Abstracts

4-6\textsuperscript{th} September 2014.
International Conference Centre (ICC)
Birmingham, UK
Thursday 4th September 2014

Paediatric Symposium
See Separate CD Rom
Friday 5th September
08:30-10:30
Plenary Sessions

Paediatric Pathology Plenary Session 1

Raghunath Ramanarasimhaiah, MD, Department of Immunology, University of Connecticut Health Center, Jason C. Burghardt, HT (ASCP), Clinical Laboratory Partners, Hartford, Connecticut, Fabiola S. Balarezo, MD, Department of Pathology, Hartford Hospital, Hartford, Connecticut, Andrew Draghi II, PhD, Department of Immunology, University of Connecticut Health Center, Anthony T. Vella, PhD, Department of Immunology, University of Connecticut Health Center and Connecticut Children's Medical Center, Francisco A. Sylvester, MD, Department of Immunology, University of Connecticut Health Center and Connecticut Children's Medical Center, Connecticut, United States.

Background: We have shown that in pediatric active ulcerative colitis, osteoprotegerin (OPG) leaves the nucleus of colonic epithelial cells (CEC), enters the cytoplasm and appears in the stool. The function of OPG in the colon is however unknown.

Hypothesis: OPG is part of CEC innate immune response to infection.

Aims: To determine OPG interactions in both an in vitro Escherichia coli model and a cohort of pediatric appendiceal resection specimens without appendicitis infected with Enterobius vermicularis.

Materials and Methods: Increasing concentrations of heat-killed E. coli (HKEC) were incubated with Caco-2 cells and OPG was measured by ELISA. In addition, HKEC was treated with fluorescent-labeled recombinant human OPG (rhOPG) or rhIL-33 (also produced by CEC). Bound fluorescence was measured by flow cytometry. Sections of appendix were stained for OPG with a specific monoclonal antibody. OPG-positive nuclei in CEC were counted and expressed as % total nuclei.

Results: Caco-2 cells constitutively secreted OPG into the culture medium. However, the concentration of OPG in the medium declined as the number of HKEC increased. Adding 0.05% Tween-20 to the medium containing HKEC significantly increased the concentration of OPG. HKEC incubated with rhOPG became brightly fluorescent, and only rhOPG, but not rhIL-33 or free dye specifically adhered to bacteria. In the appendix, the number of OPG-positive nuclei was significantly lower in the epithelial surfaces which were in direct contact with Enterobius, and free eggs were coated with OPG.

Conclusions: OPG may be part of a CEC innate immune response to inflammation, luminal bacterial and parasitic infections.
A2. Improvised double-embedding technique of minute biopsies in Hirschsprung disease: A mega boon to histopathology laboratory

Lokendra Yadav, Research scholar, Department of Pathology, St. John's Medical College, Bangalore, India, Sarega Thomas (M.Sc), Amity University, Noida, India, Usha Kini, Professor of Pathology, St. John's Medical College, Bangalore, India.

Introduction: Optimal orientation of rectal mucosal/seromuscular biopsies is essential to visualize neural plexuses for a definite diagnosis of Hirschsprung disease (HD). The problem of orientation of such biopsies when minute gets compounded when they are from neonates and mandates exhaustive strip cuts, thus delaying diagnosis.

Aim: A modified agar-paraffin technique is aimed to make tissue embedding efficient and user-friendly by inking fresh/fixed mapping biopsies followed by embedding in agar after orientation and re-embedding the agar block in paraffin wax after tissue processing.

Material & Methods: One hundred and fifty two mucosal/seromuscular biopsies from suspect HD cases were processed by this improvised double embedding method with colouring inks and sections analysed microscopically.

Results: The tissue in agar paraffin blocks were well processed, firm, held secure, easy to cut with serial sections of desired thickness and spread without folds. The inks remained permanent on the tissues in the block and on microscopic sections, thus helping in easy identification of tissues. Agar did not interfere with histological/histochemical stains or with AChE enzyme histochemistry/immunohistochemistry. Differential inking of mapping biopsies from the same patient and pooling them onto a block reduced the number of tissue blocks, the worktime and reagents markedly.

Conclusion: The improvised agar-paraffin embedding technique for mucosal/seromuscular biopsies for HD is a simple reliable user friendly method that helps to obtain perfect orientation, fast turnaround time for both frozen and formalin fixed biopsy and cost-effectiveness by economizing on the number of paraffin blocks, chemical reagents and manpower.

A3. Histopathological grading of rectum prior to ileoanal anastomosis (J-pouch) in children with refractory ulcerative colitis

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Introduction: J-pouch is a surgical procedure offered to patients with refractory ulcerative colitis (UC) that have undergone subtotal colectomy with the reconnection of the ileum to the rectum. In many cases its creation occurs without complications. Anastomotic leak and stricture may occur in some patients although the reasons for these events are not fully elucidated.

Aim: To evaluate whether histopathological indicators correlated to postoperative complications.

Material and Methods: All cases of UC that underwent J-pouch procedure during the period of 2000 – 2013 were studied. The histopathology of the resected rectum at the time of J-pouch creation was blindly reviewed by two pathologists independently. The latter involved a grading system to assess architectural change, ulceration, inflammatory infiltrate, crypt destruction and apoptotic activity on the distal rectal margin.

Results: A total of 34 pediatric patients required J-pouch procedure during the study period, of whom 13 developed postoperative complications. The mean activity score ±SD of the applied grading system was 4.7 ± 3.0 in the non-complicated cases and 7.2 ±2.9 in the cases with complications (P = 0.03). Other scoring parameters such as atrophy, regeneration and healed mucosa were not significant.
Conclusion: A direct correlation between histological inflammatory activity at the rectal margin and postoperative complications was determined. This association suggests the rectal biopsy may help prognosticate complications of J-pouch surgery. Although these patients were refractory to their medical treatments prior to subtotal colectomy, perhaps additional local therapy for those with inflamed rectal stumps prior to J-pouch creation would diminish complication rates.

A4. Sloughing esophagitis pattern of esophagitis in children

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2. Rhode Island Hospital and Alpert Medical School of Brown University, Providence, RI, USA

Aim: Sloughing esophagitis (SE) is characterized by superficial necrotic squamous epithelium and endoscopic plaques or membranes. This pattern of injury typically affects older, debilitated patients on multiple medications, but herein we describe examples in children.

Materials and Methods: The clinical presentation, endoscopic and histologic findings, and follow-up data were reviewed.

Results: Four cases, all girls aged 10-14 years, were retrieved from our files. Two patients presented with epigastric pain and two with dysphagia. White streaks and/or exudates were present in the mid (3 of 4) and distal (2 of 4) esophagus.

Coagulative necrosis of the superficial epithelium was seen in the distal (2 of 4), mid (2 of 4) and proximal (1 of 4) esophageal biopsies. There was no inflammation in case 1, neutrophils and eosinophils in biopsies from case 2, high (> 20/HPF) intraepithelial eosinophils (IEEs) count in at least one biopsy site from case 3 and 4. In all cases stains for fungi and Herpes virus were negative.

Case 1 and 2 were associated with medication and follow-up biopsies were completely normal. Case 3 was rebiopsied and treated for eosinophilic esophagitis (EoE) with steroids with up to 80 IEE/HPF IEEs and 25 IEE/HPF in one biopsy at 1 and 4 months respectively. Case 4 is a patient with severe cerebral palsy and seizures on multiple medications who has been treated for reflux esophagitis without follow-up biopsy.

Conclusion: The SE pattern of esophagitis can be seen in children and like adults is usually due to medication, but it may be seen in EoE. The latter is not surprising since completely necrotic sloughed epithelium is a recognized finding in EoE.

A5. Eosinophilic gastrointestinal disease in liver transplant patients may be associated with rejection episodes

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Background: Eosinophilic gastro-intestinal disease is a well-recognized phenomenon after solid organ transplantation. However, its pathogenesis and relationship to the post-transplant clinical course is unknown. Our aim was to analyze the characteristics of this entity in children who underwent liver transplantation.
**Study design:** We performed a retrospective study including all liver transplant patients from our institution (1997-2014). A Pathology information system search allowed identification of patients with eosinophilic gastro-intestinal disease. Eosinophilic esophagitis (EE) was defined as the presence of more than 20 eosinophils/HPF; a diagnosis of eosinophilic gastritis (EG) or colitis (EC) was established when the number of lamina propria eosinophils was markedly increased, with associated degranulation and eosinophilic cryptitis.

**Results:** 301 liver transplant patients were identified, of which 13 (4.31%) developed eosinophilia in the digestive tract: 5 EE, 3 EG, 4 EC, 1 EE and EC. Within the cohort without eosinophilic disease, 52.08% (150 of 288) patients had an episode of allograft rejection during follow-up, whereas the frequency of rejection was significantly higher among patients with eosinophilic disorder (11 of 13 cases or 84.6%; p=0.01, Fisher probability test). 10 of these patients experienced acute rejection and one chronic rejection. The rejection episode preceded the diagnosis of eosinophilic disease in 9 instances (with 133 days on average).

**Conclusion:** The majority of children who developed eosinophilic gastro-intestinal disorder following liver transplant experienced a prior episode of allograft rejection. These findings suggest an (auto)immune pathogenesis for post-transplant digestive tract eosinophilia, which is unlikely to be related to treatment since all patients received similar anti-rejection therapy.

### A6. Calretinin immunohistochemistry Vs rapid Acetylcholinesterase histochemistry in Hirschsprung disease: Who wins and when?

Lokendra Yadav, Usha Kini, Dept of Pathology, Kanishka Das, Dept. of Paediatric Surgery, Suravi Mohanty, Divya Puttegowda, Dept. of Pathology, St. Johns Medical College, Bangalore, India.

**Introduction:** Acetylcholinesterase (AChE) histochemistry on rectal mucosal biopsies considered the goldstandard for diagnosis of Hirschsprung disease (HD) is not widely employed as it requires special tissue handling and pathologist expertise. Calretinin immunohistochemistry (IHC) has been reported to be comparable to AChE staining with loss of calretinin expression correlating with aganglionosis.

**Aim:** To evaluate calretinin IHC as a primary diagnostic tool in comparison to improvised rapid AChE technique in the diagnosis of HD

**Material and Methods:** Seventy four rectal biopsies (18 fresh frozen, 56 formalin fixed) from 51 cases of suspect HD were evaluated with H&E, AChE (a 40 mins staining technique) and Calretinin using a protocol. Known ganglionated and aganglionated segment biopsies served as positive and negative controls. Two pathologists blinded to the clinical details evaluated each biopsy and their observations were statistically analyzed to assess the correlation between Calretinin and AChE and the inter observer agreement.

**Results:** AChE highlighted the dark greenish black hypertrophic nerves in HD and calretinin defined the delicate mucosal fibres in nonHD. The study confirmed HD in 26 and non HD in 25 cases. The results of calretinin were comparable to AChE with a statistically significant measure of agreement (kappa 0.973) between the two. One false positive was noted with calretinin.

**Conclusion:** Calretinin is a reliable single immune marker for ruling out HD by its specific positive mucosal staining of formalin fixed rectal biopsy in non HD. However, the improvised AChE histochemistry remains indispensable to confirm HD on fresh biopsies, thus facilitating surgical decisions based on conclusive intra-operative diagnosis.
A7. Redo Pul-Through in Hirschsprung Disease

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1. Department of Pathology, University of Michigan Health System 2. Section of Pediatric Surgery, Department of Surgery, University of Michigan Health System

Most children with Hirschsprung (HSCR) disease do well following pull-through (PT) surgery; however, a small number of patients might develop postoperative complications and need a repeat PT procedure. The complications may be technical or related to mechanical obstruction. We searched our surgical and pathology records for patients with HSCR who needed a repeat PT from 1996-2011. 150 patients with HSCR underwent PT surgery and 9 required redo PT. The original and redo PT slides were reviewed. Results detailed in the table. Technical causes were found in 5 patients, retained aganglionosis due to misinterpretation of frozen section was identified in 1 patient with total colonic aganglionosis, 1 had possible transition zone (TZ) PT and 2 had long residual aganglionic cuff. Our findings confirm that only minority of patients need redo PT. Review of the original PT material along with careful examination of the redo PT can help identify reasons of PT failure. Residual long aganglionic cuff may be related to technical error or stretching of the cuff.

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A8. Integration of Genomic and Whole-Slide Imaging Data to Diagnose Pediatric Orthotopic Heart Transplant Rejection

Ajay K. Bhatia1,2, John Phan3, Sonal Kothari3, William Mahle2, Samuel N. Jactel1, Caitlin A. Cundiff1,3, May Wang3, and Bahig Shehata1 Emory University School of Medicine, and Children’s Healthcare of Atlanta, Department of Pathology1 Emory University School of Medicine, Department of Pediatric Cardiology2 Georgia Institute of Technology, Department of Biomedical Engineering3

Aim of Study: The gold standard for diagnosing orthotopic heart transplant (OHT) rejection is direct pathologic analysis of endomyocardial biopsy samples (EMB). Current methods of hematoxylin and eosin (H&E) staining and immunohistochemistry-based diagnosis are non-quantitative, subjective, have limited early prognostic value, and, in up to 20% of cases, do not correlate with clinical status. The goal of this study was to develop a clinical decision support system that integrates microRNA (miRNA) expression profiling and histopathological image data modalities to predict pediatric OHT rejection. Methods: Samples representing various grades of rejection were identified from a large bank of formalin fixed, paraffin embedded (FFPE) EMB tissue derived from pediatric OHT recipients. Whole slide images (WSIs) were tiled and acquired digitally. WSI features were identified using techniques developed by our group to eliminate artifact and maximize extraction of biologically-relevant features. MicroRNA expression profiling of FFPE EMB was performed via deep sequencing
and processed through three high-quality pipelines. Stacked generalization machine-learning algorithms were used to integrate the image-based and genomic data modalities. Results: 461 image features were extracted from each tile. We were able to use these features to cluster image regions into distinct and clinically relevant groups. Integration of miRNA expression profiles from the correlating FFPE EMB with the WSI data was able to diagnose rejection with greater accuracy of over 95% than the current method (p<0.01).

Conclusions: Integration of histopathological image and genomic data through machine learning algorithms will provide pathologists a streamlined and standardized means to accurately diagnose and predict pediatric OHT rejection.
B1. Intrapulmonary Vascular Shunt Pathways in Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACD/MPV)

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Aim: ACD/MPV is a lethal neonatal lung disease characterized by severe pulmonary hypertension, abnormal vasculature and intractable hypoxemia. Mechanisms linking abnormal lung structure with severe hypoxemia in ACD/MPV are poorly understood. Recently, we identified prominent intrapulmonary anastomotic vessels (IPAV) in ACD/MPV, but whether bronchopulmonary vascular anastomoses form intrapulmonary right-to-left shunt pathways in ACD/MVP remains uncertain

Material and Methods: Lungs from 2 infants who died of ACD/MPV were studied postmortem with direct injections of colored ink into the pulmonary artery (PA), bronchial artery (BA) and pulmonary vein (PV). One lung was injected via the PA only, while the second lung was differentially injected through the PA, BA and PV. Extensive histologic evaluations included serial sectioning, immunostaining [CD31 (for identification of endothelium), D2-40 (for lymphatics)] and 3 dimensional (3D) reconstructions.

Results: Differential injections of ink into PA, BA and PV demonstrate IPAV that link the bronchial and pulmonary circulations and bypass the alveolar capillary bed. 3D reconstruction further highlights connections between the PA and BA, and immunostaining confirms that these vessels are exclusively vascular and not lymphatic in origin.

Conclusion: This study demonstrates striking anastomoses between systemic and pulmonary vascular circulations in ACD/MPV. We identify “misaligned pulmonary veins”, the pathognomonic feature of ACD/MPV, as bronchial veins that become pathologically dilated and congested due to accommodation of increased blood flow due to shunt in ACD/MPV. Overall, these findings support the role of prominent right-to-left intrapulmonary vascular shunt pathways in the pathophysiology of fatal hypoxemia in infants with ACD/MPV.

B2. A 25-year correlation between prenatal ultrasound and autopsy findings at a tertiary center – fetuses with developmental anomalies following termination of pregnancy (TOP)

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Background: Congenital anomalies are an important cause of prenatal, perinatal and neonatal mortality and morbidity. The aim of this study was to correlate the prenatal ultrasound diagnosis with the results of the autopsy in fetuses with developmental anomalies after termination of pregnancy (TOP), in order to see how ultrasound diagnostics has improved over time.

Material and method: The material consists of 849 TOPs, carried out between gestational week 11 and 36 over a 25-year period from 1985 to 2009. The ultrasound examination was performed at the National Center for Fetal Medicine (NCFM), St. Olav's Hospital, Trondheim, a tertiary referral center for pregnant women from all over Norway.

Results: In our study, there was full agreement between ultrasound and autopsy findings in 87.3% (n=741/849), and the main diagnosis was correct in 96.5% (819/849). In 2.2% (19/849) of the cases ultrasound findings were not confirmed at autopsy. When comparing the fifteen year period 1995-2009 with the previous decade 1985-1994, the differences in detection rate were statistically significant, with \textit{P}=0.0002 for complete agreement and \textit{P}=0.0132 for main diagnosis.

Conclusion: The ultrasound diagnostics of developmental anomalies has improved statistically throughout the 25-year period. This study gives an epidemiological overview of developmental anomalies and is a quality control on TOPs carried out because of fetal anomalies diagnosed by ultrasound examination. Finally, to increase the detection and verification rate of anomalies, other methods like postmortem MRI may be helpful in certain cases.

B3. Pathophysiological Mechanism of Abdominal Parenteral Nutrition Fluid Extravasation

Beata Hargitai, Phil Cox Pathology Department, Alison Bedford Russell, Velmurugan Ramalingam, Neonatal Intensive Care Unit; Tamas Marton, Pathology Department, Birmingham Women's NHS Foundation Trust, Birmingham, UK

Aims of the study: Extravasation of parenteral nutrition fluid is a severe and life threatening complication of parenteral nutrition (PN) administered via central venous and umbilical catheters. This study is focused on abdominal extravasation aiming to offer a possible pathophysiological mechanism of abdominal fluid accumulation.

Materials and Methods: A case series of 4 preterm neonates undergoing full post mortem examinations according to standard protocol, at Birmingham Women's Perinatal Pathology Unit. Post mortem PN fluid extravasation was a complication in all 4 babies, based on macroscopic appearances and biochemical analysis of the fluid. An injection study was performed through the umbilical vein during autopsy, using a 1:4 mixture of Tissue Marking Dye (Cancer Diagnostics Inc.) and 1% aqueous solution of agar (VWR Int. Limited), to demonstrate possible perforation and the route of extravasation. Results were documented on digital images and multiple histological sections of the porta hepatis were analysed.

Results: All four cases showed subcapsular liver injury/ necrosis, vascular micro-injury, thrombosis of portal vein branches and injection study demonstrated extravasation through injured vessel walls and hepatic sinusoids in the compromised liver parenchyma. In one case perforation of the liver parenchyma by the cannula was identified, but no obvious perforation site was apparent in the other three.
Based on our findings we suggest that abdominal leakage of PN fluid results from vascular injury of portal vein branches allowing extravasation of the fluid via damaged subcapsular liver parenchyma.

**B4. The Spectrum of Cardiac Pathology in Fetal Hydrops due to Noonan syndrome**

Phil Cox¹, Alison Foster², Beata Hargitai¹, Anastasia Konstantinidou ¹,³, Tamas Marton¹.

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**Introduction:** Noonan syndrome (NS) is an autosomal dominant, frequently de novo condition due to mutations in RAS-MAPK pathway genes. Presentation is variable, usually in infancy or early childhood, and includes poor postnatal growth, cardiac abnormalities, dysmorphic features and lymphatic dysplasia. The classic cardiac abnormalities are pulmonary valve dysplasia and hypertrophic cardiomyopathy, but other cardiac anomalies are reported. Prenatal US scan showing nuchal translucency or cystic hygroma may suggest NS, and fetal hydrops is a recognised presentation.

**Aim:** To examine the range of cardiac anomalies present in cases of fetal hydrops due to NS as a guide to future diagnostic testing.

**Results:** 5 cases of hydrops due to NS were identified in the last 3 years from 2 centres, 4 female and 1 male. In 2, NS was inherited, whilst in the other 3, PTPN11 mutations arose de novo. 3 showed cystic hygroma and 1 increased nuchal translucency on early scan. In one, scan identified congenital heart abnormality. 2 cases presented as mid-trimester intrauterine deaths, one pregnancy was terminated and 2 were neonatal deaths. At post mortem, 2 cases showed an atrioventricular septal defect and hypoplasia of the aortic arch, in 1 there was a secundum-type atrial septal defect and one had aortic valve dysplasia. In 1 the heart was normal. Cardiomegaly was not identified. 2 cases showed hepatomegaly with increased extramedullary haemopoiesis in diverse tissues.

**Conclusion:** NS should be excluded in cases of fetal hydrops without a structural chromosome abnormality and with no other explanation. Cardiac valvular dysplasia is uncommon in our series and affected fetuses can show complex cardiac anomalies or none at all.

**B5. DPP6 mutation and endocardial fibroelastosis Seeking the unknown inheritance in neonatal death by whole exome hybridization**

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Department of 1Pathology and 2Medical genetics, University of Turku and Turku university Hospital, Turku, Finland, 3Modern Diagnostics Ltd, Turku, Finland, 4Wien University Center of Bioinformatics, 5Centre of Biotechnology, Turku, Finland

The purpose of the study was to test the usefulness of whole exome hybridization in seeking hereditary causes of sudden and unexpected neonatal death. For that purpose we evaluated the archives of Turku University Hospital at years 2001-2011. During that period a total of 157 cases of neonatal deaths were investigated by autopsy. Six cases had remained totally uncertain after comprehensive post mortem examination. Of those cases four provided frozen material for DNA studies and we were able to run a total exome hybridization for their DNA. We found two significant
SNP mutations in these patients. One is able to cause carnitine palmitoyl transferase deficiency type IA (CPT1A), and another (dipeptidyl peptidase 6, DPP6) may cause idiopathic ventricular fibrillation. The results show that in some cases of neonatal death that remained uncertain after post mortem examination we may find a significant cause of death by using novel gene technologies. Such information may be of great value for the parents in their family planning.

**B6. Stem cell markers in Anorectal Malformations: an immunohistochemical study.**

Ludwig Kathrin¹, Volpe Andrea², Guzzardo Vicenza¹, Midrio Paola², Gamba Piergiorgio², Alaggio Rita¹

¹ Department of Medicine (DIMED), Surgical Pathology & Cytopathology Unit; University of Padua; Padua – Italy, ² Division of Pediatric Surgery, Department of Pediatrics Salus Pueri, University of Padova, Italy

The aetiology and pathogenesis of anorectal malformations (ARMs) are multifactorial and controversial. Our aim was to analyse the morphological and histological characteristics of patients with ARMs, investigate the role of mesenchymal stem cells and correlate these results with the expression pattern of Sonic Hedgehog (Shh), previously studied.

H&E stained sections from 30 ARM surgical specimens and five normal controls - previously investigated for Shh expression - were analysed and immunostains for CD34 and CD133 were performed. The mean of positive CD34 and CD133 staining cells was evaluated in the lamina propria (LP), the muscular layers (ML) and in the subserosa (SS) (Table).

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CD34+ cells in SS were significantly increased in ARM cases [p=0.008]; in the external ML the number of CD34+ cells correlated with a higher degree of muscular damage. In LP CD133+ cells were significantly decreased in ARM cases [p=0.014] and inversely correlated with the grade of muscular damage in the external ML. Blood vessels were increased in 85% of ARMs in the area of muscular damage.

These results might indicate the attempt of mesenchymal stem cells to differentiate in response to muscular damage. The significantly lower Shh expression levels in ARMS [p=0.008] apparently exclude the hypothesis of altered CD34 and CD133 levels as indicator of increased recruitment of late endothelial progenitor cells in angiogenesis.

**B7. Knockdown of ndufb11 in Zebrafish Model Results in Cardiac Anomalies, Confirming the Role of the Newly Recognized NDUFB11 in Histiocytoid Cardiomyopathy Pathogenesis**

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**Aim of Study:** Histiocytoid cardiomyopathy (HC) is a rare form of cardiomyopathy observed predominantly in newborn females that is fatal unless treated early in life. Previous whole exome sequencing analysis on parent-proband trios identified de novo non-sense mutations in the second exon of the X-linked nuclear gene NDUFB11, which was not previously implicated in any human disease.

**Methods:** In order to examine the role of NDUFB11 in cardiac development and function, we have employed the zebrafish vertebrate model, which is well established for the study of cardiac dysfunction. Danio rerio ndufb11 is known to be expressed ubiquitously during embryogenesis. Morpholino (MO) mediated transient translational suppression of ndufb11 in zebrafish embryos was performed with two different morpholinos injected into one-cell zebrafish embryos at a final dose of 0.4mM.

**Results:** Knockdown of ndufb11 in zebrafish embryos carrying the heart marker Tg(cmlc2:mRFP) displayed edema and abnormal heart structure in approximately 80% of injected animals. Detailed analysis revealed heart structure defects such as loss of the S-shaped heart at 3 days post fertilization, resulting in a linear heart tube consistent with defective cardiac looping. Suppression of ndufb11 expression in Tg (fli1: EGFP: gata1: dsRED) zebrafish embryos also revealed defects in angiogenic vessels.

**Conclusions:** Morpholino-mediated knockdown of Ndufb11 in zebrafish embryos generates defective cardiac tissue with looping defects, confirms a role for the gene in cardiac pathology. Although HC remains a heterogeneous disease, these results illustrate the importance of NDUFB11 in cardiac function.

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**B8. Placental Pathology in Relation to Brain Injury and Neurodevelopmental Outcome in Very Preterm Birth**

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**Introduction:** To evaluate the association between placental pathology, patterns of brain injury and developmental outcome in very preterm infants.

**Methods:** All infants with gestational age (GA) between 24 – 31 weeks reaching term equivalent age between January 2007 and July 2008 were enrolled. Cerebral MRI was performed at term equivalent age and was scored for white matter injury, IVH III and IV, cerebellar hemorrhage and ventriculomegaly. Placenta examination: The presence of inflammation and placental underperfusion was scored. Development was assessed at 2 yrs corrected age (CA) using Bayley Scales of Infant and Toddler Development (BSITD-III), at 3.5 yrs using the Griffiths’ Mental Development Scales (GMDS) and at 5.5 yrs by the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) and Movement Assessment Battery for Children (Mov-ABC).

**Results and conclusion:** Placental histology was available for 176 (75.2%) of the 234 eligible infants. Infants with placental underperfusion had a higher GA (29.1 ±1.57 vs. 27.4 ±1.92 wks, p<0.01) and were more often small for GA (14.3 vs. 0.0%, p=0.04) compared to infants with chorioamnionitis. In 110 infants with placental histology, cerebral MRI was available. Placental pathology was not predictive for MRI abnormalities. At 2 years CA (n=98), BSITD-III was not different between infants with placental underperfusion and infants with chorioamnionitis. At age 3.5 years (n=81), infants with placental underperfusion had lower scores on the GMDS compared to infants with chorioamnionitis, despite a higher gestational age in this group. Placental pathology and MRI lesions were not predictive for developmental outcome at 5.5 years (WPPSI n=28, and Mov-ABC n=60).
Classification systems for perinatal mortality need to capture multi-hit placental aetiology.

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\textbf{Aim:} There are multiple classification systems for perinatal death. The aim of this study was to assess the application of recognised classification systems in a cohort of cases of multi-hit placental aetiology.

\textbf{Materials and Methods:} Perinatal post-mortems performed at our institution over a two-year period (2012-2013) were reviewed and cases with multi-hit placental aetiology were identified. Classification of cause of death was attempted using seven recognized classification systems [Extended Wigglesworth, Aberdeen, PSANZ, ReCoDe, Tulip, Codac and National Perinatal Epidemiology Centre (NPEC) Ireland].

\textbf{Results:} In 82 perinatal post-mortems, a placental cause of death was identified in 39 cases (48%). A single placental diagnosis was present in 28/39 cases (72%). In 11 cases (28%) there were multiple significant placental diagnoses (multi-hit placental aetiology). These included the following combinations: distal villous immaturity (DVI) with chorionic plate thrombosis (n=3); DVI with meconium induced myonecrosis of chorionic plate vessels (n=4); acute chorioamnionitis with high grade chronic villitis of unknown aetiology (n=3) and umbilical artery thrombosis with maternal vascular underperfusion (n=1). The majority of the classification systems could not accurately capture the cause of death in these complex cases due to i) a lack of sufficiently comprehensive diagnostic
categories for placental pathology, resulting in the excessive use of the “other” category and/or ii) an inability to record the possible contribution of more than 1 placental factor.

**Conclusion:** As perinatal mortality classification systems evolve, methods to capture multi-hit placental aetiology need to be incorporated in order to fully reflect the complexity of placental pathology in perinatal deaths.

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**B10** The Missing Piece or A Case of Umbilical Cord Cyst and Small Bowel Atresia

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**Introduction:** Small bowel atresias are thought to result from mesenteric vascular accidents during early development resulting in infarction and subsequent organization or resorption of the necrotic segment.

**Aim of study:** We present the case of a new-born with small bowel atresia and an umbilical cord cyst that demonstrates asynchronous intestinal rotation and umbilical development as the underlying cause of small bowel atresia.

**Methods:** A 24-h new-born boy with an umbilical cord cyst diagnosed prenatally underwent surgery with complete resection of the 12 cm long umbilical cord containing the cyst. Histological examination showed the presence of a detached, viable piece of small bowel with no connection to the proximal end of the umbilical cord, with associated angiomatous proliferation. Few days later the baby presented with symptoms of bowel obstruction and a remote intestinal atresia was identified. The proximal and distal edges of the atretic process showed features similar with those seen in the severed piece of intestine.

**Results:** Our case demonstrates the mechanism of atresia in this child, as entrapment of the intestinal loop in the umbilical cord, a consequence of faulty synchronization, leading to its severance from the rest of the bowel during the process of internalization. **Conclusion:** In the absence of blood supply a detached piece of intestine would undergo necrosis and mineralization. This might explain the lack of evidence for missing pieces of an atretic small bowel. This is especially true when it is located in a segment of umbilical cord that is normally not examined histologically.

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**B11** Estimated bone lengths in fetuses younger than 14 WG

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**Aim of study:** Perinatal pathologists examine an increasing number of young fetuses, aged below 15 weeks of gestation (WG), normal or malformed, intact or fragmented, macerated or not. While statural age is well determined clinically by the foot length and by sonography by the crown-rump
length or the femoral length, radiographic femoral length chart for those ages are not published. Radiographic charts are not similar to in utero sonographic charts due to “advanced” bone visualization with sonography.

Radiographic charts are useful in assessing gestational ages of pregnancies of unknown onset, or limb shortening in abnormal fetuses.

The aim of our study is to establish bone lengths charts based on a cohort of normal fetuses.

Materials and Methods: Our fetal cohort comes mainly from voluntary termination of pregnancy. Among 125 fetuses radiographed younger than 14WG, 47 met our criterias of inclusion (a priori normal pregnancies, measurable foot, whole femur). In 35/47 cases, gestational ages established prenatally by sonography were consistent with the clinical measurement of the foot. Radiographs were obtained using a faxitron® and “high resolution” digital radiographic cassettes.

Some fetuses had ex-vivo sonography for comparison with radiography.

Results: Bone mineralization of young fetus is low in the metaphyseal region. Radiographic bone length is closest to sonographic measures when realized by including the perichondrial ring.

Conclusion: We established the first radiological femoral length chart between 10 and 14 WG allowing a statistical precision of less than one week, and a humeral length chart which can be used in absence of femur.

B12 Ganglionic Localization of the Fetal Rectum

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GIT motility disorders are often misdiagnosed due to misinterpretation of the ENS in the transitional zone, and missed detection of the indistinct or immature neurons. The aim of this study was to clearly describe normal ganglion cell colonization of the small/large bowel and rectum during the early stages of gastrulation. Histological and immunohistochemical analysis of patients ranging from 5 to 24 weeks gestation was undertaken, and the number of immature ganglia in three non-adjacent fields of the small and large bowel was determined.

Archived paraffin blocks of the GIT of miscarried foetuses were obtained and analysed by comparing H and E stained slides with immunohistochemical slides stained with s-100, CD56 and PgP 9.5. Analysis under microscope determined the number of immature and mature ganglia cells in the small and large bowel. Pictures of the small/large bowel and rectum where taken in the case of normal gut development. The large intestine had a higher colony of nerve cells, and H & E stained samples yielded a higher count of ganglion cells when compared to s-100 IHC. The transition zone between the columnar epithelium and squamous epithelium was identified at both low and high microscopic power.

H & E staining showed that in normal gut development, even at early gestation, you can expect to find a dense population of ganglia cells in the submucosal and myenteric plexus. With the knowledge that submucosal ganglion cells generally correlate with the status of ganglion cells in the adjacent myenteric plexus of the gut, anomalies in GIT development are easily identifiable.
Listen to the head, not the heart: cardiac failure secondary to a dural arteriovenous fistula in a stillborn infant.

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Introduction: Dural arteriovenous fistulas (AVF) rarely occur in the neonatal period but may present with occasionally refractory fatal cardiac failure. We attribute intrauterine death to congestive cardiac failure secondary to a large cerebral dural AVF and are unaware of other similar published cases.

Case Presentation: A 21 year old woman, para 0+0, with no known personal or family history had an unremarkable pregnancy until presentation at 33 weeks with abdominal pain. Ultrasonography confirmed intrauterine death. Neuropathological examination revealed a frontal dural arteriovenous fistula with necrosis and calcification of the adjacent brain tissue. Congestive cardiac failure with right sided atrioventricular dilatation was confirmed. Secondary microcephaly was also present. The umbilical cord contained an early non-occlusive thrombus.

Discussion: Adult dural AVFs are thought to be acquired and associated with venous sinus thrombosis. The link with thrombosis is less certain in neonates. In this case, thrombus was present in the umbilical cord but not in the dural sinus. Previous reports suggest that an embryological cause is unlikely. Dural AVFs are not associated with other vascular malformations and they do not show a familial predisposition.

Conclusion: Although rare, dural AVF should be considered as a cause of death in the neonatal period if features of cardiac failure are seen. A maternal hypercoagulable state should be excluded with a thrombophilia screen.

Pitfalls in the clinical assessment of dysmorphic features in the stillborn patient

Halit Pinar, MD; Sara Muhacir, MD, Brown University

Introduction: Diagnosis of dysmorphic features is a significant component of the clinical assessment of the pediatric age group including the fetuses. In the stillborn this process is fraught with error due to deficient knowledgebase, unclear definitions, inadequate normative data, insufficient supervision of the prosectors (technical or housestaff) and presence of maceration.

Materials and Method: This is a retrospective review of consecutive autopsies in a Perinatal Center. Dysmorphological examination included anthropometric measurements and description of minor and major abnormalities. Cases with any type or degree of congenital abnormalities were collated. During classification every case was counted once.

Findings: During the 15-year study period there were 1624 autopsies. 962 (60.4%) of them were from stillbirths ≥ 20 weeks gestational age. In this group 387 (40.22%) had developmental abnormalities. 58 (14.9%) were qualified as minor and 329 (85.1%) major. Findings such as easy-to-see abnormalities (large neural tube defects, abnormalities of the trunk and extremities, etc.) were the most useful findings in defining the phenotype (p<0.0001). More detailed findings such as dermatoglyphics, subtle malposition of the eyes, ears, and similar findings were initially missed (41%) and later recognized after a trigger from the senior faculty. Anthropometric measurements obtained were inaccurate in 23% of the cases and their contribution to the main phenotypic diagnosis was limited.
Conclusions: Dysmorphological examination of stillborns is difficult to perform and require more training and supervision of the prossecting team when compared to adult autopsies.

**B15. Efficacy of perinatal autopsy in a developing economy with limited genetic/ancillary support: An audit**

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**Aim:** To determine the utility of perinatal autopsy with limited genetic/ancillary support in the management of subsequent pregnancy.

**Materials and Methods:** All perinatal autopsies performed between May 2010 and April 2013 at Fernandez Hospital, a tertiary referral center with 7000 deliveries annually, were audited and subsequent pregnancy outcome analyzed. The autopsies were conducted by employing the Rokitansky procedure with evisceration performed in blocks and the placenta was examined using accepted criteria.

**Results:** During the study period 203 babies were received of which 29 unreported cases were excluded. The gestational age ranged from 13 to 41 weeks. Majority (n=144, 82.8%) of the deliveries were induced, either for intrauterine fetal demise (48.4%) and sonographically detected abnormalities (24.1%) or for obstetric indications (10.3%). Placental etiology contributing to fetal demise was identified in 103 (59.2%) cases, commonest pathologies being malperfusion, fetal thrombotic vasculopathy, massive perivillous fibrinoid and chorioamnionitis. Syndromic babies comprised 79 (45.4%) and growth restriction was distinguished in 33 (19.0%). There were 51 (29.3%) babies without malformation while both fetal and placental cause was detected in 19 (10.9%). Thirty-nine (22.4%) women were lost to follow-up.

**Conclusion:** The study demonstrated that post issuance of perinatal autopsy report 90 (77.6%) women conceived, ongoing pregnancy was observed in 22 and a healthy neonate was delivered by 60. The review of the cause for an adverse fetal outcome in the previous pregnancy of these 60 women showed placental pathology in 30 (50%) and malformations in 26 (43.3%). Ancillary support notwithstanding, perinatal autopsy is efficacious and warrants aggressive pursuance.

**B16. A case of I-cell disease (mucolipidosis II) presenting with prenatal lethal skeletal dysplasia and pulmonary hypoplasia**

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I-cell disease is a rare autosomal recessive lysosomal storage disorder (LSD) caused by a mutation in the GNPTA gene that leads to a deficiency in the enzyme UDP-N-acetylglucoseamine-1-phosphotransferase. The disease usually develops progressively over the first few months of life with fatal outcome most often in early childhood. We report a rare case of an infant with I-cell disease diagnosed prenatally with lethal skeletal dysplasia and abnormal narrow pear shaped thorax. A male infant was born at 37+6 weeks of gestation with a birth weight of 2000 g, dysmorphic features, coarse facial features, gum hypertrophy, and skeletal shortening. Skeletal survey revealed lack of mineralisation, pathological fractures, metaphyseal cupping and periosteal cloaking of long bones. Examination of a placenta showed diffuse foamy vacuolar changes in trophoblasts and syncytiotrophoblasts. The diagnosis of I-cell disease has been confirmed on enzymology. The infant had ongoing thrombocytopenia from birth. Sadly, he expired at the age of 7 weeks. We report this case to emphasize the role of placental examination in diagnosing storage diseases and to alert the clinicians about a proportion of lysosomal storage disorders with prenatal onset. LSDs classically
have not been considered disorders of the newborn and this diagnosis should be considered in the
differential diagnosis of disorders of the neonate. Awareness of these early presentations has
important clinical implications and can enable appropriate genetic counseling for future pregnancies.

**B17. Fine Needle Aspiration Cytology – A Novel Tool For Investigating Placental Structural Organisation.**

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**Aim:** To address the paucity of published information on the cytology of the normal, term human
placenta and to investigate syncytiotrophoblast nuclear organisation patterns using cytology
techniques.

**Materials and Methods:** Following delivery, term placentas from normal pregnancies were sampled
using fine needle aspiration (FNA) and direct scrapes. Air dried smears were stained with Giemsa;
CytoLyt suspensions prepared as cytospins and ThinPrep slides were stained with Papanicolaou.
Residual material was used to generate formalin fixed paraffin embedded cell blocks and cut as H&E
sections. Standard histological examination was also performed for correlation and to exclude
pathological changes.

**Results:** Fifty slides were generated for evaluation. Both Papanicolaou stained cytospin preparations
and air dried Giemsa slides from FNA provided high quality material for assessment due to good
cellularity and presence of villous “micro-biopsies” that allowed 3D appreciation of villus branching
patterns. The nuclear chromatin pattern of both cyto and syncytiotrophoblast appeared finely clumped
in all preparations and may represent pre-apoptotic change. Complex patterns of syncytiotrophoblast
nuclear organisation not previously described cytologically were observed including irregular spacing
of nuclei, syncytioplasm windows and linear nuclear arrangements.

**Conclusion:** We have shown that placental FNA is feasible and a) provides technically excellent
material for cytological evaluation b) confirms the presence of complex nuclear organisational
patterns in the syncytiotrophoblast while eliminating the possibility of tangential sectioning artefact c)
provides superior nuclear detail over standard histological sections and d) may be an untapped
research resource because of its potential for in-vivo placental sampling.

**B18. Unexplained stillbirths - is placenta the miscreant?**

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**Aim:** Unexplained intrauterine fetal death (UIUFD) is defined as fetal death occurring without any
evidence of fetal, maternal or placental pathology prior to the onset of labor and is classified as ‘no
relevant condition identified at birth’ by the ReCoDe classification. The aim of our study was to
evaluate the placental histopathology in women with UIUFD with a view to identify a possible
placental etiology.

**Materials and Methods:** We conducted a retrospective study of all stillbirths (after 24 weeks of
gestation) born between January 2011 and December 2012 at Fernandez Hospital, a tertiary referral
center with 7000 deliveries annually. All stillbirths were catalogued using the ReCoDe classification
and placental histopathology was categorized as maternal blood supply abnormalities (MBSA), fetal
blood supply abnormalities (FBSA), inflammatory lesions and miscellaneous conditions in unexplained fetal deaths.

**Results:** Thirty-three out of 211 (15.6%) stillbirths were unexplained. The mean maternal age was 28.6 years and 78.8% deliveries occurred at or after 32 weeks of gestation. FBSA (fetal thrombotic vasculopathy) was found in 14 (42.4%), MBSA (placental malperfusion) in 4 (12.1%), inflammatory lesions in 6 (18.2%) and miscellaneous conditions such as massive perivillous fibrinoid and villitis of unknown etiology in 5 (15.2%); there was no placental pathology in 4 (12.1%) cases.

**Conclusion:** Our study demonstrated placenta to be the miscreant, directly contributing to fetal demise in nearly 88% of the women presenting with UIUFD. Diagnosis helps in providing genetic counseling, bereavement closure and implication of possible recurrence in future pregnancies.

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**B19 Kyphomelic dysplasia in fetus: a Tunisian case**

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Kyphomelic dysplasia is a rare prenatal skeletal disease characterized by severe rhizomelic limb shortening, bowed extremities and pterygia. The first cases were reported by Khajavi in 1976, since then, less than 20 cases have been reported in English literature. We describe a further case of kyphomelic dysplasia in a 24 weeks' gestation fetus.

A 22 year-old primigravida admitted at 22 weeks' gestation. The parents were consanguineous, no family history of malformations. Ultrasonography showed dysmorphic fetus with dwarfism and bent femora, radii and ulna. Termination of pregnancy was performed.

Gross showed a fetus with symmetrical shortening of the long bones and angulated femora, radii and ulna, pterygium of elbows and knees and facial dysmorphism including micrognathia, low set ears and flattened nose. Post mortem radiography confirmed symmetric angulated radii, ulna and femora.

Histological examination of femora found thick vascularized cartilage and focal degeneration of the matrix. Ossification line was preserved. Spongyous bone was ramified and surrounded by osteoblastic reaction and fibrosis.

Features were consistent with the diagnosis of kyphomelic dysplasia.

The pathogenesis of this disease is still unknown and no gene locus is identified. However, recent studies indicate that individual cases should be reclassified as another existing chondrodysplasia such as Schwartz-Jampel Syndrome.

Differential diagnosis includes campomelic dysplasia this can be distinguishable by the predominance of tibial bowing, bell shaped thorax and extraskeletal manifestations such as macrocephaly, heart defect and hydronephrosis.

Prognosis of this syndrome is encouraging because it is associated with normal mental development and bone changes can improve with age.
Pathological and Immunohistochemical Study as a Diagnostic Method in Placentas of Pregnancy with DENV Infection

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DENV infection in pregnancy has been linked to miscarriage or fetal death intrauterine, as well as the compromising secondary neonatal to both prematurity as to transmission. By dealing with viral infection with the possibility of hemodynamic compromise of the maternal organism, can be theorized a mechanism for direct fetal organ damage, as well as secondary to impaired placental inflammation (placentitis) or ischemic as a result of maternal hemodynamic changes. Dengue infection is endemic in Brazil since 1986. In the period 2002 to 2010 was performed histopathological and immunohistochemical analysis of placental and ovular remnants of 35 patients with confirmed dengue fever in pregnancy. Thirty-four patients (97%) had some abnormality. Of these seven cases (20%) were classified as purely inflammatory pathology, 7 (20%) as ischemic disease and only 20 (59%) mixed pathology (inflammatory and ischemic). The immunohistochemical test was positive in 32/35 patients (91.4%).

Conclusion: Placental inflammatory and ischemic changes are frequent in dengue virus infection. The authors suggest the use of immunohistochemistry in placental material or ovular remains as a method of laboratory confirmation in pregnant women in endemic areas of dengue fever when only material emblocado is the only material available.
B22. Placental Correlates and Clinical Consequences of Term Fetal Intrauterine Growth Restriction

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Aim of study: Intrauterine growth restriction (IUGR) has multiple etiologies including placental vascular compromise. As few studies have investigated primarily term placentas from IUGR infants, we compared placentas from IUGR infants to placentas from infants with appropriate weights for gestational age (AGA) in this population.

Methods and Materials: With IRB approval, 67 placentas from IUGR term infants and 67 placentas from AGA infants matched for gestational age and gender were selected from 2009-2011. Placental histology was reviewed and electronic records were queried for maternal and fetal birth data, infant morbidities, and infant follow-up weights.

Results: Placentas from IUGR infants were more likely to have smaller weights and thinner umbilical cords than AGA infants. IUGR placentas had a significant increase in other uteroplacental underperfusion features, including accelerated maturation and infarctions. IUGR infants tended to have lower weights up to 7 months of age; however, low number of infants with follow-up limited the statistical significance. Rates of pre-eclampsia, infant cardiac anomalies, and infant genetic abnormalities were not statistically different between groups. Fetal and maternal inflammatory responses, non-gestational diabetes, and gestational hypertension were more common in the controls, but these are common indications for placental examination. No statistical differences were present for decidual vasculopathy, chronic villitis, intervillous hemorrhages, or meconium.

Conclusion: This study confirms that small placental size and features of uteroplacental underperfusion are more common in IUGR versus AGA term placentas. The lack of other significant differences may be due to the inclusion of only term infants, with more severe pathology leading to preterm delivery.

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Aim: Giant Congenital Melanocytic Nevi (GCMN) are rare but may undergo malignant transformation. Somatic p.Q61R and p.Q61K mutations in NRAS are highly prevalent in GCMN; however, there is no clear understanding on GCMN progression to melanoma. We describe the clinical, pathological and novel genetic features of a proven case of melanoma in utero, representing the most severe end of the neurocutaneous melanocytosis (NCM) spectrum.

Material and methods: A Caucasian male was born with NCM: GCMN, multiple “satellite” lesions, an abdominal wall tumor and Dandy-Walker malformation. MRI shortly after birth showed leptomeningeal involvement.

Results: The abdominal mass showed a “neurotized” nevus with a proliferative nodule and mosaicism: 47,XY,+der(1)(1;1)(p13;q25) and 48,XY,+der(1)(1;1)(p13;q25)x2,+6. Rapidly progressing hydrocephalus, required ventriculoperitoneal shunting and cyst fenestration of posterior fossa. Chemotherapy included sorafenib, temozolomide and pegylated interferon. The tumor progressed throughout the subarachnoid space, skull base and spinal canal, leading to death at 17 months of age. Autopsy showed melanoma in the brain/spinal cord, meninges, lung, liver, bone marrow and skin. Next-generation sequencing in tumor cells from liver, skin and brain showed heterozygous NRAS p.Q61R. In addition, three amino acid changing variants were identified: KIT p.M541L, also present in the father; KDR (VEGFR2) p.Q472H and TP53 p.P72R. Retrospective histological review showed melanoma in the placenta. DNA microarray from the original abdominal tumor showed that the +der(1) contained 1p and 1q fragments including exons 1, 2 and possibly 3 of NRAS.

Conclusion: Our findings raise questions about current theories of melanomagenesis in congenital nevi, and demonstrate that malignant transformation may occur early in gestation.
A10 Focal congenital hyperinsulinism: adenomatous hyperplasia of pancreatic endocrine cells. Immunohistochemical analysis of P57 expression.

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Aim of our study is to analyze the P57 KIP2 immunohistochemical expression in 6 cases of focal congenital hyperinsulinism (CHI). This gene, involved in regulation, as inhibitor, of cell proliferation, resides on short arm of chromosome 11.

Methods: Six children, 2 males (10- and 13-month-old) and 4 females (age range from 10- to 61-month-old) showed a clear areas of increased F-fluoro-L-Dopa PET uptake within the pancreas (4 cases) or, apparently, outside the pancreas (2 cases).

Immunohistochemistry for P57 KIP2 was performed on formalin-fixed paraffin-embedded tissue. Microsatellite markers were used to demonstrate LOH in focal lesion compared to adjacent normal pancreas.

Results: All lesion were composed of large endocrine cells with dispersed abnormal nuclei. On immunohistochemistry, in all samples P57KIP2 expression was lost in the lesion, whereas it was normal outside the islets of Langerhans. Microsatellite marker, D11S909, showed loss of maternal 150 bp allele in DNA in focal adenomatous hyperplasia compared to adjacent normal pancreas.

Conclusion: Focal CHI is caused by specific loss within affected β–cells of a portion of the maternal allele of 11p15, which contains the p57KIP2 gene. p57KIP2 has been shown to be paternally imprinted in several tissues. Loss of p57KIP2 expression within the focal CHI lesion suggests that the gene is also imprinted in human β-cells.

This relatively simple immunohistochemical stain can be used to confirm LOH of the maternal allele in these lesions and may be of use in differentiating focal HI from other forms of hyperinsulinism.

A11 Somatic Mutations of PIK3CA in Lymphatic Malformations

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Aim: Localized and regional malformations can be caused by somatic mutations that are non-heritable. We have recently identified activating mutations in PIK3CA, which encodes the catalytic subunit of phosphatidylinositol 3-kinase, in affected tissue from patients with rare malformative disorders comprised of lymphatic-venous anomalies and regional overgrowth. We hypothesized somatic PIK3CA mutations would be found in affected tissue from patients with other malformative disorders containing a lymphatic component, and well as in more commonly observed isolated lymphatic malformations (LM).
Materials and Methods: We used massively parallel sequencing and droplet digital PCR (ddPCR) to detect somatic PIK3CA mutations in archival affected tissue from 80 patients with lymphatic malformations. Appropriate controls were employed.

Results: Most individuals with isolated LM (16/18) or LM as part of a malformative disorder, such as CLOVES (Congenital Lipomatous Overgrowth with Vascular, Epidermal, and Skeletal anomalies) syndrome (27/33), Klippel-Trenaunay syndrome (19/21), and fibro-adipose vascular anomaly (5/8), were somatic mosaic for 1 of 5 PIK3CA mutations; these same mutations are common somatic mutations in cancer. The frequency of mutant cells in many malformations was less than 10%.

Conclusions: The majority of LM tissue specimens contain predominately wild-type cells with a minor population of PIK3CA mutant cells. Sensitive and inexpensive assays, such as ddPCR, that can detect very low abundance mutant cells will reduce the number of patients who require more comprehensive and expensive testing. Recognizing that common and rare lymphatic disorders are associated with PIK3CA mutations should lead to improved diagnosis and novel targeted therapeutic approaches.

A12 Improving Detection of Metastatic Neuroblastoma in Bone Marrow Core Biopsies: A Proposed Immunohistochemical Approach

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Aim of Study: Bone marrow (BM) involvement is common in stage IV neuroblastoma patients, affecting both prognosis and treatment. Currently, no standard exists for immunohistochemical evaluation of staging BMs; we therefore examined the utility of three immunostains - synaptophysin, tyrosine hydroxylase (TH), and PGP9.5 – in detecting metastatic neuroblastoma in BM.

Materials and Methods: Following IRB approval, we retrospectively selected 175 BM core biopsies from 41 neuroblastoma patients. Immunohistochemistry for PGP 9.5, synaptophysin, and TH (Leica) was performed per manufacturer protocol. These slides and the H&E-stained slide from each BM were randomized and independently scored by three pathologists as positive, negative, or indeterminate. Cohen’s kappa coefficients (interobserver agreement) and McNemar’s test for frequencies of positive and indeterminate interpretations were calculated.

Results: Interobserver agreement was higher for all immunostains (synaptophysin: 78-90%, k=0.548-0.787; TH: 77-92%, k=0.481-0.788; and PGP9.5: 83-90%, k=0.601-0.629) than for H&Es (77-84%, k=0.434-0.572). Indeterminate interpretations were more frequent with H&Es (8.9%) and synaptophysin (6.0%) than with PGP9.5 (3.5%) or TH (3.3%). TH (63%) and PGP9.5 (61%) were most likely to resolve indeterminate H&E interpretation. Compared with the “consensus” diagnosis (H&E plus three immunostains), H&E detected 79% of metastases, H&E plus one immunostain detected 91-94%, and H&E plus two immunostains identified 96-98%.

Conclusion: PGP9.5 and TH showed good interobserver agreement, fewer indeterminate interpretations, and resolved indeterminate H&E diagnoses at the highest frequency. Further, combining two immunostains with H&E significantly enhances identification of tumor. Therefore, we recommend H&E and two immunostains, specifically PGP9.5 and TH, for optimal detection of metastatic neuroblastoma in BM.
**Perinatal Pathology Plenary Session 3**

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**B23 Ex vivo Endothelin-Induced Relaxation of the Rat Remodeled Spiral Artery is Mediated via Endothelin Receptor B - Evidence from NOS Inhibition**

Ilana Ariel¹, Galina Skarzinski¹, Vitali Belzer², Zaid Abassi³, Michael Bursztyn⁴

The Departments of ¹Pathology, ²Surgery and ⁴Medicine, the Hadassah-Hebrew University Medical Center, Mount Scopus, Jerusalem, and the ³Rappaport Faculty of Medicine, Technion, Haifa, Israel

**Background and Aim:** Endovascular trophoblasts (EVasT) of the rat express smooth muscle proteins and contract ex vivo upon exposure to endothelin-1 (ET) (previously presented at the SPP meetings)[video clip]. Contraction is mediated via ET receptors A and B (ETA, ETB). In vascular smooth muscle ETB, in variance from A, exerts also relaxation through activation of nitric oxide synthase (NOS) in the endothelium. Since rat EVasT express NOS we investigated its role in reaction to ET exposure.

**Materials and Methods:** Modified spiral artery rings devoid of smooth muscle cells were studied ex vivo for ET-induced contraction in the presence of [1] L-NAME, blocking NOS and thus representing the combined contractile effect of both receptors; [2] ETA antagonist (representing the combined contractile and relaxing effect of ET through ETB); [3] ETA antagonist and L-NAME (representing the isolated contractile effect mediated by ETB). Variance statistical analysis was performed using 2-way ANOVA for repeated measures.

**Results:** Functional experiments revealed that addition of 10⁻⁷M ET and ET+L-NAME ex vivo reduced vascular lumen cross section area by 8.4% 1.4%, and 9.3±1.3%, respectively, p<0.002, compared with control. This effect was reduced to only 2.5±0.5 % in the presence of ETA antagonist, and to 5.4±0.9% by ETA+L-NAME antagonist, p<0.001.

**Conclusions:** EVasT of the rat remodeled spiral artery react to ET exposure similar to smooth muscle: contract via receptors A and B and relax via receptor B through NOS activation. We suggest that this phenomenon may play a role in situations of dysregulation of the vasoactive systems.

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**B24 Adherent Basal Plate Myometrial Fibers (BPMYO) In The Delivered Placenta: A Risk Factor For Development of Subsequent Placenta Accreta.**

Rebecca L. Linn, MD Department of Pathology, Emily Miller, MD Department of Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL, K. Grace Lim, MD Department of Anesthesiology, University of Pittsburgh School of Medicine, Pittsburgh, PA., Linda M. Ernst, MD, MHS Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL.

**Aim:** To determine if the presence of BPMYO in a previous placenta is associated with the development of subsequent placenta accreta; an important cause of massive obstetrical hemorrhage and post-partum hysterectomy.
Methods: Case-Control design: 49 Cases = patients with clinical suspicion or pathologic diagnosis of placenta accreta between Jan 2008 and Sept 2013 and a previous placenta submitted to pathology. 103 Controls = patients without accreta and a previous placenta submitted to pathology. By review of H&E slides, accreta stage and quantity of BPMYO were determined. The stages were defined as follows: Stage 0: no BPMYO; Stage 1: BPMYO with intervening decidua; Stage 2: ≤ 2 layers of decidual cells separating myometrium from villi or fibrin; Stage 3: diagnostic accreta; Stage 4: placenta increta; Stage 5: placenta percreta.

Results: Previous placentas showed diagnostic accreta (Stage 3) in 10.2% of cases versus 0% of controls (P<0.0001), findings suspicious for accreta (Stage 2) in 24.5% of cases versus 2% of controls (P<0.0001) and BPMYO (Stage 1) in 42.9% of cases versus 32% of controls (P<0.0001). Accreta stage increased from previous placenta to subsequent placenta in 75.5% of cases versus 10% of controls (P<0.0001). The average total length of BPMYO was 2.46±3.27mm in cases versus 0.40±0.82mm in controls (P<0.0001) and the average number of foci of BPMYO was 2.86±2.82 in cases versus 0.83±1.34 in controls (<0.001).

Conclusions: Accreta stage (stage 2-3) and high volume of BPMYO in a delivered placenta are risk factors for development of accreta in a subsequent pregnancy.

Eosinophilic/T-cell Chorionic Vasculitis (ETCV) Involves Fetal Inflammatory Cells by Fluorescent in Situ Hybridization (FISH)

Philip J. Katzman, MD1, LiQiong Li2, Nancy Wang, PhD2

Divisions of 1Surgical Pathology and 2Cytogenetics, Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, 601 Elmwood Avenue, Box 626, Rochester, New York 14642, USA

Background: ETCV is an inflammatory lesion of placental fetal vessels. In contrast to acute chorionic vasculitis, inflammation in ETCV is seen in the chorionic vessel wall opposite the amnionic surface. It is unknown whether inflammation in ETCV is maternal cells from the intervillous space, or fetal cells migrating from the vessel. We used FISH to identify fetal versus maternal cells in ETCV.

Methods: Placentas with ETCV that were previously identified for a published study were used for this study. Infant sex in each case was identified using electronic medical record. In cases of male infants, archived tissue blocks from the pregnancies involving ETCV were cut at 3 microns onto PLUS slides and stained with fluorescent X and Y chromosome centromeric probes. A consecutive H+E stained section was used for correlation. FISH analysis was performed on 400 interphase nuclei at the site of ETCV to determine the proportion of XX, XY, X, and Y cells.

Results: In 31 ETCV cases there were 20 females, 10 males, and 1 sex not recorded. 6 of 10 cases with male infants had recuts with visible ETCV. In these 6 cases the average percentages (and ranges) of XY cells, X only cells, Y only cells in the region of inflammation were 81 (70-90), 11 (6-17), and 8 (2-14), respectively.

Conclusions: There was a 2:1 female: male infant ratio in ETCV. Similar to acute chorionic vasculitis, the inflammation in ETCV is of fetal origin. It is still unknown, however, whether the stimulus for ETCV is of fetal or maternal origin.
Placental findings in late-onset small for gestational age births without Doppler signs of placental insufficiency and their impact in neurodevelopmental outcome

Alfons Nadal¹, Miguel Parra-Saavedra²,³, Francesca Crovetto²,⁴, Stefania Triunfo²,⁵, Stefan Savchev², Anna Peguero², Eduard Gratacós², Francesc Figueras²

¹Department of Pathology, Hospital Clinic-IDIBAPS, University of Barcelona, Barcelona, Spain, ²Department of Maternal-Fetal Medicine, Institute Clinic of Gynecology, Obstetrics and Neonatology (ICGON), Hospital Clinic-IDIBAPS, University of Barcelona and Centre for Biomedical Research on Rare Diseases (CIBER-ER), Barcelona, Spain, ³Maternal- Fetal Unit, CEDIFETAL, Centro de Diagnóstico de Ultrasonido e Imágenes, CEDIUL, Barranquilla, Colombia, ⁴Fondazione Cà Granda, Ospedale Maggiore Policlinico, Dipartimento Ostetricia e Ginecologia; Università degli Studi di Milano, Milan, Italy, ⁵Department of Obstetrics and Gynecology, Catholic University of the Sacred Heart, Rome, Italy

Aims: To evaluate if histopathologic placental analysis could provide additional information reflecting latent insufficiency in uteroplacental blood supply in late-onset small for gestational age (SGA) in absence of doppler signs of placental insufficiency, and to evaluate also the 2-year neurodevelopmental outcomes in SGA babies segregated by presence or absence of lesions reflecting placental underperfusion (PUP).

Materials and methods: A series of placentas were evaluated from singleton pregnancies of SGA births (birth weight below the 10th percentile) delivered after 34 weeks with normal umbilical artery Doppler (pulsatility index below the 95th percentile), that were matched by gestational age with adequate-for-gestational age (AGA) controls. Placental lesions were classified histologically according to Redline’s classification as maternal underperfusion, fetal underperfusion or inflammation. Neurodevelopmental outcomes at 24 months (age-corrected) were evaluated, applying the Bayley Scale for Infant and Toddler Development, Third Edition to assess cognitive, language, and motor competencies. The impact of PUP on each domain was gauged via analysis of covariance and logistic regression.

Results: A total of 284 placentas were evaluated (142 SGA and 142 AGA). Placentas were smaller and had more lesions, mainly reflecting maternal underperfusion in the SGA group. Neurodevelopmental outcomes were significantly poorer among the 46 babies born with PUP compared with 37 SGA births without PUP for the three domains of the Bayley scale.

Conclusions: In a substantial fraction of near-term SGA babies without Doppler evidence of placental insufficiency, histologic changes compatible with PUP are still identifiable. These infants are at greater risk of abnormal neurodevelopmental outcomes at 2 years of age.
Friday 5th September
15:00-16:00

John Emery Lecture
(See Separate CD Rom)
**A13 Immunohistochemical Profile of MYC Protein in Pediatric Small Round Blue Cell Tumors**

Karen M Chisholm MD, PhD (1), Chandra Krishnan MD (2), Amy Hereema-McKenney MD (3), Yasodha Natkunam MD, PhD (1)

(1) Stanford University School of Medicine, Department of Pathology, Stanford, CA, United States, (2) Dell Children’s Medical Center, Department of Pathology, Austin, TX, United States, (3) Pathology and Laboratory Medicine Institute Cleveland Clinic Cleveland, OH, United States

**Aim of study:** Deregulation of MYC oncoprotein in cancers can result from multiple oncogenic mechanisms. Although MYC translocations define Burkitt lymphoma and MYC protein expression is a poor prognostic factor in undifferentiated neuroblastomas, the distribution of MYC protein across other pediatric small round blue cell tumors (SRBCT) has not been well characterized. We undertook this study to assess MYC protein expression in a large cohort of pediatric lymphomas, sarcomas, and other SRBCT.

**Methods and Materials:** Tissue microarrays (TMA) containing 208 SRBCT were evaluated by immunohistochemistry (IHC) using anti-MYC clone Y69 (Epitomics). Nuclear positivity was scored as 0, 1-25, 26-50, 51-75, or 76-100%.

**Results:** A high level of MYC protein staining (>50% of lesional cells) was seen in Burkitt (12/20), B lymphoblastic (2/4), and T lymphoblastic (3/9) lymphomas, as well as rhabdomyosarcoma (22/84), Ewing sarcoma (3/9), and soft tissue sarcoma, not otherwise specified (1/4). Only occasional cases of neuroblastoma (5/36) showed >50% staining. Wilms tumors (11), synovial sarcoma (1), and
desmoplastic small round blue cell tumor (1) had <50% staining. Recurrences and metastases most often had the same MYC staining levels as the original tumor (17/29).

**Conclusion:** MYC protein exhibited variable expression across pediatric SRBCT and while lymphomas were most often positive, a significant proportion of rhabdomyosarcomas and Ewing sarcomas also showed staining. Overall, these findings provide a baseline for MYC expression and suggest the need for caution in the interpretation of MYC IHC in the differential diagnosis of these pediatric SRBCT. Given that MYC protein expression in undifferentiated neuroblastomas signifies a poor prognosis, further study is warranted to explore if a similar prognostic effect exists in other pediatric tumors.

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**A14** Persistent Hyperinsulinemic Hypoglycemia of Infancy (PHHI): Morphoproteomics Defines the Biology of the Focal Lesion and Provides Therapeutic Options

Robert E. Brown¹, Senthil Senniappan², Khalid Hussain², and Nina Tatevian¹.

¹Department of Pathology, University of Texas Medical School at Houston, Houston, TX 77030.  
²Developmental Endocrinology Research Group, Clinical and Molecular Genetics Unit, Institute of Child Health University College London, 30 Guilford Street, London WC1N 1EH and Department of Paediatric Endocrinology, Great Ormond Street Hospital for Children, London, WC1N 3JH

**Background:** PHHI or congenital hyperinsulinism (CHI) exists in a diffuse form or as a focal lesion in pancreas. Until recently, near total pancreatectomy was required to treat the diffuse form but morphoproteomics on such cases led to the successful application of sirolimus in four such patients thereby avoiding surgery (Senniappan, et al. N. Engl. J. Med. 2014;370:1131-7). The aim of this study was to apply morphoproteomics to tissue from the focal lesion in PHHI in an effort to define its biology and to uncover potential medical therapies.

**Materials and Methods:** Pancreas from 2 patients with paternal ABCC8 and the focal form of PHHI were studied.

Immunohistochemical probes applied to detect the expression and signal intensity of phospholipase D1 (PLD1); phosphorylated (p)-mammalian target of rapamycin (mTOR[Ser2448]); insulin; total insulin-like growth factor-1 receptor (IGF-1R[Tyr1165/1166]); p-Akt (Ser473).

**Results:** PLD1 expression in islet cells (2+) and duct cells (3+) but with essential absence from the acinar cells; 2. plasmalemmal expression of p-mTOR on intercalated duct/centroacinar cells (3+) and acinar cells (1-2+) but with nuclear translocation in islet cells; 3. correlative overexpression of total IGF-1R/nuclear p-mTOR and p-Akt in the islet cells consistent with IGF-1R/mTORC2/Akt pathway signaling.

**Conclusion:** mTORC1 pathway in acinar and intercalated duct/centroacinar cells of focal lesions raise the possibility of medical therapies with rapamycin/sirolimus or metformin. Paternal ABCC8 coincides with PLD1 expression in favoring excessive Ca++ influx and therapeutic option of calcium channel blocker with metformin. Constitutive activation of IGF-1R/mTORC2/Akt pathway raises the possibility of using metformin in the focal lesion of PHHI, as a potential alternative to surgery.
A15 Therapy-related Lymphoblastic Neoplasms: Report of Two cases and Comparison with Clinicopathologic Features of Therapy-related Myeloid Neoplasms

Jared Shows, MD, University of Colorado School of Medicine; Megan K. Dishop, MD; Csaba Galambos, MD, PhD; Mark A. Lovell, MD; Heidi Cho, MD; Brian Greffe, MD; Joanne Hilden, MD; Xiayuan Liang, MD, Children’s Hospital Colorado, University of Colorado School of Medicine;

Aim of Study: Secondary leukemia is a complication of chemotherapy or radiation therapy. Most are myeloid neoplasms (therapy related[t]-AML/MDS), an entity recognized by the WHO classification. Due to its rarity, t-ALL/lymphoblastic lymphoma (t-ALL/LBL) is not yet recognized by the WHO, and its clinicopathologic and genetic features have not been fully investigated. We report 2 cases of t-ALL/LBL and perform a 30-year literature review to analyze the characteristics of t-ALL/LBL and t-AML/MDS.

Materials and Methods: Patients ≤ 21 years old with t-ALL/LBL were identified from a search at our institution (1995-2014) and the literature (1984-2014). A large study of pediatric t-AML/MDS from MD Anderson Cancer Center (JPHO, 2009) was selected for comparison.

Results:

<table>
<thead>
<tr>
<th>Case/ Age/Sex</th>
<th>Primary tumors/Treatment</th>
<th>Latent period</th>
<th>Secondary tumor/ Genetics/Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/16y/M</td>
<td>Osteosarcoma/Topoisomerase II inhibitor</td>
<td>17months</td>
<td>B-ALL/MLL/alive</td>
</tr>
<tr>
<td>2/3y/F</td>
<td>Ewing sarcoma/Alkylating agent</td>
<td>37months</td>
<td>T-LBL/MLL/alive</td>
</tr>
</tbody>
</table>

| # of Cases | 23 | 22 |
| Mean age at secondary tumor | 12.22y | 12.68y |
| M:F | 14:7 | 15:7 |

Genetics

- MLL: 12/16(75%) 5/22(23%) 0.0026
- Monosomy 7: 1/16(6%) 10/22(45%) 0.0115
- Alkylating Agent: 58months 54months

Latent period (mean)

- Topoisomerase II inhibitor: 32months 49months
- Alkylating Agent: 58months 54months

Mortality

- 10/17(59%) 13/15(87%)

Conclusions:

1. The genetic associations between t-ALL/LBL (MLL+) and t-AML/MDS (Monosomy 7) are different.
2. The latent period following topoisomerase II inhibitor therapy in t-ALL/LBL is shorter than t-AML/MDS, suggesting lymphoid cells may be more vulnerable to topoisomerase II inhibitors than myeloid cells.
3. The mortality rate for t-ALL/LBL is lower than t-AML/MDS, suggesting a more favorable biology of lymphoid neoplasms.
A16  An evidence-based recommendation for a standardized approach to detecting metastatic neuroblastoma in staging bone marrow biopsies

LN Parsons1, G Gheorghe1,2, JA Jarzemkowski1,2
Department of Pathology, 1Medical College of Wisconsin and 2Children’s Hospital of Wisconsin, Milwaukee, WI, USA

Aim of Study: Neuroblastoma is a common malignant tumor of childhood. Accurate bone marrow (BM) evaluation for metastatic tumor is essential; however, no standard pathologic workup exists for staging BMs. We examined the diagnostic yield of various BM components and optimal core biopsy (CB) length as part of developing an evidence-based recommendation for BM evaluation.

Materials and Methods: After obtaining IRB approval, 160 BM biopsies from 51 neuroblastoma patients were retrospectively selected. H&E-stained CB, aspirates, and clot sections were recorded as positive, negative, or indeterminate. Total/trabecular CB length were measured using cellSens software and a DP71 camera (Olympus). Intraspecimen correlation and correlation between core interpretation and length (two-sided Student’s t-test) were calculated.

Results: 77/160 BMs were positive for tumor in any component. Of these, 38 (49.3%) were positive in a single portion of the specimen: 22 CB(s), 13 aspirate, and 3 clot. Two were positive in the aspirate and clot. Compared with overall diagnosis, sensitivities were as follows: CBs 87%; aspirate 82.6%; clots 82.9%; cores/aspirates combined 98.2%. Mean total CBs were longer for diagnostic CBs (9.10 mm and 6.84 mm) than indeterminate CBs (6.37 mm and 2.42 mm, p < 0.0006). Positive CBs had longer trabecular space than negative marrows (7.77 mm versus 6.43 mm, p< 0.01).

Conclusions: Nearly 50% of our positive specimens showed diagnostic discordance among the various components examined. However, combining CB and aspirate examination improved sensitivity for tumor detection. We therefore recommend bilateral CBs (>1 cm each) and aspirates for optimal evaluation of BM for metastatic neuroblastoma.

A17  CD43 Expression in Myeloid/Monocytic, Langerhan’s, and Dendritic Histiocytic Cell Tumors with A Diminished Immune Reactivity Pattern in Pediatric Patients

Michelle L McCaw, DO1,2, Xiayuan Liang, MD1,2
1Children’s Hospital Colorado, 2University of Colorado Denver School of Medicine

Background/Aim: CD43 is a useful marker for diagnosing myeloid/monocytic sarcoma (MS). Myeloid/monocytic precursors and Langerhan cells are derived from the common stem cell in bone marrow, but they undergo separate differentiation pathways and develop different functions. Langerhan cells migrate into dermis and mature into dendritic cells. Myeloid/monocytic precursors mature into monocytes which become macrophages. MS, Langerhan cell histiocytosis (LCH), and dendritic histiocytic lesions, such as juvenile xanthogranuloma (JXG) can overlap in their morphology. It is unclear if CD43 can be used to distinguish these entities. We evaluated how different CD43 expression is in MS, LCH, and JXG.

Materials/Methods: 53 cases (age ≤ 21 years) of MS, LCH, and JXG at our institution were evaluated for CD43 expression by immunostaining. Positive CD43 staining is categorized as: + (<25% positive cells), ++ (25-50% positive cells), +++ (50-75% positive cells), and ++++ (>75% positive cells). The difference of CD43 positivity is compared among each group.
Results:

<table>
<thead>
<tr>
<th>Case #</th>
<th>CD43</th>
<th>MS (Group 1)</th>
<th>LCH (Group 2)</th>
<th>JXG (Group 3)</th>
<th>p (G1 vs G2)</th>
<th>p (G1 vs G3)</th>
<th>p (G2 vs G3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>2/20 (10%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>2/21 (9.5%)</td>
<td>2/20 (10%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+++</td>
<td>3/21 (14.3%)</td>
<td>1/20 (5%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>++++</td>
<td>12/12 (100%)</td>
<td>9/21 (42.9%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total positive cases: 12/12 (100%) 14/21 (66.7%) 5/20 (25%) 0.0319 0.0001 0.0122

Conclusions: CD43 is expressed in both myeloid/monocytic and histiocytic lesions. The pattern in frequency and intensity of CD43 expression from strong to weak is MS > LCH > JXG, suggesting that CD43 expression may be down-regulated as myeloid/monocytic and histiocytic cells undergo progressive maturation along their development pathways.

A18 An updated biomarker study of embryonal (undifferentiated) sarcoma of the liver.

Amy Coffey, MD, Rosanna Abellar, MD, Helen Remotti, MD
New York Presbyterian Hospital Columbia University Medical Center in New York, NY.

Introduction
Embryonal (undifferentiated) sarcoma of the liver (ES) is a rare, aggressive pediatric malignancy. The immunohistochemical profile of these tumors is said to be nonspecific and variable; however, many new useful liver markers have become available in recent years that have not been systematically evaluated in these tumors. The aim of this study is to better define the immunoprofile of ES.

Methods
Following IRB approval, four cases of ES in patients 0 to 18 years were identified in our intradepartmental cross-files within the last 20 years. A fifth case of small cell hepatoblastoma that was originally diagnosed as ES was included. A tissue microarray (TMA) was made from selected paraffin-embedded archival tissue of tumor/normal pairs with appropriate controls. Selected immunostains were performed on the TMA (see Table 1).

Results
The TMA results are shown in Table 1. The following biomarkers showed consistent immunoreactivity in all cases of ES: vimentin, glypican-3, and p16. ES cases also showed consistent loss of e-cadherin, arginase-1, and hepatocyte. Variable immunoreactivity was seen for cyclin D1, HSP70, bcl-2, p53, and MCK. Ki67 indices were generally high, ranging from 40 to 90%.

<table>
<thead>
<tr>
<th>Case</th>
<th>Vimentin</th>
<th>p16</th>
<th>GPC3</th>
<th>HSP70</th>
<th>GS</th>
<th>Arg1</th>
<th>Hepa</th>
<th>CyclinD1</th>
<th>Bcl2</th>
<th>βcatenin</th>
<th>Ecad</th>
<th>AFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-3+</td>
<td>2+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2+</td>
<td>1+m</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3+</td>
<td>2+</td>
<td>2-3+</td>
<td>0</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1+m</td>
<td>0-1+</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2+</td>
<td>3+</td>
<td>1-2+</td>
<td>0-2+</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>fw</td>
<td>1-2+</td>
<td>2+m</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>3+</td>
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<td>1+</td>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>1-2+</td>
<td>1-2+m</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>5</td>
<td>1-3+</td>
<td>0-3+</td>
<td>0-1+</td>
<td>f1+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>fw</td>
<td>0</td>
<td>0</td>
<td>0-2+</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: Case 5 is small cell hepatoblastoma

m = membranous, f = focal, w = weak
Conclusions
This study shows a variable and nonspecific immunoprofile for ES, with some caveats. The case of small cell hepatoblastoma demonstrated a different immunoprofile from ES cases, but did not stain quite as expected for hepatoblastoma.

A19 Does Increased Chimerism in Allograft Cardiac Transplants Decrease Longevity? Utilizing Quantitative Real Time PCR in Pediatric Patients
Shri Deshpande¹, Phillip C. Quigley², Caitlin A. Cundiff³, Tiffany K. Roberts², Bahig M. Shehata³.
¹Children’s Healthcare of Atlanta and Emory University School of Medicine, Department of Pediatric Cardiology. ²Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA 30322. ³Children’s Healthcare of Atlanta and Emory University, Pathology Department, Atlanta, GA, USA

Aim of Study: Previous studies examined chimerism in sex-mismatched donor/recipient pairs using the Y-chromosome probe, which is limited in its sensitivity and applicability. The first goal of this study was to evaluate a quantitative real time PCR (qRT-PCR) assay for chimerism assessment, which is less expensive and complex than methods previously described. However, cardiac allografts are known to undergo repopulation by host cells. Although repopulation is thought to occur at relatively low levels, it can potentially be increased in cases of rejection. The second goal of this study was to determine the significance of cellular repopulation through assessment of chimerism in 15 failed pediatric cardiac allografts compared to 15 biopsies from matched controls without rejection.

Methods: A qRT-PCR assay (Allele-SEQR, Celera Corp.) using bi-allelic insertion/deletion (indel) polymorphisms was used to assess chimerism in 15 pediatric explanted cardiac allografts and 15 biopsies from stable transplant patients. Pre-transplant DNA from both the recipient and the donor were screened to identify informative markers. DNA isolated from explanted allograft tissue was then compared to pre-transplant DNA of the recipient to determine the percentage of chimerism within the allograft.

Results: Chimerism was increased in the explanted allografts (up to 15%) compared to the stable control group (<2%).

Conclusion: Increased allograft repopulation by host cells (chimerism) is potentially a marker of cellular injury and may represent a risk factor for graft survival.

A20 Congenital Melanocytic Nevi (CMN) And Neurocutaneous Melanocytosis (NCM) Show Heterogeneous Mutations of Codon 61 of NRAS.
Cláudia M. Salgado¹, Dipanjan Basu¹, Marina Nikiforova³, Bruce S. Bauer, Donald Johnson, Veronica Rundell, Lorelei J. Gunwaldt², Miguel Reyes-Múgica¹. From the Departments of Pathology¹ and Plastic Surgery², Children’s Hospital of Pittsburgh; Division of Molecular Genomic Pathology³, University of Pittsburgh School of Medicine, Pittsburgh, PA, and Division of Plastic and Reconstructive Surgery, NorthShore University HealthSystem4, Northbrook, IL, USA.

Background: NRAS and BRAF mutations have been associated with CMN, but results have been contradictory. Aim: To determine the prevalence of NRAS Q61 mutation in a large cohort of CMN and NCM specimens; and to correlate mutation status with clinical features.
Methods: Prospectively collected melanocytic lesions (frozen tissue) from 54 patients with CMN (32 giant, 18 large, 3 medium and one without size categorization) were evaluated for NRAS mutations by Next Generation sequencing. Clinical data were collected using standardized questionnaire. NCM diagnosis was established with MRI, biopsy/autopsy findings, and considered negative if MRI was normal.

Results: Mutations in the codon 61 of NRAS were detected in 43 (79.6%) cases: p.Q61R,c.182A>G in 11 cases; p.Q61K,c.181C>A in 31 cases; and one case with two nucleotide substitutions p.Q61K,c.181C>A,c.183. Mutation was present in 28 (87.5%) giant CMN, 9 (61.1%) large CMN, and 3 (100%) medium CMN (p=0.058). There are no differences in age, gender, nevus number and size when we compared NRAS positive and negative cases. NRAS mutations were present in 28 (71.8%) Caucasian, 12 (100%) Asian, and 2 (100%) native American Indian patients (p=0.083). Nine patients were diagnosed with NCM; Five (45.5%) of the 11 cases with p.Q61R,c.182A>G mutation, and 2/22 (9.0%) with Q61K,c.181C>A mutation have NCM (p=0.072). The patient with two nucleotide substitutions (Q61K,c.181C>A,c.183), and one patient negative for NRAS mutation also had NCM.

Conclusions: NRAS mutations in CMN are highly prevalent but show marked heterogeneity and no correlation between NRAS status and clinical features. NCM patients show a trend to harboring p.Q61R,c.182A>G but other mutations could also be present.

A21 Does MAP2 Immunostain Have Diagnostic or Prognostic Value in the Evaluation of Ovarian Teratomas Associated with Pediatric Anti-N-Methyl-D-Aspartate (Anti-NMDAR) Encephalitis?

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Aim of Study: Anti-NMDAR encephalitis is a potentially fatal neurological syndrome in which patients present with a spectrum of CNS deficits. Sixty-percent of these cases can be attributed to the presence of tumors, most often ovarian teratomas. Previously we reported seven pediatric patients who presented with neurological deficits associated with the presence of such tumors. It was proposed that MAP2 immunostain could be used as a diagnostic and prognostic marker for anti-NMDAR encephalitis. This study evaluates the ability of two neuronal markers (MAP2 and NeuN) to differentiate between ovarian teratomas associated with encephalitis and neurologically symptom-free tumors and test their prognostic significance.

Materials and Methods: After obtaining IRB approval, we tested fourteen samples (seven study and seven aged matched control) for the presence of MAP2 and NeuN positive cells. Additional panel of immunostains, including S-100, and GFAP was also applied to the same cohort. Two pathologist reviewed the histological and immunohistochemical findings independently.

Results: No qualitative differences were seen in neuronal markers MAP2, NeuN, S-100, and GFAP between the study cases and control group by histology (H&E) or immunostains.

Conclusions: MAP2 and other neurological markers play no role in predicting development of anti-NMDAR encephalitis. Since no qualitative differences were identified between the study cases and the control group, CSF remains the best method for diagnosis and prediction of anti-NMDAR encephalitis. Tumor banking of ovarian teratomas and CSF from patients with anti-NMDAR encephalitis with further molecular analysis may further explain the etiopathogenesis of this condition.
Significance of mild isolated increase in intraepithelial lymphocytes in pediatric duodenal biopsies

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Background: Isolated increase in duodenal intraepithelial lymphocytes (IEL) is a non-specific alteration reported in celiac disease (CD), drug-induced damage, Helicobacter pylori (Hp) infection, inflammatory bowel disease (IBD). This study aimed to determine the association between this histologic finding and various gastro-intestinal diseases in children.

Study design: We identified all duodenal biopsies from 2010 to 2013 showing mild increase in IEL (20 to 40 IEL per 100 enterocytes) without significant villous blunting or epithelial damage. The celiac panel (abnormal if any of the specific antibodies was elevated), as well as the presence of other gastrointestinal histologic abnormalities were documented for these patients. The frequency of celiac disease or other digestive disorders was determined.

Results: The study included 347 patients, of which 166 underwent celiac serologic testing and 59 had elevated antibodies. However, the panel was performed following biopsy (suggesting that the increased IEL led to testing) in only 40 instances, and was abnormal in 4 of these cases (10%). From the 288 patients without serologic evidence of CD or with low clinical suspicion for CD to warrant further work-up, 67 (23.25 %) had active/inactive Hp negative gastritis, 43 (15 %) had IBD, 33 (11.5%) eosinophilic esophagitis/gastritis (EE/EG), 19 (6.5%) chemical gastritis, 18 (6.25 %) gastro-esophageal reflux and 10 (3.5%) Hp gastritis.

Conclusions: The incidence of CD in children with mild isolated increase in duodenal IEL is very low. In many cases, this histologic finding can be explained by etiologies other than CD, the most common being gastritis, IBD and EE/EG.

The High Multiplicity Of Prenatal Nevi In Adolescents And Adults As Further Evidence Of Upward Migration Of Nevus Cells.

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Aim: Congenital melanocytic nevi (CMN) are nevi identified at birth, and occur in about 1% of newborns, with multiplicity in about 3% of cases. Prenatal nevi are defined as melanocytic nevi developed in utero, diagnosed using developmental histology criteria, regardless of age of presentation. We aimed to demonstrate that multiplicity of nevus supports an upward pattern of nevus cell migration during prenatal nevogenesis.

Methods: H&E and Melan-A stains were used to assess morphology and immunophenotype in 354 patients with prenatal nevi. There were three groups: 284 adults (age 20 to 79); 57 adolescents (age 11 to 19); and thirteen children (age 2 to 10). The number of nevi submitted and multiplicity rate of prenatal nevi were calculated.

Results: Multiple prenatal nevi were present in 102 (36%) adults, 22 (39 %) adolescents and only 3 (23%) children (2 nevi each). Multiplicity of prenatal nevi ranged from 2 to 9 in adults and adolescents.
Conclusion: Our series of 354 patients showed that >30% of both adolescents and adults had multiple prenatal nevi, a strikingly higher rate of multiplicity compared to CMN in newborns, which may reflect origin beneath the epidermis, with many prenatal nevi working their way up to the surface of the skin decades after birth. Further studies to assess other potential factors leading to multiplicity (i.e. UV damage, genetic background, etc.) are needed to better understand prenatal nevi in adults.

A24 Two Cases of Familial Wilms Tumor Reveal DICER1 and FTW1 Gene Mutation

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Aim of Study: Wilms tumor (WT) is the second most common childhood solid tumor. The familial tendency of WT is less than 1%. Two familial WT genes have been identified, FWT1 at 17q12-q21 and FWT2 at 19q13.4, however not all familial WT cases have been linked to these genes suggesting the heterogeneous nature of familial WT. Two families were identified in our institution, which prompted our investigation.

Materials and Methods: Upon IRB approval, we searched our files for familial tendency in WT. Blood was collected from the affected children and their mothers. DNA was extracted. Whole Exome Sequencing was performed using the Illumina HiSeq2000 platform. The data was analyzed using Samtools, open source VarScan and SeattleSeq software’s to annotate variants.

Results: A family was identified from our institution and a second was contributed from another institution. The first is an African-American family who had 7 children from 2 fathers, 3 of which developed WT. Sequencing of this family revealed DICER1 gene mutation in the mother and the 3 affected children. The second family had a mother diagnosed with WT at age 3.5. 30 years later, her 4-year-old daughter presented with hematuria and a renal mass was identified, resected and showed WT. Sequencing of this family revealed FWT1 gene mutation in the mother and daughter.

Conclusions: DICER1 gene syndrome is associated with pluropulmonary blastoma, cystic nephroma and Sertoli-Leydig tumors. These results are suggestive of a correlation with familial WT. Further analysis is recommended to identify other causal mutations associated with familial WT.

A25 Comparison of Intraoperative Interpretations in Pathology Reports and Operative Reports in a Large Pediatric Institution

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Aim of Study: To compare pathologists’ intraoperative diagnoses with surgeons’ interpretations in a large pediatric hospital.

Methods: Intraoperative pathology reports in a 12-month period were compared with surgeons’ interpretation in operative reports. Discrepancies were classified based upon the degree of clinical significance. Class I discrepancies occurred when the surgeon’s report was more or less specific than the pathologist’s diagnosis, but was unlikely to affect patient management. Class II discrepancies
occurred with reporting of descriptions or conclusions not present in the pathology report. Class III discrepancies involved dissimilar diagnoses, but without a change in benign versus malignant interpretation. Class IV discrepancies differed on the basis of benign versus malignant. Intraoperative pathology interpretations not documented in operative reports were labeled Class V.

**Results:** 297 diagnoses from 197 procedures with at least one intraoperative/intraprocedural pathology interpretation involving 185 patients in a 12 month period were reviewed. The patients ranged in age from 6 days to 19 years; 101 (54.6%) were male and 84 (45.4%) female. Discrepancies between the pathologist’s intraoperative diagnosis and the operative report were found in 168 (56.6%) instances. Class I discrepancies were noted in 101 (34.0%), Class II in 7 (2.4%), Class III in 2 (0.7%), and Class IV in 5 (1.7%) instances. In 53 (17.9%) intraoperative consultations, there was no record of the diagnosis in the operative report (Class V).

**Conclusion:** Discrepancies between pathologists’ interpretations and surgeons’ interpretations are frequent and demonstrate a need for better interdisciplinary communication.

**A26 Disorders of sex development, Gonadal Dysgenesis, Dysplasia: histological assessment of gonadal samples and pathological approach to the tumoral risk**

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**Aim:** to study the specific pathological features of gonads encountered in Disorders of Sex Development (DSD), their prevalence in several aetiologic groups (based on the Consensus statement on management of DSD, Chicago), clarify the nosology of histological features, and evaluate the tumoral risk in case of a conservative approach.

**Material and methods:** 175 samples issued from 86 patients with various forms of DSD were analyzed. We established a strictly pathological classification based on a reading template. We excluded the term of “gonadal dysgenesis” and introduced the concept of dysplasia to describe the architectural disorganization of the gonad. We define dysplasia as the morphological reflection of dysgenesis (abnormality of gonadal embryonic development).

**Results:** We distinguish five histological types of gonads: normal gonad, hypoplastic testis, dysplastic testis, streak, and ovotestis. Dysplasia and UGT are only seen in some aetiologies (patients with chromosomal or genic abnormalities), and never in DSD with hormonal deficiencies. Our data show an association between the presence of undifferentiated gonadal tissue (UGT), recently identified by Cools et al. as the precursor of gonadoblastoma, and dysplasia. We assume that UGT belongs to the spectrum of dysplasia, as its more severe form. Our study also enlightens some immunohistochemical characteristics of dysplasia, as well as the abnormal expression of immature germ cells markers in dysplastic areas.

**Conclusion:** Dysplasia, as well as UGT, should be considered as a potential neoplastic lesion. Pathological examination and immunohistochemical staining provide information about the tumoral risk on the surgical biopsy performed during the surveillance of patients with DSD.
A27 IgG4 sclerosing cholangitis in a child

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Aim: Report the first case of IgG4 sclerosing cholangitis in a child.

Materials and Methods: The clinical, laboratory, histologic and imaging studies are reviewed.

Results: A 16 year-old female underwent ultrasound-guided needle biopsy of the liver after she presented with a history of 8 kg weight loss and right upper quadrant pain. Her liver function tests were persistently elevated with peak values of: AST/SGOT 50 IU/L (normal 14-37), ALT 71 IU/L (normal 8-29), alkaline phosphatase 221 IU/L (normal 34-104) and GGTP 235 IU/L (normal 8-23). Prior to the biopsy her total IgG level was 2550 mg/dL (normal 562-1585), but ANA, AMA, actin antibodies, anti liver kidney microsomal antibody and viral testing were all negative.

Sections from the biopsy demonstrated dense lymphoplasmacytic infiltrates within large septae and associated fibrosis, presence of complete obliteratorive phlebitis (highlighted by elastic stain) and limited interface activity. No significant lobular activity was present. There were >50 IgG4 positive plasma cells per HPF in these areas and >30 IgG4 positive cells in the obliterated venule.

Subsequently, the serum IgG4 level was found to be 277.4 mg/dL and an MRI of the abdomen with and without contrast demonstrated moderate intrahepatic and extrahepatic biliary dilation with diffuse mural thickening and enhancement along with regions of focal narrowing and longer segment narrowing involving the intrahepatic biliary tree.

Conclusion: Although IgG4 related disease typically occurs in adult patients, this disorder must be considered and worked up in children to guide appropriate therapy.

A28 Masson’s lesion: A reactive endothelial proliferation – A pediatric series

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Background: Described on in 1923 as an endothelial neoplasm, Intravascular Papillary Endothelial Hyperplasia (IPEH) involves medium size vessels, is mostly intravascularly or rarely occurs in extravascular hematomas. Discriminating IPEH from true neoplasms is sometimes challenging.

Material and methods: After IRB exemption, we retrospectively searched our files in a 15 year period. Histology and immunohistochemistry (IHC) including CD31, CD34, Glut-1, D2-40, smooth muscle actin (SMA), muscle actin (HHF35), vWF, Prox-1 and Ki-67 were reviewed. Location, size and history of trauma were assessed.

Results: Nine cases, 6 adequate for IHC, were identified. Age ranged from 5 months to 13 years (mean age =5.3 years). The M:F ratio was 4:5. Lesions involved the head and neck (4), trunk (2), distal extremities (2) and axilla (1). Their diameter ranged from 0.5 to 2.0 cm. History of trauma, interrogated in seven patients, was positive in five. Four cases featured associated venous and/or lymphatic malformations; 5 lesions arose in organizing thrombi or hematoma. CD31, CD34 and vWF stained the endothelium in 6/6 cases; Glut-1 was negative. SMA, HHF35 were positive in the walls in all cases. One case showed D2-40 staining, while Prox-1 was focally positive in 2. Proliferative index (PI) assessed in 6 cases ranged from 2 to 5%, with only 2 cases showing 10%.
**Conclusion:** IPEH is a reactive lesion, always associated with thrombosis or organizing hematoma. Differentiation from aggressive vascular tumors (Dabska tumor and others) is based on low PI, and lack or minimal presence of lymphatic markers. IPEH should be considered in the differential of superficial or deep vascular proliferations, and may be associated with a typical vascular malformation.

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**A29 Cardiac Manifestations of Rosai-Dorfman Disease: Report of a Pediatric Case and Review of Literature**

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**Aim of study:** Rosai-Dorfman disease (RDD) is uncommon, typically presenting as massive painless lymphadenopathy. Cardiac involvement is exceedingly rare and not well characterized. At our institution, we encountered this unique presentation of RDD, and sought to compare our experience with that represented in the literature.

**Methods and Materials:** H&E-stained sections and immunostains from the case of interest were reviewed, along with the patient medical record. A PubMed search for cases of RDD presenting with cardiac manifestations was performed and the relevant literature reviewed.

**Results:** A 3 year-old girl presented with chest pain and a 2.5 x 3 cm right ventricular wall mass. Histology revealed numerous abnormal histiocytes exhibiting emperipolesis, which were positive for S100, fascin, and CD163 and negative for CD1a. Patient was treated with prednisone, rituximab, and cyclophosphamide with subsequent reduction of the mass; three years later she remains asymptomatic.

Literature review yielded 13 case reports of cardiac extranodal RDD. Presenting signs included cardiac/valve dysfunction. In the 8 cases which had a premortem diagnosis of RDD, radiographically-identified masses of the heart or great vessels lead to diagnostic tissue biopsy. Microscopy in all cases showed a proliferation of histiocytes with rounded nuclei and abundant pale cytoplasm with mixed cellular infiltrate. All cases showed emperipoiesis and S100 positivity (when performed).

**Conclusion**
Extranodal cardiac RDD is rare, and may present as isolated or multiple cardiac lesions. Significant symptoms and hemodynamic compromise necessitate treatment. Corticosteroids and chemotherapy have limited success in mass size reduction, but may prevent further progression of the disease, as with our patient.

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**A30 Correlation between p16 and p63 expression and Human Papilloma Virus infection in juvenile laryngeal papillomatosis**

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Juvenile laryngeal papillomatosis (JLP) is benign, low risk Human Papilloma Virus (HPV) linked. JLP evolution is unpredictable, from spontaneous remission to obstructive life threatening tumor. We determined immunohistochemical status of p16 and p63 and HPV status by chromogenic in situ hybridization (CISH) in 20 JLP patients (38 biopsies) in order to determine prognostic value of these data.
Patients were 1 to 21 (mean = 6) with sex ratio 9M/11F. We observed no dysplasia in 11 patients, 4 mild dysplasia, 4 moderate dysplasia and 1 severe dysplasia. Recurrences were noted whatever the degree of dysplasia. Every multifocal JLP recurred (n=7). Degree of dysplasia remained constant over recurrences.

CISH showed no HPV in 4 biopsies (without dysplasia), 15 low risk HPV (LRHPV; 7 without dysplasia, 4 mild dysplasia, 4 moderate dysplasia) and 1 high risk HPV (HRHPV) with severe dysplasia.
P16 positivity was noticed in the unique severe dysplasia biopsy. P63 was expressed in 2/3rd of the epithelium in 15 biopsies (6 without dysplasia, 5 mild dysplasia, 4 moderate dysplasia) and in the entire epithelium in 11 biopsies (3 without dysplasia, 3 mild dysplasia, 4 moderate dysplasia, 1 severe dysplasia). No HPV was identified in 6/7 biopsies with p63 expression restricted to basal third epithelium.

JLP are associated to LRHPV without or mild dysplasia. Degree of dysplasia seems stable over recurrences. HRHPV is extremely rare and here associated to severe dysplasia. The extension of p63 immunostaining seems linked to the degree of dysplasia. However p63 status cannot predict recurrences.
Saturday 6\textsuperscript{th} September
11:30-13:00

Slide Seminar
(See separate CD Rom)

Perinatal Pathology Plenary & Poster
Session 5

\textbf{B27} C4d deposition in placentas with histiocytic intervillositis

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\textbf{Aims of the Study:} Deposition of the complement split product C4d is a phenomenon extensively studied as a marker for complement activation in antibody mediated transplant rejection. C4d is also observed in placental disease processes including spontaneous abortion and villitis of unknown etiology. Chronic histiocytic intervillositis is a rare but significant placental abnormality which is associated with increased risk of growth restriction, fetal death, and recurrent fetal loss and is thought to have an immunologically mediated mechanism. In this study, we evaluated staining characteristics of C4d in placentae with chronic histiocytic intervillositis.

\textbf{Methods:} Placentas were divided into 4 groups, 15 intervillositis-complicated pregnancy, 16 intervillositis-uncomplicated, 20 villitis-uncomplicated, 11 villitis-uncomplicated, all with varying degrees of monocytic cells in the intervillous space. Representative blocks were immunohistochemically stained for C4d. The percentage of positive staining of the microvillous surface of the syncytiotrophoblast was scored by 5 pathologists and a consensus score was determined: 0 = 0-5\%, 1 = 5-25\%, 2 = 25-75\%, 3 = >75\%. Association between stain intensity and explanatory variables was estimated using proportional-odds logistic regression, adjusting for site

\textbf{Results:} C4d stain intensity did not correlate with pregnancy complications but did correlate with chronic intervillositis compared to villitis (Odds ratio 6.3, CI 2.1-18.7, p=0.001).

\textbf{Discussion:} C4d staining is evidence of fixation of complement due to humoral antibody directed to the microvillous surface of the placenta. This mechanism may in some way stimulate monocyte microvillous adhesion and monocyte to monocyte adhesion. We did not find a relationship of staining with stillbirth and/or fetal growth restriction.
**B28 Meconium Induced Myonecrosis on a background of Distal Villous Immaturity – a repeating pathological sequence in a proportion of late stillbirths?**

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**Aim:** Distal villous immaturity (DVI) is a placental disease characterised in part by inadequate vasculosyncytial membrane development. DVI has been associated with late stillbirth although the majority of pregnancies with DVI result in live infants. We investigated whether meconium-induced myonecrosis (MIM) of placental fetal vessels could be a significant compounding factor in late stillbirth associated with DVI.

**Materials and Methods:** A retrospective review was conducted of 95 consecutive perinatal post-mortems performed in our institution identifying cases of stillbirth associated with DVI. For comparison a control group of placentas was identified from sequential cases submitted for routine diagnostic analysis over a 3 month period where there was DVI at comparable gestational age but with live birth. The presence of MIM was documented in each group.

**Results:** Of 95 perinatal post-mortems 19 cases of stillbirth associated with DVI were identified and of these 6 had MIM. Of 136 sequential placentas from live-born infants 18 showed DVI but none of these DVI cases also showed MIM. This overrepresentation of MIM in the stillbirth group was statistically significant (Fisher’s exact test, p = 0.0197).

**Conclusions:** MIM is seen with increased frequency in stillbirths associated with DVI. On this basis we postulate that as these DVI complicated pregnancies approach term that fetal stress increases the likelihood of heavy meconium discharge. Resultant meconium associated vascular injury may then act as a “second hit” in a vulnerable pregnancy resulting in intrauterine fetal death. We suggest that further investigation of this potential pathological sequence is required.

**B29 Effect of Pregnancy Induced Hypertensive Disorders on Growth of Placenta Along Short and Long Axes and Neonatal Outcomes.**

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**Background:** To assess the effect of pregnancy induced hypertensive (PIH) disorders on the growth of the placenta on the short and long axes and neonatal outcomes.

**Methods:** A retrospective cohort study of gross and histological characteristics of placentas and the fetal outcomes of normotensive and hypertensive pregnancies over a 3 year period from January 2009 to December 2011 at a tertiary teaching hospital in ACT, Australia.

**Results:** Placentas and neonatal outcomes from 100 pregnancies complicated with PIH/preeclampsia were studied and compared with 51 gestational age matched placentas and neonatal outcomes from normotensive pregnancies. The median maternal age and smoking history were similar in the two groups (p=0.894; p=1.00 respectively). The median pre-pregnancy weight was significantly higher (p<0.001) and primiparity more common (p=0.001) in the study group. The median weight of the placenta was significantly lower (p<0.001) and below the 10\(^{th}\) centile (p<0.001) in the study group. Both the long and short axes of the placental disc were significantly smaller in the study...
group (p=0.002; p= <0.001 respectively). Accelerated villous maturation and placental infarcts were more common in the study group (p<0.001). The median birth weight and the number of infants with birth weight and length below the 10th centile were significantly higher in the study group (p=0.008; p<0.001; p= 0.004 respectively).

**Conclusion:** Evaluation of placenta diameters in the second trimester of women with a higher than average pre-pregnancy weight may help predict those primiparous women who may develop PIH and IUGR.

### B30 A 3 Case Report of Unusual Pediatric Cardiac Tumors

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**Children’s Healthcare of Atlanta and Emory University, Department of Pathology, Atlanta, GA, USA**

**Material and Methods:** Upon IRB approval, we searched our files for cardiac tumors from 2000 until 2014.

**Results:** 27 tumors were identified (19 benign, 6 malignant, and 2 metastatic), of which 3 were extremely rare. A 12-year-old male presented with chest pain and fever. CT scan identified an intracardiac mass originating from the interatrial septum extending into both atria, encircling the aorta. Needle biopsy showed Rosai Dorfman, which was confirmed by emperipolesis and S-100 positive immunostain. The patient underwent successful heart transplant. A 7-year-old male was admitted for heart failure. Work up revealed significant cardiomegaly and biventricular hypertrophy on echocardiogram. Acute decompensation led to cardiac arrest. Autopsy revealed an enlarged heart weighing 440g with subtotal replacement of the myocardium, SA and AV node with leukemic infiltrate exclusive to the heart. A 13-year-old with flu-like symptoms acutely decompensated and expired after a cardiac arrest in the emergency room. Autopsy revealed a large pedunculated tumor arising from the medial wall of the left atrium. A detached fragment of the tumor occluded one of the pulmonary veins and another blocked the mitral valve. Histologic examination revealed synovial sarcoma, confirmed by immunohistochemical staining.

**Conclusion:** Although rare, these cases present the need for in-depth molecular and genomic analysis of pediatric cardiac tumors to identify the etiopathogenesis of cardiac tumors.

### B31 Arrhythmogenic Right Ventricular Dysplasia (ARVD) A Review of 34 Pediatric Cases From a Single Institution Focusing on Genetic Background and a Review of the Literature

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**Aim of Study:** Arrhythmogenic Right Ventricular Dysplasia (ARVD) is a familial cardiomyopathy characterized by fibro-fatty replacement within the right ventricular myocardium. Mutations in 12 identified genes have been found to cause disruptions in desmosomal proteins of the myocardium.
Although a heritable condition, ARVD typically has no clinical manifestation at birth. Cardiac arrhythmias and sudden cardiac death (SCD) may be the first manifestations without any warning sign. The role of endomyocardial biopsy, which can provide a definitive diagnosis, continues to evolve.

**Methods:** Upon IRB approval, we identified 34 cases of pathologically diagnosed ARVD in pediatric patients from our tertiary care center. Patients' demographics, clinical presentation, genetic testing, and family history were reviewed.

**Results:** 8 patients presented with SCD, and ARVD was diagnosed at autopsy. 26 patients presented with heart failure. Initially the clinical diagnosis was unclear, with concern in the majority of these cases for myocarditis, and as such endomyocardial biopsy was undertaken. Myocardial biopsies identified fibro-fatty replacement leading to the diagnosis of ARVD in all included patients. 8 have subsequently undergone cardiac transplantation, 3 have required placement of a pacemaker or Automatic Implantable Cardioverter Defibrillator (AICD) and have been followed clinically.

**Conclusions:** ARVD should be considered in the differential diagnosis for children who are admitted for new onset or worsening heart failure. Endomyocardial biopsy can provide a definitive diagnosis to allow immediate intervention, and genetic counseling prior to progression of symptoms and thus avoid SCD.

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**B32 Eosinophilic gastroenteritis: A pediatric series**

Sabah Boudjemaa (1), Chloé Broudin (1), Linda Dainese (1), Julie Lemale (2), Joseph Aroulandom (2), Vincent Guinard-Samuel (2), Patrick Tounian (2), Aurore Coulomb (1)

Departments of (1) Pathology and (2) Gastroenterology and Pediatric Nutrition, Armand Trousseau Hospital, Paris, France.

**Background:** Eosinophilic gastroenterocolitis (EGE) is a rare condition. Diagnostic criteria include digestive symptoms, eosinophilic infiltration of digestive wall and exclusion of other eosinophilic gastrointestinal disorders (EGID).

The aim of this retrospective study was to better define morphological criteria of EGE by evaluating clinical, morphological and endoscopic findings in a pediatric series over a 7 years period (2007-2014).

**Methods:** Number and site of biopsies, average number and location of eosinophils, mast cells, crypt hyperplasia, necrosis, epithelial ulceration, villous atrophy, fibrosis, pathogens (HP) were evaluated.

**Results:** Sixteen patients with digestive eosinophilia were included: mean age 7.2 (3 months to 15 years), sex ratio 3. Nine children had digestive symptoms without underlying disease (group 1): epigastric pain (N=3), iron deficiency anemia (N=2), chronic diarrhea (N=3), vomiting and dysphagia (N=1). In 7 cases, digestive symptoms occurred in the course of an underlying disease (group 2): 2 CIBD, 1 cystic fibrosis with long term antibiotic therapy, 2 digestive malformations, 2 HP infections. Endoscopic findings were available in 10 cases: normal (N=4), inflammatory (N=4), cardia protrusion (N=2).

Location of eosinophilic infiltrate: duodenum (N=13), duodenum+stomach (N=1), oesophagus+stomach (N=1), duodenum+rectum (N=1).

Average eosinophils: 44/ HPF in group 1, 31/HPF in group 2.

In group 1, 2/9 cases had formal criteria for EGE (eosinophils 50-150/HPF). In group 2, eosinophilia (31/HPF) was relied to the underlying disease.
**Conclusion:** EGE is rare. Among the subgroup with isolated digestive eosinophilia, only 2 patients have strict criteria of EGE. The remaining 7 patients need further investigations.

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**Soft tissue malignant rhabdoid tumor arising after 25-year remission**

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Malignant rhabdoid tumor (MRT) is described as a rare high-grade sarcoma generally arising in the neonatal and early childhood population. MRT is frequently characterized by the loss of expression of the SMARCB1/INI-1 protein. Our two goals are 1) to report a rare case of a mandibular soft tissue MRT diagnosed in a 9-month-old infant whose disease free survival exceeded 25 years and 2) to discuss long-term treatment-associated complications that occur when an infant with MRT outlives the disease’s survival expectancy.

Material and Methods: HES, immunohistochemical panel, including SMARCB1/INI-1 protein analysis.

Histological examination showed a solid proliferation of irregular round polygonal cells with high nucleus to cytoplasm ratio, prominent nucleoli and intra cytoplasmic eosinophilic inclusion, consistent with rhabdoid features. The neoplastic cells showed loss of expression SMARCK1/ INI-1 protein. Long-term complications were closely related to the extensive surgery and local radiotherapy. Complications included: severe maxillofacial problems due to muscular atrophy, dental problems, primary hypothyroidism secondary to thyroidecumenty, severe headaches and dizziness. On follow-up, at the age of 26, multiple hepatic lesions ranging from 0.5 to 2.5 cm in size were discovered. Ultrasound-guided needle biopsies of the hepatic lesions were performed. Hepatic lesions were similar to the initial mass, both morphologically and immunohistochemically.

MRT of soft-tissue is an unusual and highly aggressive neoplasm rarely encountered in pediatrics pathology. To our knowledge, this is the longest remission time documented in a patient with soft tissue MRT. This unusual case raises a question regarding these hepatic lesions’ origin: are they metastases or primary tumors?

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**Sclerosing rhabdomyosarcoma with collagen rosettes, a case presenting with multiple lesions in an infant.**

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**Case:** A previously healthy 7-month-old boy presented with a rapidly enlarging subcutaneous lump on his lower back. Imaging revealed a 4 cm deep-seated mass in the left lower back, and two similar lesions in the right buttock and left thigh.

**Histology:** Biopsies from two of the lesions showed areas typical of embryonal rhabdomyosarcoma (ERMS) and hypocellular areas showing small groups/trabeculae of cells embedded in a hyaline matrix, as described in sclerosing rhabdomyosarcoma (SRMS). Cells were arranged in different patterns: tubular, single-file or diffuse. Chondroid areas were also recognized. A striking feature was
the presence of large rosettes made up of radiating collagen fibers. Tumor cells were focally positive for desmin and myogenin. MyoD1 was diffusely expressed. FOX01 was negative (FISH).

**Discussion:** SRMS is an unusual type of RMS characterized by a hyalinizing matrix-rich stroma, diffuse positivity for MyoD1, and focal or no expression of desmin and myogenin. Collagen rosettes have never been described in RMS. Currently, it is unclear whether SRMS is a variant of ARMS or ERMS or represents a new subtype. At least some SRMS are associated with MyoD1 mutations. A recent study suggests that pediatric MYOD1-mutant RMS follow an aggressive behavior with high mortality. There is no consensus on the treatment of SRMS, however, radical excision combined with adjuvant chemotherapy and/or radiotherapy remains the mainstay for most cases.

**Conclusion:** We describe a unique case of SRMS presenting as multiple soft tissue masses in a very young patient. Collagen rosette formation has not been previously described in RMS.

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**B35 Establishing Quantitative Parameters for the Identification of Ganglion Cells in Cases of Hirschsprung’s Disease.**

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The diagnosis of Hirschsprung’s Disease is based on a finding of a lack of mature ganglion cells in the distal colon. However, this diagnosis is can be difficult due to the subjective nature of identification of ganglion cells. Currently the identification of ganglion cells relies strongly on pathologists’ experience. This is further complicated by the relative sparcity of ganglion cells and variability of the maturity of ganglion cells. To date, no quantitative parameters have been established by which to positively identify ganglion cells. Our study aimed to compare ganglion cells, both mature and immature, with Schwann cells, endothelial cells, and fibroblasts. The parameters measured were area, perimeter, width, circularity, angle, Feret diameter, nucleus to cytoplasm area ratio, skewness, and kurtosis. Measurements were performed using ImageJ and new plugins. Double blind data was collected off routine hematoxylin and eosin stained rectal slides. The results showed mature ganglion cells to have significantly greater areas, perimeters, width, and Feret diameter in comparison to all other cell types. Mature ganglion cells were also found to have significantly lower nucleus to cytoplasm ratios in comparison to all other cell types. Immature ganglion cells showed significant differences amongst other cell types on all parameters measured. The results suggest that maturing ganglion cells can be differentiated from other cell types using quantitative parameters, which can then be employed to develop a more objective method for the identification of ganglion cells. This would allow for more confidence in the reliability and validity of diagnoses where ganglion cells are concerned, as in the case of an original diagnosis or a pull through procedure for Hirschsprung’s disease.