.

RESULTS

Liver cells during embryogenesis diffusely express a granular cytoplasmic positivity for WNT1 (**Fig. 1**). Proliferating cells present a weaker expression of the same marker. Also HCC cells express a granular and diffuse cytoplasmic positivity for WNT1; some of these cells show marked cytoplasmic positivity for this marker (**Fig. 2**).
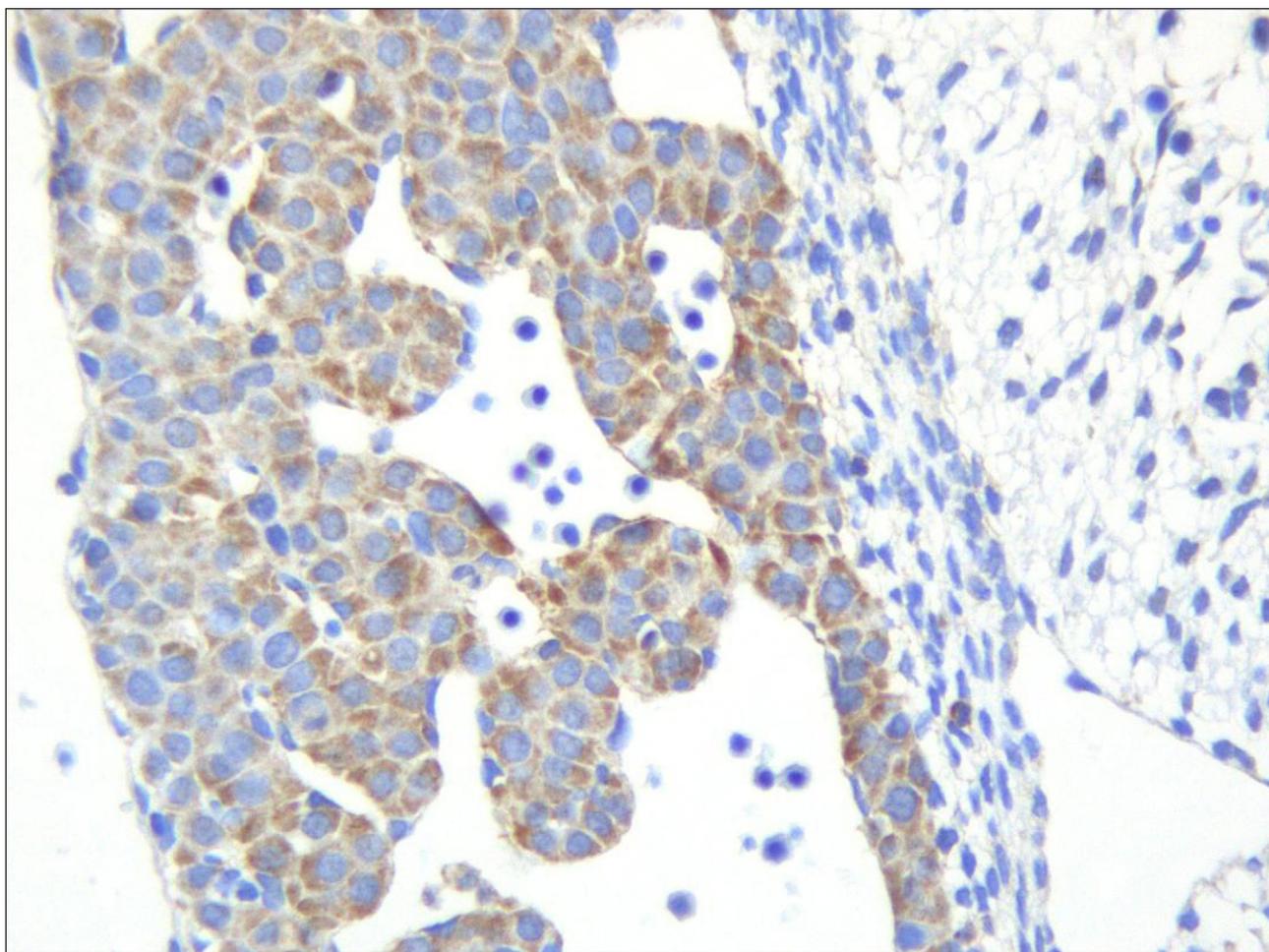
Figure 1. Human embryo liver cells diffusely express a cytoplasmic granular positivity for WNT1.
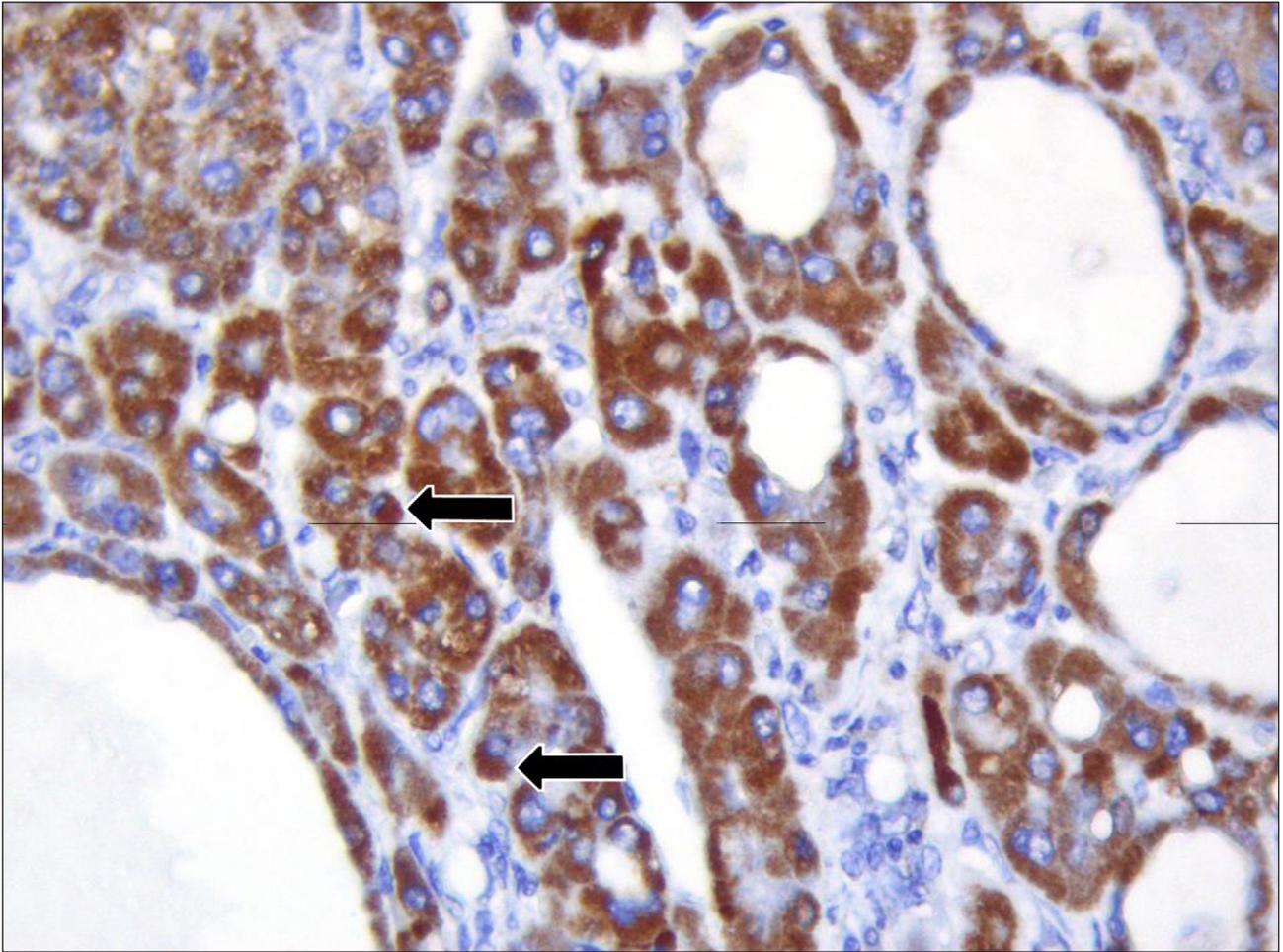
Figure 2. HCC express a granular and diffuse cytoplasmic positivity for WNT1. Some of these cells show marked positivity (arrows).

DISCUSSION

Our data show, for the first time, the expression of WNT1 in the early phases of liver development, suggesting a major role for this signaling protein in liver development around seventh week of gestation. Further studies will clarify the role of WNT1 during all the stages of gestation till birth and in postnatal period. The finding of a similar expression pattern for WNT1 in the embryonic liver cells and in HCC reinforces the hypothesis of a reactivation of fetal programs in cancer stem cells.

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ABS 45

EPIDERMOLYSIS BULLOSA

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DEFINITION

Hereditary epidermolysis bullosa (EB) is a group of genetically determined bullous diseases of the skin that share skin fragility as a common feature. This

fragility is caused by mutations in various structural proteins of the epidermis and the dermoepidermal junction. The clinical symptoms and prognosis depend on which adhesive structure is missing, and on the location of the skin disruption. We describe four major types of EB, transmitted in AD or AR:

1. simple EB or epidermolytic;
2. junctional bullous epidermolysis;
3. dystrophic Bullous epidermolysis or dermo-lytic EB (DEB);
4. Kindler Syndrome.

All the illness are characterized by the onset of blisters, following minimal trauma than in normal subjects do not cause any injury.

EPIDEMIOLOGY

The overall prevalence of simple EB, junctional EB, dystrophic EB in the population is estimated at 1/130,000 in the United States, 1/100,000 in Italy, 1/20,000 in Scotland. Kindler Syndrome is very rare, it is probably underdiagnosed, and currently about 200 cases have been described.

CLASSIFICATION

Simple Epidermolysis bullosa or epidermolytic

Intraepidermal bullous are the most common lesions, representing the 50% of cases. The EBs are divided into two subtypes: basal E, due to cytolysis of basal keratinocytes, with the presence of predominantly cutaneous bullous lesions that resolve without scarring more; suprabasal E, where the lesions are formed in the suprabasal layers of the epidermis and include three different types extremely rare. They are transmitted in AD and AR form and are due to mutations in the genes KRT5, KRT14 (keratin 5 and 14), PLEC1 (plectin), PKP1 (Placofilin1), DSP (Desmoplachin).

Junctional bullous epidermolysis

It is characterized by blisters between the epidermis and dermis at the level of the lamina lucida of the basement membrane. The lesions heal with the formation of hypertrophic tissue and/or atrophy. They originate from mutations transmitted in AR or AD. There are several variants due to mutation of genes LAMA3, LAMB3, LAMBC2 (laminin 3-3-2), COL17A1 (collagen type XVII), ITGB4 (integrine 4).

Dystrophic Bullous epidermolysis or Dermolytic BE

Bullous lesions are localized under the dense lamina of the skin basement membrane in the papillary dermis. They are characterized by slow healing with scarring nend formation of milia. This form is about 25-35% of cases of EB. There are two major subtypes based on the mode of transmission AD or AR, with different clinical variants. All variants of Dystrophic EB are due to mutations in the COL7A1 gene coding for type VII collagen, the main component of the anchoring fibrils that ensure the adhesion of the basement membrane of stratified epithelia to the underlying mesenchyme.

Kindler Syndrome

It is characterized by fragility of skin and mucosa, photosensitivity, progressive poikiloderma with extensive atrophy.

DIAGNOSIS

Clinical features combined with immunofluorescence antigen mapping and/or electron microscopy examination of a skin biopsy allow to define the EB type and subtype. Molecular diagnosis is nowadays feasible in all EB subtypes and is required for prenatal diagnosis. The extent of skin and mucosal lesions varies greatly depending on EB subtype and patient age. In the more severe EB subtypes lifelong generalized blistering, chronic ulcerations and scarring sequelae lead to multiorgan involvement, major morbidity and life-threatening complications. In the absence of a cure, patient management remains based on preventive measures, together with symptomatic treatment of cutaneous and extracutaneous manifestations and complications. Owing to its nature and severity, DEB presents unique challenges for developing successful therapies that simultaneously alleviate the plethora of complications while having a significant impact on survival and quality of life. Recent approaches such as allogenic cellular therapy, gene therapy, and protein therapy seem promising.

DIFFERENTIAL DIAGNOSIS

Congenital ichthyosis bullosa, incontinentia pigmenti, Staphylococcal pyoderma Bullous, Pemphigus luetic, autoimmune bullous diseases (pemphigus, herpes gestationis), Bullous mastocytosis, transient dermatolisis of newborn.

ABS 46

PLATELETS ALLOIMMUNE ANTIBODIES: DIFFERENT OUTCOME IN TWIN PREMATURE INFANTS

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INTRODUCTION

Neonatal alloimmune thrombocytopenia (NAIT) is a critical condition which may severely affect both the foetus and the newborn, potentially leading to

intracranial haemorrhage during the intrauterine life or soon after birth. Some of these patients may suffer severe neurological sequelae or even die.

CASE REPORT

We report the case of a twin infant (monochorionic biamniotic), born at 33 gestational weeks by caesarean section for suspected anaemia (twin to twin transfusion, donor twin). Apgar Index: 9/10, weight 1,542 g. Haemoglobin at birth was 15 g/dl and stable afterwards. Perinatal course was uneventful. At 6 days she was intubated during severe sepsis by *Kl. pneumoniae*. Platelets count was normal at birth and decreased during sepsis (3,000/mm^3), requiring multiple transfusions. PT and PTT were normal and no frozen plasma was administered. Antibiotics therapy was started and after three days clinical condition improved so the newborn was extubated. Unfortunately cerebral tetraventricular haemorrhage (IVH) was detected. After few days, as multiple platelets transfusion produced unsatisfactory results (< 30,000/mm^3) we assumed an immunological cause and decided to administer i.v. immunoglobulins and cortisone, avoiding other transfusions. Platelets count slowly improved with no rebound. IVH remained stable. Interestingly, alloantibodies against paternal platelets antigens HPA5b were detected in maternal and newborn's blood samples. The other twin developed mild sepsis with no specific pathogen detection. Of note, she had constantly normal platelets count and no IVH.

DISCUSSION

In the literature, reviews on neonatal alloimmune thrombocytopenia usually include only low risk term neonates and report IVH as possible consequence. In preterm babies, main causes of thrombocytopenia are associated with sepsis and intrauterine growth retardation, both predisposing to IVH. In our cases, we speculate that for unknown reasons maternal antibodies had quite a different effect on twins, as only one of them did present severe sepsis and IVH. Further research is needed to better clarify this issue. Meanwhile, in case of preterm infants with severe sepsis and persistent very low platelets count, we suggest to consider NAIT in the differential diagnosis.

ABS 47

AN UNEXPECTED ENEMY

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INTRODUCTION

Neuroblastoma (NBL), originally described by Virchow in 1863, is an embryonal tumor of the autonomic nervous system, arising from sympathetic cells derived from the neural crest. It is the most common solid extracranial malignancy of childhood and the most common malignant tumor in infants [1, 2]. The overall incidence of NBL is about 130 newly diagnosed patients per year in Italy [3]. NBL has been described as an enigmatic tumor because of its highly variable biologic behavior. Tumors may spontaneously regress, differentiate into benign ganglioneuromas or follow an unrelenting progressive course with ultimate fatal outcome [4-8]. Early diagnosis is essential because this tumor can exhibit a very aggressive malignant phenotype. High level of catecholamine metabolites such as vanillylmandelic acid (VMA) and homovanillic acid (HVA) can be found in the urine. Abdominal ultrasound may be used initially to characterize the mass. MRI, TC, MIBG scan can be utilized for secondary assessment. However, definitive diagnosis can only be confirmed pathologically with tissue obtained from tumor or bone marrow.

Risk stratification is based on the age, stage at diagnosis, histopathology and molecular abnormalities [2, 9-12]. Nearly half of patients present with metastatic disease, and have 5-year event-free survival of less than 50% [13].

Surgery alone offers definitive therapy with excellent outcome for patients with low-risk disease, while patients at high-risk for disease relapse are treated with intensive multimodality therapy [2, 12-14].

CASE DESCRIPTION

We report the case of a male infant born at 38 weeks of gestation by caesarean section due to sudden rupture of the uterus. His weight at birth was 3,750 g with Apgar scores of 0 and 5, respectively. He was hospitalized in NICU for a neurological impairment caused by respiratory distress and pulmonary hypertension.

He came at our Emergency Unit at three months of age because of refusal to feed and abdominal pain which lasted from 24 hours.

The physical examination revealed good general condition, normal vital signs, apyrexia, nontender abdomen instead the neurological examination demonstrated axial hypertonia without meningeal signs. Complete blood count (CBC), emogasanalysis, glycemia, ALP, CPK, liver, pancreatic and renal function, C-reactive protein, were normal instead LDH was increased (1,288 UI/l). Stool culture and fecal occult blood were negative, fecal calprotectin was positive and urine culture was positive for *E. coli*.

Ammonium level in blood was slightly increased. Abdominal ultrasound detected an inhomogeneous mass behind the right kidney. Suddenly body temperature increased (peak: 39.7) and appeared restlessness, inconsolable crying with suffering aspect, hypohydratation for incipient diarrheal stools and profuse sweating. The MRI subsequently performed confirmed a right retroperitoneal lumbar mass compressing left ureter (resulting in early hydronephrosis), and extended to spinal canal with contralateral dislocation of the spinal cord and the *cauda equina*. Neurogenic tumor was suspected and the patient was immediately sent to the Department of Pediatric Hemato-Oncology for further in-depth analysis and adequate treatment.

CONCLUSIONS

It is very important never to underestimate an infant with feeding refusal associated to neurological signs. Altough mean age of diagnosis of NBL is 2 years, it must be suspected also in a child of few months. Early diagnosis is necessary because NBL can be very aggressive.

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ABS 48

MAY THE HIF-1α POLYMORPHISMS BE HELPFUL IN UNDERSTANDING THE NEONATAL HYPOXIC-ISCHEMIC EVENTS?

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Hypoxic and ischemic complications during the pre- and peri-natal period are common causes of acquired neonatal brain damage [1] associated with severe long-term neurodevelopmental disabilities. Several patterns of HI brain injury are known, mainly due to the brain selective vulnerability to hypoxia-ischemia related to the stage of brain maturation and to the severity of hypoxia [2]. Inflammatory (e.g. IL-6, IL-8, TNF-α), excitotoxic (e.g. glutamate) and apoptotic pathways are involved in the complex neurotoxic cascade following HI brain injury and activate a process of self-sustaining secondary neurodegeneration in vulnerable CNS region [3]. In addition, the availability of endogenous adaptive mechanisms modifying early and delayed stages of hypoxia-induced molecular cascade has been proposed as a crucial factor in the pathophysiology of HI damage of the developing brain.

Among these adaptive systems, Hypoxia-Inducible transcription Factors (HIFs) seem to be of particular interest because of a) their crucial adaptive role during immediate cerebral response to HI, and b) their ability to induce vasoactive and metabolic cytoprotective mechanism during late stages of HI brain damage.

The hypoxia-inducible factor-1 (HIF-1) is the master regulator of the cellular response to hypoxia and its expression levels are tightly controlled through synthesis and degradation. Its role is related to the ability to activate the transcription of more than 100 genes crucial for adaptation to hypoxia [4].

The HIF-1 transcription factor is a heterodimer of the regulated HIF-1α subunit and the constitutively expressed HIF-1β subunit [5]. HIF-1α is continuously transcribed and translated in hypoxia statuses despite an overall decrease in global protein translation.

To date, three HIF-a isoforms (HIF-1α, HIF-2α and HIF-3α) have been described, and HIF-1α and HIF-2α are the best characterized. HIF-1α is expressed ubiquitously, whereas HIF-2α displays tissue-specific expression. HIF-3α can also dimerize with HIF-1β to activate transcription.

The HIF-1 heterodimer binds to a conserved HIF-binding sequence within the Hypoxia-Responsive Element (HRE) in the promoter or enhancer regions of target genes, thereby eliciting their trans-activation and an adaptive hypoxic response [6].

The induction of HIF-1 activity during hypoxia can be attributed to a variety of factors.

PHYSIOLOGICAL ROLE OF HIF-1

During normoxia, HIF-1α is bound to the chaperone molecule Hsp90. In the presence of oxygen, 2-oxoglutarate and ascorbate, HIF-1α is hydroxylated by prolyl hydroxylases (PHD1, 2 and 3), at two conserved proline residues (Pro402 and Pro564) situated within its oxygen-dependent degradation (ODD) domain.

In addition, HIF-1α is acetylated by an acetyltransferase named arrest-defective-1 (ARD1). The hydroxylated, acetylated HIF-1α protein is easily recognized and bound by the von Hippel-Lindau protein (pVHL). The binding of pVHL leads to ubiquitination of HIF-1α. The ubiquitinated pVHL/HIF-1α complex targets the HIF-1α protein to the proteasome where it is degraded [7]. Although the transcription and synthesis of HIF-1α are constitutive and seem not to be affected by oxygen, HIF-1α has a short half-life (several minutes), and it is rapidly degraded in normoxic condition, resulting in essentially no detectable HIF-1α protein under these circumstances [5].

Furthermore, during normoxia, the transcriptional activities of HIF-1α target genes are also inhibited. This inhibition effect is completed by hydroxylation of HIF-1α. Oxygen together with 2-oxoglutarate activate asparaginyl hydroxylase (also known as factor-inhibiting HIF), which hydroxylates an asparagine on the HIF-1α protein. The hydroxylation prevents HIF-1α binding to hypoxia response elements in the promoters of HIF target genes. As a result, the transcription of target genes is prevented [7].

Hypoxia leads to an almost immediate shut down of general protein translation to decrease energy consumption during hypoxic energy starvation [8]. In addition to enzymatic inhibition of the PHD, hypoxia causes perturbations in the mitochondrial electron-transport chain, increasing the levels of cytoplasmic reactive-oxygen species (ROS). ROS can alter the oxidation state of Fe^{2+} (a cofactor for PHD activity) to Fe^{3+}, thus inhibiting PHD activity. However, the role of ROS in hypoxia and HIF-1 regulation remains controversial owing to discrepancies in different model systems, a lack of tools for accurate detection of ROS and variability in the severity and length of hypoxia applied.

Moreover, hypoxia appears to activate mitogen-activated protein kinase (MAPK) that phosphorylates HIF-1α and thus stabilizes the molecule. Another aryl hydrocarbon nuclear translocator (ARNT) called HIF-1β is also phosphorylated at the same time. These two proteins are dimerized to form HIF-1. HIF-1 acts on hypoxia response elements (HRE) in the promoter of a variety of hypoxia responsive genes [5]. Studies have shown that HIF-1α protein levels increased immediately after the hypoxic exposure, peaked at 3-4 h after hypoxic-ischemic injury, and the elevated level of HIF-1α persisted up to 24 h after the insult [9].

Once stabilized, HIF-1 is responsible of a complex cellular response to the hypoxia, which is summarized below.

ANAEROBIC METABOLISM

HIF-1 promotes both the uptake and metabolism of glucose through anaerobic glycolysis by upregulating the expression of glucose transporters (GLUT1 and GLUT3) and of glycolytic enzymes (6- phosphofructo-2-kinase and fructose-2,6-biphosphatase). To maintain the metabolic flux through glycolysis, HIF-1 activation also leads to the inhibition of the Kreb's cycle by upregulating pyruvate dehydrogenase kinase 1, which decreases the availability of pyruvate and lactate dehydrogenase A, thus, increasing the conversion of pyruvate into lactate. This activity tips the balance towards lactic acid production and away from the mitochondrial Kreb's cycle and oxidative phosphorylation, both of which require oxygen. This shift from aerobic to anaerobic metabolism is frequently observed in cancer cells, even in normoxia, and is known as the Warburg effect.

PH REGULATION

The metabolic shift towards anaerobic glycolysis results in potentially toxic intracellular acidosis owing to the increased production of lactic acid and CO_2. To counter this, hypoxia-induced HIF-1 also upregulates the expression of monocarboxylate transporter 4, which mediates lactic acid efflux, and of membrane-bound carbonic anhydrase IX, which catalyses the conversion of extracellular CO_2 to carbonic acid (H_2CO_3). The latter contributes to the acidification of the extracellular space and enables an increase in intracellular pH through the subsequent uptake of HCO_3 (a weak base).

ANGIOGENESIS

HIF-1 directly activates the expression of several pro-angiogenic factors, the best characterized of

which is the vascular endothelial growth factor (VEGF). This event promotes the formation of new blood vessels, thus restoring the supply of oxygen and nutrients. Increased angiogenesis is one of the key HIF-1-dependent protumorigenic events that enable continued tumor growth.

OTHER RESPONSES

In addition to the roles mentioned above, HIF-1 increases oxygen transport by promoting erythropoiesis (EPO) and has been linked to changes in cell proliferation and survival through its effects on c-Myc and on components of the cycle cell and cell-death machinery.

SINGLE NUCLEOTIDE POLYMORPHIMS (SNPS) AT THE HIF-1A GENE (*HIF1A*)

The human *HIF1A* gene is located on the chromosome 14q21-q24 and consists of 14 exons and 13 introns. Up to now, several Single-Nucleotide Polymorphisms (SNPs) have been identified in the *HIF1A* gene. Among these SNPs, C1772T (also note as p582S or rs11549465 C>T) and G1790A (T590A or rs11549467 G>A) are the most common and widely investigated polymorphisms either in healthy individual and in pathological situations (some meta-analysis showed an association between the mutant allele at both the polymorphisms and several types of cancer; pancreas, head and neck, digestive, prostate, renal and so on). Both of these two missense mutations cause amino acid substitutions within oxygen-dependent degradation (ODD) domain and they have been related to an increase trans-activation capacity of HIF-1α either in normoxia than in hypoxia conditions [10], although the exact molecular mechanism of this functional effect has not been elucidated yet. Overexpression of HIF-1α has been identified in multiple types of human cancers [11] and increased HIF-1α expression occurs in the early stages of carcinogenesis, before histological evidence of angiogenesis or invasion can be available [12]. It is therefore biologically plausible that the overexpression of this protein (HIF-1α) may be responsible of subsequent changes in the expression of the more than one hundred downstream target genes related to angiogenesis, glucose transport and cell proliferation/survival.

No direct inference may be drawn by the abovementioned evidence by the presence in a newborn of one or both the mutant alleles in a heterozygous state (being the mutant homozygous state very rare, being probably not viable).

It is well known that HIF-1α expression in the brain may be responsible either of a prodeath or of a prosurvival effect [13], due to the different regulation depending upon the different type of stimuli, the severity of the insult, the presence of hypoxia alone or hypoxia-ischemia and the developmental state of the brain. Both hypoxia and free radicals may activate HIF-1 and the opposing effects of the HIF-1α may depend upon the duration and the severity of hypoxia and free radicals level (ROS). Chen et al. [13] hypothesized that a mild hypoxia is able to induce adaptive gene (such as EPO, Glut1 and VEGF) expression whereas in severe or in sustained hypoxia HIF-1α may lead to activation of prodeath genes (such as BNIP3, COX2 or p53 stabilization). The progressive accumulation of ROS may be responsible – even due to the newborn immature antioxidant defenses – of secondary transcription factors responsible for ensuing cell death. Baranova et al. [14] described two phases of HIF-1α activation after cerebral ischemia. The first one occurred immediately after injury up to 12 hours which correlated with the upregulation of most prodeath genes, while in the second phase of HIF-1α activation most prosurvival genes are involved.

Being both the described mutant alleles at the *HIF1A* gene responsible of a "permanent" HIF-1α overexpression in some CNS cells or in all of them – with the subsequent activation of the genetic cascade – and being the 'physiological' expression of HIF-1α modulated by the type and severity of the hypoxic (-ischemic) stress, the genetic constitution of the newborn may play a pivotal role in the early and delayed response to this insult.

An in-depth analysis of possible correlation between newborns carrying mutant alleles at the *HIF1A* gene who underwent an hypoxic-ischemic event and clinical outcomes may be helpful in unraveling the inter-individual response to perinatal hypoxia. Knowing before the delivery the presence in the fetus of a wild or of a mutant type may be an important issue in order to adequately manage a perinatal hypoxic event with a more aggressive physical and/or pharmacological therapy in the "golden hours".

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ABS 49

IMMUNE THROMBOCYTOPENIA DUE TO RIFAMPICIN: DESCRITION OF A PEDIATRIC CASE

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CLINICAL CASE

A 13-year-old Pakistani girl was admitted in our Department with subclinical fever, chest pain, asthenia, long-lasting loss of weight (three months). She was immigrated with her mother and her 18-year-old brother from Pakistan and lived in a low socio-economic condition.

Past medical history was negative and the members of their family were in good clinical conditions.

The auscultation revealed rare crepitations on the right basal lung zone without cought and the restant examination advertised an important lateral cervical bilateral limphoadenopathy, hepatomegaly, dehydration and underweight.

Chest radiography showed a thickening basal in the right lung like a flogosis (**Fig. 1**).

TC total body showed the same lesion with pneumothorax of the lung bilaterality and pericardic deposit, hepatomegaly, over and under diaphragmatic ilo-mediastinic lymphopaties (**Fig. 2**).

The laboratory investigations revealed a microcytic ipochromic anemia, CRP, ALT, AST, GGT weakly altered, high LDH value for age, low level of total seric proteins sieric and cholinesterase.

Mantoux test and Quantiferon were positive, as well as the cultures performed on the biological samples and on the lateral cervical lymph node biopsy of the neck.

The tests (CD30 and CD15) carried out on lymph node biopsy ruled out any lymphoma.

The susceptibility testing showed resistance only to streptomycin.

Antitubercolosis treatment was started with isoniazid 300 mg, rifampicin 450 mg, ethambutol 800 mg and pyrazinamide 1,500 mg daily, according to clinical guidelines [1].

There were a clinical improvement and a good compliance at the therapy.

After two weeks from the beginning of the therapy, diffuse petechial lesions and purpuric rash in the extremities appeared. Thrombocytopenia was observed (9,000/mm³), while other blood series and general laboratory parameters were normal.
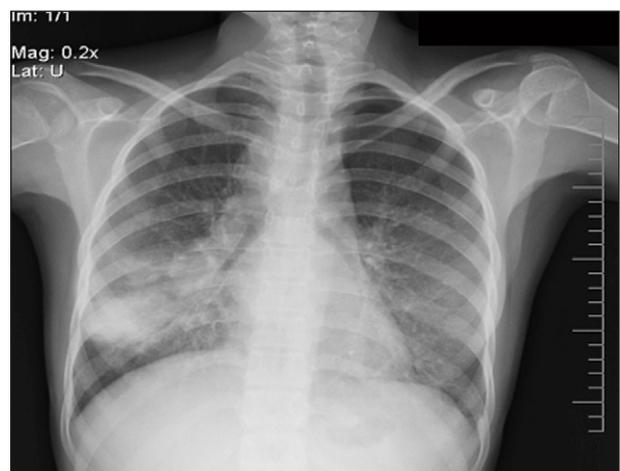
Figure 1. RX CHEST: pulmonary opacity structured equipped with air trapped; mediastinal lymphadenitis. Apical and subclavian pneumothorax on the left.
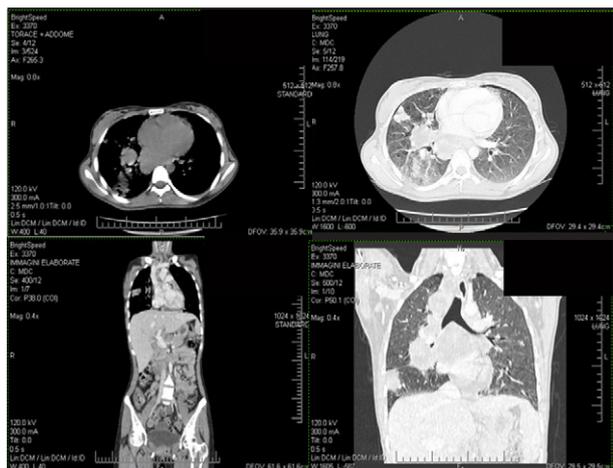
Figure 2. TC ms volumetric total body: Multiple pulmonary consolidations until global hepatization. Lymphonodal masses are combined and colliquate around,over and under the mediastinum with pericardial effusion and bronchial compression. Hepatosplenomegaly. Bilateral pneumothorax.

According with the literature, rifampicin is the main drug associated with thrombocytopenia during the course of anti-tubercolosis therapy [2].

It was decided to stop rifampicin and oral prednisolone treatment was started, together with platelet transfusion and intravenous immunoglobulin at a dose of 1 g daily.

The platelet count steadily rose to 200,000/mm^3 in three days (**Fig. 3**).

The patient continued therapy with the other three drugs without any problems. Thrombocytopenia was not observed any more.

The girl presented high transaminases values and elevation of the GGT (maximum AST 115 U/L and ALT 88 U/L, GGT 60), risolving spontaneously after about one month.

After two months, according with guidelines [1], the treatment included only two drugs: pyrazinamide 1,500 mg and isoniazid 300 mg daily; ethambutol was suspended.

At the last check, the girl shows blood tests into the normal value, including platelet and ALT and AST; her clinical conditions are good and she had a weight gain.

Chest X-ray has improved (**Fig. 4**).

CONCLUSIONS

Rifampicin-induced thrombocytopenia is an uncommon but potentially life threatening complication of antitubercolosis treatment, described for the first time in 1970 by Blajchman and co-workers [3].

To the best of our knowledge this is the first pediatric case. The few reported cases are in adults [4].
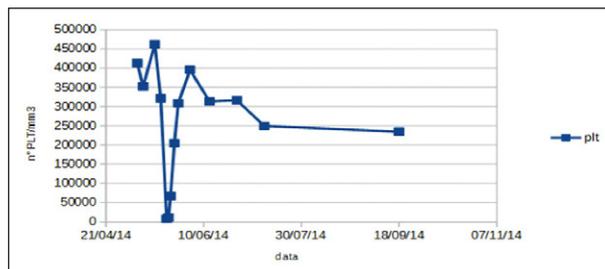
Figure 3. Graphic: Trend of platelets before the start of anti-tuberculosis therapy, during and after Rifampicin therapy until the last check.

Figure 4. RX CHEST (last control after four months of therapy): Progressive reduction of the opacity complex and mediastinal masses.

It has been suggested that rifampicin-induced thrombocytopenia is caused by presence of anti-rifampicin antibodies; these antibodies fix the complement on the platelet causing their distruction [5].

It has been recommended that rifampicin-induced thrombocytopenia is an absolute contraindication to further therapy with the drug [6].

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ABS 50

THE EFFECTS OF NEONATAL RESUSCITATION TRAINING ON THE APGAR SCORES OF BABIES BORN AT THE NATIONAL HOSPITAL ABUJA (NHA), NIGERIA

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INTRODUCTION

Perinatal asphyxia accounts for 28% of Nigeria's very high neonatal mortality rate [1]. The majority of deaths occur within the first week of life, and are mainly due to complications during pregnancy and delivery, reflecting the intimate link between newborn survival and the quality of maternal and perinatal care [2].

In some settings resuscitation training has improved Apgar scores [3].

Because randomized trials of training are difficult to perform in a single setting, use of improvement science methods provides an appealing alternative.

HYPOTHESIS

Using all births at National Hospital Abuja (NHA) (**Tab. 1**), structured neonatal resuscitation training will reduce the incidence of birth asphyxia by 20%, by June 2014, as measured by 1-, 5- and 10- minute Apgar scores.

METHODS

1-, 5- and 10-minute Apgar scores were aggregated weekly beginning February 2012. All births at NHA were potentially affected by the intervention. Babies with congenital malformations were excluded.

Birth asphyxia was defined as any Apgar score less than 7 at 5 minutes.

Statistical method

Standard improvement science methods and statistical process control analyses were used including special cause rules that identified probabilities of less than 2% ($p < 0.02$).

Table 1. Demographic and delivery characteristics.

Characteristics	Range	Mean (SD)
Maternal age (years)	15-50	31.4 (4.8)
Parity	1-7	3.3 (1.8)
Gestational age (weeks)	25-43	37.4 (4.2)

Baseline data were collected for 16 months prior to initiating interventions. To ensure reliability and validity of Apgar scoring, trainings on scoring were held for labour and delivery and Paediatric staff by NRP instructors.

Interventions

- Several meetings with the NHA management, faculty and staff of Obstetrics and Gynaecology and Paediatrics in July 2013 to highlight birth asphyxia.
- Additional resuscitation equipment were supplied to Labour room in August 2013.
- Training sessions to 70 doctors and nurses on neonatal resuscitation using Helping Babies Breathe tools initiated in September 2013.

Apgar scores were aggregated weekly over 25 months. Control charts with 3 standard error confidence limits were used to monitor the proportion of scores greater than ≥ 7.

RESULTS

- From February 2012 through March 2014, there were 3,427 births at NHA, approximately 33 births per week.
- 52.1% of babies were delivered vaginally, 17.8% by elective Caesarean section and 28% by emergency Caesarean section.
- The mean gestational age was 37.8 ± 1.9 weeks and mean birth weight was 3.2 ± 0.8 kg. Preterm babies (postmenstrual age < 37 weeks) constituted 22.7%.
- The incidence of birth asphyxia as defined *a priori* was 33%, 17% and 10% while post intervention the incidence was 18%, 17 % and 6% at 1-, 5- and 10- minutes Apgar scores respectively (**Fig. 1**).

CONCLUSIONS

- Increased communication and training of delivery personnel is associated with reductions in measures of birth asphyxia of 45%, and 40% at 1 and 10 minutes, respectively.
- Our study demonstrates the feasibility and utility of using improvement science methods to assess and improve perinatal outcome in low-resource settings.
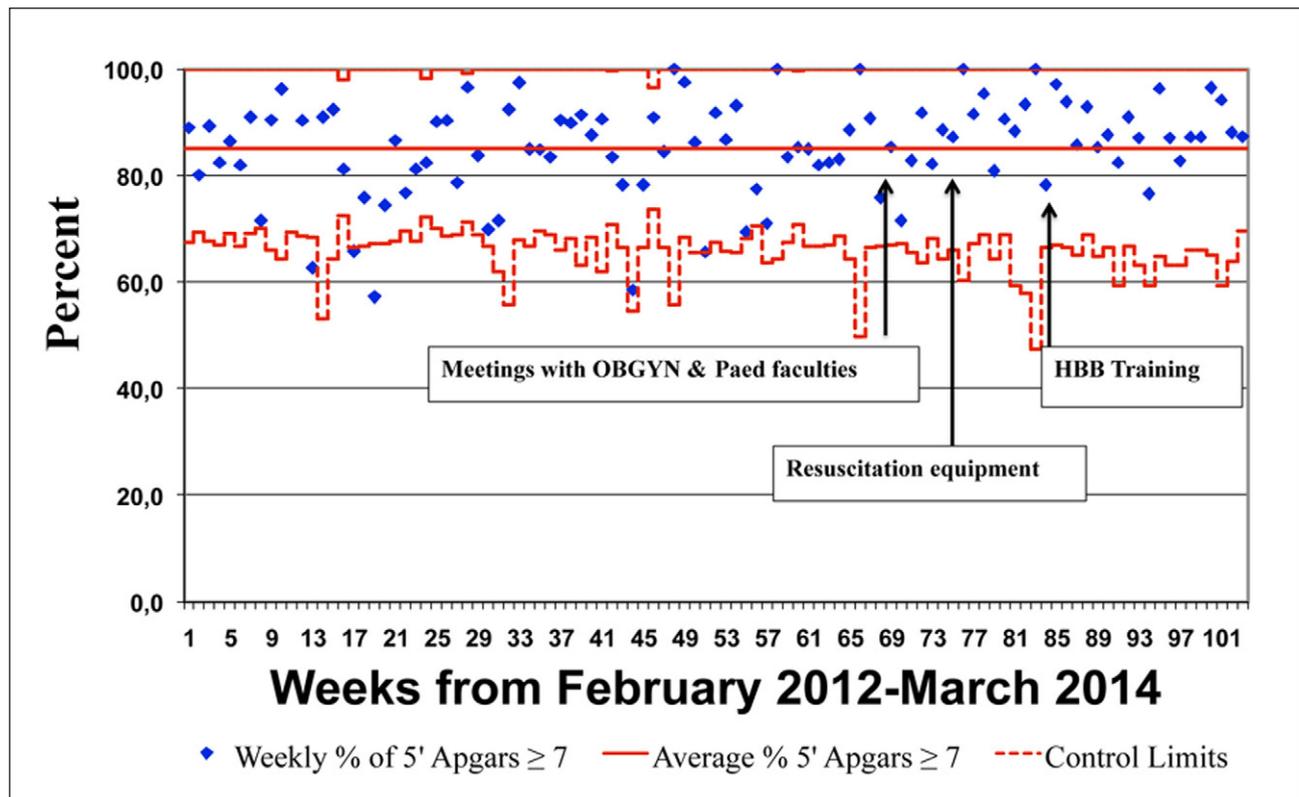
Figure 1. Weekly percentage of 5' Apgar scores ≥ 7 at NHA.

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