1 Mitosis-Karyorrhexis Index (MKI) and MYC Status in Neuroblastoma, with a Special Reference to Intermediate MKI: A Report from the Children’s Oncology Group (COG)

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Background: MYCN status is reported to have a reproducible relationship to mitotic and karyorrhectic (M/K) activities in neuroblastoma. According to the International Neuroblastoma Pathology Classification (INPC), one of 3 MKI classes is assigned for prognostic evaluation: low MKI (L-MKI, <100/5,000 cells, indicating better prognosis), intermediate MKI (I-MKI, 100-200/5,000 cells, better prognosis for pts <18 mos of age and poor prognosis for pts > 18 mos), or high MKI (H-MKI, >200/5,000 cells, poor prognosis).

Design: MKI classes of 4,282 neuroblastomas filed at the COG Neuroblastoma Pathology Reference Laboratory between 8/1/2001 and 3/31/2012 were determined by averaging the activities from representative microscopic fields of the tumor tissues. (1) Prognostic effects by 3 MKI classes, (2) Relationship between MYCN status (amplified-A or non-amplified-NA) and MKI, and (3) Effective age cut-off distinguishing two prognostic groups by I-MKI, were analyzed statistically. Immunostaining for N-myc and C-myc protein was performed on 50 selected I-MKI tumors (24 MYCN-A, 26 MYCN-NA).

Results: Outcome was significantly worse as MKI increased (p<0.0001): L-MKI (N=2,365; 81.2+/1.0% 3-yr EFS, 92.0+/0.7% 3-yr OS); I-MKI (N=1,068; 68.6+/1.8% EFS, 81.0+/1.6% OS); H-MKI (N=849; 51.2+/2.2% EFS, 64.4+/2.1% OS). A significant association was found between increasing MKI class and MYCN amplification (p<0.0001): MYCN-A tumors (N=814, L-MKI 9%, I-MKI 20%, H-MKI 71%); MYCN-NA tumors (N=3,468, L-MKI 66%, I-MKI 26%, H-MKI 8%). 85% (907) of I-MKI tumors were MYCN-NA. The exploratory Cox PH models for pts with I-MKI tumor demonstrated that age cut-offs of 12-18 mos effectively differentiated 2 prognostic groups in terms of increased risk of event for older pts. Furthermore pts <18 mos having I-MKI tumor had comparable EFS (83.8+/2.0%) and OS (93.7+/1.3%) with pts having L-MKI tumor while pts >18 mons having I-MKI tumor had comparable EFS (51.4+/2.9%) and OS (66.7+/2.7%) with pts having H-MKI tumor. Immunohistochemically, MYCN-A & I-MKI tumors often (22/24) expressed N-myc protein strongly (15) or faintly (7); while 6 of 26 MYCN-NA & I-MKI tumors expressed N-myc faintly. In this series, C-myc protein was expressed in 12 MYCN-NA tumors and 1 MYCN-A tumor. 3 tumors were positive for both C-myc and N-myc. 12 tumors were negative for both proteins.

Conclusions: As suggested molecularly, MYCN amplification was proven to be a driving force for increasing M/K activities. This study confirmed the INPC system using the age cut-off of 18 mos for prognostic distinction of the pts with I-MKI. Since MYCN-A is rare in I-MKI neuroblastomas, other mechanisms, either MYC-related (C-myc expression or faint N-myc expression in MYCN-NA cases) or -unrelated, should be considered for intermediately increased M/K activities.

2 ALK Point Mutations in Neuroblastoma: Association with Poorly Differentiated Morphology

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Background: ALK mutations are present in 8-9% of neuroblastomas (NB) and recent studies indicate that survival for patients with ALK mutations is significantly worse compared to those with wild-type ALK. Knowledge about ALK status of tumors is becoming more relevant as new small molecule inhibitors against ALK such as Crizotinib are increasingly being explored in targeting tumors harboring ALK mutations, including NB. Little is known about the pathologic features of NB harboring these mutations. We retrospectively analyzed the association of pathologic features of NB with their ALK mutation status.
Design: We searched for NB cases that were sequenced for ALK mutations in exons 23, 24, and 25. All samples were formalin fixed and paraffin embedded. We reviewed tumors that harbored mutations and compared the pathological findings with a group of samples where mutations were not detected. Overall we reviewed 25 tumors, 7 of which were pre-chemotherapy and 18 were post-chemotherapy.

Results: Of 83 cases tested for ALK point mutations, 10 (12%) harbored mutations in exon 23 (F1174L, 6 cases), exon 25 (R1275Q, 3 cases) or exon 24 (L1240V, 1 case), of which 3 were pre-chemotherapy and 7 were post-chemotherapy. Of 10 tumors with ALK mutations, 9 (90%) were poorly differentiated NB and one tumor had histology of differentiating NB. In the latter tumor, most of the sample consisted of poorly differentiated tumor but contained barely enough differentiating neuroblasts to qualify as differentiating NB. In comparison, 8 of 15 tumors without mutations (53%) were poorly differentiated NB and 7 tumors (47%) had differentiating or differentiated morphology including differentiating NB, ganglioneuroblastoma, and ganglioneuroma. All 7 pre-chemotherapy tumors were poorly differentiated NB (3 with ALK mutations and 4 without). However, of the post-chemotherapy samples, 6 of 7 (86%) with ALK mutations were poorly differentiated NB compared to 4 of 11 (36%) samples without mutations. Also, post-therapy poorly differentiated NB without mutations showed foci of marked nuclear pleomorphism and hyperchromasia, whereas none of the tumors with ALK mutations contained such pleomorphic foci.

Conclusions: ALK point mutations are present in a minority of neuroblastomas. These mutations are associated with poorly differentiated morphology with uniform neuroblasts even in post-chemotherapy setting in vast majority of cases. Lack of maturation following chemotherapy raises a possibility that traditional chemotherapy may not be appropriate for NB with ALK mutation, and should be a subject for future investigations on a larger cohort.

3 Clinicopathologic Features of Wilms Tumors with WTX Gene Mutation
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Background: A proportion of Wilms tumors (WT) are characterized by genetic and molecular abnormalities involving tumor suppressor genes (WT1) and oncogenes in Wnt pathway (CTNNB1 mutations). A recent body of literature shows that mutation or deletion of WTX gene is responsible for the tumorigenesis in Wilms tumor by dysregulating the beta-catenin and TP53 pathway. The goal of this study is to characterize the clinical and pathologic findings in WT with WTX gene mutation/deletion in comparison with sporadic WT and WT with WT1 and CTNNB1 mutation.

Design: Consecutive cases of WT with available frozen tissue were included. Clinical, gross and microscopic features were examined. Karyotype was examined in a subset of cases. All cases had been previously analyzed for WTX, WT-1 and CTNNB1 gene mutations and deletions via a detailed genome-wide scan for DNA copy-number changes; deletions were confirmed via quantitative PCR. Features of patients with a WTX gene abnormality (deletion or mutation) were compared with patients without. Clinical and pathologic variables were assessed using Fisher’s exact test with 2-tailed p values and student t-test.

Results: Eleven of 35 patients had a WTX abnormality. No significant differences were identified between patients with mutated versus nonmutated WTX with respect to gender (45 versus 33% male), age (mean age 3.9 versus 4.1 years), tumor size (mean size 12.7 versus 12.8 cm), anaplasia (10 versus 13%), rhabdomyoblastic differentiation (18 versus 8%), cartilage differentiation (9 versus 4%), mucinous epithelial differentiation (9 versus 4%), nephrogenic rests (28 versus 21%), or disease-free survival (89 versus 75%).

Conclusions: Our study revealed no appreciable clinicopathologic distinctions between WT patients with and without WTX gene abnormalities. This similarity lends support to the concept that there is a final common tumorigenic pathway among WTX-mutated WT and those with other mutations. Further studies, enriched for rare “outcomes” such as anaplasia or relapse/death, would have more statistical power to detect slight differences in tumor behavior.
4 Revisiting the Morphological Spectrum of Pediatric Cystic Nephromas: The COG Experience
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Background: Cystic nephromas (CNs) are uncommon renal neoplasms that almost exclusively affect either infants/young children or adult females. Although differences between the CN presenting in adults versus children have been suggested, large studies detailing the morphological spectrum of pediatric CN have not been conducted. Recent recognition of the association of pediatric CN with the pleuropulmonary blastoma syndrome that has been linked to DICER-1 mutations supports the notion that pediatric and adult CN may have different pathogeneses and biology, and indeed represent distinct entities.

Design: H&E slides from 37 patients registered on the COG study AREN03B2 with central pathology review of CN from 1/1/2007 to 3/1/2012, and 3 additional consult cases were reviewed.

Results: The typical multicystic architecture with thin septae seen in adult CN was also present in all pediatric cases. In addition, three histologic features within the pediatric cases that have not been frequently described in adult CN were identified. First, while a defined fibrous pseudocapsule surrounded the entire lesion in 19 cases, 21 cases were poorly demarcated from the normal parenchyma due to partial (11) or complete (10) lack of a pseudocapsule, often with intermingling of the cysts with the normal renal parenchyma. Second, many of the pediatric CN contained areas with increased amounts of septal stroma, greater variation in cyst size and shape with more numerous small cystic structures, and frequent pericyctic stromal condensation. These features conferred a more complex architecture reminiscent of multicystic dysplasia. These complex areas were a prominent feature in 21 cases, and had a statistically significant association with lack of encapsulation (p=0.0001). No immature elements, glomerular elements, hypercellular or ovarian-type stroma were seen. Third, in all cases with sampling of pelvicaliceal structures (36), the lesion appeared to abut the latter. In 18 cases, the lesion arose in direct contiguity to the pelvis, calices or papillae.

Conclusions: Pediatric CNs can be distinguished from those in adults by dysplasia-like morphologic features and lack of hypercellular or ovarian-type stroma. In our series, 42% of pediatric CN are characterized by both lack of encapsulation and prominent complex areas with smaller cysts and frequent pericyctic stromal condensation, and all show involvement of the renal pelvis. Molecular studies for DICER-1 mutations in these cases are in progress in order to establish a possible correlation with these histopathological findings. In particular, the contiguity with pelvicaliceal structures is intriguing in light of published evidence of aberrant development of the collecting system in DICER-1 negative animal models.

5 Somatic DICER1 Mutations in Cystic Nephroma
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Background: Cystic nephroma (CN) is a rare, benign tumor of the kidney. It has a bimodal age distribution with a male predominance in early childhood and a female predominance after the fifth decade of life which represent disparate morphologies. CN is commonly associated with the Pleuropulmonary Blastoma (PPB)-DICER1 syndrome. Heterozygous germline DICER1 mutations are identified in nearly 70% of patients with PPB. Recurrent somatic missense DICER1 mutations that significantly reduce production of miRNAs from the 5p arm of precursor miRNA molecules are also seen in the majority of PPBs and have also been described in ovarian stromal tumors. We sought to determine the incidence of DICER1 mutations in cases of CN referred independent of a history of familial PPB.
**Design:** De-identified DNA samples from 15 CN and 8 cystic partially differentiated nephroblastosoma (CPDN) tumors were obtained from the Cooperative Human Tissue Network (CHTN) from 2007 to 2010. Samples were annotated with age in years, site and a pathologic description. Sanger sequence analysis was performed.

**Results:** Ages ranged at diagnosis from 7 days to 8 years 10 months with a median age at diagnosis of 18 months. There were 14 females (61%) and 9 males (39%). Tumors were unilateral in all cases: 13 (57%) right and 8 (35%) left, unknown in 2 (8%). We identified deleterious DICER1 mutations in 8/15 (53.3%) CN samples and in 0/8 CPDN samples. Missense mutations in so-called “hotspot” regions were found in 9/15 CN (60%) and 2/8 (25%) CPDN; 5/15 (33.3%) CN had both deleterious and hotspot mutations, presumed to be biallelic.

**Conclusions:** Biallelic deleterious and missense hotspot DICER1 mutations are associated with CN similar to that seen in the PPB-DICER1 syndrome. Somatic hotspot mutations are also seen in CPDN confirming that it is likely related to CN and PPB. The percent of tumors with missense “hotspot” mutations may be underestimated in this pilot study as CN and CPDN often contain a mixture of normal-appearing and abnormal-appearing tissue. Massively parallel sequencing may be more sensitive in identifying somatic missense mutations in small populations of tumor cells within a sample. CN in a pediatric patient should prompt genetic testing for DICER1 mutations as it may represent the first clinical finding within a family harboring the PPB-DICER1 syndrome. The presence of “hotspot” missense mutations provides additional parallel with the pathogenesis of PPB and indicates that this second “hit” does not necessarily lead to cancerous transformation.

**6 Immunohistochemical Detection of Prognostically Significant Drug Targets in Pediatric Brain Tumors**

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**Background:** Brain tumors cause more deaths than any other childhood malignancy. In recent decades, dozens of novel therapeutic drugs, particularly kinase inhibitors, have been developed which have the potential to treat these tumors. In many cases, immunohistochemical stains can indicate whether a target is present that may respond to a particular drug.

**Design:** Archived formalin-fixed paraffin-embedded tissue was used to create tissue microarrays (TMA) for four tumor types: high-grade glioma (n=15), medulloblastoma (n=36), ependymoma (n=35), and primitive neuroectodermal tumor (n=8). Each tumor was sampled in duplicate or triplicate. In some instances, primary and recurrent tumors from the same patient were represented. Each TMA was stained with immunohistochemical antibodies to potential therapeutic drug targets (EGFR, PDGFR, HER2, CD117, pERK, and pS6). Staining was independently scored on a 0-4+ scale by two pathologists, with the exception of HER2, for which the standard 0-3+ breast scoring system was used. Immunohistochemical expression patterns were analyzed with detailed clinical information to determine survival.

**Results:** Mean three year survival by tumor subtype ranged from 58-83%, with no statistically significant difference in patient survival by tumor type (p = 0.68). For all tumor types, 78% of patients with EGFR - tumors were alive at 3 years, versus 37% of patients with EGFR + tumors. Univariate analysis confirmed that EGFR expression negatively impacts survival (p= 0.011). Preliminary data by tumor subtype indicates that HER2 is a significant predictor of poor survival in patients with ependymomas (p = 0.007). There were trends in survival with several other markers, including PDGFR expression (p = 0.09) and pS6 (p = 0.07), but they did not reach statistical significance in this study.

**Conclusions:** EGFR expression by diverse types of pediatric brain tumors is associated with a poor prognosis. Additionally, HER2 may be a novel predictive marker of poor survival in ependymomas. In the future, target-specific immunohistochemical stains may be used to individualize therapy for pediatric brain tumor patients.
7  Beta-HCG Positive Tumor Cells are Common in Pre-Treatment Osteosarcoma
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Background: Beta-hCG (b-hCG) expression has been reported in a variety of cancers including osteosarcoma, with some cases associated with elevated serum b-hCG levels. No large series of pre-treatment biopsies have been studied to determine the frequency of b-hCG immunoreactivity in osteosarcoma, the extent of paraneoplastic b-hCG expression, or association with clinical parameters.

Design: Pathology files from 2006-2012 at The Children’s Hospital of Philadelphia were searched for pre-treatment osteosarcoma. Fifty-one cases with viable tumor were selected for b-hCG immunohistochemistry (IHC). Placenta served as positive control. The percentage of cells positive for cytoplasmic b-hCG was evaluated for each case. Two cases with extensive hemosiderin deposition were excluded from scoring. Data on patient and tumor characteristics were collected for the remaining 49 cases. Two-tailed Student’s t-test and Fisher’s exact test (significance level 0.05) were used for statistical analysis.

Results: All 51 biopsies showed high-grade osteosarcoma (34 conventional, 3 epithelioid, 1 periosteal, 1 periosteal chondroblastic, 1 small cell, 1 telangiectatic, and 10 high-grade not otherwise specified). Of the 49 scored biopsies, 28/49 (57%) were positive for b-hCG by IHC. Twenty-seven of 28 (96%) positive cases showed rare cells (<2%) scattered throughout the lesion or in small clusters. The histology of the positive cells was similar to that of the negative cells. One epithelioid osteosarcoma in a female patient had b-hCG staining in 10% of cells. Twenty-three of 24 female patients had negative urine and/or serum b-hCG values. The case with 10% tumor staining demonstrated a positive urine pregnancy test and serum b-hCG of 64.6 mIU/mL which resolved to <1 mIU/mL after tumor resection. Beta-hCG values were not available on the 25 male patients.

There was no statistical difference in patient age, sex, presence of metastases, live/dead status, tumor size, or treatment response (% necrosis), between b-hCG positive and negative IHC groups.

Conclusions: Sparse b-hCG positivity is common in pre-treatment osteosarcoma specimens; however, rare cases show significant b-hCG expression with a secondary paraneoplastic syndrome. This is similar to previous case reports of elevated serum b-hCG levels in osteosarcoma patients. In contrast to a previous smaller case series, we found no difference in treatment response between b-hCG IHC positive and negative groups; however, there is limited follow up time. Recognition that osteosarcomas can express b-hCG can prevent confusion with other b-hCG positive tumors or tumors with scattered syncytiotrophoblasts that may metastasize such as choriocarcinoma, epithelioid trophoblastic tumor, dysgerminoma/seminoma and yolk sac tumor. Furthermore, recent Phase II clinical trials of b-hCG vaccines have shown promising results in colon and bladder cancer, and these vaccines may serve as an additional treatment modality for a subset of osteosarcomas.

8  Evaluation of Intestinal Biopsies for Pediatric Enteropathies: A Proposed Immunohistochemical Panel Approach
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Background: Congenital enteropathies are rare disorders with significant clinical consequences. Definitive diagnosis is often difficult or impossible based on morphologic assessment of intestinal biopsies with routine stains alone. To determine the role of immunohistochemistry in the evaluation for possible enteroendocrine cell dysgenesis, microvillous inclusion disease, and tufting enteropathy, we retrospectively reviewed a series of biopsies from pediatric patients with congenital refractory diarrhea.

Design: Twenty-six pediatric patients with chronic diarrhea and available duodenal biopsy materials were identified from our institutional database from 1996 to 2012. All patients were under the age of 24 months at the time of presentation, or there was otherwise clinical or histologic suspicion for a congenital enteropathy. Two patients were diagnosed with microvillous inclusion disease at the time of initial evaluation. The remaining 24 patients had no definitive diagnosis. Immunohistochemical stains for chromogranin, CD10, and EpCAM were performed on all biopsies, and the results were
correlated with H&E and ultrastructural findings, when available.

**Results:** The 2 patients diagnosed with microvillous inclusion disease at the time of initial evaluation both demonstrated diffuse CD10-positive cytoplasmic inclusions in duodenal epithelial cells with normal expression of EpCAM and chromogranin. Biopsies from three patients, including two siblings with confirmed EPCAM mutations, demonstrated complete loss of EpCAM expression with normal CD10 and chromogranin staining; electron microscopic evaluation revealed characteristic ultrastructural findings of tufting enteropathy. One patient with confirmed NEUROG3 mutation demonstrated an absence of intestinal enteroendocrine cells by chromogranin staining with normal expression of CD10 and EpCAM, consistent with enteroendocrine cell dysgenesis. Biopsies from 1 patient exhibited focal loss of EpCAM expression, as well as focal large, CD10-positive cytoplasmic inclusions, with ultrastructural analysis revealing mixed features of tufting enteropathy and microvillous inclusion disease. Three biopsies demonstrated patchy loss of EpCAM expression, and two biopsies exhibited small, CD10-positive cytoplasmic globules. Fifteen biopsies exhibited normal expression for all 3 markers.

**Conclusions:** The routine use of an immunohistochemical panel of EpCAM, CD10, and chromogranin may be warranted in patients meeting specific age and/or clinical criteria, as the morphologic findings of congenital pediatric enteropathies may be subtle, focal, or inapparent on routine stains.

9   Villin Immunohistochemistry is an Optimal Method for Diagnosing Microvillous Inclusion Disease

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**Background:** Microvillous inclusion disease (MID) is a rare congenital disorder that manifests as intractable watery diarrhea. It is characterized by a deficient brush border and microvillous inclusions within the apical cytoplasm of absorptive cells due to misplaced assembly of brush border proteins. The diagnosis is based upon histopathology, immunohistochemistry (IHC), and electron microscopy. Currently, CD10 IHC is commonly used as an adjunct, but like the PAS, stains the brush border and a variety of apical cytoplasmic structures including microvillous inclusions, multivesicular bodies, lysosomes and autophagic vacuoles interfering with recognition of microvillous inclusions. Villin is a protein that specifically binds to the actin core bundle of microvilli. We have investigated by IHC whether villin is superior to CD10 in diagnosing MID.

**Design:** We utilized antibodies to villin (clone SP67, Ventana, Tucson) and CD10 (clone CWWB1, Ventana, Tucson) on formalin-fixed paraffin-embedded duodenal biopsies from 5 children with ultrastructurally confirmed MID, 5 with celiac disease and, as a control group, 5 children with ill-defined intestinal symptoms whose biopsies were normal.

**Results:** In the control group, both villin and CD10 IHC showed a distinct, uniform and densely stained brush border, but CD10 IHC also displayed granular staining of the apical cytoplasm. In MID, both villin and CD10 IHC showed an attenuated and focally absent brush border, but villin IHC also exquisitely delineated the microvillous inclusions whereas they were largely obscured by the globular and coarse granular staining with CD10 IHC. In celiac disease, both villin and CD10 IHC showed a generally thinned brush border but the staining pattern was otherwise similar to the control group, respectively.

**Conclusions:** Villin IHC not only depicts the deficient brush border, but also clearly defines the cytoplasmic microvillous inclusions in MID, making it the optimal adjunct for diagnosis and likely obviating the need for electron microscopy.
10 Pediatric Gastrointestinal Graft-Versus-Host-Disease: Use of Paneth Cell Population as a Predictor of Patient Prognosis
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**Background:** Allogeneic hematopoietic-cell transplantation (HCT) is a common therapeutic method for many childhood malignancies. However, Graft-versus-host disease (GVHD) is a major obstacle to such potentially life-saving transfusions, and occurs when donor T cells respond to host human leukocyte antigens (HLAs). GVHD mainly affects three main organ systems including skin, liver, and gastrointestinal tract (GI). GVHD of the GI tract affects more than 60% of patients who undergo an HCT, leading to many complications including nausea, GI hemorrhage, and secretory diarrhea. Currently, endoscopic biopsy is used to determine the presence of GVHD. However, current histological grading (GVHD I-IV) fail to constantly correlate with severity or clinical outcome. Recent studies on animal models found a strong correlation between an islet-derived 3-alpha (REG3α), a C-type lectin secreted by Paneth cells with the clinical outcome in bone marrow transplant patients (BMT). Paneth cells contribute to mucosal innate immunity by releasing host defense proteins and antimicrobials including lysozymes and α-defensins. GVHD of the GI tract specifically affects Paneth cells, causing an increase in intestinal flora, which leads to an increase in denudation of the intestinal mucosa, providing the characteristic histology seen in GVHD.

**Design:** After IRB approval, we reviewed our files from (2002-2012) and found 71 pediatric GVHD patients with 119 biopsies. We evaluated the number of Paneth cells under 40X high power field by two pathologists independently utilizing immunostains for lysozyme. The charts were reviewed for the number of GI biopsies, recurrent rate of GVHD, and morbidity/mortality.

**Results:** We divided the patients into two groups, those with 10 or more Paneth cells and those with less than 10 Paneth cells per high power field. 42 children showed 10 or more Paneth cells while 29 children showed less than 10 Paneth cells. 9 out of 42 in the first group (21%) while 13 out of 29 (44.8%) in the second group showed an aggressive course and died, with a p-value of (0.04). The current grading system (GVHD-I through GVHD-IV) showed no consistent correlation with mortality in these patients.

**Conclusions:** Our results showed that patients with less than 10 Paneth cells per high power field demonstrated a statistically significant higher mortality rate. These results could have a tremendous impact on predicting the clinical outcome of patients affected with GVHD by correlating the number of Paneth cells with the prognosis of GVHD patients. This new parameter will provide clinicians with an additional tool to determine appropriate treatment.

11 Role of WNT/Beta-Catenin Pathway in TPN-Induced Liver Injury: Does IHC Have a Role in Assessing the Need for Transplant?
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**Background:** Cholestatic jaundice is the major complication of total parenteral nutrition (TPN) in infants and children. Liver disease develops in 40-60% of infants who require long-term TPN for intestinal failure with development of portal hypertension and liver failure. This liver injury and degree of fibrosis noted on biopsies determines the need to transplant. The mechanisms that lead to liver failure are not well understood and the role of liver regeneration in TPN livers has not been studied. The Wnt/beta-catenin pathway is known to play a significant role in liver regeneration and we hoped to study its role in TPN induced liver damage. Our hypothesis was that significant liver regeneration caused by beta-catenin (BC) activation would result in increased glutamine synthetase (GS) (a known activation marker of BC) expression and possibly GPC3. This may result in a favorable outcome without significant irreversible liver damage and hence these patients may not need liver transplant.

**Design:** A retrospective study of TPN liver biopsies and explants (55 specimens from 49 patients)
was carried out with IRB approval. 2 groups were represented in the sample Group 1: Patients with no liver Tx, but with biopsies - includes intestinal Tx only (25 patients); Group 2: liver Tx with SI or multivisceral (30 specimen from 24 patients). A representative histologic slide was reviewed and stains for BC, GS, GPC3 (all cases) were performed. Histology was evaluated for typical features and extent of fibrosis while IHC was interpreted for localization and intensity (BC-memb, cyto or nuclear); GS (normal pericentral, expanded zonal, diffuse or loss) and GPC3 (positive 1+-3+ or negative) with appropriate stain controls.

**Results:** Short gut syndrome was the main cause for TPN therapy. Group 1: All showed strong GS staining except 2 which had focal/weak staining. GPC3 showed strong staining in 16/25 patients in Group 1. BC was membranous to cytoplasmic in all cases with GS staining. Group 2: In 6 cases biopsy and explants were available and stained. GS was strongly positive in 5/6 biopsies but was negative in 5/6 corresponding explants; In the 18 single Tx specimens, 4/18 had strong GS while 14/18 had focal or loss of staining. GPC3 was negative in 19/24 cases in explants, while in 3 of these the biopsies was positive but explants were negative. Beta-catenin showed strong membranous staining in all those cases with GS staining, with some cytoplasmic staining also which was difficult to evaluate.

**Conclusions:** Wnt/BC pathway appears to be upregulated in TPN induced liver injury. Activation results in overexpression of GS which appears to correlate with preservation of liver function and the lesser likelihood of need for transplant. Loss of GS staining in biopsy (implying lack of BC induced liver regeneration) appears to increase the risk for liver failure and/or portal hypertension and need for transplant. GPC3 staining correlates and supports this regeneration and may explain elevated AFP levels.

**12 Hyaline Droplets in Kupffer Cells; A Novel Diagnostic Clue for Autoimmune Hepatitis**

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**Background:** Pediatric autoimmune hepatitis (AIH) is relatively common and has a characteristic but non-specific histopathology with a usually prominent lymphoplasmacytic infiltrate. Immunoglobulin immunofluorescence in mesenchymal cells was described 50-years-ago in unclassified hepatitis and cirrhosis. Herein, we describe for the first time the presence of characteristic hyaline droplets in the cytoplasm of Kupffer cells on routine H&E section in autoimmune hepatitis.

**Design:** The medical records and pathologic material from children with AIH (n=30), hepatitis B (n=30), and hepatitis C (n=30) from the pathology files at Boston Children’s Hospital over a 20-year-period (1992-2012) were reviewed. All children had percutaneous needle liver biopsies. We reviewed the H&E, PAS and PAS with diastase stained sections for the presence of hyaline droplets in all 90 biopsies. We also performed immunohistochemistry for IgG, IgA and IgD in 6 selected biopsies with AIH. Serum IgG levels, when available, were correlated with biopsy findings. Statistical analysis utilizing a two-tailed T-test was performed in the children with AIH to compare their serum IgG levels with the presence or absence of droplets in their liver biopsies.

**Results:** The average age of patients with AIH was 15 years (range from 6-23, 16 males and 14 females). Of these patients, 17 had type 1, 2 had type 2, 7 had overlap syndrome and 4 were unclassified. Hyaline droplets were identified in Kupffer cells throughout the lobules in 16 of 30 biopsies (easily found in 14 and rare in 2); conversely no droplets were identified in 14. They were identified in 10 AIH type 1 biopsies, 1 in AIH type 2, 3 in overlap syndrome and 2 in unclassified. Seventeen patients had serum IgG levels available for review. The average IgG level in patients without droplets in their liver biopsies was 1459 mg/dl, in contrast to 3189 mg/dl in patients with droplets (p-value 0.069). IHC performed in 6 biopsies revealed that droplets were nearly always positive for IgG, occasionally for IgA, and rarely for IgD. The average age of patients with hepatitis C was 15 years (range of 2-30, 17 males and 13 females); none of the biopsies contained hyaline droplets. The average age of patients with hepatitis B was 13 years (range of 2-23, 18 males and 12
females); a single biopsy revealed hyaline droplets. The latter biopsy had an unusually prominent plasmacytic infiltrate raising the possibility of overlap AIH / viral hepatitis syndrome.

**Conclusions:** As far as we are aware, hyaline droplets in Kupffer cells on routine H&E sections have never been described. They should be distinguished from the nonspecific granular lysosomal structures frequently found in Kupffer cells in a variety of chronic liver diseases. The presence of hyaline droplets may occur in AIH regardless of the type and correlates with a greater than 2-fold increase in serum level of IgG as compared to patients without droplets in their liver biopsies. Identification of hyaline droplets in Kupffer cells provides a useful diagnostic clue to distinguish AIH from other forms of chronic hepatitis.

13 **Whole Exome DNA Sequences of Histiocytoid Cardiomyopathy (HC) Patients and First Degree Family Members – Reporting Two New Genes Confirming Inheritance Patterns in HC**

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**Background:** HC is a rare arrhythmogenic disorder. The underlying molecular genetic etiology has eluded researchers for decades. In our continuing efforts to identify potential causal mutations, we now describe whole exome sequencing data of two family trios, searching for genomic variations that are observed in these families.

**Design:** After obtaining IRB approval, two families of HC patients were contacted and consented for participation in this study. Whole blood was obtained from the HC patients, their parents, and siblings. DNA extraction was performed, followed by whole exome DNA sequencing using the Illumina HiSeq2000 platform. The data was analyzed using Samtools, open source wAnnovar and SeattleSeq softwares to annotate variants.

**Results:** The two families yielded seven (7) samples (family #1 - patient*, father, mother, sibling and, family #2 - patient*, father, and mother). *The two HC female patients are surviving, post heart transplant. Preliminary analyses identify two genes that are potentially contributory to the pathology of histiocytoid cardiomyopathy. Both children are homozygous for an identical rare missense mutation in the NWD1 gene, that is carried by both parents, but previously undescribed. The function of the protein that encodes for the NWD1 gene (NACHT and WD repeat domain protein) is unknown, though expression has been noted in heart muscle. Additionally, both children carry premature stop codons due to two different de novo mutations in the same gene, NDUFB11, which encodes a mitochondrial NADH dehydrogenase subunit component of electron transport complex I.

**Conclusions:** Initial observations of a mutation in the NWD1 gene suggests the NACHT and WD repeat domain protein plays a role in the pathogenesis of HC. Additionally, the mutations in the NDUFB11 gene are X-linked, which may explain the female-bias of HC if hemizygous males (with one copy of the mutation) tend not to be viable. Since mutations affecting other components of complex I have previously been associated with cardiomyopathy, neural degeneration, and lethal neonatal disease, NDUFB11 also deserves close consideration for having a role in HC pathogenesis. These observations may allow new opportunities for therapeutic interventions in affected children and genetic counseling to carrier parents.

14 **Fibrous Hamartoma of Infancy (FHI): Analysis of 59 Cases with Emphasis on Clinicopathologic Variability and Diagnostic Pitfalls**

Saab ST, McClain C, Coffin CM. Vanderbilt University Medical Center, Nashville, TN

**Background:** FHI, a benign superficial soft tissue neoplasm of early childhood, typically displays a triphasic organoid proliferation of mature adipocytes, fibroblastic/myofibroblastic cells, and primitive round or spindled mesenchymal cells. Occurrence in unusual sites or in older children and morphologic variability, especially a prominent collagenized pseudoangiectoid or neuroid pattern, can lead to diagnostic challenges. The purpose of this study is to demonstrate the clinicopathologic
variability, immunohistochemical features, and diagnostic pitfalls in FHI.

**Design:** 59 cases of FHI were retrieved from surgical pathology files. All reports, slides, and medical records were reviewed. Immunohistochemistry (IHC) for SMA, S100, CD34, HMB45, MelanA, Ki67, and Bcl2 was performed with standard techniques.

**Results:** The male:female ratio was 2(39m:20f) with mean age of 18 months(16d-8y at diagnosis). Tumor size was 2-9 cm(mean 4cm). Sites included: extremities(19), axilla(10), back(10), external genitalia(9), chest(5), and abdominal wall(6). All cases had triphasic elements in varying proportions. A pseudoangiectoid or neuroid pattern was seen in 1/3 of cases and was sometimes dominant to the point of obscuring the diagnosis. Diagnostic considerations for cases with pseudoangiectoid pattern prior to consultation included vascular neoplasms, giant cell fibroblastoma, neurofibroma, and solitary fibrous tumor. IHC showed CD34 and variable SMA in fibroblastic/myofibroblastic foci, S100 in adipocytes, variable CD34 in immature mesenchymal foci, and diffuse CD34 in pseudoangiectoid foci. No areas of storiform pattern, pigmented cells, or HMB45 or MelanA staining were noted. Immature mesenchymal foci had the highest Ki67 staining with diminution of staining in mature tissues. Bcl2 staining was restricted to mesenchymal and pseudoangiectoid foci, reflecting their proliferative nature.

**Conclusions:** FHI is a straightforward diagnosis when it occurs in infancy or early childhood and has a classic triphasic organoid appearance. Presentation in older children or in unusual sites (head, lower back, abdomen), large size, and unusual histologic features can lead to diagnostic challenges, including consideration of more locally aggressive tumors or sarcoma. IHC highlights subtle triphasic foci and is useful to exclude mimics. The pseudoangiectoid/neuroid pattern is a potentially significant diagnostic pitfall, especially for locally recurrent or rarely metastasizing mimics which require more extensive treatment.

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15 The Value of Fetal Autopsy for Midtrimester Dilation and Evacuation Specimens: What the American Board of Pathology is Missing

Ernst LM, Fritsch MK. Northwestern University, Feinberg School of Medicine Department of Pathology, Chicago, IL.

**Background:** The American Board of Pathology (ABP) issued a statement that for a fetal autopsy to satisfy the requirement for Anatomic Pathology (AP) board certification, there must be signed autopsy consent and the fetus must be intact. Increasingly patients are choosing dilation and evacuation (D&E) over labor induction for midtrimester pregnancy termination. Patients and providers frequently desire a more in-depth fetal and placental examination than typically performed in surgical pathology and request autopsy examination. The ABP policy has engendered considerable debate among Pathology trainees regarding the value of these examinations since they fail to satisfy the ABP definition of an autopsy and can no longer be used to satisfy the minimum autopsy requirement. Our goal was to determine the value of autopsies on D&E specimens.

**Design:** We reviewed consecutive fetal autopsies performed from September 2008 to August 2012 and recorded gestational age, degree of maceration and fragmentation, clinical diagnoses and final autopsy diagnoses. All cases were performed by a Pathology resident directly supervised by a board certified Pediatric Pathologist and included the 7 elements of an autopsy required by the ABP. Examination included radiographs, cultures when indicated, external examination of all fetal tissues, weight and histological examination of as many internal organs as possible, and a detailed report. Clinical diagnoses were compared with pathologic diagnoses and categorized by the level of new information added by the autopsy.

**Results:** 262 fetal autopsies were performed with 34% (N=89) performed on fragmented fetuses ranging from 14 to 24 wks gestation (mean 19.5 wks). The percentage of autopsies on fragmented fetuses increased over the study period from 18.5% in 2009 to 39% in 2012. 66%(59/89)of D&E fetuses had no significant maceration changes, and the rest showed variable levels of maceration. The most common clinical diagnoses were MCA (44/89) and IUFD (31/89). For MCA, a cytogenetic abnormality was found in 11% (10/89). The autopsy added information about additional anomalies,
defined a syndrome, diagnosed a tumor, changed the clinical diagnosis, or answered the clinical question in an additional 28% (25/89). A significant placental pathologic finding was seen in 28% (25/89). In only 7% (6/89) of cases were the pathologic findings unable to confirm clinical findings or add any significant information.

Conclusions: Autopsy examination on fragmented fetuses provides important data for genetic counseling, family planning, and education of residents regarding perinatal disease. Our data show that the autopsy on the fragmented fetus should not be undervalued by the ABP. Considering that partial adult autopsies limited to a single organ such as the brain satisfy the ABP definition of an autopsy, we recommend that any fetal autopsy performed with a valid autopsy permit under the direct supervision of a board certified Pediatric Pathologist should fulfill the requirement for AP board certification.

16 Development of Novel Software to Generate Anthropometric Norms at Perinatal Autopsy
1Cain MD, 2Siebert JR, and 1Faye-Petersen OM. 1University of Alabama at Birmingham, Birmingham, AL, 2Seattle Children’s Hospital and University of Washington, Seattle, WA

Background: Autopsy examination of previable fetuses, stillborns and live born infants provides information regarding cause of death, recurrence risks for future pregnancies, and helps give closure to parents. The pediatric pathologists who perform these autopsies reside primarily in large children’s hospitals or academic centers. However, a significant number of perinatal evaluations are performed by general practice pathologists or trainees who often find them time consuming and/or intimidating. We sought to create a program that would enable specialists as well as general pathologists and trainees to conduct these examinations with greater ease and produce reliable, informative reports.

Design: Using Visual Basic for Applications, we developed a program that automatically generates the full set of normal, expected anthropometric and organ weight ranges by gestational age (GA)/postnatal age (PA) after entry of case infant foot length or body weight and a correlative table with the GA/PA that best matches the observed anthropometry. The program highlights measurement and organ weight discrepancies, enabling users to identify abnormalities such as growth restriction or relative organomegaly. The program permits data export to Word and databases, enabling detection of institutional trends.

Results: First and second year pathology residents tested the program to determine usage ease and benefits. The average time, using conventional methods (i.e., reference books and internet sites), for a novice resident to acquire infant GA norms was 26.7 minutes (min) compared to 15 min for a seasoned resident. Using the program, first time users averaged 7.2 min and, thereafter, 3.2 min to acquire normal values. Mean time for novices’ acquisition of values using standard methods versus first-time program users was statistically significant (p= 0.046). The difference between mean acquisition times for seasoned residents using conventional methods versus our software was also statistically significant (p = 0.02). Testing further revealed that several autopsy reports would have benefited from the program’s corrective features. All participating residents found the program simple to use and preferred it to conventional methods.

Conclusions: This novel application saves time and improves the accuracy and quality of infant autopsy reports. The software and automatic storage features also allow data exportation to reports, derivation of institutional norms, and trend analyses, by gender, ethnicity, or other variables. A web-based version of our program is in progress to facilitate usage by both universities and private practice groups.
**In Utero Embryonic Intracardiac Injection Under Biomicroscopy Guidance: A Cutting-Edge Technology to Study Embryonic Lung Development in Vivo**

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Children’s Hospital Colorado, Aurora, CO, *Children’s Hospital of Pittsburgh of University of Pittsburgh Medical Center, Pittsburgh, PA#

**Background:** Approximately 14 per 10,000 live-born humans suffer from pulmonary hypoplasia (PH) often causing neonatal death. Although the pathomechanism of PH is poorly understood, emerging data suggest a key role for pulmonary vessels and blood flow in regulating lung development. Therefore, there is a pressing need to utilize novel tools that permit in vivo manipulation of embryonic vasculature at early development stages. We aimed to develop a method that allows us to study the role of blood flow in lung development in vivo.

**Design:** We devised a novel technique involving in utero fetal intracardiac injection under high-resolution ultrasound biomicroscopy guidance (IHB) to identify perfused pulmonary vessels. We injected wild-type mouse embryos at early embryonic stage (E10.5-12.5) using three different tracers: endothelial specific FITC-tomato lectin (FITC-TL), erythrocyte specific FITC-wheat germ lectin (FITC-WGL) and FITC-labeled microbeads (FBs). Intracardiac injection guaranteed that these tracers only highlight perfused vessels. We captured images of whole-mount fluorescent labeled peripheral lung buds with confocal microscope and 3D reconstructions were performed by IMARIS software. We analyzed FITC-TL vs. PECAM labeled peripheral vasculature. Identification of FITC-WGL erythrocytes and FBs within peripheral vessels confirmed perfusion.

**Results:** IHB provides direct vascular access to the developing mouse embryo at early time points; the technique is survivable and highly reproducible. Significant overlap was seen on merged PECAM and FITC-TL images of pulmonary vessels at all ages. FITC-WGL erythrocytes and FBs were present all throughout the lung vasculature including the developing peripheral microvessels around the growing lung buds.

**Conclusions:** Our technique allows, for the first time, a performance of an embryonic “pulmonary angiogram” to show true embryonic blood flow in living, developing mouse lung. Our data suggest that most pulmonary vessels are perfused at a very early gestational age. This surprising finding led us to postulate that a blood perfused pulmonary vascular network is, in fact, necessary for proper lung growth and altered blood flow may play a role in the pathomechanism of PH. With this technique we are not only able to further study the role of embryonic pulmonary blood flow but we can also deliver a variety of agents directly to the embryo (bypassing the placental barrier) including genes, viruses, stem cells, and drugs to address the developmental pathobiology of the lung.

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**An Evidence-Based Reassessment of the 1997 CAP Criteria for Placenta Examination**

Szabo S, Suchi M, Jarzembowski JA. Medical College of Wisconsin and Children’s Hospital of Wisconsin, Milwaukee, WI

**Background:** Placental examination can yield information about the existence and effects of maternal, fetal, or placental disease, the cause of stillbirth, and potential risks in future pregnancies. Unfortunately, such examinations are not uniformly done even in cases where the need is evident. In 1997, a College of American Pathologists (CAP) task force issued recommendations of which clinical criteria should trigger a pathologic examination of a placenta. The proposed criteria were based on the group members’ knowledge and experience but not on any specific large data sets or studies. To date, though, neither the American College of Obstetrics & Gynecology nor any other professional medical organization has issued its own guidelines on the matter. Thus, the CAP criteria remain the standard of clinical practice today. Multiple studies and surveys have found that at most hospitals, only 10-30% of placentas undergo complete pathologic examination, despite over 40% of all placentas meeting the CAP criteria.

**Design:** From 2009-2012, every placenta from every delivery at our institution was submitted to the laboratory along with a custom requisition form that included a checkbox list of the CAP-recommended maternal, fetal, and placental indications for examination, completed by clinical
providers. Pathology staff then reviewed the forms to determine which placentas would be examined. Therefore, we were able to guarantee – limited only by the completeness and accuracy of the requisition forms – that the CAP criteria were strictly followed.

Results: Reports from 1963 of these placentas were reviewed for potentially clinically significant histologic pathologic findings, including funisitis, chorioamnionitis, villitis, moderate meconium staining, vasculopathies, and ischemic changes. Overall, 1253 (63.8%) had significant pathologic findings – 63% of those submitted for maternal indications, 69% of those for fetal indications, and 74% of those for placental indications. The lowest pathologic yields were from submissions for repeat cesarean deliveries (47.4%), cesarean deliveries for obstructed labor (54.6%), and multiple gestation pregnancies (56.0%). The highest pathologic yields were for peripartum fever or suspected infection (86.3%), IUGR (81.7%), and fetal demise (80.8%).

Conclusions: The pathologic yield for all of the CAP criteria for placental examination was excellent, ranging from 47.4-86.3%. Almost half of placentas from repeat cesarean deliveries showed significant pathologic findings. A novel submission system can ensure that the CAP criteria for placental examination are strictly followed, thus increasing the clinical utility and reliability of the service. Future studies of placentas from lower risk obstetric populations, including “normal” deliveries, will help further assess the practical utility of the CAP criteria demonstrated here.

19 Maternal Obesity and Villitis: Gender-Specific Differences in Placental Pathology and Fetal Growth
Leon-Garcia S, Knepe K, Roeder H, Laurent L, LaCoursiere Y, Parast M. University of California, San Diego CA

Background: Obesity in pregnancy increases the rates of several complications, including gestational diabetes, fetal macrosomia, and stillbirth. Adverse effects of obesity in non-pregnant populations have been linked to inflammation in various tissues and organ systems, including adipose tissue and pancreas. However, studies on placental inflammation in obesity have shown conflicting findings. We set out to investigate 1) the relationship between maternal obesity, diabetes, hypertensive disease, and chronic villitis (CV) and 2) whether there is a connection between these entities and macrosomia.

Design: Singleton pregnancies without fetal anomalies, consisting of patients consented for our Placental Banking study, were included in this analysis. Data included detailed prenatal history including medical comorbidities and early pregnancy BMI, neonatal outcomes including birth weight, and placental pathology. Obesity was defined as pre-pregnancy BMI ≥ 30; normal weight patients had a BMI between 20 and 24.9. Diabetes was defined as either type 2 diabetes or gestational diabetes requiring medication (GDM2). Hypertensive disorder was defined as chronic or gestational hypertension or preeclampsia. Birth weight was reported as centiles, controlled for gestational age and sex; SGA was defined as <10th and LGA as >90th centile. Data was analyzed using Chi square and multiple logistic regression; odds ratios and 95% confidence intervals (CI) are reported.

Results: 155 obese and 140 normal weight patients were included in this analysis. Diabetes, hypertensive disease, and LGA were more common in the obese population (53.5%, 55.4%, and 20.6% vs. 11.4%, 20.7%, and 5%, respectively, p<0.001 for all). Percent CV was similar in both obese and normal weight groups (20.6% vs. 20%). Among obese patients only, univariate analysis revealed that CV was over twice as prevalent in the placentas of female neonates (28.4% vs. 13.6%, p=0.029). Finally, multivariate analysis controlling for diabetes and hypertension showed that, compared to male neonates, female neonates born to obese mothers were 2.6 times more likely to have CV in their placentas (CI=1.15-5.92, p=0.022), and showed a trend toward being less likely to be LGA (OR=0.47, CI=0.21-1.07, p=0.073).

Conclusions: The effect of obesity on inflammation as manifested by CV in the placenta is independent of diabetes and hypertension, and is gender-specific. Since CV is known to be associated with fetal growth restriction, we propose that the presence of this lesion in female
placentas may contribute to the reduced incidence of LGA in female neonates born to obese mothers. These data underscore the importance of placental histopathology in understanding mechanisms of abnormal fetal growth in the setting of maternal obesity.

20 Comparison of Placental Findings in Type 1 vs Type 2 Diabetic Women
Starikov R, Chen K, Lopes V, Inman K, Pinar H, He M. Women Infants Hospital of Rhode Island / Alpert Medical School of Brown University, Providence, RI

Background: Abnormal glucose metabolism is one of the most common medical complications of pregnancy, which has significant impact on fetal and placental growth and development. Type I and type II diabetes have different underlying pathogenic mechanisms. It has been recognized that risk and outcomes of pregnancy are different in the two types of diabetes. This study aimed to compare placental pathology between the 2 types of diabetes.

Design: This was a retrospective cohort study. Women were identified through registration in the Diabetes in Pregnancy Program from 2003 to 2011. Data analysis was performed with the SAS program. Categorical variables were compared by Chi-square1 or Fisher's exact test2. Continuous variables were compared using t-test3. P<0.05 is considered statistically significant.

Results: Type II diabetic women (n=176) has smaller BMI, lower fasting blood glucose and Hgb A1c level. Their placentas harbor significantly more decidual vasculopathy, increased synytial knots, and delayed villous maturity, than those in type I diabetes (n=117). More type II diabetic pregnancy are associated with <10th percentile fetal/placental weight ratio, suggestive of lower placental efficiency. More fetal inflammatory response is observed with type I diabetes.

Table 1. Clinical information and placental pathology in type I and II diabetic women

<table>
<thead>
<tr>
<th>Variables</th>
<th>Type I Diabetes (n=117)</th>
<th>Type II Diabetes (n=176)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age: Median (Min-Max)</td>
<td>28 (17-43)</td>
<td>33 (19-44)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI: Median (Min-Max)</td>
<td>26.4 (16.4-50.7)</td>
<td>36.5 (20-63.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GA (wks): Median (Min-Max)</td>
<td>37 (24-40)</td>
<td>38 (23-40)</td>
<td></td>
</tr>
<tr>
<td>Glucose testing results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hgb A1c levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>8.6 (2.0)</td>
<td>7.6 (1.8)</td>
<td></td>
</tr>
<tr>
<td>Median (Min-Max)</td>
<td>8.3 (5.7-14.2)</td>
<td>7.1 (4-13.4)</td>
<td></td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>119.7 (26.6)</td>
<td>97.4 (16.3)</td>
<td></td>
</tr>
<tr>
<td>Median (Min-Max)</td>
<td>116.8 (66-210.7)</td>
<td>94.3 (73.9-169.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Placental pathology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decidual vasculopathy</td>
<td>27 (23.1%)</td>
<td>72 (40.9%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Tenney-Parker changes or increased syncytial knots</td>
<td>27 (23.1%)</td>
<td>58 (32.9%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Delayed villous maturity</td>
<td>8 (6.8%)</td>
<td>22 (12.5%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Parenchymal infarcts</td>
<td>20 (17.1%)</td>
<td>31 (17.6%)</td>
<td>0.90</td>
</tr>
<tr>
<td>Chorangiosis group of pathology</td>
<td>5 (4.3%)</td>
<td>10 (5.7%)</td>
<td>0.59</td>
</tr>
<tr>
<td>Acute chorioamnionitis</td>
<td>17 (14.5%)</td>
<td>30 (17.1%)</td>
<td>0.56</td>
</tr>
<tr>
<td>Acute vasculitis of fetal vessels</td>
<td>18 (15.4%)</td>
<td>15 (8.5%)</td>
<td>0.053</td>
</tr>
<tr>
<td>Placenta weight percetile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGA</td>
<td>22 (18.8%)</td>
<td>39 (22.2%)</td>
<td></td>
</tr>
<tr>
<td>LGA</td>
<td>32 (27.4%)</td>
<td>47 (26.7%)</td>
<td>0.78</td>
</tr>
<tr>
<td>Fetal/placenta ratio</td>
<td>6 (5.1%)</td>
<td>23 (13.1%)</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Conclusions: Despite better glycemic control, type II diabetes have higher incidence of placental pathology related to uteroplacental underperfusion, while more fetal inflammatory response were
observed in type I diabetes. These results suggest possible different mechanisms, such as vascular
dysfunction or inflammation, leading to different pregnancy outcomes in different types of diabetes.

21 Hemophagocytic Lymphohistiocytosis: A Single Institutional Experience
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Children’s Hospital, Houston, TX
Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare, but life threatening condition,
characterized by immune dysregulation with uncontrolled and ineffective immune response. HLH
primarily affects infants and children, but is increasingly found in adults as well. Early recognition is
challenging due to variable presentation with non-specific symptoms, but is essential for favorable
outcome. For diagnosis of non-genetic HLH, 5 of 8 diagnostic criteria (fever; splenomegaly;
cytopenias; hypertriglyceridemia or hypofibrinogenemia; hemophagocytosis [bone marrow, lymph
node or liver]; low or absent NK cell activity; elevated ferritin level; and elevated soluble CD25)
must be met. According to current paradigm, HLH develops secondary to a spectrum of triggers
(infection, autoimmune disorder, or malignancy) in the background of variable genetic susceptibility.
The goal of our study was to identify morphologic characteristics that might help differentiate
infection associated and non-infection associated HLH cases.

Design: Under institutional IRB approval, our anatomic pathology database was searched for
patients with a clinical diagnosis of HLH between the years 2002 and 2011. The patients' clinical and
laboratory features as well as histological features of the bone marrow biopsies were evaluated.

Results: A total of 39 patients were identified with an age range of 6 months to 17 years (median of
5 years). There were 14 male and 23 female patients. The majority of the cases were infection
associated (62%; n=24; 23 EBV and 1 CMV related), followed by malignancy (10%, n=4),
autoimmune disease (13%, n=5), and post-transplantation state (5%; n=2). Interestingly, seasonal
variation was seen in viral associated cases showing a peak incidence in September compared to
non-infection associated cases. Malignancy associated cases developed in the background of CD8
positive T-cell lymphomas (anaplastic large cell lymphoma and subcutaneous panniculitis T cell
lymphoma). Histological evaluation of the bone marrow showed hemophagocytic activity in all
cases. The marrow cellularity, myeloid to erythroid ratio, as well as the relative lymphocyte count
were analyzed in relation to the etiology (infection associated vs other etiologies) to see if features
varied depending on the primary trigger. The most common findings were erythroid and
megakaryocytic hyperplasia followed by increased numbers of eosinophils, in 45, 30, and 12.5% of
the cases, respectively. No statistically significant difference was seen in any of the histologic
features between infection associated versus non-infectious associated cases.

Conclusions: Histologic evaluation is valuable to direct and help the clinician in establishing the
diagnosis of HLH, however based solely on the morphologic findings, the underlying process
triggering the HLH can not be predicted.

22 Incidental EBV-Positivity in Pediatric Post-Transplant Patients and Tonsillectomy
Specimens Demonstrates the Need for Stringent Criteria for Diagnosing PTLD
King RL, Paessler ME, Wertheim G. The Children's Hospital of Philadelphia, Philadelphia, PA
Background: Post-transplant lymphoproliferative disorders (PTLD) associated with EBV infection
are common in pediatric organ transplant recipients (OTR) due to a high rate of EBV seronegativity
at transplant, and can present at sites (e.g. tonsil) that are unusual in adult PTLD. Early PTLD can be
challenging to diagnose due to a lack of architectural distortion, absence of cytologic atypia, and
polyclonality. The minimal diagnostic criteria for pediatric PTLD are not well established and rely
heavily on the demonstration of EBV infection. This situation is problematic given the implications
of PTLD therapy. To start to address this concern, we determined the rate of EBV positivity found
incidentally in tissue from OTR in whom PTLD was not suspected and from routine tonsillectomy
specimens.

Design: EBER in situ hybridization was done retrospectively with appropriate controls on sections
of colon and lung from 38 pediatric autopsies of OTR (lung, heart, kidney, liver, stem cell) and pediatric tonsillectomy specimens from non-OTR (96) and OTR (6). Patients with a history of PTLD were excluded from both data sets. Slides were reviewed by two authors independently.

**Results:** Median age at autopsy was 7.5 yr (range 6 mo – 26 yr). Median time from transplant to death for all patients was 9 mo (range 0.1 – 153 mo). EBER expression was found incidentally in 3/38 autopsy cases (7.8%) whose clinical characteristics are described in Table 1. Median time between transplant and death in EBER positive cases was 37 months. EBER was positive in 26/102 tonsils (25%). Among tonsils from OTR, 4/6 (67%) were EBV positive.

Table 1. Clinical features of autopsy cases with incidental EBV positivity and no morphologic concern for PTLD.

<table>
<thead>
<tr>
<th>Case</th>
<th>Transplant</th>
<th>Reason for Transplant</th>
<th>Age at Death (yr)</th>
<th>Months Transplant to death</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Autologous stem cell</td>
<td>Neuroblastoma</td>
<td>7</td>
<td>37</td>
<td>Influenza A</td>
</tr>
<tr>
<td>2</td>
<td>Heart</td>
<td>Idiopathic cardiomyopathy</td>
<td>7</td>
<td>31</td>
<td>Chronic graft rejection</td>
</tr>
<tr>
<td>3</td>
<td>Lung</td>
<td>Idiopathic pulmonary fibrosis</td>
<td>6</td>
<td>37</td>
<td>Aspergillus pneumonia</td>
</tr>
</tbody>
</table>

**Conclusions:** Results of this study indicate that EBV-positivity can be found incidentally in reactive infiltrates of the GI tract and lung in pediatric OTR, and does not negatively impact survival. In addition, reactive tonsils from OTR in whom there was no concern for PTLD are EBV positive at a rate higher than in non-OTR (p=0.036, Fisher’s exact test). These findings suggest the need for further, prospective studies to evaluate the significance of EBV-positivity in lymphoid infiltrates from OTR and to define strict criteria for early PTLD lesions in the pediatric population.

23 Assessment of Benign Lymphoid Aggregates in Pediatric Bone Marrow Specimens

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**Background:** Benign lymphoid aggregates (BLAs) in bone marrow biopsies are frequently seen in adult patients and are well known to be associated with advanced age. Although BLAs are occasionally seen in pediatric patients, their significance and any associations with underlying diseases have not been investigated. We examined a series of pediatric patients with BLAs in their bone marrow biopsy specimens at our institution to elucidate these questions.

**Design:** 43 cases of bone marrow biopsies with BLAs at Children’s Hospital CO from 2000 to 2012 were evaluated. All patients were < 20 years of age. All lymphoid aggregates are non-paratrabecular and well circumscribed. All cases are divided into the following subgroups according to patients’ underlying disorders: (1) solid tissue tumor, (2) hematopoietic neoplasm, and (3) non-neoplasm.

**Results:** (1) BLAs are more frequently seen in patients with neoplastic disease versus non-neoplastic disease (27/43, 62.79% vs 16/43, 37.21%) as well as in patients with hematopoietic disorders than patients with non-hematopoietic disorders (35/43, 81.4% vs 8/43, 18.60%). (2) Solid tissue tumor is less often associated with BLAs in bone marrow than hematopoietic neoplasms (8/43, 18.6% vs 19/43, 44.19%). (3) Myeloid leukemia (9 cases) is associated with BLAs more often than ALL (4 cases), although the incidence of ALL is much higher than myeloid leukemia in children. (4) All ALL patients with BLAs had B-ALL, no T-ALL with BLAs is identified. (5) Most of the patients in the lymphohistiocytic neoplastic group are Hodgkin lymphoma patients (4/6, 67%). No BLAs were seen in non-Hodgkin lymphoma. (6) All patients with BLAs in the non-neoplastic group (16 cases) had autoimmune disorders or a non-specific lymphoid proliferation. (7) There were no differences in the age and gender distributions.
Conclusions: In pediatric patients, the formation of BLAs in bone marrow appears to be associated with (1) underlying neoplastic disorders: hematopoietic neoplasms more often than solid tissue tumors, myeloid leukemia more often than lymphoid leukemia, B-cell leukemia more often than T-cell leukemia, and Hodgkin lymphoma more often than non-Hodgkin lymphoma; and (2) autoimmune and non-specific lymphoproliferative processes. These findings suggest that certain types of neoplasms affect the lymphoid system more than other types of neoplasms and that BLAs are associated with autoimmune and lymphoproliferative processes in pediatric patients, in contrast what is seen in the aging adult population.

24 Poor Diagnostic Yield of Fungal Culture in Histology-Proven Pediatric Invasive Fungal Infection
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Background: Invasive fungal infections carry a high mortality rate. Targeted antifungal therapy is the foundation of treatment and requires accurate genus-level classification of the causative fungus. Identification of the invasive fungus is typically based on morphology seen in tissue sections in combination with fungal culture. The objectives of this study were to examine the diagnostic yield of fungal culture in invasive fungal infections and correlate histology findings with culture results.

Design: The laboratory database at The Children’s Hospital of Philadelphia was queried to identify all surgical pathology specimens in which invasive hyphal elements were identified on histology between January 2001 and December 2011. Histologic findings and concurrent fungal culture results were abstracted where available. Slides (H&E, PAS, GMS) in cases with fungal growth were reviewed and histologic findings were compared with the identity of the fungus isolated in the microbiology laboratory.

Results: We identified 100 tissue samples with histologically-confirmed hyphal invasion. In 29 of the cases (29%), parallel specimens were not submitted to the microbiology laboratory for fungal culture; 6 of the 29 were duplicate specimens. Of the 71 cases submitted for culture, 28 (39%) did not grow fungi. Yield on fungal culture for sinonasal specimens was 100% (12/12); 70% for skin (21/30); 44% for lung (8/18); 0% for liver, spleen, kidney (0/8); and 67% for other specimens (2/3). Positivity rate was stable at 2 to 9 cases per year. There were no trends over time with respect to incidence, genus or specimen type. Histology on 9 specimens (4 skin, 5 sinonasal) showed small hyphal fragments that were insufficient for definitive morphologic characterization. Culture results were concordant with histology findings except for one discordant case in which mediastinal biopsy showed budding yeast which was inconsistent with concurrent growth of a dematiaceous fungus.

Conclusions: 100 histologically-proven invasive fungal infections were identified in our tertiary pediatric care hospital over an 11-year period. Parallel fungal cultures were not submitted in 29% of cases. Of the remaining specimens submitted to microbiology, only 60% grew fungi underscoring the importance of histologic evaluation as the primary modality for fungal identification. The poor yield of fungal culture in visceral specimens should be noted considering the invasive nature of the sampling procedure. Systematization of specimen collection and submission for both histology and microbiology studies may be beneficial to improve timely and accurate fungal identification.

25 Light Microscopic Hair Abnormalities in Children: Retrospective Review of More Than 120 Cases in a 10-Year Period
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Background: Abnormalities in the hair shaft and hair follicles may be congenital or acquired conditions. Genetic conditions with associated hair abnormalities include Menkes Kinky Hair syndrome, Netherton’s syndrome, uncombable hair syndrome, trichothiodystrophy, loose anagen hair syndrome and monilethrix. Acquired hair abnormalities are mostly associated with grooming or use of various hair products. There are many patterns of hair abnormalities that can be readily identified under a light microscope. Patients with Menkes Kinky Hair syndrome typically have
multiple twists of hair shaft at irregular intervals (pili torti). Nodular swelling with a cup-like expansion of the proximal hair cortex that surrounds the distal segment (trichorrhexis invaginata) is considered a diagnostic pearl of Netherton’s syndrome. Longitudinal grooves along the hair shaft (pili canaliculi et trianguli) are seen in uncombable hair syndrome. Alternating light and dark banding under polarized light (tiger tail banding) is a characteristic hair finding in patients with trichothiodystrophy. An example of hair follicle abnormality commonly seen in young, blond-haired girls is loose anagen hair syndrome in which loose anagen hairs are easily pulled out with gentle pulling.

**Design:** We performed a retrospective review of 127 hair mount samples from 122 patients that were submitted to pathology department of our hospital over a 10-year span (from 2001 to 2011). We also reviewed the patients’ medical records to collect clinical information including molecular genetic testing results at the time of presentation and follow-up visits for correlation with the microscopic hair findings.

**Results:** Of the 122 patients, sixty-six (54%) had abnormal hairs. Twenty-five patients met the diagnostic criteria of loose anagen hair syndrome. Seven patients had uncombable hair syndrome. Two patients were diagnosed of Netherton’s syndrome, three patients with Menkes Kinky Hair syndrome confirmed by molecular testing. One patient with neurologic symptoms was diagnosed of trichothiodystrophy after her hair sample showed tiger tail banding pattern and decreased sulfur content. One patient with alopecia was diagnosed of monilethrix. The remaining abnormal hair samples showed non-specific or non-diagnostic changes with presence of loose anagen hairs suggestive of loose anagen hair syndrome (11 patients), trichorrhexis nodusa (15 patients) or pili torti (1 patient with ectodermal dysplasia). We describe and demonstrate the characteristic light microscopic features of different patterns of hair abnormalities in children. The typical clinical findings and molecular defects of some of the genetic conditions are also discussed.

**Conclusions:** Our study confirms that light microscopic examination of hair samples is a quick, simple, noninvasive and valuable tool. It should be used more widely in pediatric hospitals as a first line of investigation on various pediatric conditions.

### 26 Variability of Characteristic Ultrastructural Findings in Genetic Disorders of Surfactant Dysfunction

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**Background:** Over the past two decades, mutations have been identified in genes (SP-B, SP-C, ABCA3, TTF1) that result in surfactant dysfunction with respiratory failure and interstitial lung disease. Lamellar bodies (LB), found in type II pneumocytes, are lysosome related organelles specialized for the storage and secretion of surfactant. Normal LB (NLB) are classically described as tightly packed concentric bi-layer membranes surrounded by a limiting membrane, with or without an electron dense core. Electron micrographs (EM) of human lung, however, typically show wavy concentric membranes with variable spacing between membranes (“onion skin”). NLB are reported to be absent in SP-B and ABCA3 mutations, replaced by multivesicular bodies (MVB) and dense bodies (DB), respectively. In the present study, we describe variability of LB ultrastructure within individual genetic disorders of surfactant dysfunction (GDSD).

**Design:** The archives of the Department of Pathology at Washington University Medical School were searched for lung specimens with EM (1994-2012). Cases from 28 patients with GDSD were identified (16 ABCA3, 7 SP-B, 3 SP-C, 2 TTF1). Controls included infants with congenital heart disease (6), alveolar capillary dysplasia (2), pulmonary hypoplasia (1) and choanal atresia (1). EM was systematically reviewed (blinded to diagnosis) for the presence of NLB, MVB, DB (including DB with “fried egg appearance”), composite bodies (CB), and heterogeneous cytoplasmic inclusions (HCI). LB size, compactness of membranes, and artifact (collapse) were noted.

**Results:** MVB were identified in 5 of 7 SP-B cases, including 4 cases with 121ins2 mutation. One patient with this mutation had HCI, but lacked MVB. NLB were present in 3 SP-B cases, including 2
patients with novel mutations. DB were rare in the SP-B group. In contrast, DB were present in 15 of 16 ABCA3 cases. NLB were present in 15 ABCA3 cases; compared to controls, they were small, more compact and lacked collapse artifact. Within individual ABCA3 cases, there was heterogeneity of NLB and DB within and among individual pneumocytes.

Conclusions: Genetic mutations of SP-B and ABCA3 have characteristic ultrastructural findings of MVB and DB, respectively. Individual cases, however, may lack these characteristic structures or have a variety of structures, making diagnosis by EM more challenging. Small NLB are seen in a majority of ABCA3 cases, a finding that has not been previously emphasized in the literature.

27 Peripheral Insertion of Umbilical Cord
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Background: Numerous studies have highlighted the importance of marginal and membranous umbilical cord (UC) insertion. In view of recent reports suggesting that eccentrically inserted UC may also be significant, we have begun for the past 2 years to report UC insertion <3cm from the nearest margin as peripheral insertion of UC (PIUC). In this study we assessed the significance of this parameter in terms of maternal, placental and fetal abnormalities of pregnancy.

Design: Singleton placentas (N=1418) examined over an 18 month period were evaluated in a case control study. Cases of PIUC (n=119) were matched with a control of the same gestational age. Multiple pregnancies were excluded. Clinical and pathologic data were compared using information from clinical records and pathology reports. Chi square and student t test were used as appropriate for categorical and continuous data.

Results: The overall prevalence PIUC was 8.4% and was significantly increased (21.4% vs 7.6%, p<0.001) in extremely low gestational age (<28 wk) newborns (ELGAN). PIUC was associated with decreased placental weight Z-scores (-0.69 + 0.92 vs -0.22 + 1.3, p=0.006), but not fetal weight Z-scores suggesting increased placental efficiency or increased utilization of placental reserve. PIUC was also associated with long/narrow placentas (length – width), (1.0 + 3.1 vs 2.6 + 3.2, p=0.006 ). PIUC tended to be more frequent in young (<20 yo), primiparous mothers and was significantly less common in women with a history of prior curettage (50% vs 66%, p=0.013). This combined with equivalent prevalence in women with prior C-sections, multiparity, and advanced maternal age supports a primary developmental disorder as opposed to a secondary abnormality related to underlying uterine abnormalities (“trophotropism”). Aside from a borderline significant association with findings suggestive of maternal malperfusion (p=0.078), PIUC was not associated with other placental diagnoses (e.g. abortion, chorioamnionitis, avascular villi, VUE). Aside from the increased prevalence in ELGAN, there was no association with other adverse pregnancy outcomes including stillbirth, fetal growth restriction, malformations, recurrent pregnancy loss, low Apgar scores.

Conclusions: Our data support PIUC as a primary developmental abnormality occurring more commonly in early preterm deliveries and in young women without previous uterine instrumentation. Although associated with abnormal placental weight and shape, PIUC was not related to other adverse outcomes or placental lesions.

28 Comparison of Antemortem Imaging and Autopsy Findings in Pediatric Blunt Force Injury Deaths
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Background: Radiology is relied on heavily for the assessment of injuries in both the clinical and forensic settings. Experience in forensic autopsy shows that there are often discrepancies between radiographic interpretation of injuries and the true findings on postmortem examination. However, there are only a few references in the literature that quantify this discordance, particularly with a focus on pediatric deaths.

Design: We reviewed all injury-related deaths at Children’s Medical Center in Dallas between 2005 and 2009 in which the injuries were documented by pertinent antemortem imaging performed in the
hospital and an autopsy was subsequently conducted by the Dallas County Medical Examiner. In the 84 cases included, autopsy findings were compared to radiology findings with respect to skull fractures, epidural hemorrhages, subdural hemorrhages, subarachnoid hemorrhages, brain contusions and rib fractures. The presence or absence of each injury type by each modality and discordance between the two modalities were tabulated for each case.

**Results:** Imaging was 91% sensitive and 98% specific for skull fracture. In the cases with skull fracture seen by imaging and confirmed at autopsy there was a 19% discordance rate with regards to location and or number. Imaging was 64% sensitive and 99% specific for epidural hemorrhage. Discordance in the reporting of epidural hemorrhage was due solely to the sensitivity and specificity of imaging. There was 100% concordance with respect to location and number of those hemorrhages that were seen by imaging and confirmed at autopsy. Imaging was 81% sensitive and 75% specific for subdural hemorrhage. In the cases with subdural hemorrhage seen by imaging and confirmed at autopsy there was a 29% discordance rate with regards to location and or number. Imaging was 70% sensitive and 88% specific for subarachnoid hemorrhage. In the cases with subarachnoid hemorrhage seen by imaging and confirmed at autopsy there was a 42% discordance rate with respect to location and or number. Imaging was 62% sensitive and 95% specific for brain contusion. In cases with brain contusion seen by imaging and confirmed at autopsy there was a 22% discordance rate with respect to location and or number. Imaging was 91% sensitive and 94% specific for rib fracture. In cases with rib fractures seen on imaging and confirmed at autopsy there was an 84% discordance with regard to location and or number.

**Conclusions:** This study confirms our experience that there is a high level of discordance between radiology and autopsy findings in cases of blunt force injury in children. Review of the records suggests that differences in style and terminology may account for some degree of discordance. Moreover, specialized training of forensic radiologist and the increased detail afforded by post mortem imaging may resolve some of the issues. Close cooperation between radiologists and forensic pathologist is necessary to standardize the reporting of injuries which will allow better comparisons in future studies.

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**How Does Placental Histology Correlate with Bowel Pathology in the Premature Neonate with Necrotising Enterocolitis or Spontaneous Intestinal Perforation?**

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**Background:** Many risk factors for necrotising enterocolitis (NEC) and spontaneous intestinal perforation (SIP) are already recognised. However, few studies have identified potential associations between placental histological findings and the later development of NEC and SIP in the premature neonate. We performed a retrospective review of placental histology from NEC/SIP cases from our institution.

**Design:** Patients with a diagnosis of SIP/NEC were identified from over the past 9 years. Criteria for entry to the study were that: the diagnosis of NEC/SIP was made within the first week of life, the neonates were born prematurely (<37 weeks), the diagnosis was confirmed (clinically or histologically), and the placenta had been submitted for histological examination. Placental histology was retrieved and analysed for histologic variables including acute chorioamnionitis (ACA), placental insufficiency and fetal thrombotic vasculopathy (FTV). CD34 immunostain was performed to assess the integrity of the endothelial lining in cases with ACA. Martius scarlet blue (MSB) stain was used to demonstrate fibrin deposition/early thrombus formation FD/ET.

**Results:** Between 2003-2012, 21 c were identified which met all the defined inclusion criteria. Of the 21 c, 12 had NEC, 8 SIP and in 1 c the diagnosis was clinically indeterminate. 16/21 c (76.2%) had histologic evidence of ACA, 3/21 (14.3%) had placental insufficiency and 2/21 (9.5%) had focal FTV. Of the 16 cases of ACA, 13 (81.3%) were found to have umbilical phlebitis, 12 (75.0%) umbilical arteritis, 6 (37.5%) funisitis and 12 (75.0%) chorionic vasculitis. In total, 75% of both NEC and SIP c had ACA, however all cases of SIP demonstrated umbilical phlebitis and arteritis,
versus 66.7% of NEC cases. Similarly, a greater proportion of cases with SIP were found to have funisitis and chorionic vasculitis. Of those with ACA, 8/16 c (50%) showed evidence of FD/ET formation within the chorionic vasculature or umbilical vessels (confirmed with MSB). In these instances, the fibrin/early thrombus formation appeared to be associated with a damaged endothelial lining in vessels with prominent vasculitis. Of these cases, 4/8 (50.0%) included FD/ET in chorionic plate vessels, 2/8 (25.0%) in the subchorionic stem villi and 3/8 (37.5%) in the umbilical cord vessels (some cases showed more than one location). This finding was seen in 5/9 NEC cases with ACA, and 2/6 SIP cases with ACA. The other was the patient with indeterminate diagnosis.

**Conclusions:** We confirm an apparent association between ACA and SIP/NEC occurring within the 1st week of life in premature neonates. A striking feature was the frequent occurrence of endovascular fibrin deposition/early thrombus formation in this population. Early thrombus formation was located in vessels with vasculitic involvement. We propose that the endothelial damage and thrombus formation caused by the vasculitis may have a role in the pathophysiology of NEC/SIP presenting in the first week of life.

30 **Histopathology of the Pediatric Breast: Fifteen Years Experience of The Vanderbilt Breast Consultation Service**


**Background:** Invasive breast cancer (IBC) in the pediatric population is very rare, <0.1% of all breast cancers. Similarly ductal carcinoma in situ (DCIS) and atypical ductal hyperplasia (ADH) are uncommon. In contrast, fibroadenomas (FA) are the most common breast lesion in adolescent girls. Phyllodes tumors are significantly less prevalent, with only 5-10% occurring in teenaged girls.”

**Design:** The authors report the variety of pediatric breast pathology seen in consultation by the Vanderbilt Breast Consultation Service over a 15 year period from December 1997 to June 2012.

**Results:** Cases from 257 pediatric patients averaging 14.9 years of age (range 1-18 years) were received in consultation. 95% of cases were in girls. 12% of cases were atypical or malignant including 5 IBC (3 secretory, 2 no special type), 1 metastatic neuroendocrine tumor, 5 DCIS (2 involving FA), 10 ADH (2 in FA, 2 in gynecomastia), 3 borderline phyllodes tumors (PT) and 7 malignant (PT). 68% of cases were benign fibroepithelial lesions: 152 FA (including lactating and tubular variants), 20 benign PT and 2 hamartomas. The remaining cases (20%) consisted of 25 benign proliferative lesions (papillomas, epithelial hyperplasia without atypia, complex sclerosing lesions, sclerosing adenosis, fibrocystic change), 7 benign stromal lesions, 5 cases of macromastia, 3 benign vascular lesions, 2 neurofibromas, 1 granular cell tumor, 1 myofibroblastoma, and 4 miscellaneous benign lesions. Five percent of cases (13) were from boys including gynecomastia (6), ADH (5, 2 cases in the setting of gynecomastia), myofibroblastoma (1), FA (1), and neurofibroma (1).

**Conclusions:** FA was the most frequent pediatric breast lesion seen in consultation. The most common reason for referral was to distinguish a cellular fibroadenoma from a benign phyllodes tumor. Malignant PT was the most frequent malignant lesion, followed by DCIS and secretory carcinoma. ADH and DCIS were often encountered in the setting of other lesions, most commonly FA. The significance of ADH in the pediatric population is not established. Careful attention to histopathologic criteria should help avoid unnecessary surgery that can have an impact on breast development.

31 **Validation of a Transcription Mediated Amplification Assay for Chlamydia Trachomatis and Neisseria Gonorrhoeae in a Predominantly Pediatric Population**

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**Background:** Chlamydia Trachomatis (CT) and Neisseria gonorrhoeae (NG) are common causes of asymptomatic sexually transmitted infections (STIs) in adolescents, which if left untreated can lead to adverse clinical sequelae such as pelvic inflammatory disease and ectopic pregnancies. Previous
studies have shown that the accurate and timely diagnosis of pediatric STIs can be a challenge for physicians particularly in emergency care settings. Urine samples are a less invasive testing method especially in adolescent and pediatric populations when compared to endocervical swabs. The platform under evaluation is currently FDA approved for swabs in ages 16 and above and requires clinical validation for urine samples.

**Design:** In a prospective study, 199 consecutive samples, 170 urine specimens and 29 endocervical swabs from a predominantly adolescent female population were analyzed by transcription mediated amplification for ribosomal RNA from a specific region of the 23S rRNA from CT and a specific region of the 16S rRNA from NG via DNA intermediates on the PANTHER APTIMA Automated Assay, a platform recently released in the US, and the APTIMA DTS Combo 2 Assay. The detection is based on a Dual Kinetic Assay that enables simultaneous measurement of both targets.

**Results:** The PANTHER system when compared to the APTIMA DTS had identical performance characteristics with both the clinical sensitivity and specificity at 100% for the detection of NG. However the PANTHER had higher detection rates of CT in 2 samples, 1 urine and 1 swab (1.05%). The prevalence rates in urine samples were CT, 28 cases (16.4%), NG 7 cases (4.1%) and dual positive in 12 cases (7.05%) and in swabs CT 4 cases (13.7%), NG 2 cases (6.8%) and dual positive in 1 case (3.4%).

**Conclusions:** This study shows that the PANTHER APTIMA system has nearly identical analytical sensitivity and specificity to the more widely used APTIMA DTS COMBO 2 Assay. For pediatric and adolescent patients urine sampling is less invasive and more acceptable than an endocervical swab. The platform reduces the potential for technical errors and contamination, and demonstrates excellent performance characteristics on urine specimens from pediatric patients.

32 Correlation of Oncogene Expression and its Regulator in HPV Associated Recurrent Laryngeal Papillomatosis by RT-PCR of Frozen Tissue

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**Background:** Recurrent laryngeal papillomatosis (RLP) is the most common benign pediatric neoplasm of the larynx. Despite its benign histology, it is frequently associated with a protracted clinical course and substantial childhood morbidity. RLP has been linked to infection with human papilloma virus (HPV) subtypes 6 and 11. Although infection with HPV11 is more commonly associated with an aggressive disease course, HPV subtype is not entirely predictive of clinical outcome. Increased expression of viral oncogenes E6 and E7 are implicated in malignant transformation of high risk HPV subtypes. Oncogene overexpression is thought to involve loss of the transcriptional regulator E2. In our previous study during which RNA was extracted from paraffin embedded tissue, the E2 expression could not be detected. In this study, we examined the relationship between E2 and oncogene expression (E6 and E7) in HPV 6 and 11 from frozen tissue to better understand possible contribution of E2 in the clinical outcome of RLP patients.

**Design:** After obtaining IRB approval, a retrospective case review between January 2000 and October 2012 identified 31 patients treated for RLP. Ninety fresh frozen tissue biopsies from the patients were analyzed for expression of E6 and E7 oncogenes and the E2 transcriptional regulator by RT-PCR. Patient charts were reviewed for clinical outcome data including: demographic information, age at RLP diagnosis, approximate frequency of surgical intervention and absolute number of surgical procedures. P values were calculated from a Wilcoxon rank-sum test with significance defined as P <0.05.

**Results:** 28/31 (90%) of patients express E6 and E7 oncogenes and E2 from both HPV 6 and 11 subtypes. All patients with interval serial samples that initially expressed a single HPV subtype eventually convert to a mixed HPV profile. Also, a statistically significant relationship was found between E2 and E6 in HPV6 subtype with a p value (0.015).

**Conclusions:** The correlation between transcriptional regulator E2 and E6 in the HPV6 subtype explains its less aggressive clinical outcome as compared with HPV11. Additionally, the successful
determination of E2 expression emphasizes the importance of using fresh frozen tissue in RT-PCR analysis as compared with paraffin embedded tissue. Given that the incidence of RLP is rare compared to the exposure of HPV6 or 11, it is likely that additional factors such as immune function or other infections play a role in determining the clinical course of RLP.

33 Differences in Liver Fibrosis: Post-Fontan vs. Biliary Atresia
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Background: Significant liver fibrosis is a complication following the Fontan procedure for congenital heart disease and is also associated with biliary atresia (BA). In order to characterize differences in the mechanism of fibrosis associated with the two diseases, we determined differences between myofibroblast localization, extracellular matrix content, and vessel proliferation in cases of post-Fontan (FA), BA, and control (CO) livers.

Design: Liver tissue was obtained from patients with a history of the Fontan procedure prior to death (mean 2804 days, range 183-6386) who underwent autopsy (n=7), patients with BA (n=8) at the time of Kasai, and (for comparison) patients with normal liver histology (n=5). Immunohistochemistry was performed using antibodies against alpha-smooth muscle actin (SMA), VE-cadherin (VEC, blood vessel marker), versican (VER), and D2-40 (lymphatic marker). Sirius Red (SR) staining was also performed. Slides were analyzed using image analysis software.

Results: Total liver fibrosis (SR staining) was significantly higher in BA compared to FA (p=0.02) and CO (p=0.005). Fibrosis was centered around portal tracts in BA and central veins/hepatic lobules in FA. The total percentage of SMA staining was significantly higher in FA and BA cases compared to CO (p≤0.03) and the percentage of SMA positive cells within the hepatic lobe was notably higher in FA than BA (p=0.005). Total staining for VER, a large extracellular matrix proteoglycan, revealed that FA and BA livers had significantly increased levels compared to CO (p≤0.001). However, the pattern of VER staining was more diffuse in FA livers compared to BA, where staining was heaviest in the portal tracts. FA cases also showed less VEC and D2-40 staining compared to BA (p≤0.04) with BA cases notable for both vascular and lymphatic proliferation within portal areas.

Conclusions: Whereas BA livers have more periportal myofibroblasts and expanded vasculature, FA livers have more myofibroblast proliferation in the hepatic lobe and less portal lymphovascular expansion. Proteoglycan expression in both diseases was elevated above normal, but was peri-portal in BA and more diffuse in FA. These results suggest that matrix production in BA is portal based and portal expansion is accompanied by dramatic increases in blood vessels and lymphatics, whereas matrix production in FA is peri-central with a prominent lobular component and without significant increases in blood vessels and lymphatics. Collectively, these data suggest significant differences in the mechanism of fibrosis in these two diseases.

34 Different Etiopathogenesis of Macerated and Nonmacerated Stillbirth
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Background: Stillbirth–related regressive placental changes can obscure the pre-existing placental pathology. This analysis intends to show, if despite that, any conclusions regarding the etiopathogenesis of stillbirth could be drawn based on placental pathology.

Design: Twenty seven clinical and 47 placental variables were analyzed among 520 consecutive stillbirths (329 macerated [MS] and 191 nonmacerated [NMS]), autopsied by JS in years 1995-2012. The differences were assessed with the analysis of variance and the chi-square test with the Yates correction with the Bonferroni correction for multiple comparisons, as well as with the hierarchical clustering analysis with the Ward dendrogram and the Jaccard similarity index. P values less than 0.05 were regarded as statistically significant.

Results: There were no statistically significant differences in gestational age at delivery between the
MS and NMS. Retroplacental hematoma, preterm premature rupture of membranes, and acute chorioamnionitis (fetal inflammatory reaction), were statistically significantly more common in NMS than in MS, 20.4% vs. 7%, 19.4% vs. 8.2% and 20.9% vs. 9.7%, while induction of labor was less common, 7.9% vs. 23.7%, respectively. Umbilical cord hypercoiling (18.8% vs. 2.1%) and other umbilical cord abnormalities (23.1% vs. 9.9%), maternal diabetes mellitus (7.9% vs. 0.5%), fetal growth restriction (20.1% vs. 3.1%), clusters of avascular chorionic villi (12.5% vs. 2.1%), villous hemosiderosis in a lobular distribution (22.2% vs. 6.8%), stem luminal vascular abnormalities (58.1% vs. 4.7%), and diffuse villous fibrosis (58.1% vs. 8.4%) were statistically significantly more common in MS than in NMS, respectively. There were no statistically significant differences in frequencies of hypertensive diseases of pregnancy or fetal or placental hypoxic lesions other than the above mentioned postuterine hypoxic patterns. MS statistically significantly clustered with the Genest’s regressive villous changes (multiple luminal stem vessel abnormalities with villous fibrosis) but not with clinical factors, while the NMS clustered with the 2nd trimester deliveries, small placentas and acute chorioamnionitis, maternal inflammatory reaction.

Conclusions: The ascending infection manifesting as acute chorioamnionitis is the single most common cause of stillbirth, particularly the NMS. Investigation of the MS is likely to identify either maternal diabetes mellitus or, more frequently, the clinically unsuspected various umbilical cord abnormalities with secondary fetal thrombotic vasculopathy. The diffuse stem luminal vascular abnormalities and diffuse villous fibrosis identified in a small proportion of NMS can also be due to an underlying fetal thrombotic vasculopathy; otherwise, the NMSs were associated with the premature rupture of membranes/acute chorioamnionitis, maternal inflammatory reaction.

35 Adenomas and Adenomatosis: Spectrum of the Disease, Patient's Background and Associations

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Background: Hepatic adenomas (HA) are rare benign liver neoplasms classically associated with oral contraceptives in young woman but with diverse etiology in the pediatric population. Less well understood is hepatic adenomatosis (HAS), which is characterized by having greater than or equal to ten adenomas within an otherwise normal liver. Few case reports showed association of HAS with genetic and metabolic diseases. The link between adenomas/adenomatosis subtypes and their complications, and association with other abnormalities remains largely unclear. Therefore the aim of this study was to determine clinical and histological features that could shed some light on the etiology of these lesions and help to optimize their management.

Design: The histopathological findings of human liver biopsies and resections of HA (n=6) and HAS (n=6) were retrospectively reviewed (1994 to 2012). The clinical history and clinical follow-up were obtained from the patient's medical record.

Results: The median age of the patients with HA was 9.6 years (1-14) and HAS 13.9 (7-20) years. HA was more frequent in females 4F:2M and HAS in males 4M:2F. Half of the patients with HA had an underlying disease: sickle cell (n=1), bile salt export pump deficiency (n=1) and hepatoblastoma with APC mutation (n=1), whereas all but one patient with HAS had an underlying disease: glycogen storage disease (n=2), maturity onset diabetes of the young (n=1), sickle cell anemia (n=1) and Fanconi anemia. The treatment varied from partial hepatic excision (n= 7) and liver transplant (n=3), to clinical follow-up alone (n=2). One HAS patient died of sepsis fifteen days after the liver resection but, all the others are alive and in clinical surveillance. The histological features were similar in both lesions with hepatocytes arranged in cords of 2-3 cells with a relatively preserved sinusoidal arrangement but no recognizable lobular architecture. Immunohistochemical stains were performed in three HA cases and showed one case with glypican granular cytoplasmic stain and rare positive nuclear beta catenin stain, one case with glypican granular cytoplasmic and cytoplasmic beta catenin positive stain and one case was negative for both stains. The remaining patient's slides are being currently stained and the results will be added to this data.
Conclusions: Although our sample is small and limited by numerous variables we demonstrate a tendency of HAS to occur more frequently in male patients with basic underlying diseases than HA. As far as treatment, it seems that both can be cured by resection. Liver transplant was performed in patient's with end-stage liver diseases or complicated HAS. Because these benign liver neoplasms are rare; cases series like ours are necessary to improve understanding of these entities and better assist in the management of these patients.

36 Preterm Deliveries in a Unique Miami Inner-city Population: Connecting Histopathological and Demographic Risk Factors
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Background: Preterm deliveries (PTD) occur before 37 weeks gestational age. Preterm (PT) infants have a great perinatal risk of morbidity and mortality and an increased risk of long term neurodevelopmental and behavioral disabilities. In 2005, the annual cost of PTD in the United States was estimated to be more than $26 billion; $16 billion in medical care. PTD is a multifactorial phenomenon that includes differences in socioeconomic status, prenatal care, maternal risk behaviors, sociocultural believes, nutrition, infection rate, stress, and genetic factors. Currently, PTD accounts for 11.72% of all live births; approximately 1:5 infants born to non-Hispanic black mothers was born PT compared to 1:10 births to non-Hispanic white and Hispanic women. Our goal in this study is to characterize our black and Hispanic-centered population to identify possible risk factors associated to PTD, notably SPTD.

Design: All PTD in the year 2007 were recorded in a data base. We selected 196 singleton deliveries (116 Hispanics; 80 blacks) between 24-36 gestational weeks; demographics and clinical characteristics were recorded; and placentas were histologically evaluated. The data set was stratified for indicated preterm deliveries (IPTD) and spontaneous preterm deliveries (SPTD) and statistically analyzed for risk factor association using χ2 tests and common odds ratio estimates (SPSS v21.0).

Results: Consistent with the literature, there is an increased odds of black women having SPTD compared to Hispanics (OR=2.26, 95%CI=[0.941-5.267]). An increase in maternal age and BMI are inversely and directly associated to SPTD, respectively. Infants born by SPTD have higher birth weights compared to IPTD (p<0.05) because they usually present later in pregnancies (p>0.05). Socioeconomically, we found higher frequencies of IPTD in more educated women (≥ 12 Grade) (60.5%, p>0.05). Women with preeclampsia have higher frequencies of IPTD compared to SPTD. Interestingly, we found low frequencies of chorioamnionitis in women with SPTD as opposed to IPTD. On the other hand, there is comparatively higher frequency of umbilical cord vasculitis in SPTD (p>0.05). Placental histological findings in SPTD shows a possible association with chronic villitis of unknown etiology (OR=2.10, 95%CI=[0.526-8.412]) and infarcts (OR=1.353, 95%CI=[0.273-6.699]), postulating that both may be due to an immunological response.

Conclusions: In this study, we selected a distinctive population combination (blacks and Hispanics) because of our unique population composition. We saw a bias in the amount of IPTD which is related to our high risk population. Due to racial disparities, more research and a higher sample size are needed to tease out modifiers and risk factors in PTD and to lessen the psychosocial, economical and emotional impact on the families and society.

37 Rapid Whole Genome Sequencing and Analysis Reveals Compound Heterozygous Nonsense Mutations in the NEB Gene in Two Fetuses with Lethal Multiple Pterygium Syndrome
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Background: Mutations in the nebulin gene (NEB) are associated with congenital nemaline myopathy, a rare autosomal recessive hereditary disorder that, in the severe form, presents in
neonates with severe myopathy and death. Multiple pterygium syndrome (MPS), a syndrome of akinesia, multiple pterygia, amyoplasia and arthrogryposis, can cause fetal death in lethal cases. The less severe form, the Escobar type, has been described in association with Nemaline myopathy and truncating mutations of the Beta-tropmyosin gene (TPM2). Mutations in the NEB gene have not been previously reported in stillborn fetuses with MPS.

**Design:** We report similar autopsy findings of 2 siblings who were stillborn at 26 and 27 weeks of gestation. Tissue from both autopsies was submitted for whole genome sequencing.

**Results:** There were multiple pterygia, arthrogryposis, cystic hygroma, dysmorphic facies, and scoliosis. Both siblings demonstrated bilateral syndactyly with webbing of the fingers and equinovarus deformities. There was complete lack of skeletal muscle development with replacement of muscle by adipose tissue, confirmed by histology and immunohistochemical staining with desmin, noted in multiple sections from the ilioptosas and quadriceps muscles. Pathologic diagnosis was consistent with lethal multiple pterygium syndrome. Rapid, 50 hour whole genome sequencing and analysis was performed on both fetuses and the parents, revealing compound heterozygous nonsense mutations in the NEB gene, c.18786 C>G (p.Tyr4561X) and c.18981 C>G (p.Tyr6327X). Neither mutation has been reported in the literature but this genotype is expected to be disease-causing. The mutations were confirmed clinically by Sanger sequencing in both patients and parents.

**Conclusions:** This is the first report of NEB gene mutations associated with lethal multiple pterygium syndrome. These cases demonstrate the utility of genome sequencing in testing for disorders with phenotypic and locus heterogeneity.

38  Gata-6 Expression in Congenital Acinar Dysplasia
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**Background:** GATA-6 is a transcription factor expressed in the distal pulmonary epithelium during the pseudoglandular stage of lung development, but significantly decreases towards term, remaining confined to type II cells. It has been implicated in alveolar cell differentiation and induction of surfactant protein synthesis, through activation of TTF-1. Although GATA-6 appears to be fundamental for normal pulmonary morphogenesis, its overexpression has been associated with defective branching, a block of differentiation to mature pneumocytes (type I and II) and inhibition of alveolarization. This study examines the potential role of GATA-6 expression in congenital acinar dysplasia (CAD), in which the acini fail to develop and maturation arrest occurs at a pseudoglandular stage with no alveolar formation.

**Design:** The study consisted of 9 autopsy cases from one institution diagnosed as CAD. In 5 cases, pre-mortem lung biopsy was also available. Immunohistochemistry for GATA-6, TTF-1, surfactant proteins SpA, SpB were performed on the autopsy and/or biopsy lung samples. Lung from a morphologically normal term infant, who died soon after birth, was used as control. Histology and Micrographs were also reviewed.

**Results:** All patients were term mature and died from respiratory failure between 10 hours and 5 weeks. Five patients had severe pulmonary hypoplasia, and four enlarged lungs. Histologically, all cases showed maldevelopment of the distal airways with cuboidal pneumocytes and pseudoglandular structures. The septa were particularly thickened in the cases with pulmonary hyperplasia. Electron Microscopy (EM – 6 cases) showed alveoli lined by immature cuboidal cells, with variable number of lamellar bodies. No type I pneumocytes were identified in the samples studied. In all lung biopsy samples, GATA-6 was strongly expressed in the alveolar cells and in the interstitial fibroblasts. TTF-1 had a similar epithelial expression. In the autopsy samples, expression of both was variable, depending on the tissue preservation. In the control case, only rare pneumocytes were GATA-6 and TTF-1 positive. All cases showed expression of SpA and SpB with variable intensity.

**Conclusions:** This series of CAD demonstrated immature phenotype of pneumocytes, with strong GATA-6 immunoreactivity. Whether this aberrant expression is a primary cause of CAD or simply part of the retained fetal pneumocyte phenotype remains to be resolved.
Pauci-Eosinophilic Esophagitis Variant of Eosinophilic Esophagitis: Fact or Fiction?
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**Background:** Eosinophilic esophagitis (EoE) typically occurs in patients with a history of food allergies and dysphagia. The endoscopic findings are characterized by white plaques, patches, rings and/or furrows. The histopathologic features on biopsy (Bx) include marked basal cell hyperplasia (BCH) usually involving the full thickness of the epithelium, intraepithelial eosinophils (IEE) >15/HPF, evidence of stratification of eosinophils to the surface, eosinophilic clustering and subepithelial fibrosis (SEF). More recently, the pauci-eosinophilic (PE) variant of EoE has been described in abstract form in which the typical clinical features and endoscopic findings are present but the IEE count is low (usually <10/HPF) while characteristic BCH is present. In our practice, we have noticed that some biopsy fragments may be PE while others have the requisite IEE count suggesting that PE variant of EoE might be a result of sampling.

**Design:** We retrieved examples of esophagitis in patients 18 years and younger from our surgical pathology files for a 3-year period. The clinical features and endoscopic features were reviewed. Cases having typical clinical features and endoscopic findings of EoE were selected and the biopsies were assessed for number of biopsy fragments, BCH, IEE count and SEF.

**Results:** Fifty-four patients (10 females, 44 males) with a median age of 12 years (range 1-18 years) fulfilled first time clinical and endoscopic criteria for EoE. One 14-year-old male with food impaction had an IEE count of <10/HPF in all 5 Bx fragments of his biopsies from the distal and mid esophagus. Another 24 patients had at least 1 biopsy fragment (range 1-6) with a count of <10/HPF from 2-10 biopsy fragments. All of these cases demonstrated prominent SEF.

**Conclusions:** PE variant of EoE does exist, is usually associated with SEF and is likely as a result of sampling. From a practical point of view it may be prudent in such cases to alert clinicians to this likelihood and recommend rebiopsy so that patients are appropriately treated.

Immunohistochemical Analysis of Epithelial Components of Hepatoblastoma
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**Background:** Hepatoblastoma (HB) is usually a heterogeneous tumor displaying one or more of the following main epithelial components: fetal, embryonal and small-cell undifferentiated (SCU). Surgical resection is often curative for patients with only pure fetal component while those with an embryonal component require chemotherapy, and those with a SCU component need more aggressive treatment. Accurately identifying HB epithelial components is not only essential for objectively subtyping HB and appropriate patient care but also challenging histologically. So far, no single immunohistochemical (IHC) marker is capable of distinguishing these components.

**Design:** Forty-five HB cases (34 primary tumor biopsies, 10 post-treatment resection and 1 lung metastases) from Children Hospital Los Angeles over the last 15 years were studied by retrospective review of H&E slides. One representative block from each case was selected for H&E and IHC staining with the following markers: Glypican 3 (GPC3), beta-catenin, claudin 1, forkhead box protein G1 (FOXG1) and delta-like protein (DLK). The staining patterns (canalicular, membranous (M), cytoplasmic (C) and nuclei (N)), extensity (0, <5%, 1+, 6-25%, 2+, 26-50%, 3+, 51-75%, 4+, >75%) and intensity (0, negativity, 1+, faint, 2+, moderate, 3+, strong) of each marker were evaluated. The final score was determined after multiplying the extensity and intensity. The data was presented as mean ± SD.

**Results:** 24 cases have a fetal component, 30 cases with an embryonal component and 7 cases with a SCU component. The GPC3 demonstrated three distinctive staining patterns in HB: granular canalicular pattern in fetal components, diffuse cytoplasmic staining in embryonal components and complete negativity in SCU. Beta-catenin showed strong nuclear and cytoplasmic staining in SCU (12 ± 0 for both), mainly membranous staining in fetal components (M, 7.2 ± 1.4, C, 2.6 ± 2.3, N,
1.3 ± 1.3), and mainly cytoplasmic staining in embryonal components (M, 1.6 ± 1.4, C, 7.0 ± 2.0, N, 4.1 ± 2.7). Claudin 1 demonstrated completely negativity in SCU, stronger membranous staining in embryonal than in fetal components (5.9 ± 2.8 vs. 4.4 ± 2.4, p<0.05). FOXG1 showed complete negativity in SUC, similar cytoplasmic staining in fetal and embryonal components (4.7 ± 2.8 vs. 4.4 ± 2.4, p<0.05). DLK also showed completely negativity in SCU, similar cytoplasmic and canalicular staining in fetal and embryonal components (2.2 ± 3.0 vs. 3.8 ± 0.1, p>0.05).

Conclusions: The use of a small panel of IHC markers including GPC3, beta-catenin and claudin 1, provides a simple and effective tool to identify HB epithelial components.

41 Pediatric DCM with EFE: A Distinct Clinico-pathological Entity?
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Background: Dilated cardiomyopathy (DCM) is characterized by an enlarged left ventricular (LV) chamber and reduced systolic ejection, without a concomitant increase in LV wall thickness. Heart failure due to DCM is a major indication for cardiac transplantation (CTx) in the pediatric age group. Endocardial fibroelastosis (EFE) represents a non-specific reaction to myocardial stress. Although considered to be a common finding in pediatric DCM there is a striking paucity of reports which detail pathological features, such as EFE, in this age group. At the Hospital for Sick Children (HSC), all explanted hearts from transplant recipients undergo detailed pathological examination. Our study objective was to analyze the surgical pathology reports of explanted pediatric DCM hearts in order to: (1) characterize the pathological features of DCM; and (2) compare the findings in pediatric age sub-groups so as to provide insight into possible etiologic variation.

Design: We conducted a retrospective review of all DCM cardiac explant pathology reports at HSC from 1990 to 2010, with follow-up of DCM-CTx recipients from time of their transplantation until the end of study period. Assessed pathological parameters included: heart weight; ventricular chamber dilatation and wall thickness; and presence of endocardial fibroelastosis (EFE). Data analysis entailed comparison of the pathology findings in 3 age groups (<1 year, 1-10 years, 11-18 years).

Results: Of the 246 HSC files reviewed, 70 (37 males; 33 females) related to explants from infants and children diagnosed with heart failure due to DCM. The age at time of transplantation ranged from 2 days to 18 years (mean: 7.3 years) with 17 patients aged <1 year; 25 between 1 to 10 years; and 28 between 11 to 18 years. 18 died before study completion while 52 remained alive. There was a proportionately greater number of hearts with LV EFE in the <1 age group than in the other two older age groups. Using the chi-square test, this difference between the age groups was statistically significant (p=0.0024; Standardized Residual for cell=+2).

Conclusions: Our study detailed the pathologic characteristics of pediatric DCM cardiac explants. LV EFE is significantly more common in explants from DCM-CTx recipients <1 year of age. We propose that DCM with EFE in infants represents a clinico-pathological entity distinct from DCM without EFE in older pediatric age groups. The reason for the difference in LV EFE presence between these groups in our study remains elusive, but may relate to genetic variation in signalling pathways during periods of rapid development.

42 Pineoblastomas in Pediatric Patients
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Background: Pineoblastomas are rare, highly malignant pineal parenchymal tumors encountered predominantly in pediatric patients. They are distinct from PNET at other sites in that they exhibit photosensory differentiation including Flexner–Wintersteiner rosettes and fleurettes. Diagnosis can be challenging since these tumors share morphologic and immunohistochemical features with other embryonal tumors and the developing pineal gland. Pineal anlage is a rare variant of pineoblastoma defined by divergent neuroepithelial and ectomesenchymal differentiation without an endodermal
The published cases of anlage tumors behaved aggressively. We describe the clinical, radiological and pathological characteristics of pediatric pineoblastomas.

**Design:** Pathology records from 1980 to 2012 were searched for pineoblastomas at a tertiary pediatric hospital. Cases were reviewed and classified using the presence/absence of anlage/divergent differentiation component, rosette formation, pigment production, mitoses and necrosis. Radiology reports and clinical data were reviewed.

**Results:** We report thirteen cases of biopsy-proven pineoblastomas including one case of anlage tumor. The female to male ratio is 2.2 and the age ranges from 8 months to 17 years. The radiological findings (by CT scan, MRI) showed enhancing, lobulated pineal gland lesions with dimensions ranging from 2.5 to 5 cm. Hydrocephalus was identified in 69% of patients. Five cases had spinal cord metastasis, three showed no evidence of dissemination and no information was available in the rest of the cases. The majority of patients (9) received a combination of surgical resection, irradiation and chemotherapy and one patient had a combination of resection and irradiation. Three patients had surgical resection but no other information was available with regards to therapeutical management. Nine patients are alive with survival times between 6 months and 25 years. Four patients died, with an average survival time from the diagnosis of 4.25 years (range 2-6 years). The anlage tumor was diagnosed in an 8-month-old girl. The tumor had unusual ganglioglioma features, an embryonal component, pigmented epithelium and mesenchymal differentiation. The patient is alive and well, 8 months after the diagnosis.

**Conclusions:** Pineoblastomas are uncommon pediatric tumors. Diagnosis can be difficult as the surgical biopsy material is often limited and the tumor lacks a distinctive immunophenotype. In our study the majority of patients (69%) did well with appropriate therapy. Adequate follow up in order to detect the possibility of recurrence or dissemination is warranted.

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**43 Myxoinflammatory Fibroblastic Sarcoma in Children and Adolescents: A Rare and Diagnostically Challenging Locally Aggressive Neoplasm**

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**Background:** Myxoinflammatory fibroblastic sarcoma (MIFS), originally described as a low-grade malignant acral soft tissue tumor in adults, has recently been described in non-acral sites such as the head, neck, thigh, and shoulder, and in younger patients. This study reports the clinicopathologic features of MIFS in children and adolescents and emphasizes the diagnostic challenges.

**Design:** MIFS in patients in the first two decades of life were retrieved from surgical pathology files. Medical records, pathology reports, and slides were reviewed. Immunohistochemical stains were performed using standard techniques. One case had been previously published (Alaggio R et al 2012).

**Results:** Four MIFS occurred in two males and two females, aged 5-17 years (one in the first decade, three in the second decade). Clinical findings were nonspecific, with impressions including dermatofibroma, tendon sheath fibroma, ganglion cyst, and a reactive process. The slowly growing, superficial soft tissue mass had been present for months to years. All involved the deep dermis or superficial subcutaneous tissue; sites included the scalp, middle finger, forearm, and thigh; diameter was 1.0-2.5 cm. The well circumscribed masses had focal honeycomb infiltration of the adjacent adipose tissue, a spindle cell morphology with prominent nuclear pleomorphism and pseudo-inclusions, myxoid foci, and an inflammatory infiltrate. Three had <1 mitosis/10 hpf; one had 6 mitoses/10 hpf. One had focal necrosis (<10%). Immunohistochemistry revealed reactivity for CD34 (3/4, all focal) and smooth muscle actin (3/4), with <5% nuclear Mib-1 (2/2), with no reactivity for desmin (4), S100 (4), cytokeratin(4), EMA (3), HMB45 (3), or CD30 (2). Follow-up (2 cases) revealed no evidence of recurrence or metastasis. None of the cases had cytogenetics done as part of the diagnostic evaluation.

**Conclusions:** MIFS is a locally recurrent, rarely metastasizing neoplasm originally described in the
distal extremities of adults. This study reports an expanded age range and topographic distribution. The differential diagnosis includes reactive processes and a variety of atypical spindle cell neoplasms with inflammation, such as inflammatory myofibroblastic tumor, inflammatory liposarcoma, fibrous histiocytoma, atypical fibroxanthoma, pleomorphic undifferentiated sarcoma, a melanocytic neoplasm, and Hodgkin disease. Our series increases awareness of this tumor, includes children in the demographics, expands the location of the tumor, and suggests that immunohistochemistry is useful in differential diagnosis. Recent reports of repetitive cytogenetic abnormalities in MIFS suggest potential future diagnostic applications for genetic analyses.

44 Etiopathogenesis of Stillbirth Varies with Gestational Age at Delivery
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**Background:** Patterns and frequency of normal gross and histological placental features and lesions and predictive significance of placental diagnosis vary with gestational age at delivery, being the strongest for the second trimester severe preeclampsia and preterm severe ascending infection. The impact of gestational age at delivery on the placental diagnosis has not been thoroughly studied in stillbirths yet. Therefore, a retrospective analysis of gestational age at delivery dependence of maternal and fetal clinical conditions and placental variables has been performed using two statistical tools.

**Design:** 26 clinical maternal-fetal and 48 placental variables were analyzed in 3 gestational age study groups: G1 (16-27 weeks, 330 cases), G2 (27-36 weeks, 102 cases), and G3 (37+ weeks, 88 cases) among 520 stillbirths consecutively autopsied by JS in years 1995-2012. 2 statistical tools were used: the chi-square or Fisher exact test after the Bonferroni correction for multiple comparisons, and the hierarchical clustering analysis with the Ward dendrograms.

**Results:** By conventional statistics, statistically significantly (p<0.05) differences in frequencies (%) among Groups 1-3, respectively were found in: premature rupture of membranes (16.7, 4.9, 4.5), abnormal cardiotocography (0.6, 3.9, 8.0), thick meconium (0, 2.9, 9.1), acute chorioamnionitis, fetal reaction (19.1, 1.0, 9.1), placental edema (18.2, 11.8, 3.4), retroplacental hematoma (14.8, 11.8, 1.1), venous intimal cushions (0.9, 10.8, 6.8), fetal vascular thrombi (3.0, 11.8, 14.8), uterine (2.1, 12.7, 3.4) and preuterine (0.9, 5.9, 11.4) patterns of diffuse chronic placental injury, deep (decidual) meconium penetration (0.6, 6.9, 22.7), stem obliterator endarteritis (0, 3.9, 5.7), and chronic villitis of unknown etiology (4.2, 10.8, 18.2). By hierarchical clustering analysis, placental lesions/patterns clustered statistically significantly (approximately unbiased value >95): premature rupture of membranes/fetal inflammatory reaction in G1; antepartum hemorrhage, fetal growth restriction, and maternal diabetes mellitus/umbilical cord compromise/induction of labor in G2; and fetal growth restriction/nonmacerated stillbirth/oligohydramnios/thick meconium/abnormal cardiotocography/cesarean section in G3.

**Conclusions:** In stillbirth placentas, only the statistically significant association of premature rupture of membranes and acute chorioamnionitis, fetal inflammatory reaction, corresponded to that of the overall placental population in the 2nd trimester. In the 3rd trimester, other clinicoplacental associations, including the placental abruption, uteroplacental insufficiency, and stasis-induced fetal thrombotic vasculopathy were dominating, but not those associated with hypertensive complications of pregnancy. Multifactorial clusters were typical in the 3rd trimester, particularly in the stillbirths at term.

45 Placental and Fetal Hypoxic and Thrombotic Overlap Lesions
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**Background:** Placental lesion multiplicity portends poorer prognosis for pregnancy than single lesions. This analysis is aimed to study clinical and placental associations of placental hypoxic acute, chronic, and acute-on-chronic hypoxic lesions.

**Design:** Frequencies of 32 clinical (maternal and fetal) and 47 placental (gross and microscopic)
phenotypes were statistically compared among 2831 consecutive placentas from ≥16 weeks gestations: 778 placentas with chronic hypoxic lesion(s) (diffuse patterns of hypoxic placental injury, chorangiosis, excessive extravillous trophoblasts, microscopic chorionic pseudocysts, clusters of decidual multinucleate trophoblasts)[Group 1, G1], 481 placentas with acute hypoxic lesion(s) (infarction, intravillous hemorrhage, decidual meconium penetration) [Group 2, G2], 585 placentas with hypoxic overlap lesion(s) (coexisting at least one lesion from each of above groups)[Group 3, G3], and 987 placentas without placental hypoxic lesions, adjusted for gestational age (Control Group, CG). The differences among the 4 groups were evaluated using the Yates Chi-square, 3df, using the Holm-Bonferroni correction for multiple comparisons.

Results: For G1-G3 and CG, respectively, statistically significant differences (p<0.05) were found for the following variables: poor prenatal care (1.8, 2.7, 3.2, 6.3%), maternal substance abuse (6.5, 5.8, 8.5, 3.9%), severe preeclampsia (4.1, 2.5, 9.7, 1.4%), HELLP (1.8, 0.4, 2.9, 0.4%), maternal diabetes mellitus (8.5, 4.2, 4.8, 3.8%), premature rupture of membranes (12.5, 12.5, 8.5, 18.6%), thick meconium (0.9, 5.2, 13.1, 1.8%), abnormal Dopplers (4.5, 1.6, 5.3, 1.4%), induction of labor (8.9, 9.1, 13.8, 6.1%), fetal growth restriction (13.6, 8.3, 20.3, 7.8%), acute chorioamnionitis (31.4, 38.3, 28.9, 42.5%), erythroblastosis of fetal blood (11.3, 7.7, 17.1, 3.8%), decidual arteriolopathy (19.9, 20.3, 36.9, 15.1%), intervillous thrombi (11.9, 14.5, 17.1, 9.9%), retroplacental hematoma (3.2, 10.6, 8.7, 4.8%), diffuse villous fibrosis (9.5, 10.2, 12.1, 6.2%), clusters of avascular chorionic villi (8.6, 7.7, 10.6, 5.2%), intimal cushions of fetal veins (5.4, 5.2, 7.5, 3.9%), abnormal coiling of umbilical cord (14.4, 14.5, 22.0, 9.2%), dilatation of fetal veins (3.3, 2.7, 5.1, 1.0%).

Conclusions: Of two major placental injury patterns, the inflammatory pattern dominated the CG (more frequent poor prenatal care, premature rupture of membranes, and acute chorioamnionitis), while the fetal (erythroblastosis) and placental hypoxic patterns were associated not only with the clinical hypoxia-associated conditions, but also with the clinical (abnormal Dopplers) and the placental features of fetal thrombotic vasculopathy (villous fibrosis, fetal vein intimal cushions, clusters of sclerotic chorionic villi), particularly in the G3 (hypoxic overlap features/patterns). Therefore, it seems that placental hypoxic overlap lesions predispose to thrombotic lesions, some most likely stasis-induced (abnormal coiling of the umbilical cord).

46 Neonatal Alloimmunization and a Potential Link to Histoplasmosis?
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Background: The rate of alloimmunization in immunocompetent adults is 2.6-2.9%, however neonatal alloimmunization is rarely reported. Relative immunodeficiency of the neonatal state may be causative. A state of increased inflammation may induce alloantibody formation with crossreactive epitopes of microbes/allergens.

Design: We report a 4 month old female with disseminated histoplasmosis who developed 3 alloantibodies in response to blood product transfusion. At admission, an antibody screen was negative. The patient received 5 transfusions of packed red blood cells from three separate units. 11 days after her first transfusion, anti-Kell (K1) and anti-E alloantibodies were identified in the patient’s plasma. A direct antibody test was positive (4+ IgG, 1+ complement) and the eluate was positive with anti-Jka specificity. Passive antibody transfer was ruled out by negative antibody screens of each donor and the absence of any allo- or autoantibodies in the mother’s plasma. Retained red blood cell segments were antigen typed and positive for the E, Jka, and K1 antigens. Molecular genotyping showed the patient was K1, E, and Jka-antigen negative. An electronic medical record database query was performed at Vanderbilt Medical Center, over 12 years, to identify patients with a diagnosis of histoplasmosis and concurrent alloantibody(ies).

Results: A review of the medical record database showed a 5% alloimmunization rate in patients with a history of Histoplasma infection. Multiple antibodies were made in 2 of 5 cases. 4 of 5 patients developed at least one antibody to the RhCE or RhD blood groups.

Conclusions: A 4 month old female with disseminated histoplasmosis developed three distinct alloantibodies within 11 days of exposure to 3 packed red blood cell units. While the exact
The physiologic mechanism of neonatal alloimmunization is unclear, severe inflammation and infection appear to potentially induce alloantibody formation in certain patients.

47 Histologic Differences in Placentas of Eclamptic/Preclamptic Gestations by Birthweight, Placental Weight and Time of Onset
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**Background:** With eclampsia/preeclampsia (E/PE), infants more often are either LGA or SGA. We explored whether the differences in the infant birthweight (BW) or placental weights (PW) are more often associated with specific histologic features of maternal vascular underperfusion. We assess whether time of symptomologic onset is important in frequency of histologic features.

**Design:** A retrospective chart review was conducted to identify E/PE mothers between 2007 and 2010. Neonate BW, gestational age, race, and time of E/PE onset, before or after 34 weeks gestational age, were obtained. The cases were assigned to normal, high or low 10th percentile for PW and BW. Gestational age but not PW or BW was made known at slide review. Investigated features included increased syncytial knots, villous agglutination, increased intervillous fibrin, distal villous hypoplasia, acute atherosis, mural hypertrophy of membrane arterioles, muscularized basal plate arteries, increased placental site giant cells, increased number of immature intermediate trophoblasts, infarcts, and villitis. The results were correlated with BW and PW and onset of E/PE.

**Results:** 138 E/PE mothers were identified with 76 normal, 45 low, and 17 high BW infants, 76 normal, 28 low, and 17 high PWs. 86 gestations had early E/PE onset, with 52 normal, 28 low, and 6 high BWs, and 46 normal, 20 low, and 20 high PWs. 52 gestations had early E/PE onset, with 24 normal, 17 low BW and 29 normal, 8 low, and 15 high PWs. High BW infant placentas had decreased syncytial knots (<0.01), less increased placental site giant cells (<0.05) compared to other groups, and increased mural hypertrophy of membrane arterioles (<0.05) compared to the normal BW. Low PWs had increased syncytial knots (<0.05) compared to high PWs, increased intervillous fibrin (<0.05) and increased acute atherosis (<0.01) compared to other groups. High PWs had decreased distal villous hypoplasia (<0.05) compared to other groups. Early onset disease had increased syncytial knots, distal villous hypoplasia, villous agglutination (<0.01) and infarcts (<0.05).

**Conclusions:** Fewer placental histologic features of maternal vascular underperfusion in high BW and PW placentas, more in the low BW and PW placentas, and more features in the early onset E/PE suggest that maternal vascular underperfusion may have more effect on the E/PE pregnancies with early onset and those pregnancies with low infant BWs and PWs.