

**Society for Pediatric Pathology (SPP)**  
**Fall Meeting – September 29-October 2, Milwaukee, WI**

**Platform Presentations Session 1**  
**Friday, September 30, 2011 8:30 – 10:00 am**

**Abstracts are listed in presentation order, beginning with Platform Presentations.**

**1 Peripheral Neuroblastic Tumors with Genotype-Phenotype Discordance: A Report from Children's Oncology Group and International Neuroblastoma Pathology Committee**  
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**Background:** Among 4,706 peripheral neuroblastic tumors (pNTs) centrally reviewed by the COG Neuroblastoma Pathology Reference Laboratory at Children's Hospital Los Angeles, 4 prognostic groups were identified according to the Histopathology Classification (Favorable Histology-FH vs. Unfavorable Histology-UH) and MYCN status (Amplified-A vs. Non-amplified-NA). They were FH&NA (2,582 cases, 54.9%: 88.3±1.1% 5-year EFS, 96.9±0.6% 5-year OS), FH&A (51 cases, 1.1%: 65.2±11.6% EFS, 72.6±11.0% OS), UH&NA (1,339 cases, 28.4%: 56.0±2.3% EFS, 66.0±2.2% OS) and UH&A (734 cases, 15.6%: 41.4±3.2% EFS, 48.2±3.2% OS).

**Design:** In a previous study, presence of prominent nucleoli in undifferentiated/poorly differentiated neuroblastoma was demonstrated as an additional morphologic marker of aggressive clinical behavior. After reviewing H&E slides from the genotype-phenotype discordant group (FH&A), 2 subsets, A & B, were identified based on the nuclear morphology: A - tumors with conventional "salt-and-pepper" type nuclei, and B - tumors having unique nuclear feature containing one or few prominent nucleoli (site of RNA synthesis for protein expression). Clinicopathological characteristics were compared between the subsets. Immunostaining for MYCN protein was performed on selected cases from both subsets.

**Results:** FH&A tumors made an extremely rare group in pNTs and included 35 cases in Subset A and 16 in Subset B. Subset A was composed of Neuroblastoma (NB), poorly differentiated (PD) subtype (26 cases); NB, differentiating subtype (4 cases); Ganglioneuroblastoma-intermixed (3 cases); and Ganglioneuroma, maturing subtype (2 cases). All tumors in Subset B were NB-PD. There was no significant difference in distribution of prognostic factors such as age at diagnosis, clinical stage, DNA index, 1pLOH, 11qLOH and MKI between the subsets. However, survival rates for patients in Subset A (85.7±12.2% EFS, 89.3±10.3% OS) were significantly better than those in Subset B (31.3±13.0% EFS, 42.9±16.2% OS) (P=0.0010 & 0.0008). Immunohistochemically, all tested tumors (11/11) in Subset A were negative for MYCN protein. In contrast, 10/11 tumors in Subset B expressed MYCN protein.

**Conclusions:** Among the genotype-phenotype discordant tumors (FH & amplified MYCN), presence or absence of prominent nucleoli, putative site of MYCN RNA synthesis/accumulation leading the protein expression, can determine 2 prognostic subsets. The results of this study will make a significant contribution to patient stratification and treatment choice in this rare but important group of pNTs.

## **2 A Re-review of Alveolar Rhabdomyosarcoma: Looking Back at COG Study D9803**

Erin Rudzinski, MD (1), James Anderson, PhD (2), Julie Moore, HT (3), Stephen Skapek, MD (4), Doug Hawkins, MD (5), and David Parham, MD (6), 1)Oregon Health and Science University, Portland, OR; 2)University of Nebraska Medical Center, Omaha, Nebraska; 3)Nationwide Children's Hospital, Columbus, OH; 4)University of Chicago, Chicago, Illinois; 5)Seattle Children's Hospital, Seattle, WA; 6)The University of Oklahoma Health Science Center, Oklahoma City, Oklahoma

**Background:** The diagnostic criteria for alveolar rhabdomyosarcoma (ARMS) have shifted over recent decades. Historically, a diagnosis of ARMS required >50% alveolar morphology; however, from about 1998 to 2004 the criteria were reduced to any amount of alveolar pattern, and solid variants were added. Correspondingly, the number of ARMS diagnosed by central pathology review increased from 30% to 41%. Simultaneously, a new variant, sclerosing RMS, that mimics ARMS was described. This raised the concern that some patients may have been misclassified as ARMS and over-treated with more intensive ARMS chemotherapy, prompting a "look-back" of older cases in order to identify possible discrepancies from current classification.

**Design:** The COG D9803 intermediate-risk rhabdomyosarcoma (RMS) study enrolled 616 patients from 1999 to 2005. From this study, two pathologists re-reviewed 245 of 278 ARMS cases, based on availability of diagnostic material at the Biopathology Center in Columbus, Ohio. A randomly chosen subset of 38 embryonal rhabdomyosarcomas (ERMS) was also reviewed as a control group. Archived material included H&E and immunohistochemical stains (including myogenin for 244/283 total cases of ARMS and ERMS reviewed). We diagnosed ARMS based on >50% classical alveolar or solid variant morphology, with special attention to newly described RMS variants and myogenin staining patterns.

**Results:** Of ARMS originally enrolled on D9803, we reclassified 80 (33%) as ERMS and 18 (7%) as mixed RMS (10-90% ARMS pattern). The highest rates of reclassification were with primary tumors of GU/non-bladder/prostate (66%), perineum/retroperitoneum/trunk (43%), and orbit/head and neck (36%) origin. GU, non-bladder/prostate cases were overrepresented among the sclerosing and mixed RMS subtypes. None of the 38 control ERMS were re-classified. Of the 131 "original ARMS" for which fusion data is available through D9803, 34 (26%) were re-classified as ERMS and 12 (9%) were re-classified as mixed RMS. No gene fusion was demonstrated for any cases reclassified as ERMS, but evidence of gene fusion was found in 69 (81%) of 85 confirmed ARMS (53 PAX3, 16 PAX7).

**Conclusions:** Recognition of sclerosing RMS and application of the >50% alveolar morphology diagnostic criteria with strong, diffuse myogenin expression resulted in a return to the historic rates of 25-30% ARMS. This also reduced the number of fusion negative pure ARMS from 52% to 19%, on par with historic rates of 22-23% fusion negative ARMS of prior Intergroup Rhabdomyosarcoma Study protocols.

### **3 Four Cases of Pediatric Deep-seated/subcutaneous Pyogenic Granuloma and Review of Literature**

Amar Agadi and Anita Gupta, Dept. of Pathology and Lab Medicine, Cincinnati Children's Hospital

**Background:** Pyogenic granulomas are benign, reactive, typically superficial vascular lesions which can be idiopathic or arise secondary to trauma, underlying vascular malformations, infections, physiologic or pathologic endocrine changes, & hormone therapy. Pyogenic granulomas occasionally present in children (CPG) and occur at a mean age of 5.9 years. CPGs are slightly more common in males, average 7.3 mms in size, and primarily located in the head and neck region. Only a handful of deep-seated/subcutaneous pyogenic granulomas (DSPG) case reports are found in the literature. DSPG can be a clinical & pathologic challenge as these lesions mimic other vascular lesions including kaposiform hemangioendothelioma, and infantile hemangiomas.

**Design:** Using the 1996 Mulliken and Glowacki vascular anomaly classification which is now accepted by the International Society for the Study of Vascular Anomalies (ISSVA), and immunohistochemical stains for GLUT-1, MIB-1, & PROX-1, we report four cases (3.8%) of DSPG from 106 pediatric cases of CPGs excised over the last year (June 2010-June 2011) at Cincinnati Children's Hospital. Literature search was performed on PUBMED for "deep seated" and "subcutaneous pyogenic granulomas" and "subcutaneous lobular capillary hemangiomas." Data from each of the four patients' charts were compiled & compared with other DSPG pediatric cases (3 reports) that were previously documented in literature.

**Results:** Summarized data of the 4 cases demonstrates the average age of the child to be 10 yrs. with a male to female ratio of 1:1. Average size of the lesions is 1.7 cms. Lesions are located in the following areas: 2 - extremities, 1-back, and 1- nose. None of the children reported any history of trauma & 2 children are taking prescribed medications for mood disorders. On histology, DSPG are ovoid with vague, varying sized lobules composed of capillaries lined by plump endothelial cells intermixed with occasional scattered larger vessels occasionally lined by hobnailed endothelial cells. Lesional stroma in between and surrounding the lobules is fibrotic. Rare scattered lymphocytes and mitosis are present & one case has several organizing thrombi. There is no evidence of a neutrophilic or eosinophilic inflammatory infiltrate. Platelet microthrombi and spindle cells are absent. Endothelial cells are negative for PROX-1 and GLUT-1. MIB-1, proliferative index ranged from 5-15%. The lesions did not recur after excision.

**Conclusions:** We describe 4 additional pediatric cases of DSPG including the histopathology features differentiating DSPG from more common pediatric vascular lesions like infantile hemangioma, kaposiform hemangioendothelioma, and others. In addition, DSPG accounted for 3.8% of all our pyogenic granulomas within a single year implying these lesions may not be as rare as suggested in literature, but rather underdiagnosed.

#### **4 Alveolar Soft Part Sarcoma: Morphoproteomic Analysis of Fatty Acid Synthase and STAT3 Pathways with Therapeutic Implications.**

N Tatevian, D Lopez-Terrada, WL Wang, M Bhattacharjee, RE Brown., UT Health Science Center at Houston, Houston, Texas; MD Anderson Cancer Center, Houston; Baylor College of Medicine, Houston, Texas, USA.

**Background:** Alveolar soft part sarcoma (ASPS) is a rare tumor that affects primarily infants, children, adolescents and young adults. It is relatively indolent but eventually results in death in a high percentage of cases. ASPS is associated with a chromosomal translocation that produces a fusion gene and a chimeric fusion transcript, ASPL/TFE3. The latter has been associated with the activation of the c-MET gene and the expression and activation of its tyrosine kinase, c-Met. Fatty acid synthase (FASN) serves as a facilitator in the post-translational activation of c-Met, whose downstream signaling includes activation of the signal transducer and activator of transcription (STAT)3 pathway. Additionally, the platelet-derived growth factor receptor (PDGFR) and interleukin(IL)-6 family of signal transducers have been identified in ASPS specimens. These signal through the src/STAT and JAK/STAT pathways, respectively. A computer-assisted search of the National Library of Medicine's Pubmed data base revealed that neither the FASN nor the STAT3 pathway appear to have been analyzed in ASPS.

**Design:** Objectives: To study the expression levels of FASN protein and the state of activation of the STAT3 pathway in cases of ASPS using morphoproteomic analysis. Study Group: With Institutional Review Board approval, formalin-fixed paraffin-embedded material of six (6) ASPS cases with documented ASPL/TFE3 fusion transcripts were studied by applying immunohistochemical probes with appropriate controls to detect FASN and phosphorylated (p)-STAT3 (Tyrosine 705).

**Results:** The intensity and subcellular compartmentalization of the chromogenic signal for each of these analytes in the tumor cells was graded on a scale of 0-3+ by three (3) pathologists.

Results: All cases showed variable cytoplasmic expression of FASN in the tumor cells with 5 out of 6 showing up to 2 to 3+ intensity. Similarly, p-STAT3 (Tyr 705) was noted in tumoral nuclei in all cases, at up to 2 to 3+ intensity.

**Conclusions:** The expression of FASN together with the finding of ASPL/TFE3 fusion transcripts in our cases accords with the previously published report of constitutive activation and expression of phosphorylated c-Met tyrosine kinase in ASPS. The nuclear expression of p-STAT3 (Tyr705) in these cases is consistent with downstream signaling by both the FASN/c-Met pathway and the aforementioned IL-6 and PDGFR pathways. This latter observation accords with the relative efficacy of sunitinib, a multi-tyrosine kinase inhibitor in treating ASPS patients. Moreover, it opens the possibility of using combinatorial targeted therapy for ASPS patients with other agents such as crizotinib which inhibits c-Met tyrosine kinase, metformin, which has been shown to downregulate FASN and the phosphorylation of src, a statin, which inhibits IL-6 signal transduction through the JAK/STAT pathway and sorafenib which inactivates p-STAT3.

## 5 Epithelioid Sarcoma is Associated with SMARCB1 Deletions and p16 Pathway Alterations

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**Background:** SMARCB1 gene alterations were first described in malignant rhabdoid tumor (MRT), an aggressive childhood tumor occurring in the kidney, soft tissue and CNS. A growing number of tumors show loss of SMARCB1 protein expression by immunohistochemistry (IHC) including the majority of epithelioid sarcomas (ES). MRT and ES express epithelial and mesenchymal markers and may show overlapping patient demographics and histologic features making their distinction challenging. However, the distinction is important due to worse prognosis and associated germline mutations in 35% of MRTs. The mechanism by which SMARCB1 loss induces malignancy is unclear. Cell lines and animal models for MRT suggest that loss of SMARCB1 leads to decreased levels of p16. There is conflicting data on the mechanism of SMARCB1 loss in ES and very limited data evaluating members of the p16 pathway in these tumors. The aim of this study was to investigate SMARCB1 gene alterations and downstream regulation of the p16 pathway in ES to aid in diagnosis and provide insight into tumorigenesis.

**Design:** Twenty-one distal and proximal type ES cases were selected from the Department of Pathology, Mayo Clinic for SMARCB1 gene testing and SMARCB1 and p16 IHC. SMARCB1-negative cases with available formalin-fixed paraffin embedded tissue scrolls with at least 50% tumor were chosen for SMARCB1 mutation analysis and multiplex ligation-dependent probe amplification (MLPA). The percentage of positive nuclei was scored for p16. Nuclear staining for SMARCB1 was evaluated as positive or negative. Results were compared to appropriate positive and negative controls.

**Results:** Nineteen of 21 (90%) ES were SMARCB1 negative by IHC. Twelve of the negative cases (63%) met inclusion criteria and had adequate DNA recovery for evaluation. Ten of 12 (83%) showed homozygous deletions by MLPA. Two cases showed heterozygous deletions and sequencing showed polymorphisms, but no mutations. For the SMARCB1 negative cases, the average p16 score was 10.7% (range 0-25%). Of the 2 SMARCB1 positive epithelioid sarcomas, p16 was 0% and 80%.

**Conclusions:** The majority of SMARCB1-negative ES cases showed homozygous gene deletions with no coding sequence mutations. In contrast, 37% of soft tissue MRT reportedly demonstrate gene mutations. In the SMARCB1-negative cases, p16 staining was weak to absent as predicted by MRT models. The 2 SMARCB1 positive cases showed discordant results and are difficult to interpret due to the small sample size. Taken together these results support a low frequency of SMARCB1 gene mutations in ES and suggest dysregulation of the p16 pathway.

## 6 Digital Pathology is a Valid Surrogate for Glass Slide Microscopy in Neuroblastoma Central Case Review

JA Jarzembowski, K Nicol, R Sukanuma, T Barr, H Shimada, Children's Hospital of Wisconsin, Milwaukee, WI; Nationwide Children's Hospital, Columbus, OH; COG Biopathology Center, Columbus, OH; Children's Hospital of Los Angeles, Los Angeles, CA

**Background:** Each year in the U.S., about 650 children are diagnosed with neuroblastoma. The majority of these cases are enrolled in COG trials with each tumor centrally reviewed and diagnosed according to the International Neuroblastoma Pathology Classification for patient risk-stratification and protocol assignment. The INPC involves complex pattern recognition and is difficult to implement consistently and accurately without significant training and case-based experience. Digital imaging and virtual microscopy systems might allow for expedited case review, remote-training, and mentoring. We compared the utility of a digital pathology system for classifying neuroblastic tumors to the standard method of reviewing glass slides.

**Design:** Four pathologists independently reviewed scanned images of 150 cases using Aperio™ XT robots through the BPC VIPER system and a subset of 50 also by glass slides, assigning a tumor category, grade of neuroblastic differentiation, MKI, and INPC classification (favorable/unfavorable histology). The software in this project allowed pathologists to review digital images and digitized pathology reports and to complete review forms via a web-based interface. Inter- and intraobserver correlations were calculated using kappa statistics.

**Results:** Diagnostic accuracy was similar between glass and digital slides, with majority agreement (3/4 or 2/3 pathologists matching gold standard diagnosis) present for tumor category (95.3% by glass vs. 94.5% by digital images), differentiation (92.2% vs. 91.1%), MKI (89.1% vs. 93.6%), and INPC classification (96.9% [ $\kappa=0.74-0.91$ ] vs. 94.1% [ $\kappa=0.67-0.93$ ]). However, unanimous agreement was modestly higher using glass slides with regard to tumor category (92.2% vs. 81.2%), differentiation (85.9% vs. 78.2%), and INPC classification (90.6% vs. 76.2%). Intraobserver correlations between glass slides and digital images were acceptable for tumor category (range, 88.5%-98.1%), differentiation (84.3%-98.1%), and INPC classification (84.3%-95.6%;  $\kappa=0.65$  to 0.91); MKI was substantially more variable (64.7%-94.3%). Discordant categorizations and classifications frequently involved polyclonal tumors such as composite neuroblastomas and nodular ganglioneuroblastomas. The most inconsistently evaluated feature by digital imaging was the same area known to be problematic by conventional microscopy - MKI.

**Conclusions:** Central review and INPC classification of neuroblastic tumors using digital pathology is reproducible, accurate, more efficient, and less expensive than traditional glass slide review. Virtual microscopy may represent the future of pathology central review not only for neuroblastoma, but also for other pediatric tumors under investigation by COG and other cooperative groups.

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**Platform Presentations Session 2 (Perinatal Abstracts)**  
**Friday, September 30, 2011 10:30 am – 12:00 pm**

**Abstracts are listed in presentation order, beginning with Platform Presentations.**

**7 Development of a DNA Microsatellite Genotyping Test for Aneuploidy Detection in Paraffin Embedded Tissue from Products of Conception**

LV Furtado, M Jama, CN Paxton, AE Gardiner, AR Wilson, E Lyon, KB Geiersbach.,  
Department of Pathology, The University of Utah and ARUP Laboratories, Salt Lake City, UT  
**Background:** Autosomal trisomy is the most common type of genetic abnormality observed in pregnancy loss, and diagnosis of trisomy is relevant for genetic counseling. Cytogenetic analysis is sometimes problematic due to culture failure and maternal cell contamination, and fresh tissue is often unavailable for chromosome analysis. A molecular technique applicable to fresh or formalin fixed, paraffin embedded (FFPE) tissue is highly desirable in this setting. Mini-short tandem repeats (m-STRs) with small amplicons (< 200 bp) can potentially be employed for trisomy detection even on small quantities of partially degraded DNA. The objective of our study was to determine whether an m-STR-based assay can provide sufficient quantitative information for aneuploidy detection in FFPE samples.

**Design:** A panel of primers was designed targeting highly polymorphic m-STR loci on chromosomes 13, 18, and 21 from sub-optimal DNA samples. Criteria for STR locus selection and primer design included rate of heterozygosity (>70%), PCR product size (< 200 bp), low stutter artifact, and position on the chromosome, with a minimum of 4 loci spread widely across each targeted chromosome. FFPE POCs previously characterized by chromosome analysis were tested, including trisomy 13 (n=2), trisomy 18 (n=4), trisomy 21 (n=3) and 6 without aneuploidy. Three trisomy 21 samples from fresh tissue were also tested. DNA was extracted from microdissected villous or fetal somatic tissue selected by histologic examination of an H&E stained section. Unpurified crude lysate was subjected to single-plex PCR amplification using fluorescently labeled primers, and products were separated by capillary electrophoresis (ABI 3100) and analyzed with GeneMarker software (Softgenetics). Loci informative for trisomy were expected to show either a triallelic pattern (1:1:1) or a biallelic pattern with a skewed (1:2 or 2:1) peak area ratio. Reference ranges were established using previously tested FFPE POCs.

**Results:** Microdissection yielded adequate DNA free from maternal cell contamination. All samples showed robust amplification of the tested STR loci, and concordance between cytogenetics and genotyping was 100%.

**Conclusions:** In this initial study, we designed and successfully tested a panel of m-STR markers for aneuploidy detection in chromosomes 13, 18 and 21 on FFPE samples obtained from POCs. This groundwork demonstrates that m-STR analysis is a viable and cost-effective molecular technique for aneuploidy detection in FFPE tissue. Further work is necessary in order to optimize the assay for clinical use.

## **8 Resilience of the Human Fetal Lung Following Stillbirth. Potential Relevance for Pulmonary Regenerative Medicine.**

ME De Paepe, S Chu, N Heger, S Hall, Q Mao., Women and Infants Hospital, Providence, Rhode Island.

**Background:** Recent advances in pulmonary regenerative medicine have increased the demand for alveolar epithelial progenitor cells. Fetal lung tissues from spontaneous pregnancy losses may represent a neglected, yet ethically and societally acceptable source of alveolar epithelial cells, provided these tissues are obtained with full informed parental consent. The aim of this study was to determine the regenerative capacity of fetal lungs obtained from second trimester stillbirths.

**Design:** Lung tissues were harvested from 11 stillborn fetuses (13-22 weeks' gestation) at post-delivery intervals ranging from 10 to 41 hours. Lung tissues were grafted to the renal subcapsular space of immune suppressed rats to provide optimal growth conditions. Histology, epithelial and alveolar type II cell proliferation, and surfactant protein-C mRNA expression were studied in preimplantation lung tissues and in xenografts at post-transplantation week 2. The study was performed with informed parental consent and approved by the Institutional Review Board and the Institutional Animal Care and Use Committee.

**Results:** All xenografts displayed advanced architectural maturation compared with their respective preimplantation tissues, regardless of gestational age and post-delivery interval. The proliferative activity of the grafts was significantly higher than that of the preimplantation tissues (Ki-67 labeling index  $26.7 \pm 7.7\%$  in grafts versus  $14.7 \pm 10.5\%$  in preimplantation tissues,  $P < 0.01$ ), and did not correlate with post-delivery interval or presence of inflammation/chorioamnionitis. All grafts displayed robust surfactant protein-C mRNA expression, indicative of recuperation of type II cell function.

**Conclusions:** Fetal lung tissues from mid-gestation stillbirths can recuperate, and even exceed, the normally high proliferative activity and surfactant production of fetal lungs in situ following short-term culture, even when harvested more than 24 hours after delivery. Highly proliferative and metabolically active fetal alveolar epithelial cells, obtained after spontaneous second trimester miscarriages, may represent an abundant and powerful source of cells for regenerative and tissue engineering purposes. Importantly, the altruistic act of fetal tissue donation may offer some degree of emotional closure to the bereaved parents.

## **9 Tufting Enteropathy Revisited: Value of MOC-31 (EpCAM) Antibody Staining.**

S Ranganathan, L Schmitt., Department of Pathology, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA

**Background:** Tufting enteropathy (TE) is an uncommon disease causing intractable diarrheas starting in early childhood and resulting in failure to thrive, dependence on total parenteral nutrition and eventually requiring transplantation for treatment. The diagnosis has been based on histology showing the presence of epithelial "tufts" in the small bowel and colonic mucosa and variable villous alterations with mild to no inflammatory changes and preserved brush border. More recently, the gene for TE has been identified to be the EpCAM gene on chromosome 2p21. The aim of this study was to determine if the MOC31 antibody will be helpful in diagnosis of TE in paraffin tissue even when classical epithelial tufts were not apparent on biopsies and to see its distribution in the bowel.

**Design:** With IRB exemption, all cases of intractable diarrhea in children where TE was suspected or diagnosed were retrieved from the pathology files (15 patients). Other cases of infantile, neonatal and childhood diarrhea were also retrieved to serve as controls for the staining (total 30 patients). These included microvillous inclusion disease -2, autoimmune enteropathy -16, allergic enteritis-1, celiac disease-2, allograft bowel-1, adenoviral-2 and EBV enteritis-3, and Crohn's-3. EpCAM/MOC31 (1:50; Cell Marque Corporation, Rocklin, CA) antibody staining was performed (Ventana Benchmark XT Immunohistochemistry Stainer) after mild antigen retrieval, followed by detection with the Ventana iView DAB detection system and hematoxylin counterstain. Positive (appendix) and negative controls were run simultaneously.

**Results:** 15 patients (11 males, 4 females) with a diagnosis of TE ranged in age at diagnosis from 3 months to 9 years, all presenting with protracted diarrhea and/or failure to thrive usually since birth. The histology in most showed epithelial tufts in various portions of small bowel and colon. MOC31 was done in all but 2 patients (both consults) and was completely negative in the epithelium irrespective of the site of biopsy or resection. Many cases had multiple sections stained including duodenum, ileum, colon and appendix, and all showed absent staining of epithelium and crypts. In contrast, MOC31 was positive in all other cases tested giving a sensitivity and specificity of 100% for loss of staining. 2 of these children had confirmatory genetic studies and showed both classic and variant mutations.

**Conclusions:** MOC31 is a diagnostic stain for tufting enteropathy and should be included in the panel in any case of prolonged diarrhea in children to exclude this possibility. It is helpful in the diagnosis of cases where tufts are not evident on biopsy specimen and also help exclude the diagnosis of tufting when small epithelial cell clumps are seen in reactive processes or due to damage. The absence of staining appears to correspond to the mutations in the EpCAM gene.

## **10 C4d Immunoreactivity and Cardiac Allograft Vasculopathy Leading to Death in Pediatric Heart Transplant Recipients**

MK Mirza, S Fedson, AN Husain., The University of Chicago Medical Center

**Background:** Endomyocardial biopsy (EMB) is the gold standard to detect acute cellular rejection after cardiac transplantation. C4d is widely accepted as a marker for antibody-mediated rejection in cardiac allografts; however its usefulness in pediatric heart transplantation has yet to be independently evaluated. The aim of this prospective study was to determine the significance of C4d immunoreactivity in pediatric EMBs by correlating with cardiac allograft vasculopathy (CAV) and death.

**Design:** 276 biopsies from 22 pediatric heart transplant patients (< 18 years) were stained prospectively by IHC for C4d deposition on paraffin-embedded tissue using anti-human C4d polyclonal antibody. Only strong diffuse endothelial staining was considered positive.

**Results:** The mean age at transplantation was 7.9 years (range 2 months to 17 years). Positive staining of C4d was present in 9 biopsies (41%) from 4 patients (18%). The average time from transplant to the first episode of C4d positivity was 628 days (ranging from 7 to 1135 days). Five of the 22 patients (23%) died, 4 of whom (80%) had a history of positive C4d staining. Of these 4, 3 had post-mortem examinations which showed CAV as the cause of death in all (100%). The fifth patient died of complications of respiratory failure and post transplant lymphoproliferative disorder and had no CAV.

**Conclusions:** In conclusion, C4d positivity correlated with CAV and poorer outcomes. 4 out of 4 patients who showed C4d positivity died (100%) (institutional 5-year post-transplant survival is 78%). This prospective study points to a prognostic role for C4d immunostaining in the outcome of pediatric heart transplantation.

## 11 Gross Patterns of Umbilical Cord Coiling: Correlations with Placental Histology and Perinatal Outcome.

M Huang, E Curry, LM Ernst., Northwestern University, Chicago, IL.

**Background:** Hypercoiled umbilical cords have been associated with adverse perinatal outcomes; however, the majority of pregnancies with hypercoiling, as determined by the umbilical cord coiling index, have normal outcomes. Beyond number of coils in the umbilical cord, the gross patterns of the hypercoiling and their effects on fetal outcome have not been elucidated. The purpose this study is to define gross patterns of umbilical cord hypercoiling and determine correlations between specific coiling patterns and histological features in the placenta and/or perinatal outcomes such as stillbirth.

**Design:** The authors established a priori definitions of 4 major gross coiling patterns: undulating, rope-like, segmented, and linked. The undulating pattern was defined as a cord with a serpentine or loose S-shape without significant indentations between the coils. The rope-like pattern was characterized by relatively tight coils with a generally preserved/linear external surface to the cord. The segmented umbilical cord showed coils with indentations involving < 50% of the diameter of the cord between each coil, and the linked pattern displayed deeper indentations (> 50% of the cord diameter) between each coil. Gross images of placentas with hypercoiled umbilical cords (>3 coils/10cm) between Jan 2009 and December 2010 were reviewed in a blinded fashion and for each case a major umbilical coiling pattern and the direction (right or left) of the coiling was assigned. Outcome variables obtained from placental pathology reports included histological abnormalities indicative of significant chronic fetal vascular obstruction, such as fetal vascular thrombi, avascular villi, villous stromal-vascular karyorrhexis, and fetal thrombotic vasculopathy, and stillbirth.

**Results:** 322 placentas were identified with adequate pictures of the umbilical cord. The rope-like pattern was the most common (52%), followed by the undulating (25%), segmented (19%) and linked (4%) patterns. The segmented and linked gross coiling patterns were significantly correlated with evidence fetal vascular thrombi (34% vs 19%,  $p=0.007$ ), avascular villi (33% vs 16%,  $p=0.001$ ), fetal thrombotic vasculopathy (15% vs 6%,  $p=0.008$ ) and stillbirth (14 % vs 4%,  $p=0.001$ ), when compared with the rope-like and undulating patterns. Number of cord coils per 10 cm did not correlate with any of the outcome variables. Cords with right twists had significantly higher incidence of fetal vascular thrombi (36% vs 19.5%,  $p=0.006$ ), avascular villi (29.5% vs 17%,  $p=0.03$ ), fetal thrombotic vasculopathy (15% vs 6%,  $p=0.023$ ), and stillbirth (11.5% vs 5%,  $p=0.04$ ) than cords with left twists.

**Conclusions:** Among hypercoiled umbilical cords specific gross patterns of coiling can be recognized, and patterns with the most significant indentation or pinching of the cord diameter are associated with histological evidence of chronic fetal vascular obstruction and stillbirth. Further investigation is needed to determine if these coiling patterns can be recognized antenatally to identify fetuses at risk for adverse outcome.

## 12 Correlation Between Cord Insertion Type and Superficial Choriovasculature in Diamniotic-Monochorionic Twin Placentas: It's the Company We Keep.

ME De Paepe, S Shapiro, LC Hanley, S Chu, FI Luks., Women and Infants Hospital, Providence, RI.

**Background:** Non-central cord insertion has been associated with diminished chorionic vascular distribution in singleton placentas. The choriovascular correlates of peripheral cord insertion in monochorionic twin placentas remain undetermined. The aim of this study was to investigate the association between type of cord insertion and choriovascular distribution of both twin territories in diamniotic-monochorionic twin placentas.

**Design:** A prospective cohort of 138 monochorionic placentas was examined at our institution between 2008 and early 2011. 35/138 cases (25%), including disrupted placentas, placentas from higher order multiples and placentas from pregnancies complicated by twin-to-twin transfusion syndrome, were excluded. The correlation between cord insertion and superficial choriovascular distribution was studied in the remaining 103 dye-injected diamniotic-monochorionic placentas. Cord insertion was categorized as paracentral, marginal or velamentous. The choriovascular distribution of both individual twin territories was assessed by analysis of number and density (number per surface area) of perforating chorionic arteries (PCAs).

**Results:** Unexpectedly, there was no correlation between a twin's cord insertion type and its own choriovascular distribution, assessed by analysis of PCA density. However, a strong correlation was found between the choriovascular distribution of one twin and the cord insertion type of the opposite twin. For twins with paracentral or marginal cord insertion, the PCA density was significantly higher if the co-twin had a velamentous cord insertion than if the co-twin had a paracentral cord insertion ( $P < 0.001$  and  $P < 0.05$ , respectively). Similarly, the PCA density of twins with velamentous cord insertion tended to be higher in the presence of a co-twin with velamentous, rather than paracentral cord insertion ( $P = 0.09$ ).

**Conclusions:** This is the first study to suggest that in diamniotic-monochorionic twin gestations, a twin's choriovascular architecture is correlated with the cord insertion type of the co-twin, rather than its own. In general, velamentous cord insertion of one twin is associated with increased choriovascular distribution of the opposite twin territory. Our observations likely reflect novel manifestations of twin interdependence in monochorionic pregnancies.

**Society for Pediatric Pathology (SPP)**  
**Fall Meeting – September 29-October 2, Milwaukee, WI**

**Poster Discussion**  
**Friday, September 30, 2011 1:30 – 3:00 pm**

**Abstracts are listed in presentation order, beginning with Platform Presentations.**

**13 The Value of a Standardized Pathologic Examination of Non-Intact, Second Trimester Fetal Demise**

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**Background:** The objective of this study is to assess the findings of pathologic examination of non-intact, second trimester fetal demise specimens and determine if a careful, standardized anatomic examination, performed by a perinatal pathologist, yields clinically useful information for clinician counseling.

**Design:** We conducted a retrospective chart review of fetal demise cases between 14 and 24 weeks gestation from May 2006 to October 2010. Data collected included evacuation method, gestational age and size, suspected abnormalities, chromosomal diagnoses and final pathologic diagnoses. A general surgical pathology examination was performed on non-intact specimens between May 2006 and October 2008, while a perinatal pathologist examined the specimens in a standardized anatomic manner between October 2008 and October 2010. Statistical analysis consisted of Analysis of Means (ANOM) and Chi-square tests by Stata/SE 10.0.

**Results:** 158 patients met inclusion criteria: 32 underwent intact vaginal deliveries and 126 patients (132 fetuses) underwent non-intact, Dilation and Evacuation (D&E). Mean gestational size of D&E patients was 16.17 weeks (SD 2.17). Specialized perinatal pathologic evaluation diagnosed significantly more abnormalities than general pathologic examination on non-intact specimens [87.8% vs. 10.4% ( $p < 0.005$ )]. 42 specimens had 75 abnormalities identified on pathology. 77.3% (n=58) were placental (infection, maternal or fetal vascular abnormalities), 8% (n=6) had possible aneuploid findings with confirmation on 4 of 6, and 14.7% (n=11) had fetal abnormalities (growth restriction, hydrops, anatomic). A total of 74.6% (94/126) underwent chromosomal analysis. 12.8% were (n=12) abnormal, with 11 of 12 evaluated by surgical pathology. The perinatal pathologist confirmed abnormalities on 66.7% of those examined (4/6) and the general pathologist did not confirm any abnormalities (0/5).

**Conclusions:** In addition to the pathologic documentation of the presence of fetal and placental tissue, implementation of a standardized, anatomic examination on non-intact, second trimester fetal demise specimens more frequently confirms clinically suspected anomalies and also uncovers unanticipated fetal anomalies and other gestational abnormalities which may yield clinically useful information for patient counseling.

#### **14 Semi-quantitative Comparison of Autopsy Diagnoses between Late Preterm and Term Infants Dying in the First Year of Life: A Case-Control Study**

CK Steigman(1), X Tang(2), S Bai(3)., (1) Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR; (2) Department of Pediatrics (Biostatistics), University of Arkansas for Medical Sciences, Little Rock, AR; (3) Department of Statistics, Ohio State University, Columbus, OH.

**Background:** Late preterm infants, defined as those born between 34 and 36 weeks gestation, suffer more medical complications than those born at term (38-42 weeks). Although clinical studies have documented increased medical issues in late preterm infants, to date there has been no published systematic review of autopsy findings in this special subgroup of preterm infants.

**Design:** This case-control study is based on retrospective review of autopsy records from Arkansas Children's Hospital between 1994 and 2008. A single reviewer identified autopsies performed on infants under one year of age who were born between 34-36 weeks (late preterm) and 38-42 weeks (term). Autopsy diagnoses were grouped into six broad categories: cardiovascular (CV) abnormalities with or without other malformations, congenital malformations not involving the CV system, infections, metabolic disorders, perinatal insults, and "others". Gender and causes of death were summarized as number and proportion for the two groups. We used Fisher's exact test to compare gender between the two infant groups. Fisher's exact test was also used to evaluate whether there was an overall difference in the cause of death between term and late preterm infants.

**Results:** Of the 205 infants studied, 162 (79%) were term and 43 (21%) were late preterm. Males outnumbered females in each group; however, this difference was not statistically significant ( $p=0.23$ ). Furthermore, there was no significant difference in the causes of death between the term and late preterm infants ( $p=0.09$ ). Although the proportion of late preterm infants with congenital anomalies was twice that of term infants, and oppositely, the proportion of term infants with CV anomalies was more than twofold that of late preterm infants, the differences between the two groups were not statistically significant with the sample size we had.

**Conclusions:** Although we found no significant difference in causes of death in this preliminary study, there were potential trends in the CV abnormality group and the group with congenital malformations not involving the CV system. Further studies with a larger sample size would be needed to achieve better statistical power. Although most cases clearly fit into one of the six broad diagnostic categories, approximately 20% of the cases did not due to diagnostic ambiguity of the autopsy diagnoses or the presence of co-morbid conditions; in these cases, despite the use of additional clinical information to clarify the cause of death, potential bias on the part of the reviewer cannot be excluded. Finally, since only a small proportion of infants who die receive autopsies, selection bias may influence our results as well.

## **15 Calretinin Immunohistochemistry - A Useful Method in the Evaluation of Suboptimal Rectal Biopsies Performed for Hirschsprung's Disease: An Institutional Experience.**

S Alexandrescu, A Al-Ibraheemi, H Rosenberg, N Tatevian., Department of Pathology, University of Texas Health Science Center at Houston.

**Background:** The diagnosis of Hirschsprung's disease (HD) is based on evaluation of ganglion cells status on suction rectal biopsies. Immaturity of ganglion cells, proximity to squamo-columnar junction, insufficient quantity of submucosa, and paucity of ganglion cells makes the hematoxylin-eosin microscopical interpretation of the specimens difficult, and sometimes impossible. Calretinin was shown to increase specificity of the diagnosis in such situations. We implemented the use of calretinin stain in questionable cases of HD in our institution.

**Design:** Two pediatric pathologists at UT HSC at Houston evaluated 28 rectal biopsy specimens from 2010-2011 to rule out or confirm the diagnosis of HD. The patients' age ranged from 15 days - 8 years. Twenty-three cases were suction biopsies, and five were resection specimens for HD. Hematoxylin-eosin stain was performed on at least 80 levels for the suction biopsy specimens. Calretinin immunohistochemical stain was performed on levels 40-42 in all cases, with adequate controls. Ten of the biopsies examined (10/28) had inadequate amount of submucosa.

**Results:** Thirteen (13/28) (eight suction and five intraoperative biopsies) had no ganglion cells on hematoxylin-eosin examination. Calretinin immunohistochemistry showed non-specific submucosal cytoplasmic and nuclear staining in few cells, but no nerve fibrils in the lamina propria. Fourteen cases (14/28) had normal or limited number of ganglion cells identified in the submucosal plexus, and calretinin highlighted the nerve fibrils in the lamina propria in a granular, linear pattern, ruling out HD. Two of the cases with suboptimal amount of submucosa (2/10) did not show ganglion cells on hematoxylin-eosin stain, but calretinin showed nerve fibrils in the lamina propria. One case lacked mucosa and submucosa, and a diagnosis could not be made.

**Conclusions:** Our institutional experience with calretinin supports the previously published data; calretinin was found to be useful in ruling out HD, when having a suboptimal amount of submucosa, and when the ganglion cells are immature and/or decreased in number. This is especially important in the newborns or preterm infants with other pathophysiology to avoid unnecessary re-biopsy. If HD still cannot be ruled out, clinical correlation, and possible re-biopsy is suggested. The advantage of calretinin over other ancillary studies is that it is performed in continuity with hematoxylin-eosin stain, and the pathologic correlation of the findings is accurate.

## 16 Classification of Preterm Birth with Placental Correlates

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**Background:** Classification of disease can better elucidate pathophysiology. Premature birth lacks a widely accepted classification that unites features of the clinical presentation with placental pathology. We present associations between the clinical categories of preterm birth and placental histology.

**Design:** Placentas of 109 infants (including 15 twin sets, 2 triplet sets) in the NICU from March 2008-March 2009 with gestational age < 34 weeks and birth weight < 2000 grams were analyzed for 73 gross and microscopic variables. After slide review, we queried electronic records for classification as preterm labor with intact membranes (PTL), prelabor premature rupture of membranes (PPROM), preeclampsia (PE), indicated preterm birth for maternal factors (IM), indicated preterm birth for fetal factors (IF) and the clinical diagnosis of abruption. The diagnoses of PE, IF and abruption defined their respective categories, and may have had PTL or PPRM as well. Select placental features were grouped into amniotic fluid infection (AFI), lymphocytic inflammation (LI) for cases with chronic chorionitis, chronic chorioamnionitis and chronic villitis, and maternal underperfusion (MU) for cases with maternal decidual vasculopathy, or villous ischemic changes. Individual variables were analyzed using two-tailed t-tests and Fisher exact tests.

**Results:** Placental features seen in the clinical classification are tabulated below:

	<i>AFI</i>	<i>LI</i>	<i>MU</i>	<i>AFI/LI/MU</i>	<i>AFI/LI</i>	<i>AFI/MU</i>	<i>LI/MU</i>
PTL (n=23)	2	2	5	3	1	3	5
PPROM (n=27)	6	3	2	2	3	8	3
Abruption (n=10)	1	2	1	2	1	1	2
PE (n=27)	0	0	20	0	0	0	7
IF (n=10)	0	0	4	0	0	2	1

Overlapping categories: PE = 7 PTL, 2 PPRM, 2 Abruption; Abruption = 8 PPRM, 6 PTL; IF = 2 PTL, 1 PPRM. Others not in table: 7 IM, 2 twins premature without PTL/PPROM, 2 siblings of PPRM triplet, 1 sibling of IF twin.

Chronic chorionitis was significantly correlated with any PPRM ( $p=0.007$ ), and no PE ( $p=0.026$ ). Basal chronic villitis correlated with no PE ( $p=0.039$ ), but severe extensive chronic villitis was weakly associated with PE ( $p=0.046$ ). Increased perivillous fibrin correlated with IF ( $p=0.006$ ). Plasma cell deciduitis was not significantly associated with any one clinical category. Statistical significance was reached for multiple individual variables common to the categories above with expected correlates (e.g. distal villous hypoplasia and preeclampsia).

**Conclusions:** Changes of maternal underperfusion are pervasive in all clinical categories, commonly associated with AFI and LI. AFI is predominantly associated with PPRM. LI is seen in PTL, PPRM, Abruption and PE without AFI, suggesting immunologic "rejection" as a mechanism of preterm birth. The common pathologies among these clinical categories, with exception of preeclampsia, suggests the clinical preterm birth categories may be more related at their root cause than previously suspected, and that susceptibility to amniotic fluid infection or "maternal rejection" may both be mechanistically related to vascular remodeling for pregnancy and maternal perfusion.

## **17 Pathologic Findings in Placentas with Sickled Maternal Erythrocytes: An Institutional Experience.**

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**Background:** Sickle cell disease and sickle cell trait are known to be associated with placental changes, including infarcts, abruption, prematurity, accelerated villous maturation, and villous edema. Surprisingly high number of placentas with incidental finding of sickling of maternal erythrocytes in patients with no previous history of sickle cell disease/trait prompted us to perform comprehensive microscopic review in search for other abnormalities, and to tailor the pathologic diagnosis in a way that can improve the outcome of future pregnancies for these patients.

**Design:** 36 placentas with sickling of maternal erythrocytes (13.8% of all placentas submitted for pathologic examination in April 2010-April 2011) were reviewed. Demographic data, gestational age, macroscopic and microscopic findings were recorded. The patients' charts were reviewed in collaboration with the clinicians.

**Results:** Of the thirty-six placentas reviewed, 68% of the patients had no prior clinical diagnosis of sickle cell disorder. 32% of patients were known to have sickle cell trait (16%) or disease (16%). Preterm delivery was recorded in 22% of cases. 13 (36%) of placentas with sickle cells were small for gestational age, 6 (16%) were large for gestational age. Other pathologic findings included lack of physiologic conversion of decidual vessels and occasional fibrinoid necrosis of the vascular media (22%), villous edema, mainly focal (50%), focal villous infarct and intervillous hemorrhage (27%). Acute chorioamnionitis and necrotizing deciduitis were noted in 38% of the cases, in combination with acute vasculitis and funisitis in 14%. Retroplacental hemorrhage was found in 14% of the cases. Only two placentas (5%) showed no macro- or microscopic abnormalities.

**Conclusions:** 68% of all reviewed placentas with extensive sickling of maternal erythrocytes had no previous diagnosis of sickle cell disorder. These finding alone, with/without other pathologic placental changes, should trigger further testing for definitive diagnosis and close monitoring of patients to achieve better outcome of future pregnancies.

## **18 Exencephaly in the Setting of Amniotic Band Syndrome: A Review of Four Cases With Discussion of Pathogenesis**

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**Background:** Exencephaly is defined as partial or complete absence of the calvaria associated with protrusion of abnormally developed brain tissue through the defect. A small proportion of cases of exencephaly have been associated with amniotic band syndrome, but the pathogenesis of this association has not been fully elucidated.

**Design:** Cases of exencephaly associated with amniotic bands that had undergone full post mortem examination between 1 January 2005 and 1 July 2011 were identified and reviewed.

**Results:** Three cases were identified; they were 21, 22, and 25 weeks gestational age respectively. In two of the cases, examination of the membrane covering the brain showed amnion with a hypervascular subepithelial layer. Two cases showed features of early malformative change (arhinencephaly, abnormal basal vascular pattern) and in all three cases there was evidence of reactive phenomena (leptomeningeal heterotopias, leptomeningeal giant cells, and old haemorrhage). In at least one case, extra-amniotic development of the central nervous system was demonstrable. In all of the cases there was evidence of typical local amniotic band injury to the limbs.

**Conclusions:** These features suggest that mechanical, vascular, and induction events all probably contribute to abnormal brain development in amniotic band syndrome with exencephaly. It is likely that in early gestation disruption of the development of the brain structure is impacted, and at later gestation haemorrhage is a significant factor.

## **19 19 Month-old Male with Clinical Diagnosis of Congenital Lamellar Ichthyosis and Multiorgan Involvement by Langerhans Cell Histiocytosis.**

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**Background:** Congenital Lamellar Ichthyosis is a rare disorder of skin keratinization. Langerhans cell histiocytosis is neoplastic process of CD1a positive Langerhans cells. To the best of our knowledge association between Langerhans cell histiocytosis and Congenital Lamellar Ichthyosis has not been previously reported.

**Design:** We incurred a case of LCH in congenital lamellar ichthyosis and collected the clinical information. Here we present a case of a 19 month old boy with congenital lamellar ichthyosis who presented with fever, hepatosplenomegaly, anemia and thrombocytopenia. Differential diagnosis included infection, malignancy and hemophagocytic lymphohistiocytosis (HLH). Viral and fungal cultures were negative. Blood chemistry showed normal serum ferritin and mildly elevated triglycerides. The patient had a prolonged prothrombin time and elevated EBV IgM. A CT scan showed diffuse hepatomegaly and massive splenomegaly (15.6 cm). PET CT scan showed diffuse and strong FDG uptake in the spleen and non-significant uptake in the liver. Bone marrow biopsy was normal for age.

**Results:** Spleen biopsy showed clusters and sheets of CD1a and S100- positive cells with large amphophilic cytoplasm and folded nuclei, compatible with Langerhans cell histiocytosis (LCH). Tissue cultures and special stains for microorganisms (GMS and Acid Fast) were negative. A skin biopsy showed excessive keratosis (ichthyosis). Liver biopsy showed portal tract involvement by LCH. Unusual features were lack of ductular infiltration, paucity of eosinophilic infiltrate and large Golgi zone surrounded by numerous lysosomes (PAS, PASD, electron microscopy) in lesional cells. The patient was treated with Methylprednisolone and Vinblastine with excellent response and is alive and free of disease 4 months after the presentation. Unusual cytology of Langerhans cells with large Golgi apparatus and numerous lysosomes containing PAS-positive diastase-resistant material was identified in our case.

**Conclusions:** Hepatosplenomegaly in congenital Lamellar Ichthyosis can be due to involvement by Langerhans cell histiocytosis. Langerhans cells may have some unusual and/or exaggerated features. Perhaps this may reflect an unknown underlying metabolic defect contributing to both conditions. Whether there is a common link between congenital lamellar ichthyosis and LCH remains to be elucidated.

## 20 Clinicopathological Factors as Predictors of Outcome in Term Infants with Hypoxic Ischemic Encephalopathy Undergoing Therapeutic Hypothermia.

LM Ernst, RO deRegnier, L Boswell, MH Huang, JY Khan., Prentice Women's Hospital, Northwestern University, Chicago, IL.

**Background:** Despite the similar severe degree of insult, neurological outcome of infants surviving hypoxic ischemic encephalopathy (HIE) treated with hypothermia varies. Placental examination can provide useful information about the antenatal environment which may shed light on the nature and severity of in utero processes contributing to hypoxia. The purpose of this study is to investigate the role of placental pathology in the context of clinical factors known to predict outcome in patients treated with selective head cooling.

**Design:** Placentas of 30 consecutive term infants with HIE treated with hypothermia were collected both retrospectively and prospectively between 2006 and present. Placentas were examined grossly, weighed, and sampled with sections of membranes, umbilical cord, and fetal/maternal surfaces. Gross and microscopic pathology was categorized. Major inflammatory/infectious pathology (both maternal and fetal inflammatory responses) and major vascular pathologies (maternal vascular underperfusion, fetal thrombotic vasculopathy, pathologic evidence of abruption, high grade chronic villitis, meconium-induced myonecrosis) were recorded. Logistic regression models, odd ratios (OR) and 95% confidence interval (CI), were used to select and evaluate factors predictive of poorest outcomes, defined as death or severe neurological impairment (NI).

**Results:** Seven (23%) infants died and 23 (77%) survived. Among survivors, 6 (26%) had severe NI and 17 (74%) had normal or near-normal outcome. Major vascular pathology and major inflammatory/infectious pathology was found in 53% and 43% of infants respectively. Baseline covariates are shown in table below. Multivariate models suggest that major vascular pathology (OR, 11.61) and any abnormal placental weight for gestational age (OR, 3.69) may be important factors, while major inflammatory pathology had a modest effect (OR, 1.4); however, none of these factors were statistically significant, and only 10 minute Apgar score was found to be an independent predictor of adverse outcome (OR, 52.43; P=0.009).

Table: Factors associated with death/severe NI

<i>FACTOR</i>	<i>OR</i>	<i>95% CI</i>	<i>P-value</i>
Major Vascular Pathology(+/-):	1.8	0.43-7.53	0.431
Maternal Vascular Underperfusion(+/-):	3.33	0.57-18.85	0.197
Fetal thrombotic vasculopathy(+/-):	0.85	0.14-5.16	0.869
Clinical Abruption (+/-):	2.03	0.44-9.32	0.377
Pathologic Abruption (+/-):	2.25	3.37-13.41	0.41
Major Inflammatory Pathology(+/-):	0.7	0.17-2.95	0.638
Intense Chorionic Vasculitis (+/-):	0.85	0.14-5.16	0.869
10 min Apgar score ( < 5 / ≥ 5):	15.56	2.74-88.49	0.0012

**Conclusions:** Although limited by small sample size, our results show that despite frequent abnormal placental pathology, only Apgar score < 5 at 10 minutes is an independent predictor of outcome. Findings also suggest that vascular pathology may be relatively more important than inflammatory/infectious pathology.

## 21 Upregulation of the Endothelin System in the IUGR Rat Model Induced by Maternal Hyperinsulinemia

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**Background:** Exogenous hyperinsulinemia causes intrauterine growth restriction (IUGR) and abrogation of the normal gestational blood pressure (BP) decline in pregnant rats. This is associated with shallower trophoblastic invasion in the implantation site, designated the mesometrial triangle (MT). We have previously demonstrated altered expression of the 3 isoforms of nitric oxide synthase (NOS) in the placenta and MT of hyperinsulinemic dams (HD). Endothelin counteracts the vasodilating effect of NO. Hyperinsulinemia may increase production of endothelin-1 (ET-1), produced by sequential proteolysis of its precursor big endothelin by endothelin-converting enzyme (ECE)-1. Our aim was to examine the expression of the endothelin system in the placenta and other organs in our IUGR rat model.

**Design:** Rats were rendered hyperinsulinemic by implantation of a subcutaneous insulin pellet, mated and followed to pregnancy day 21. ECE-1 expression was examined by western blot in the placenta and MT, kidneys, heart and liver of HD. Immunostaining for ECE-1, endothelin receptor A (ET-A) and ET-B was quantified by an automated image analysis system.

**Results:** HD had higher BP than normal pregnant dams (NPD) ( $130\pm 17$  mmHg in HD vs.  $115\pm 16$  mmHg in NPD,  $p < 0.05$ ), lower placenta weight ( $0.44\pm 0.08$  g in HD vs.  $0.47\pm 0.08$  NPD,  $p < 0.05$ ) and reduced fetal weight (males  $4.9\pm 0.4$  g in HD vs.  $5.5\pm 0.4$  g in NPD,  $p < 0.0001$ ; females  $4.7\pm 0.4$  g in HD vs.  $5.2\pm 0.4$  g in NPD,  $p < 0.0001$ ). ECE-1 protein expression was significantly increased in the placenta and the MT of HD by 46% and 48%, respectively. In the kidney and heart of HD ECE-1 protein expression was increased by 230% and 220%, respectively, but its level in the liver was similar in both groups. Immunostaining revealed ECE-1 expression in endothelial cells and in trophoblastic cells of the placenta and MT. ET-A, a mediator of vasoconstriction by ET-1, was also expressed in endothelium and in trophoblasts of the placenta and MT. The expression of both ECE-1 and ET-A was significantly stronger in HD. In contrast the expression of ET-B was similar in both groups.

**Conclusions:** ECE-1 and ET-A, mediators of vasoconstriction, are expressed in trophoblast cells in the placenta and MT. Their overexpression in HD may affect local endothelin levels, thus BP, and may be one of the factors leading to IUGR.

## 22 Congenital Anomalies in Patients with Multiple Nephrogenic Rests

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**Background:** An association between intralobar or perilobar nephrogenic rests (ILNR, PLNR) and several constitutional syndromes has been established. However, the significance of the presence of multiple nephrogenic rests within a kidney removed for Wilms tumor has not been thoroughly investigated. This results in questions regarding the need for ancillary laboratory tests in search of constitutional genetic changes.

**Design:** This study describes congenital anomalies (CA) identified in patients with multiple, single, and no nephrogenic rests registered on National Wilms Tumor Study -5 (NWTS-5 ). This protocol required the provision of data regarding the presence of CA and other clinical conditions which were documented with ICD-9 codes. The presence of nephrogenic rests, Beckwith medulla and dysplastic medullary ray nodules (features previously associated with Beckwith-Wiedemann syndrome, BWS) were prospectively recorded at the time of central review.

**Results:** 618/2550 evaluable patients had multiple nephrogenic rests. CA were identified in 28%, 19%, and 12% of patients with multiple, single, and no nephrogenic rests, respectively. PLNRs were commonly multiple; CA were identified in 23% and 22% of patients with multiple and single PLNRs, respectively. In contrast, CA were identified in 35% and 18% of patients with multiple and single ILNRs, respectively and in 34% of patients with both ILNR and PLNRs. Five percent of all patients had clinical or pathologic evidence of an overgrowth syndrome. While the majority of these had multiple PLNRs, many had both ILNRs and PLNRs or ILNRs alone.

Dysplastic medullary ray nodules and/or Beckwith medulla were identified in 2% of all patients, and 28% of these carried the clinical diagnosis of an overgrowth syndrome. 18 patients (0.7%) presented with Denys-Drash (DD) and 21 (0.8%) presented with aniridia/WAGR spectrum. The majority of these contained single or multiple ILNR. More patients with multiple ILNRs had clinical evidence of BWS or isolated hemihypertrophy (15) than either DD (12) or WAGR (8).

**Conclusions:** All patients with nephrogenic rests have an increase in CA. Patients with multiple ILNRs and patients with both ILNRs and PLNRs have the highest percentage of CA, particularly constitutional syndromes associated with Wilms tumor. Patients with BWS may show both ILNR and PLNRs, and often show dysplastic medullary ray nodules or Beckwith medulla.

## 23 Exstrophy Polyps or Polypoid Cystitis in Exstrophy Bladders as a Unique Pathology Entity

Rong Fan, David J. Grignon, Liang Cheng, Department of Pathology, Indiana University, Indianapolis, Indiana

**Background:** Exstrophy of bladder is a congenital malformation, with estimated incidence between 1 in 50,000 to 30,000 live births. It can happen in both genders, but typically has a male predominance. The polyps arising from these everted bladders are also relatively common and well-known to the specialists in pediatric urology; however, they are less well-known or even poorly recognized among the pathologists. This research is an effort from the pathologist's perspective to describe and define the unique gross and histopathology features of these exstrophic bladder associated polyps and establish a unique pathological entity. Some correlations with clinical features are made and tentative conclusions are drawn to guide clinical management.

**Design:** Thirteen cases of the bladder polyps arising from patients with classic bladder exstrophy from a single major pediatric institution files were retrieved. The operational notes, pathology reports, slides were examined and reviewed by one pediatric pathologist and two genitourinary pathologists. The gross and histopathology features of the polyps were reviewed and correlated with major clinical parameters.

**Results:** The exstrophic bladders polyps were unique in their gross and microscopic appearance. Grossly, they were mostly round sessile polyps with a red angry surface; the size range varied from 0.5 to 2 cm. The patient age range of patients was from 2 days to 17 years old, with a male predominance 2:1. On histopathology, the exstrophy bladder polyps have the all the features of the conventional chronic cystitis, showing variable degree of acute and chronic inflammation with occasional lymphoid aggregates, edema, fibrosis, squamous metaplasia, von Brunn's nests, cystitis cystica, cystitis glandularis and cystitis glandularis with intestinal metaplasia; however, in contrast to conventional cystitis cases, these exstrophy polyps have some very unique features. The von Brunn's nests, cystitis cystica and cystitis glandularis in these polyps typically go very deep in the stroma, with very prominent fibrotic concentric cuffing; the background stroma typically has rich vasculature and fascicles of smooth muscle bundles (muscularis propria) present. Cystitis glandularis with intestinal metaplasia is considered as more advanced change in chronic cystitis and happens more frequently when surgery occurs at more later stage of life.

**Conclusions:** Exstrophy polyp or polypoid cystitis in exstrophy bladder should be considered as separate pathological entity due to their unique gross and histopathology features. When correlated with the age at surgery, the longer the exposure time is, the more advanced changes will show up, such as cystitis glandularis and intestinal metaplasia, which are considered pre-malignant as we understand them. It seems reasonable to do the repair of the exstrophy bladders as early as possible, not only in term of immediate quality of life issues with the patients, but also justified because of possible decreased risk of malignant potential.

## **24 Primary Intrarenal Neuroblastoma- A Clinical Pathologic Study of 8 Cases**

Rong Fan, Department of Pathology, Riley Hospital for Children, Indiana University, Indiana

**Background:** Neuroblastoma is the most common extracranial solid tumor in infancy and early childhood. However, primary intrarenal neuroblastoma is rare and only scattered case reports exist in the English Medical Literature. We report 8 cases accumulated in our institution over the past years and summarized their clinicopathological features.

**Design:** After retrieving the 8 cases from an electronic database(Copath)of our institution, a major state pediatric hospital, the pathology slides and reports, operative notes, radiology images, lab results and extensive hospital charts and clinical notes have been reviewed to extract the relevant clinical and pathological information. Neuroblastoma cases with secondary renal involvement are excluded.

**Results:** The composite picture of a patient of intrarenal neuroblastoma is a male child of 17 month of age, presents with a large renal mass, about 9 cm in size, accompanied by hypertension. It usually lacks of sufficient amount of calcification to facilitate the radiology detection. The mass is typically hemorrhagic but can be either encapsulated or unencapsuated and infiltrating; an intrarenal neuroblastoma can be undifferentiated, poorly differentiated or differentiating, fall into either Favorable or Unfavorable Histology category with presentation at a higher stage. The N-myc is typically unamplified and bone marrow usually is not involved at presentation. Unless the tumor is undifferentiated or very poorly differentiated, patients with intrarenal neuroblastoma fare well, though not without improved and new modalities of treatment.

**Conclusions:** Primary intrarenal neuroblastoma is perhaps more common than people realized and higher level of awareness and early recognition is important in terms of prognosis and management as they are very different from that of Wilms tumor.

## 25 PAX Immunoreactivity Pattern in Rhabdomyosarcoma

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**Background:** PAX gene products belong to a family of nuclear transcription factors involving embryogenesis and many developmental controls such as kidney, eye, ear, nose, and brain. Their patterns of expression in rhabdomyosarcoma were rarely studied in the past; the exception being PAX5, which has been shown to be useful in identifying alveolar rhabdomyosarcoma (ARMS). We aim to explore the immunoreactivity pattern of three PAX gene expressions (PAX2, PAX5, and PAX8) in rhabdomyosarcoma and their possible useful diagnostic and therapeutic applications.

**Design:** With the Indiana University Institutional Review Board approval, 34 cases of rhabdomyosarcoma were collected from our surgical pathology files from 2005-2010. Twenty of the 34 cases were of embryonal type. The remaining 14 cases were of alveolar type. Original H&E slides and immunostains for PAX2, PAX5, and PAX8 were independently reviewed by two pathologists. The intensity of nuclear staining was evaluated for each marker and was assigned an incremental score of 0, 1+, 2+, or 3+. The extent of staining was categorized as focal (< 25%), multifocal (25% to 75%), or diffuse (>75%).

**Results:** All 14 ARMS were diffusely and strongly positive for PAX2. 10 of 20 embryonal rhabdomyosarcoma (ERMS) displayed variable immunoreexpression for PAX2. PAX8 immunoreexpression was noticed in five and three cases of ARMS and ERMS, respectively. PAX5 showed only weak reactivity in one case of each variant.

**Conclusions:** PAX2 appears to be a useful diagnostic marker for ARMS. Together PAX2 and PAX8 are immunoreactive to most of the rhabdomyosarcomas; however, PAX2 has greater sensitivity and specificity for ARMS as compared to PAX8. Neither marker is specific or sensitive for ERMS. PAX5 is generally nonreactive based on our results, contrary to the previously published literature, and needs to be further studied in a larger sample. PAX2 and PAX8 expression in rhabdomyosarcoma provide additional diagnostic tools and possible novel therapeutic targets.

## 26 An Immunohistochemical Analysis of Hepatoblastomas - Is There a Pattern?

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**Background:** Hepatoblastoma (HB) is the most common pediatric liver tumor and presents in early childhood with most cases in the first decade. The outcome is determined by Stage of the disease and specific morphologic subtypes. Well-differentiated fetal (WDF) Stage 1 tumors have excellent prognosis while small cell HB (SCU) has the worst prognosis. Immunohistochemical studies using beta-catenin, glypican-3, cyclin D1 and glutamine synthetase has been done in a few studies but specific correlation with histologic subtypes and variations of HB have not been attempted. The aim of this study is to use a panel of 4 stains to see if there is a pattern to staining of different morphologic subtypes of HB.

**Design:** With IRB exemption, a retrospective review of 87 cases of hepatoblastomas was done in a 25 year period with selection of 37 cases with adequate material for all staining. In some cases both biopsy and post-treatment resection were evaluated while in some only one specimen was available. In 4 cases metastases were also studied. Histology was reviewed in each case with determination of various components in each and specific areas of WDF, fetal with mitoses (Crowded fetal), embryonal, mesenchymal, SCU, cholangioblastic and teratoid areas were documented. All cases were stained with Glypican 3 (GPC3), beta-catenin (Bcat), glutamine synthetase (GS) and cyclin D1 (CCD1). The staining intensity and pattern (membrane, cytoplasmic or nuclear) was graded cumulatively as 0-3 and documented in each component. Adequate positive and negative controls were run.

**Results:** 21 patients were in the first 2 years of life (range 6 mths-8 years), the M:F ratio was 21:16. 28 cases were stage 1 disease and 4 were stage 4. The majority of cases showed a multiple pattern within the same tumor. The table summarizes the staining reaction and the patterns will be highlighted.

Histology	GPC3	GS	Bcat	CCD1	Total
Fetal - WDF+CF	35 pos	32 pos; 3 neg	35 pos	15 pos	35
Embryonal	28 pos	26 neg, 2 pos	27 pos	20 pos	28
SCU	13 neg	13 neg	13 pos	8 pos	13
Mesen	10 neg	All Neg	9 pos	4 pos	10
Cholangio	3 neg	All Neg	3 pos N	Neg	3

The GPC3 staining for WDF was fine granular while it was coarse and strong for crowded fetal and embryonal patterns. Bcat was mainly membranous in fetal with 8 cases showing nuclear staining. GS was cytoplasmic in all fetal subtypes.

**Conclusions:** The above staining panel is helpful in identifying specific HB histologic subtypes. All fetal HB show staining for GPC3 with GPC3 helping differentiate WDF and CF, most are positive for GS, a differentiation marker, show largely membranous Bcat staining although variably associated with nuclear Bcat staining and usually weaker but positive CCD1 staining. A negative GPC3 and GS with a strong nuclear Bcat brings out the SCU population. Identifying these low and high risk subgroups may help in predicting behavior and chemotherapeutic regimen when applied to prospective studies. Cyclin D1 correlated in most cases with activation of Bcat as does GS.

## 27 Morphologic Changes in Upper Gastrointestinal Tract Biopsies in Children with Prolonged Use of Proton Pump Inhibitors.

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**Background:** Although proton pump inhibitors (PPIs) have shown a remarkable tolerability profile, there are concerns regarding their long-term safety. Several studies have shown that PPIs are associated with an increased risk of development of fundic gland polyps, parietal cell hyperplasia, and nutritional complications. Non-specific gastritis has been reported without any identified cause.

**Design:** IRB-approved chart and pathology reports review of all the upper endoscopy procedures done in our institution from July 2009 to June 2010 was performed. Demographic data, PPI use (duration and dosage), and biopsy results were recorded. Subsequently, all biopsies were blindly reviewed by a single pathologist. Patients with H. pylori infection, as well as acute or severe chronic gastric inflammation were excluded. Altogether, 310 charts and 205 gastric biopsies were reviewed. Esophageal, gastric, and duodenal biopsies were evaluated for architectural changes, eosinophilic inflammation, chronic and acute inflammation, and organisms. Gastric biopsies were examined for parietal cell and neuroendocrine cell hyperplasia. Peak eosinophil count was recorded for all biopsies.

**Results:** A total of 193 patients were included; 88 (46%) had a history for PPI use, and 48 (25%) of those were found to have non-specific gastric inflammation (NSGI). The odds of NSGI were higher in patients who took PPI compared to those who did not (OR: 2.81, 95CI: 1.36-5.93)  $p=0.007$ . A longer duration of PPI use (>3 months) gave an even higher odds for having NSGI (OR: 5.44, 95CI: 1.46-20.28). PPI dosage was not independently associated with NSGI when adjusted for duration. Association with NSGI was highest for the combination of high dosage and prolonged use. No statistically significant difference was found upon evaluation of other morphologic parameters. Gender, ethnicity, age, and obesity were not associated with NSGI in multivariable models.

**Conclusions:** Prolonged PPI use was associated with NSGI, with the highest ORs among patients using high doses for a long time. Our results did not establish cause-and-effect correlation.

## 28 "Precursor" Lesions Incidentally Found in Two Liver Resections for Hepatoblastoma from Two Children with an APC Germline Mutation

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**Background:** Carcinogenesis is a multistep process in many human solid tumors encompassing successive genetic and histological alterations ultimately leading to cancer. An example of such an algorithm is seen with in Wilms tumor, thus it would not be surprising to find such a process in other pediatric solid tumors. Hepatoblastoma (HB) is the most common hepatic neoplasm in children. There is a known association between HB and familial polyposis syndrome (FAP). Compared to the general population, the risk of developing HB is 800x higher in children with germline mutations in the APC tumor suppressor gene. This mutation results in  $\beta$ -catenin translocation to the nucleus with transcription of oncogenic molecules, such as glutamine synthetase (GS), thus making the background liver fertile for precursor lesions. Overexpression of GS can be seen in FNH,  $\beta$ -catenin mutated adenomas, HB and majority of hepatocellular carcinomas. Recent literature has shown diffuse overexpression of GS +/- glypican 3 (GPC-3) in neoplastic liver lesions. We report two cases of a 9 and 18-month-old girls with APC germline mutations, who underwent a liver resection for HB. In addition to the main tumor, several independent lesions were grossly present in both livers. We underwent a detailed analysis of these nodules.

**Design:** Conventional H&E analysis and immunohistochemistry for GPC-3,  $\beta$ -catenin, CKAE1/AE3, CD34, MIB-1, GS and fatty-acid binding protein were performed on all 16 grossly visible independent lesions, background liver, and hepatoblastoma.

**Results:** These independent lesions were tan (slightly paler than the background), round to oval, bulging, & varied in size from 0.2 to 1.5 cms. Histology demonstrated ill-defined, unencapsulated, round-irregular nodules with rare to no portal tracts. Occasional lesions demonstrated foci of pseudoacinar transformation, binucleation, and anisocytosis. One of the lesions measuring 1.1cm in diameter demonstrated a nodule (0.9 cm) within a larger nodule. Diffuse glutamine synthetase reactivity was present in all the nodules and an additional 2 mm nodule (lesion 17) was recognized in a section of normal background liver. 59% of these lesions demonstrated at least focal reactivity to Glypican-3, and 17% demonstrated focus of nuclear  $\beta$ -catenin expression.

**Conclusions:** Although the morphology in the majority of these lesions was benign, the diffuse glutamine synthetase overexpression in all the nodules and occasional foci of glypican-3 expression in over half of the lesions suggests these lesions are neoplastic "in-situ/dysplastic/precursor" in nature. To our knowledge, this is the first case report of "precursor" lesions found along with hepatoblastoma in liver resections of children with an APC gene mutation.

## **29 Atypical Clinical Presentation of Primary Hemophagocytic Lymphohistiocytosis with a Novel Perforin1 Gene Mutation.**

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**Background:** Familial hemophagocytic lymphohistiocytosis, an inherited form of HLH syndrome, is an autosomal recessive immune disorder that most commonly presents with fever, hepatosplenomegaly and cytopenia. The median age of the patients at presentation is 3 months.

**Design:** Here we report a 3 year old boy who is the only common son of a non-consanguineous couple from Honduras without any history of hemophagocytic lymphohistiocytosis or other hematologic disorders in any of his step-siblings. He first presented to the emergency department with left leg pain. He underwent a left inguinal hernia repair four days prior to his initial presentation. He had several consequent visits to ER with abdominal pain, emesis and headaches and was admitted when his symptoms did not resolve. On admission he was found to have a splenomegaly, elevated C reactive protein, indirect bilirubin, C3 fraction and pancytopenia. Bone marrow aspirate revealed paucicellular trilineage hematopoiesis with rare atypical histiocytic cells and no evidence of malignancy. An unequivocal diagnosis of HLH could not be made at that time, but was strongly considered. MRI of the brain showed extensive, infra and supratentorial white matter disease.

**Results:** The patient's neurologic status progressively deteriorated leading to brain death. Noticeable, there were scattered infiltrates of CD8+ T lymphocytes and macrophages showing lymphohistiocytosis in multiple areas of the brain. Subsequently, genetic analysis showed a patient to be a compound heterozygote with a novel Perforin1, PRF1, missense mutation 659 G>A (G220D) in addition to previously described 681 C>T(R232C) mutation.

**Conclusions:** This case demonstrates an atypical clinical presentation of genetic/primary HLH due to patient's age and recent surgery. This case also documents a novel missense mutation of PRF1 which could be associated with the atypical clinical presentation.

### 30 Primary Mastoid Presentation of Acute Megakaryoblastic Leukemia: A Case Report and Comparison of Extramedullary M7 and Non-M7 Cases in the Literature

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**Background:** Extramedullary involvement of acute megakaryoblastic leukemia (M7) is rare among acute myeloid leukemia (AML) occurrences outside the bone marrow (BM). M7 often has complex clinical presentations such as lytic bone lesions. In children, it is frequently confused with bone tumors or metastatic solid tumors. Primary AML infiltration of the mastoid is also scarce. Notably, primary manifestation of M7 in the mastoid has never been reported. The extreme rarity of M7 involvement in the mastoid, the tendency of extramedullary M7 to mimic solid tumor, and the unusual morphology of megakaryoblasts can create a substantial diagnostic challenge. Here, we describe a case of M7 which initially presented with a mastoid lesion and review the literature comparing the characteristics of extramedullary M7 and Non-M7 AML.

**Design:** Primary extramedullary AML cases  $\leq$  18 years old were searched from Children's Hospital Colorado archives (1990 - 2011) and English literature (1971 - 2011). All cases were divided into M7 and non-M7 groups. Comparisons of clinical characteristics were made between the two groups and statistical significance was calculated. **Case Report:** A 12-month-old, karyotypically normal male presented with cranial nerve VII palsy and fever. He was treated with antibiotics for otitis media without improvement. CT scan revealed lytic changes in the mastoid. Biopsy showed a collection of CD99+CD1a- atypical cells. EWS/ETS translocation is not present. BM aspirate tumor cells were CD34+CD13+CD33+ CD117+CD56+CD41+CD61+ by flow cytometry. The diagnosis of AML-M7 was made. He was treated with chemotherapy and achieved complete remission for 6 months.

**Results:** 8 cases of extramedullary M7 and 80 cases of extramedullary non-M7 were identified. 1 AMKL patient and 4 non-AMKL AML patients were found to have mastoid leukemic infiltration.

	<i>Patients with Extramedullary M7</i>	<i>Patients with Extramedullary Non-M7</i>	<i>p-value</i>
# of Cases	8	80	-
Cases with mastoid involvement	1/8 (12.5%)	4/80 (5.0%)	0.3864**
Mean Age(age range)	1.62 (1-2.42) yrs	7.16 (0-18) yrs	< .0001*
$\leq$ 2 yrs	8/8 (100%)	27/80 (33.8%)	0.0004**
Male:Female	3:5	45:35	0.4601**
BM Lesion	8/8 (100%)	55/76 (72.4%)	0.1918**

\*t-test, \*\*Fisher's exact test

**Conclusions:** All pediatric patients with primary extramedullary M7 were  $\leq$  2 years of age and were significantly younger than those with extramedullary non-M7 AML. - While the incidence of primary mastoid presentation is low among extramedullary AML in children (5/88, 5.68%), mastoid involvement of M7 is even more rare (1/88, 1.14%). - AML involvement should be considered in the differential diagnosis of mastoid lesions in children. M7 should be especially considered in patients  $\leq$  2 years of age.