

## SPP/PPS Combined Fall Meeting, Philadelphia

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

### Platforms

#### **1-A Novel GATA1 Mutation in Transient Myeloproliferative Disorder Leading to a Lack of Protein Expression**

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**Background:** The zinc-finger transcription factor GATA1 plays a decisive role for the development of trisomy-21 associated Transient Myeloproliferative Disorder (TMD). Importantly, mutations described so far cluster in exon 2 and encode a shortened, functionally impaired GATA1 protein, leading to enhanced hematopoietic proliferation and inhibited maturation.

**Design:** We diagnosed a congenital myeloproliferative disorder in a placental specimen of a preterm boy (32nd gestational week) in the setting of cytogenetically confirmed trisomy 21. The leukemic placental specimen was compared with two normal placental specimens (37nd and 32nd gestational week, respectively) and normal fetal liver hematopoiesis (24nd gestational week). Circulating white cell populations were microdissected from umbilical vein and chorionic plate vessel paraffin blocks and screened for GATA1 exon 2 mutations. The entire GATA1 exon 2 was sequenced by nested polymerase chain reaction. GATA1 protein expression was assessed by immunohistochemistry.

**Results:** We detected a previously undescribed point mutation resulting in a premature stop codon at codon 2 (E2Term). Since the mutated GATA1 sequence is predicted to encode for only a single methionine amino acid with complete loss of GATA1 protein, this precludes any anti-GATA1 antibody to be able to target the translated product of the mutated GATA1 DNA. As a consequence, immunohistochemically, the leukemic cells harbouring this novel point mutation were all negative for the nuclear signal of GATA1, whereas the nuclear staining was present in some maternal lymphocytes in the intervillous space. In contrast, in normal placenta, nuclear GATA1 expression was detectable in a proportion of maternal as well as fetal lymphocytes. Normal fetal liver showed nuclear GATA1 expression in some of the hematopoietic precursors, implicating its role in non-neoplastic hematopoiesis.

**Conclusion:** The novel GATA1 mutation represents the earliest known premature stop codon and points towards the leukemogenic importance of the complete loss of GATA1 protein expression in Transient Myeloproliferative Disorder.

#### **2-ALK Expression in Neuroblastic Tumors Does Not Correlate with Unfavorable Clinicopathologic Features**

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**Background:** Anaplastic lymphoma kinase (ALK) functions as a major oncogene in several

pediatric neoplasms including anaplastic large cell lymphoma and inflammatory myofibroblastic tumor. Amplification of the ALK gene locus occurs in approximately 5% of primary neuroblastic tumors, whereas ALK kinase domain mutations are found in another 6-11%; these activating mutations have also been described in the germline of patients with a familial predisposition to neuroblastoma. Recently, small molecule inhibitors of ALK have been shown to inhibit the growth of NB cell lines harboring ALK mutations. These novel findings led us to investigate ALK protein expression in neuroblastic tumors.

**Design:** We examined tissue microarrays comprised of 59 previously untreated neuroblastic tumors accrued from multiple institutions. These included 43 neuroblastomas (NB; 7 differentiating, 30 poorly-differentiated, and 6 undifferentiated), 9 ganglioneuroblastomas, intermixed (GNB), and 7 ganglioneuromas (GN). Immunohistochemistry was performed on automated stainers using commercially available anti-ALK antibodies according to the manufacturers' recommended protocols. Results were simply scored as positive (>5% of cells) or negative based on a consensus of evaluable cores. Relationships between ALK expression and clinicopathologic data were examined using univariate statistical analysis to calculate Spearman correlation coefficients and Student's t-distribution to calculate significance.

**Results:** Eleven of the 59 cases showed positivity for ALK (19%), mostly as diffuse or granular cytoplasmic staining in neuroblastoma or ganglioneuroblastoma. No case had nuclear or membranous localization, and all ganglioneuromas were negative. Positive ALK staining trended with worse classification (NB and GNB versus GN), unfavorable histology, MYCN amplification, younger age at diagnosis, shorter event-free survival, and higher mortality, but none of these relationships were statistically significant ( $p > 0.05$  in all cases).

**Conclusion:** ALK immunoreactivity was found in approximately 19% of NB, similar to the expected combined frequencies of ALK gene amplification or mutations combined (approximately 17%). Measurable ALK protein expression, as detected by immunohistochemistry, was seen more frequently in cases with unfavorable clinicopathologic parameters, but the correlations were weak and unlikely to be prognostically useful in isolation. These results are similar to those described for ALK gene mutations, with mutated cases more commonly presenting as high stage disease, but without independent prognostic significance. Correlation of ALK protein immunohistochemical staining with ALK gene mutations or amplifications may prove informative.

### **3-INI-1 Expression in High Grade Brain Tumors: A Retrospective Review**

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**Background:** Atypical teratoid/rhabdoid tumors (ATRT) are characterized by at least focal rhabdoid morphology, loss of INI1 expression by immunohistochemistry, and detection of monosomy 22 or mutations in the INI1 gene. The wide use of INI1 immunohistochemistry over the last several years has also led to the discovery that high grade brain tumors, including medulloblastomas and choroid plexus carcinomas, may show loss of INI1 expression. These neoplasms are now best classified as ATRTs. Less well described are tumors with classic rhabdoid morphology and immunophenotype with retained INI1 expression. We examined INI1 expression in high grade brain tumors in which ATRT was an important diagnostic consideration.

**Design:** We searched the SCH surgical pathology database between the years 1991 and 2009 for all diagnoses of ATRT, medulloblastoma in children less than 5 years of age, and choroid plexus

carcinoma (CPC). We stained representative sections with BAF47 antibody and correlated nuclear INI1 expression with tumor morphology, immunophenotype including expression of vimentin and epithelial markers (CK or EMA), and available cytogenetic studies.

**Results:** We identified twelve cases each of ATRT, medulloblastoma and CPC. INI1 expression was absent in one medulloblastoma with large cell, anaplastic morphology and in one choroid plexus carcinoma with extensive epithelial differentiation and focal rhabdoid features. Although all cases with an original diagnosis of ATRT showed co-expression of vimentin and CK or EMA, four cases retained INI1 expression. Each of these cases showed rhabdoid morphology with classic eosinophilic cytoplasmic inclusions in three and glandular and cartilaginous differentiation in a fourth. Karyotyping in this latter case showed isochromosome 17 and trisomy 7 (more commonly identified in medulloblastomas), but karyotyping or FISH studies in the remaining three cases showed no abnormalities.

**Conclusion:** Detection of INI1 expression by immunohistochemistry is considered critical in diagnosing ATRTs. Using loss of INI1 expression as the criteria for diagnosis, two cases previously diagnosed as medulloblastoma or CPC could now be diagnosed as ATRT. The finding of retained INI1 expression in 4 of 12 cases with classic ATRT morphology and immunophenotype raise the dilemma of appropriate diagnosis of these tumors. In a recent report, 15% of morphologically diagnosed malignant rhabdoid tumors retained INI-1 expression, suggesting an as yet unidentified mutation could result in a rhabdoid phenotype. Our four cases further support this hypothesis.

#### **4-INI1 Expression is Decreased in Synovial Sarcomas, with no Evidence of INI1 Gene Alteration**

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**Background:** Loss of INI1 expression is a diagnostically useful hallmark of rhabdoid tumors, and has also been demonstrated in epithelioid sarcomas. Previous studies have shown almost universal expression of INI1 in other sarcomas, but with variability in the few synovial sarcomas tested. This study was designed to comprehensively study synovial sarcoma INI1 expression.

**Design:** 23 specimens from a total of 12 pediatric patients with synovial sarcoma were immunohistochemically stained for INI1. Expression was separately evaluated in spindle cell and epithelial components, and graded as fully retained, weakly retained or not retained. 9/12 patients had diagnostic confirmation by demonstration of t(X;18); the other three had classic morphologic and immunohistochemical features of synovial sarcoma. In 10 specimens from 7 patients, alterations of the INI1 gene were sought for using multiplex ligation-dependent probe amplification, standard sequencing, and quantitative gene expression assay. In addition, 12 adult synovial sarcoma specimens were stained for INI1.

**Results:** INI1 expression was weak or absent in at least one specimen from all 12 of the pediatric patients. 9 of the patients had multiple specimens (biopsies, resections, recurrences), and these showed variability in INI1 staining. In 3 patients INI1 was initially retained but lost in subsequent specimens, and in 4 patients INI1 was consistently weak or absent in primaries and recurrences. One patient had INI1 loss in the primary but not in the recurrence. In biphasic tumors, INI1 was always retained in the epithelial components. In addition, INI1 expression was weak or absent in all 11 adult specimens tested. Molecular genetic analysis found no evidence of gene deletions or mutations in any of the specimens tested.

**Conclusions:** Total or partial loss of INI1 expression is a common but not universal event in

synovial sarcomas, and is more likely to be seen in the spindle cell components of the tumor than in the epithelial components. Unlike rhabdoid tumors there is no evidence for a primary INI1 gene mutation or deletion in synovial sarcomas, and the reduced INI1 expression is most likely an epigenetic phenomenon involving interaction of the fusion protein and INI1.

**5-Well Differentiated Hepatocellular Neoplasms in Children: Are immunostains helpful?**

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**Background:** Well differentiated hepatocellular neoplasms in children (WDHNC), hepatocellular carcinoma (HCC) and hepatic adenoma (HA), are a diagnostic challenge. Unlike hepatic adenomas in adults which are mostly benign neoplasms associated with oral contraceptive usage, WDHNC appear to have a higher rate of malignant transformation for which histologic criteria are imperfect. Recent literature suggests subclassifying hepatic adenomas on molecular alterations independent of age. The categories in this subclassification include: Bcatenin mutated, HNF1-alpha mutated, and inflammatory adenoma with or without mutations. We applied these criteria to WDHNC and focal nodular hyperplasia in children and compared these results to conventional criteria using H&E appearance and reticulin stain.

**Design:** Twelve cases of WDHNC identified in our pathology archives originally were diagnosed as HCC or hepatic adenoma. The 8 males and 4 females had a mean age of 9.6 years (Range 1-18 years). All twelve patients had excisions (2 explants and 10 segmental resections). Underlying disease in 6 cases included: GSD-2, Fanconi anemia – 2, and idiopathic neonatal hepatitis – 2. Formalin-fixed tissue blocks were retrieved from all 12 WDHNC and 3 cases of focal nodular hyperplasia. Primary classification was based on H&E and reticulin stain. Immunohistochemical stains were performed using monoclonal mouse antibodies against Glypican 3, CD34, ER, PR, P53, Ki-67,  $\beta$ -catenin, Glutamine synthetase, CK7, and SAA, and polyclonal rabbit antibody against Fatty Acid Binding Protein (inactivation of Hepatocyte Nuclear Factor 1- $\alpha$ ).

**Results:**

Diagnosis: H&E and Reticulin		# of cases	Glypican-3	FABP	$\beta$ -Catenin	Glutamine Synthetase Reactivity/Patterns	
HA	Intact: 1-3 cell layers	5	0/5	3/5	1/5	1/5	Homogenous
HCC	Fragmented, Pericellular, > 3 cell layers	5	3/5	3/5	5/5	5/5	Homogenous
HCC arising in HA	Fragmented, Pericellular, > 3 cell layers	2	1/2	0/2	2/2	2/2	Homogenous
FNH	Intact: 1-3 cell layers	3	0/3	0/3	0/3	3/3	Heterogeneous

**Conclusions:** Four of five HA immunohistochemically demonstrate molecular mutations in  $\beta$ -catenin or hepatocyte nuclear factor 1 –  $\alpha$ . The former is reported to increase risk for malignant transformation. All of the HCC cases including the two cases apparently arising from adenomas have a  $\beta$ -catenin mutation. Immunopanel composed of the combination of  $\beta$ -catenin, glutamine

synthetase, and FABP can be of prognostic use in identifying a HA with an increased risk for developing HCC. In addition, the pattern of glutamine synthetase expression in  $\beta$ -catenin mutated adenomas differs from FNH suggesting this immunostain may be helpful in differentiating these two lesions especially on needle core biopsies.

### **6-NUT Midline Carcinoma in a Newborn with Multiorgan Disseminated Tumor and a Two Year Old with a Pancreatic/Hepatic Primary**

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**Background:** NUT midline carcinoma (NMC) is defined by chromosomal rearrangement of the gene NUT (chr. 15), which often results from t(15;19), forming a BRD4-NUT fusion oncogene. NMCs are poorly differentiated carcinomas that primarily affect midline structures of young individuals, with the youngest reported in a 3 year old. Reported cases always have a rapidly lethal clinical course despite intensive multimodality therapy. Regions most commonly involved include the upper aerodigestive tract and mediastinum. The etiology and cellular origin of this tumor is currently unknown. BRD4 acts as a transcriptional regulator, but the function of NUT is currently unknown.

**Design:** The authors investigated the clinical and pathologic findings in two pediatric patients. The first was a newborn who presented with a supraorbital mass and had extensive multiorgan involvement, including spine, lungs, liver, pancreas, adrenal glands, and subcutaneous tissue. The clinical diagnosis was presumed stage IV neuroblastoma. The second patient was a two year-old male with a large abdominal mass involving the liver and pancreas and a small pulmonary metastasis. This was clinically presumed to be hepatoblastoma metastatic to lung. Immunohistochemical staining, cytogenetic analysis, and fluorescent in situ hybridization (FISH) were performed on biopsies of the patients' tumors.

**Results:** Histopathological analysis of both tumors showed markedly undifferentiated malignant neoplasms. Immunohistochemistry showed positivity for epithelial markers. Both tumors demonstrated t(15;19) by chromosome analysis. Immunohistochemistry and FISH for the BRD4/NUT rearrangement were performed on the tumors of both patients, with positive results. Both patients had fatal outcomes.

**Conclusion:** To our knowledge, this is the first reported case of congenital NMC and a 2 year old with primary involvement of the liver and pancreas. Further studies of this recently identified malignancy are needed for a better understanding of its nature and possible therapeutic approaches.

### **7-Targeting the Epigenome in NUT midline carcinoma.**

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**Background:** NUT midline carcinomas (NMC) are defined by chromosomal rearrangements of the NUT gene that result in the formation of chimeric fusion oncogenes, most commonly BRD4-NUT. These poorly differentiated, highly aggressive, lethal cancers occur predominantly in the mediastinum and upper aerodigestive tract, and affect patients of all ages. Median survival

is 6 months despite aggressive chemo/radiation therapy, necessitating development of alternative therapies. BRD-NUT acts to block differentiation and maintains the growth of NMC cells, and knockdown of these oncoproteins results in differentiation and arrested proliferation.

**Design and Results:** With the knowledge that BRD-NUT binds chromatin, we sought to determine how BRD-NUT affects histone acetylation. Gain-of-function and loss of function experiments established that BRD-NUT results in global de-acetylation of chromatin that was reversed by the HDACi trichostatin A (TSA). Strikingly, TSA treatment of NMCs also resulted in squamous differentiation and arrested proliferation, a phenotype identical to that produced by knockdown of BRD-NUT. A screen of an HDACi library (n=30 small molecules) revealed a variety of class I HDACi produced the same phenotype, suggesting that HDACi can bypass BRD-NUT-induced de-acetylation, and thus might be used to treat NMC. In vitro studies were conducted with five NMC cell lines and primary cells derived from the index patient, JT. The effects on growth and differentiation of NMCs by a library of HDACi compounds (n=30) were assessed by quantitation of chromatin acetylation, proliferation and differentiation. Analysis of in vivo tumor response in the patient, JT, to daily 400mg dosing of the FDA-approved HDACi, Vorinostat (SAHA, Merck), was assessed over a 5-week period by positron emission tomography (PET) and CT scanning. A 10 year-old male was diagnosed with mediastinal NMC based on in situ hybridization showing BRD4-NUT rearrangement and aberrant speckled nuclear immunohistochemical staining for NUT. After expedited approval by the Dana-Farber Cancer Institute IRB, oral Vorinostat was initiated. Follow-up CT-PET evaluation following 5 weeks of therapy revealed a marked decrease in FDG uptake in both mediastinal and metastatic adrenal lesions. In this interval, tumors showed little change in apparent size, suggesting a primary consequence of differentiation by Vorinostat in vivo, corroborating in vitro preclinical studies.

**Conclusion:** We conclude that Vorinostat shows promise as an active, single agent for the treatment of NMC. To our knowledge, this is the first demonstration of response of a solid tumor to an HDACi, and represents a new rationale for targeted therapy for this uncommon, but lethal, carcinoma.

### **8-Infantile Rhabdomyofibrosarcoma: Rhabdomyosarcoma or Congenital Fibrosarcoma with Aberrant Expression of Myogenic Markers?**

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**Background:** The infantile rhabdomyofibrosarcoma (RMFS), first reported in 1983, is a rare, controversial entity, morphologically overlapping with CIFS and spindle-cell rhabdomyosarcoma. It has been recently suggested a possible biologic, cytogenetic link with CIFS. Nonetheless the few reported RMFS have shown an aggressive clinical course. We retrospectively investigated a series of CIFS and RMFS, comparing their clinico-pathologic and molecular features in order to explore biological relationships.

**Design:** Clinical records and histopathological specimens from 16 spindle-cell lesions, with diagnosis of CIFS and RMFS: data on RT-PCR for the ETV6-NTRK3 gene fusion were retrieved from the surgical pathology files of University of Padua between 1997 and 2008. Histologic features and immunostains were analyzed. Immunostains for desmin, myogenin (myf4) and myoD were performed in all cases. RT-PCR for myogenin was done in selected cases.

**Results:** 13 cases were diagnosed as CIFS, 3 as RMFS. One case of infantile RMFS had a

previous diagnosis of fibrosarcoma. RMFS and CIFS were histologically undistinguishable. Both showed an uniform proliferation of spindle cells with elongated, slightly hyperchromic nuclei, in a fascicular pattern, with intercellular collagen. Typical rhabdomyoblasts were not detected. In one RMFS occasional, very atypical cells with prominent nuclei, resembling rhabdomyoblasts were seen. Mitoses were frequent. All CIFS were negative for myf4, MyoD1, desmin. ETV6-NTRK3 transcript was detected in 10/13. RMFS showed a focal staining for myf4 and myoD1 (mean 20% of cells) in all cases, whereas desmin was positive in all but one, that had been originally diagnosed as CIFS. All RMFS showed negative RT-PCR for ETV6-NTRK3 transcript and positive for myogenin. All patients with CIFS are alive with no evidence of disease (ANED), 3 after multiple relapses. Two RMFS, treated as rhabdomyosarcomas, had a good response to therapy according to RMS protocol. One is ANED after 2 years, the second is recent. The child with the diagnosis of CIFS died of disease after many relapses.

**Conclusions:** RMFS may be histologically indistinguishable from CIFS. The expression of myogenic markers at immunohistochemical and molecular level and the absence of ETV6-NTRK3 transcript support its classification as a variant of RMS. The absence of evident myogenesis at histology and the lower immunohistochemical expression of myogenin suggest a peculiar, primitive variant of rhabdomyosarcoma, with potentially aggressive behaviour.

### **9-Fusion-negative Alveolar Rhabdomyosarcomas: a Second Look**

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**Background:** A subset of rhabdomyosarcomas with alveolar histology lacks PAX3/FOXO1 or PAX7/FOXO1 gene fusions. These neoplasms have been the source of extensive debate, and recent investigations suggest that they lack the biological signature that characterizes fusion-positive tumors. Alveolar rhabdomyosarcomas exhibit a strong diffuse expression of myogenin on immunohistochemical stains, but this has not been systematically correlated with fusion negativity. We therefore performed a retrospective review of a set of fusion-negative rhabdomyosarcomas that had been stained for myogenin expression, in order to further characterize these lesions.

**Design:** We compiled a series of 44 alveolar rhabdomyosarcomas that were submitted for COG histology review between 1996 and 2004 and also found to be negative for PAX3-FKHR and PAX7-FKHR fusions by reverse transcription-PCR testing. All of the histologic sections submitted for COG protocol entry were re-reviewed. Cases were selected because of availability of sections for myogenin immunostains, which were performed at the Biopathology Center using a standard avidin-biotin-peroxidase technique. We evaluated histologic parameters including pattern, cytologic features (cell shape, nuclear shape, and chromatin pattern), and mitoses per 20 high power fields. Myogenin expression was characterized as 1+ (only occasional positive cells); 2+ (<50% of cells positive); 3+ (>50% and <90 % positive); and 4+ (all cells positive).

**Results:** Of the 44 cases, 16 had a pure histologic pattern typical of alveolar rhabdomyosarcoma (including solid variant), and 9 showed strong myogenin expression (3+ : 6 cases; 4+: 3 cases). Eight cases showed both typical features; one case showed strong myogenin expression but had a mixed pattern with anaplasia. The remainder showed the following patterns: botryoid (1), embryonal (8), mixed embryonal-alveolar (6), PNET-like (1), rhabdoid (1), sclerosing (6), pleomorphic (1), and spindle (2); 6 had anaplasia. Other features had little correlation with

histology or myogenin expression.

**Conclusions:** Tumors previously characterized as fusion-negative alveolar rhabdomyosarcomas comprise a heterogeneous group including those with vague embryonal, mixed, sclerosing, and anaplastic features. Strong myogenin expression appears to correlate with a characteristic alveolar histology.

## **10-The Effect of Pre-operative Chemotherapy on Histological Sub-typing of Wilms' Tumours: the UKW3 Experience**

### **Tumours: the UKW3 Experience**

G.M. Vujanic, A. Kelsey, C. Mitchell, S. Popov, N. Sebire, K. Pritchard-Jones, on behalf of the UK CCLG Renal Tumours Working Group

**Background:** There are two principal approaches to Wilms' tumour (WT) treatment: primary surgery (as followed in the NWTs/COG trials) or pre-operative chemotherapy (as followed in the SIOP trials). Both groups use post-operative chemotherapy and sometimes radiotherapy in a risk-adapted approach that includes histological sub-classification of the tumour. In the UK Children's Cancer and Leukaemia Group (CCLG) Wilms' Tumour Trial 3 (UKW3) these two approaches were applied in a randomized fashion to the subgroup of patients with operable localized disease, presence of metastatic or bilateral disease, clinician preference or parental consent.

**Design:** The aim of this study is to compare the effect of pre-operative chemotherapy on histological features and sub-typing of WTs.

**Material and Method.** The cases were identified from the UKW3 Trial database. The criteria for inclusions were: age 6 months or more; non-anaplastic and non-metastatic WTs; reviewed by the CCLG Panel of Pathologists; with an adequate number of blocks submitted. The tumours were sub-classified according to the SIOP 9301 and NWTs criteria.

**Results.** There were 244 WTs in the immediate surgery (IS) and 182 WTs in the pre-operative chemotherapy (pre-op Cx) group. They were sub-typed as follows: blastemal 86/244 (35.2%) vs. 9/182 (4.9%), epithelial 34/244 (14%) vs. 12/182 (6.6%), stromal 12/244 (4.9%) vs. 25/182 (13.7%), mixed 112/244 (45.9%) vs. 45/182 (24.7%), in IS and pre-op Cx groups, respectively, plus 72/182 (39.6%) regressive and 19/182 (10.4%) completely necrotic WTs in the pre-op Cx group. The differences between the identical types in both groups were highly significant (Fisher exact test for comparison of proportions  $P < 0.0001$ ).

**Conclusion:** Pre-operative chemotherapy significantly alters histological features and sub-typing of WTs. There were fewer blastemal and epithelial WTs in the pre-op Cx group in which 50% of WTs showed marked chemotherapy-induced changes (regressive and completely necrotic WTs). Accurate sub-typing is important for risk adaptation of post-operative treatment intensity in the SIOP WT trials, where histological response to pre-operative chemotherapy is being refined as a prognostic factor.

## **11-Anaplasia in Nephroblastoma: Findings from the UKW3 and UK SIOP WT 2001**

### **Datasets**

N.J. Sebire, A. Kelsey, K. Pritchard-Jones, C. Mitchell, S. Popov, R. Hobson, G.M. Vujanic, on behalf of the UK CCLG Renal Tumours Working Group

**Background:** Anaplasia in nephroblastoma represents a high-risk histological feature, but there remains uncertainty regarding the effect of preoperative chemotherapy on its incidence and stage distribution. This study presents results from the most recent UKW3 and UK SIOP WT 2001 trials in the UK.

**Design:** All included cases underwent central pathology review as part of the trial protocols.

Cases were centrally classified as anaplasia present / absent, focal/diffuse, and age and stage distributions compared between anaplastic and non-anaplastic cases using modified chi-squared tests.

**Results:** The frequency of anaplasia in UKW3 and UK SIOP WT 2001 was as follows: 51/595 nephroblastomas (8.6%) or 51/641 paediatric renal tumours (8.0%), and 51/492 nephroblastomas (10.4%) or 51/590 paediatric renal tumours (8.6%), respectively. The combined frequency was 102/1087 nephroblastomas (9.4%) or 102/1231 paediatric renal tumours (8.3%), with 30/102 (29%) representing focal anaplasia and 72/102 (71%) diffuse anaplasia. In UKW3, the frequency of anaplasia was lower in those undergoing primary nephrectomy than those undergoing pre-operative chemotherapy: 17/280 (6.0%) vs. 34/315 (10.8%;  $Z=-2.1$ ,  $p=0.04$ ), respectively, though this comparison may be confounded by larger tumours preferentially receiving pre-operative chemotherapy. Only 8/102 (7.8%) of anaplastic cases were <2yrs vs. 204/650 (31%) of non-anaplastic cases ( $Z=-4.9$ ,  $P<0.0001$ ) whereas 39/102 (38.2%) anaplastic cases were >5 years vs. 136/650 (20.9%) non-anaplastic cases ( $Z=3.8$ ,  $P<0.0001$ ). In UK SIOP WT 2001, significantly more anaplastic vs. non-anaplastic cases were stage IV: 16/51 (31%) vs. 91/442 (21%) respectively ( $Z=1.8$ ,  $P=0.04$ ), but the proportion of stage II and stage III cases were similar: 9/51 (18%) vs. 71/442 (16%) and 12/51 (24%) vs. 100/442 (23%) respectively.

**Conclusion:** Anaplasia is present in almost 10% of UK nephroblastomas, two-thirds of cases representing diffuse anaplasia. Cases with anaplasia are generally older and are more likely to demonstrate metastatic disease, but the frequency of local stages II and III are similar.

## **12-Pediatric Salivary Gland Neoplasms: a 20 Year Experience of a Single Children's Hospital**

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**Background:** Salivary gland neoplasms are rare in both adults and children, accounting for 3% of all head and neck tumors and 8% of head and neck tumors in children. Previous studies have demonstrated that pleomorphic adenoma is the most common benign lesion and mucoepidermoid carcinoma the most common malignancy. Surgical excision with adequate margins is the mainstay of therapy for salivary gland lesions, but local recurrence is common.

**Design:** The laboratory information system was searched for all salivary gland neoplasms diagnosed at CHOP from 1988 to 2007. Glass slides, pathology reports and patient charts were reviewed. Inflammatory/infectious lesions, mucoceles, and vascular lesions were tallied for relative incidences only.

**Results:** 56 neoplasms were found arising in the salivary glands, of which 45 were salivary gland epithelial neoplasms. The mean patient age was 13 years with a female predominance of 1.8:1. The mean lesional size was 1.9 cm. The majority of epithelial neoplasms arose in the parotid gland (76%) with the remainder in the submandibular gland (24%). Of note, no epithelial neoplasms were identified in the sublingual gland. (In contrast, ~76% of sublingual gland lesions were mucoceles, but none were identified in the parotid gland.) 30 cases of pleomorphic adenoma (67%), 9 cases of mucoepidermoid carcinoma (20%), and 6 cases of acinic cell carcinoma (13%) were identified. Interestingly, no cases of adenoid cystic carcinoma or Warthin's tumor were seen; the latter being the second most common lesion in adults. In addition, there were a number of miscellaneous cases (20% of total), which included neurofibroma (5%) and lymphoepithelial lesions (4%). A large proportion of epithelial tumors (33%) were not thought to be of salivary gland origin at the time of surgery, and many underwent inadequate initial surgery (incisional biopsy, "shelling," positive margins). Seven

patients underwent immediate revision surgery and five had surgery for recurrence performed at CHOP at a later date (average time to recurrence 5.7 months for malignant, 10 years for benign). **Conclusions:** Salivary gland neoplasms are rare in the pediatric population, the majority being pleomorphic adenomas, mucoepidermoid carcinomas and acinic cell carcinomas. As salivary gland origin is frequently unexpected, inadequate initial surgery may be performed. Although most of these neoplasms follow an indolent course, recurrence is a major complication. All masses in the region of the major salivary glands should be handled as possible neoplasms, with adequate margins obtained by surgery and appropriate evaluation by surgical pathology.

### **13-Centrality of the Umbilical Cord Insertion in a Human Placenta Influences the Birth Weight.**

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**Background:** We hypothesize that trophotropism, considered to underlie eccentricity of umbilical cord insertion, results in a deformed placenta, less functionally efficient, and that the more eccentric the cord insertion, the less efficient the placenta.

**Design:** The model is based on a random fractal growth process (DLA). By placing the initial seed asymmetrically, the model produces placental vascular trees with a non-centrally placed umbilical insertion point, whereas the overall shape of the tree remains round to oval. To test this hypothesis, we calculated a measure of roundness by taking a mean radial distance to the perimeter. The sector was rotated in 2.5 degree increments to produce 24 radial measurements; the mean square deviation sigma was calculated for both real and model placentas. In the photographs of the UNC placentas the surface vascular branches were traced and, for each pixel in the chorionic surface, the minimal distance to a traced vessel was calculated. The resulting number is dimensionless (relative chorionic vascular distance delta). A lower value means a better coverage of the chorionic surface by the blood vessels.

**Results:** The correlation of the standard deviation of the placental radius (a measure of nonroundness of the placenta) with cord displacement was negligible at 0.01. Numerical simulations of the vascular growth model with cord displacement show no noticeable deviation from a normal round-to-oval placental shape for cord displacement of 10-50% of placental radius. Thus, non-central cord insertion has little measurable effect on the placental shape. However, placentas with a displaced cord show a markedly reduced placental function, reflected in birth weight. The correlation of the metabolic scaling exponent  $\beta$  (defined in [3]) with cord displacement is 0.158 (significance < 0.001). The correlation of  $\beta$  with the displacement measured by Fourier analysis is 0.2 (significance < 0.001). Analysis of the chorionic vascular density in two sets of traced images shows a high correlation of the relative vascular distance (a measure of “gaps” in the vascular coverage) with the cord displacement: 0.59 in one set of 12 images, and 0.20 in the other set of 28 images.

**Conclusion:** “Trophotropism”, the directional growth of the placenta due to variations in the intrauterine environment, is considered the most common basis for asymmetrical cord insertions. Our data suggest that even relatively “mild” eccentricity is associated with abnormal development of chorionic surface vessels. “Compensation” for a problematic intrauterine environment is incomplete, and does not restore the placenta to an “optimally transporting” structure.

### **14-Model for Oxygen Transport in the Human Placenta**

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**Background:** The mature placenta is a complex vascular network extending ultimately to the capillaries of the terminal villi, site of all oxygen and nutrient exchange between the mother and fetus. Respiratory transfer across the placenta to the fetus occurs in three steps: (i) maternal blood bathes the chorionic villi in oxygen, (ii) oxygen permeates the villus surface and diffuses into fetal capillaries, and (iii) oxygen is transported to the fetus via the bloodstream.

**Design:** Step (ii) has been modelled by the diffusion of oxygen from the villous membrane and the fetal capillaries. The stationary concentration  $c(x,t)$  of oxygen within each of the villi is the solution of the two-dimensional Laplace's equation,  $\Delta c = 0$ , with a fixed concentration  $c_v$  at the villous surface and a Robin boundary condition at the capillary boundary:  $D\partial c/\partial n = Kc$ , where  $\partial/\partial n$  is outward normal derivative,  $D$  is the oxygen diffusion constant and  $K$  is the permeability of the capillary. These equations are solved in regions determined by the villus and capillary boundaries obtained from digitized images.

**Results:** The solution for the oxygen concentration determines the diffusive current of oxygen across the capillaries. Many factors are expected to influence this current, including the numbers and shapes of villi and capillaries. Our initial analysis indicates that larger capillary calibers yield the largest flux per unit perimeter length and area of the villi; to date, these features are only able to be qualitatively described. These methods will allow numerical quantification of the key structural variabilities that affect villous function.

**Conclusion:** The geometrical shapes and spatial distributions of the villi and capillaries are important placental characteristics for the transport of oxygen to the fetus. Once the main factors that determine oxygen transport have been identified, this approach, applied to digitized placental slides that allow analysis of many hundreds of villi per slide (and multiple slides per placenta) should provide a quantitative basis for measuring placental oxygen fluxes.

### **15-Mutations in Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase and Placental Maternal Floor Infarct/Massive Perivillous Fibrin Deposition**

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**Background:** Maternal floor infarction, also known as massive perivillous fibrin deposition of the placenta, (MFI/MPVFD) is a rare disorder with no definitive etiology or pathophysiology, associated with significant fetal morbidity and mortality. A single case report in 2002 discussed an 8 month old child diagnosed with long-chain 3-hydroxyacyl-coenzyme A dehydrogenase (LCHAD) deficiency whose pregnancy was complicated by a maternal floor infarction of the placenta (Mol Genet Metab, 2001 Mar;72(3):265-8). Since this study was published, no further investigations have been reported to provide evidence that confirms or refutes the validity of this observation. The objective of this study is to investigate placentas with MFI/MPVFD for the presence or absence of LCHAD mutations.

**Design:** Between the years of 2000 and 2006, three cases of MFI/MPVFD associated with fetal or neonatal demise were documented at the Hospital of University of Pennsylvania. Review of the H&E slides and gross pictures confirmed the diagnosis as MFI/MPVFD. Paraffin blocks of placental tissue were retrieved, tissue scrolls were harvested and DNA samples were extracted.

The LCHAD gene was subsequently amplified using specific primer sets and directly sequenced by the dideoxy chain termination method.

**Results:** All three samples demonstrated heterozygous mutations in the LCHAD gene. The first sample, placental tissue obtained from a 25 4/7 week gestation growth restricted female infant delivered via cesarean section for premature rupture of membranes, revealed a heterozygous mutation in exon 11, C1072A (Glutamine → Lysine) with a heterozygous sequence difference in the intron following exon 6 (insertion T position +9). The second sample, placental tissue obtained from a 32 4/7 week gestation stillborn, growth retarded male fetus, revealed a heterozygous mutation (A+3G after exon 3) as well as a clear homozygous sequence difference in exon 17. The third case, placental tissue obtained from a 31 week gestation African American infant, also revealed heterozygosity (A+3G mutation after exon 3).

**Conclusion:** All three placentas with MFI/MPVFD demonstrated heterozygous mutations in the LCHAD gene. Given a background incidence of heterozygosity in the general population for LCHAD mutations of approximately 1 in 220, these findings lend further support to the hypothesis that LCHAD mutations may be associated with MFI/MPVFD.

### **16-Expression of AP-2 $\alpha$ Transcription Factor Is Abnormal in Villous Trophoblast in Severe Preeclampsia**

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**Background:** In vitro studies showed that the expression of the transcription factor AP-2 $\alpha$ , critical in the villous cytotrophoblast (CT) differentiation to form syncytiotrophoblast (ST), is abnormal in preeclampsia (PE). This retrospective analysis has been designed to study the AP-2 $\alpha$  trophoblastic expression in severe PE in clinical material by immunohistochemistry.

**Design:** Paracentral sections from grossly unremarkable areas of 10 placentas from patients with severe PE (study group, SG) and 10 sections from control placentas from patients without hypertension in pregnancy matched for gestational age (control group, CG) were double immunostained for AP-2 $\alpha$  and E-cadherin. E-cadherin immunostain highlights the CT membrane and the inner ST membrane, thus distinguishing the CT and ST cells from themselves and from all other cells of the chorionic villi. The AP-2 $\alpha$  positive and negative CT and ST cells were blindly counted in five representative 40x fields (hpf) by two independent observers in SG and CG. The differences were evaluated with ANOVA, single factor, and Yates chi-square, where appropriate.

**Results:** The average gestational age in CG and SG was 31.7 $\pm$ 5.1 weeks. Eight SG placentas featured diffuse villous hypoxia (uterine or post uterine) and decidual arteriopathy, compared with only 1 CG placenta with diffuse villous hypoxia and decidual arteriopathy ( $p < 0.05$ ). The numbers of chorionic villi and syncytial knots per hpf were higher in SG than in CG (29.0 $\pm$ 7.1 vs. 25.5 $\pm$ 7.6 and 14.6 $\pm$ 9.6 vs. 10.6 $\pm$ 8.9,  $p < 0.001$  and  $< 0.05$ , respectively). There were no statistically significant differences in the absolute numbers of villous CT and ST cells, but the AP-2 $\alpha$  positivity differed significantly both for CT (33.6 $\pm$ 29.7 vs. 44.8 $\pm$ 28.2) and ST (106.9 $\pm$ 140.1 vs. 48.8  $\pm$ 69.4) nuclei per one hpf in SG and CG ( $p < 0.05$  and  $< 0.001$ ), respectively.

**Conclusion:** Chronic hypoxia, manifesting in most of our SG cases by placental hypermaturity (increased number of chorionic villi per hpf, i.e. smaller size thereof, and Tenney-Parker change) and the histologic patterns of diffuse placental hypoxia, may be a regulating factor of the abnormal AP-2 $\alpha$  gene expression in CT and ST in severe PE. The reverse pattern of AP-2 $\alpha$  positivity in CT and ST may be explained by the accelerated villous CT differentiation in PE.

Abnormalities in the AP-2 $\alpha$  cascade of transcription factors and signaling molecules may be responsible, at least in part, for the abnormal villous CT differentiation in PE.

### **17-IUGR and Shallower Implantation Site in Rats with Maternal Hyperinsulinemia Are Associated with Altered Nos Expression**

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**Background:** There is ample evidence linking hyperinsulinemia in humans to hypertension (HT) and gestational HT. Pregnant rats treated chronically with insulin do not develop the normal gestational blood pressure decline. Fetuses of hyperinsulinemic dams (HD) are significantly growth restricted (GR), and like human GR newborns, once born develop HT in adulthood. Nitric oxide synthase (NOS) plays an important role in gestational HT. Blunting of the normal increase of urinary NO metabolites in HD, and abrogation of the gestational hypertension and IUGR by treatment with L-arginine (an NOS substrate) suggest involvement of the NO system in gestational HT in our model. Placentas from rat models are widely used to study human gestational disease, despite their labyrinthine structure, based on the similarities to human placenta, i.e. the hemochorial placental barrier and the deep invasion of endovascular and interstitial trophoblasts in the implantation site, designated the mesometrial triangle (MT).

**Design:** Our aim was to investigate the morphology and the expression of NOS isoforms in the placenta and the MT in HD.

**Results:** Fetuses of HD were significantly smaller than those of normal pregnant dams (NPD) (4.8 $\pm$ 0.5g in male fetuses of HD vs. 5.4 $\pm$ 0.4g and 4.5 $\pm$ 0.5g vs. 5.1 $\pm$ 0.4g in females, p<0.0001). Their placentas weighed less than those of NPD (0.44 $\pm$ 0.08g in HD vs. 0.50 $\pm$ 0.09g, p<0.0001) and their mesometrial triangles were also smaller. Endovascular trophoblasts (EVT) were found more often and in greater depth in NPD. Possibly as a compensatory mechanism, the EVT formed cell groups rather than a monolayer and occupied a larger portion of the arterial perimeter in arteries of HD. iNOS expression increased by 80% (p<0.0001) and 180% (p=0.045) in placenta and MT of HD, respectively. The expression of eNOS was reduced by 17% (p=0.048) in the placenta and did not change in the MT (p>0.05). nNOS expression, which was generally low, was decreased by 37% (p=0.03) in the placenta and increased by 53% (p=0.035) in the MT of HD. Prominent expression of iNOS in the placental junctional zone and in interstitial and EVT in the MT was observed by immunohistochemistry. Assuming a role in trophoblastic invasion, the increased expression of iNOS in HD may explain the "compensatory" pattern of trophoblastic invasion. Expression of eNOS was prominent in endothelial cells and weak in EVT.

**Conclusions:** Our IUGR rat model of maternal hyperinsulinemia exhibits gestational HT and shallower implantation similar to humans with preeclampsia. The divergent up- or down-regulation of the 3 isoforms of NOS suggest that each isoform may have a distinct function in the placenta and placental bed.

### **18-Placental Abnormalities in Term Neonates Transported to a Tertiary Care Facility**

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**Background:** Despite continuing advances in prenatal care and fetal monitoring, the placenta remains a valuable source that may explain the underlying pregnancy risk factors and conditions that result in adverse pregnancy outcome. Although placental examination in sick neonates is considered as standard of care, it is frequently not performed especially in local hospitals with

limited resources. As a result, when the neonatal transport team does not take the initiative for retrieving the placenta specimen, this valuable resource is lost.

**Hypothesis:** We hypothesize that placental examination of neonates transferred to a level III neonatal intensive care unit (NICU) is underutilized.

**Design:** We retrospectively reviewed the data for inbound neonatal transfers to Women and Infants hospital (WIH) of Rhode Island level IIIb NICU for the year of 2004. There were 185 inbound neonatal transfers from 20 community hospitals from the catchment area of WIH, which consists of Rhode Island, Southeastern Massachusetts, and Northeastern Connecticut. All but four of the hospitals had level I nurseries. The placental reports and slides were reviewed.

**Results:** 112 (60%) of the transported cases were term (>37 weeks gestational age). Only 12 (6.5%) placentas from the transported cases were submitted for pathologic examination. These were all term. The distribution of significant macroscopic and microscopic abnormalities in the examined placentas is summarized in Table 1. There were significantly more male (75%) than female (25%) neonates among the cases reviewed. The most common placental findings were evidence of meconium exposure (75%), evidence of intrauterine infection involving both maternal and fetal compartments (66%), and fetal erythroblastemia (50%). One placenta showed six of the described lesions. Rest of the placentas had multiple lesions and their numbers ranged between three and four.

**Conclusions:** We conclude that the placental examination is underutilized in the care of neonates transported to a level III NICU. Since the majority of these placentas show potentially significant pathology, centers that are responsible from these transports should make a conscious effort to retrieve these samples.

Table 1.

Lesion	Number of placentas
Disorders of growth	
Large for gestational age	3
Terminal villous hypoplasia	3
Small for gestational age	2
Inflammatory disorders	
Evidence of intrauterine Infection involving maternal and fetal compartments	8
Circulatory disorders	
Evidence of meconium exposure	9
Erythroblastosis	6
Decidual vasculopathy	3
Infarcts (one or more)	3
Avascular villi	3
Increased perivillous fibrin/fibrinoid	3
Multifocal villous edema	2
Velamentous insertion of umbilical cord	1

### **19-Fetal Neuroaxonal Dystrophy: a New Entity in the group of Neuroaxonal Dystrophy (NAD)**

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**Background:** Neuroaxonal Dystrophies (NAD) encompass a group of neurodegenerative diseases occurring clinically in different age groups and anatomically characterized by particular axonal lesions or « spheroids » occurring in both central and peripheral nervous system. The identification of causative genes has led to refine the phenotypes and to delineate new clinicopathological and molecular entities in this group of disorders. We report pathological and

molecular data of a new form of NAD, characterized by a particularly precocious prenatal onset, clinically different from classical Infantile Neuroaxonal Dystrophy (INAD) and also from its rare connatal form.

**Design:** We studied 4 fetuses ( 21-25 weeks) examined after a prenatal diagnosis and a medical pregnancy termination in accordance with French laws, and 1 neonate, without US follow-up of pregnancy, born at term and died a few minutes after birth. All 5 subjects presented identical clinical features consisting of a severe fetal akinesia sequence with microcephaly. In 3/5 cases, PLA2G6 gene, mutated in classical INAD, was studied.

**Results:** “Spheroids” with typical morphological and immunohistochemical features were identified in all cases, suggesting the diagnosis of NAD. They showed variable density, topography and spreading in the central and peripheral nervous system. However, they involved constantly, basal ganglia, brainstem, cerebellum and spinal cord. Different CNS malformations, unusual in typical INAD, were also observed, in each case, including hydrocephalus due to aqueduct atresia (1), callosal agenesis/hypoplasia (2), olfactory agenesis (1), anomalies of neocortical cytoarchitecture (2), abnormally pigmented retina (1). Mutations of PLA2G6 were not found.

**Conclusion:** The clinical and neuropathological features observed in these fetal cases are different from those of INAD, included Connatal INAD. The absence of mutations of PLA2G6, in addition, suggests that the Fetal NAD is a new entity, distinct from INAD, with different molecular basis, in the group of NAD. Different associated malformations suggest a rather wide phenotypic spectrum with a possible genetic heterogeneity. The precocious onset allows a prenatal US diagnosis in the 2nd trimester. Finally, Fetal NAD is an additional aetiology of Fetal Akinesia Sequence.

## **20-Membrane Lipid Raft Alterations In 7-Dehydrocholesterol-Enriched Hep-2 Cells: Implications For Pathogenesis Of Smith-Lemli-Opitz Syndrome**

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**Background:** Disorders of the cholesterol biosynthetic pathway are single-gene metabolic defects that give rise to severe multiple congenital anomaly syndromes such as Smith-Lemli-Opitz (SLO) syndrome, which is caused by a defect in the enzyme 7-dehydrocholesterol reductase that catalyzes the conversion of 7-dehydrocholesterol (7-dhc) to cholesterol. Disruption of hedgehog signaling has been shown to occur in these disorders; however, their pathogenesis is not completely understood. While most investigations are geared toward understanding the biological effect of cholesterol deficiency, preliminary studies suggest that intracellular accumulation of sterol precursors of cholesterol may also have profound biologic effects.

**Design:** We grew laryngeal carcinoma cell line, HEp-2, in media rich in 7-dhc (3 mg/dL) and found that the cells take up 7-dhc. Thus, we created a simple in vitro model to specifically study the effects of increased intracellular levels of 7-dhc in the presence of normal cholesterol biosynthetic pathway. Since cholesterol is an important component of membrane lipid rafts, we studied the sterol composition of lipid rafts isolated from 7-dhc-enriched HEp-2 cells. In order to study the impact of increased intracellular 7-dhc on membrane function, we compared calcium permeability between 7-dhc-enriched HEp-2 cells and HEp-2 cells grown in regular media.

**Results:** The membrane lipid rafts isolated from 7-dhc-enriched HEp-2 cells showed the presence of 7-dhc, demonstrating that 7-dhc can replace cholesterol in lipid rafts. 7-dhc-enriched HEp-2 cells showed a consistent increase in calcium permeability, when compared to HEp-2

cells grown in regular media, demonstrating altered membrane function in 7-dhc-enriched HEp-2 cells.

**Conclusion:** Using a simple in vitro model, we have demonstrated that an isolated intracellular elevation of the cholesterol precursor 7-dhc, in the background of normal cholesterol biosynthesis, results in 1) altered sterol composition of membrane lipid rafts, and 2) altered membrane function in the form of increased calcium permeability. We plan to use this model to study the Hedgehog pathway proteins and the proteomic changes in lipid rafts, which may help further our understanding of the pathogenesis of SLO syndrome and other cholesterol biosynthetic disorders, and to discover novel avenues for therapeutic intervention.

### **21-Fog2 and Myogenesis Proteins in Congenital Diaphragmatic Hernia (Cdh)**

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**Background:** CDH is a major birth defect, characterized by high mortality. Whether it is determined by a malformation of the pleuro-peritoneal folds or by a defective migration of myocytes is debated. Defects of genes involved in diaphragmatic development, such as friend-of-GATA2 (Fog2), may play an important role in its pathogenesis. We investigated the expression of Fog2 and proteins of myogenesis in a series of CDH and in diaphragms at different stages of embryogenesis, in order to clarify the role of Fog2 in diaphragmatic development and its involvement, together with the muscular component, in the pathogenesis of CDH.

**Design:** the study includes 7 surgical biopsies at the edge of the diaphragmatic hole, 2 entire diaphragms from autopsies for CDH and 5 from controls without genetic or malformative diseases (16, 17, 22, 32, 40 weeks (w) of gestational age) collected at University of Padova School of Medicine. Diaphragmatic thickness was measured in CDH (edge of defect, controlateral emi-diaphragm) and in controls. HE stained sections were reviewed and immunohistochemical expression of muscular markers (Desmin, Myf4, Pax7), stem cell markers (CD117, CD34 and CD105), Mib1 and Fog2 were evaluated.

**Results:** Mean thickness at the edge of the defect was 4.14 mm. Controlateral emi-diaphragm in 2 autopsies and in controls at 32 and 40w measured 2 mm; histology showed a higher density of desmin-positive muscular cells at the edge of defect. CDH displayed scattered Myf4-positive cells (range 0-10%, mean 2.4%), numerous Pax7-positive cells (range 0-24%, mean 12.1%) and less than 1% Mib1-positive cells. Controls showed a reduction of positive cell with the progression of gestational age for Myf4 (30% at 16w, 20% at 17w, 5% at 22w, 1% at 32 and 40w), Pax7 (85% at 16w and 17w, 35% at 22w, 11% at 32w) and Mib1 (20% at 16w, 8% at 17w, 7% at 22w, 2% at 32w). Fog-2 was diffusely positive in mesenchymal, mesothelial and muscular cells, in diaphragms from 16 to 22w, decreasing to 20% of positive muscular cells in 32w diaphragm. In CDH only mesothelial and mesenchymal cells were positive. Stem cell markers were negative in cases and controls.

**Conclusion:** CDH show a thick muscular border with a high number of mature muscle cells and a significant increase of quiescent satellite cells (PAX7+, Mib1-), suggestive of a maturative arrest of muscle cells. Mesenchymal stem cells are absent. The expression of Fog2 in mesothelial and mesenchymal cells in CDH demonstrates the absence of a genetic defect involving Fog2 in our cases. Being Fog2 one of the genes involved in the pathogenesis of some CDH, its expression in muscle cells during the embryogenesis supports a participation of muscle cells in the genesis of diaphragmatic defect.

## **22-The Role of Pericytes In Pulmonary Microvessel Development And In Neonatal Lung Disorders.**

EC Castro, WT Parks, C Galambos, Children's and Magee Women's Hospitals of Pittsburgh of UPMC.

**Background:** Pericytes, specialized vascular supporting cells are known to play a crucial role in the process of angiogenesis by remodelling newly formed vessels. The pathology of certain neonatal lung disorders (NLDs) shows microvascular alterations. We hypothesized that normal microvessel-pericyte interaction is defective in certain NLDs. Thus we studied pericyte ontogeny in relation to lung microvessels, and pericyte marker expression in NLDs.

**Design:** Human fetal autopsy lung tissue representing all three trimesters (12, 13, 16, 18, 24, 28, 34, 39 and 40 weeks of gestational age WGA), as well as from 1, 2, 5 and 10 years of age with no significant lung pathology were used. In addition lung sections of patients with congenital diaphragmatic hernia (CDH, n=5) and bronchopulmonary dysplasia (BPD, n=5) were selected. Although pericytes lack a unique marker, smooth muscle actin immunostain (SMA) is regarded a consistent one. SMA expression profile around the lung microvessels was established by grading SMA staining distribution and intensity.

**Results:** No SMA staining was noted until 28 WGA when early capillaries were apparent. From 34 WGA until term a strong, diffuse capillary expression of SMA was noted, but it began to decrease soon after birth. By 1 year of age SMA expression was weak and focal and at 10 years of age SMA staining was virtually absent. In the lungs of all BPD patients (mean age 26.5 WGA at birth, 472 days at death) and CDH patients (37.1 WGA, 26 days at death) the expression of SMA remained diffuse and strong around the microvessels throughout life.

**Conclusion:** During lung development the appearance of pericytes follows capillary development. Pericytes remain present until birth and gradually disappear postnatally as lung maturation completes, which implies a role in preparation of microvessels for the transition to an oxygen rich environment at birth. In BPD and CDH lungs, an excess of pericytes was noted that points to improper remodelling of pulmonary microvessels. This finding raises the possibility of pericyte-mediated disordered capillary blood flow as part of the pathomechanism of certain NLDs.

## **23-Alterations in the Nitric Oxide and Prostaglandin Signaling Pathways in Neonates with Pulmonary Hypertension**

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**Background:** Reversible and irreversible pulmonary hypertension is seen in critically ill neonates with persistent pulmonary hypertension of the newborn (PPHN) and congenital diaphragmatic hernia (CDH) respectively. Both prostaglandins [PG] and nitric oxide [NO] play a significant role in modulating pulmonary vascular tone in the perinatal period. Vasodilator mediators (NO, PGI<sub>2</sub>, and PGE<sub>2</sub>) are synthesized by respective synthases (eNOS, PGIS, PGES) in the lung. EP<sub>2</sub> and EP<sub>4</sub> receptors mediate the vasodilator effects of PGE<sub>2</sub>. Thromboxane A<sub>2</sub> is a vasoconstrictor PG synthesized by TXA<sub>2</sub> synthase [TXS]. The purpose of this study is to evaluate the expression of eNOS, PGIS, PGES, EP<sub>2</sub>, EP<sub>4</sub> and TXS in the lungs of neonates with PPHN and CDH compared to controls using semi quantitative immunohistochemistry.

**Design:** Formalin-fixed, paraffin-embedded, postmortem lung tissue from infants with PPHN (13) and CDH (8) were stained with eNOS, PGIS, PGES, EP<sub>2</sub>, EP<sub>4</sub> and TXS antibodies. Staining intensity and distribution in the bronchi, alveoli, vascular endothelium and media of arterioles and venules were evaluated. Control population consisted of gestational and postnatal

age matched infants (14) who died of non-respiratory causes.

**Results:** Histological evidence of chronic lung disease and pulmonary hypertension were seen in infants with CDH but not in infants with PPHN or controls. Expression of PGIS and PGES was comparable across the three groups. In CDH infants, there was a significant increase in staining intensity and distribution for eNOS in the alveolar lining cells and arteriolar endothelium, EP4 in alveoli, EP2 in alveoli, arteriolar endothelium and media and TXS in the bronchial and alveolar lining as compared to controls ( $p < 0.05$ ). In PPHN infants, there was significant increase in staining intensity and distribution for eNOS, EP2 and TXS in the alveoli and EP4 in the arteriolar endothelium as compared to controls ( $p < 0.05$ ). Infants with CDH had greater staining intensity and distribution for eNOS in the alveoli, arteriolar and venular endothelium, EP4 in the arteriolar endothelium, EP2 in arteriolar endothelium and media and TXS in the arteriolar media ( $p < 0.05$ ) compared to infants with PPHN.

**Conclusions:** Upregulation of eNOS, EP4, EP2 and TXS was observed in infants with CDH and PPHN but was more marked in the former. Upregulation of vasoconstrictor mediators may play a role in the pathophysiologic mechanism of pulmonary hypertension in the neonate. Upregulation of the vasodilator mediators may represent a compensatory mechanism and may have implications for response to treatment and development of novel therapies for PPHN.

#### **24-The Ontogeny Of Angiotensin-Converting Enzyme (ACE) And Its Aberrant Apxression May Contribute To The Pathology of Bronchopulmonary Dysplasia (BPD).**

EC Castro, WT Parks, C Galambos, Children's and Magee Women's Hospital of Pittsburgh of UPMC.

**Background:** The mammalian lung possesses the highest level of ACE of any organ. The well-established function of this enzyme is to generate angiotensin (AT)-II from AT-I, thereby regulating systemic blood pressure. Recent data, however, indicate a role for AT-II in the pathomechanism of pulmonary hypertension, and newborn rats treated with ACE inhibitor develop lungs with features of BPD. The ontogeny of ACE in humans has not been investigated. We studied ACE expression during human lung development and in human BPD lungs.

**Design:** Human fetal autopsy lung tissue representing all three trimesters (12, 13, 16, 18, 34, 39 and 40 weeks of gestational age WGA), as well as from 1 and 10 years of age with no significant lung pathology were used. In addition lung sections of patients with bronchopulmonary dysplasia (BPD,  $n=5$ ) were selected. The slides were stained with routine immunohistochemical method utilizing monoclonal antibody to ACE (Millipore). ACE expression profile was established by grading arterial and capillary endothelial staining distribution and intensity.

**Results:** Strong, diffuse small arterial and capillary expression of ACE was seen in the human fetus as early as 12 weeks. ACE expression remained high throughout gestation and postnatally. No venular ACE expression was noted. In the BPD lungs capillary ACE endothelial staining was largely absent, and when focal staining was observed the intensity was very weak.

**Conclusion:** We established that ACE expression is present in the human fetal lung as early as 12 WGA. Moreover, the early expression of ACE suggests a role in the process of lung development. This role was further substantiated by our findings in the BPD lungs, which showed essentially no capillary ACE expression. These findings suggest that ACE may play a role in alveologensis.

#### **25-Hepatic Granulomas in Immunocompromised Pediatric Patients.**

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**Background:** Hepatic granulomas occur in primary liver diseases and as well as in generalized processes as infections, drug induced injury, autoimmune disorders.

**Design:** From the total number of 2600 liver biopsies examined in the period between 2003 and 2008 we selected retrospectively forty five liver biopsy specimens with the presence of hepatic granulomas. The age of patients ranged from 7 months to 18 years (mean age 8 years). Forty patients were immunodeficient: immunosuppression after liver transplantation (LTx), treatment in autoimmune hepatitis (AIH), X linked lymphoproliferative syndrome (XLP), immunodeficiency in severe combined immunodeficiency (SCID), common variable immunodeficiency (CVID) and Burkitt lymphoma. Ten patients underwent liver transplantation because of initial liver damage following biliary atresia, autoimmune hepatitis and alpha 1 antitrypsin deficiency and later on developed hepatic granulomas with recurrent autoimmune hepatitis, and post transplant lymphoproliferative disorder (PTLD) respectively. All biopsy specimens were fixed in 4% buffered formalin for a period of 24 hours, embedded in paraffin blocs and routinely stained: H&E, PAS with and without diastase, Azan and Gomori silver. Histological interpretation was performed using internationally accepted criteria: the extend of inflammation (inflammatory infiltrates in portal tracts and lobules) and fibrosis (portal and periportal fibrosis, portal to portal and portal to central septa) was assessed by grading and staging of the classification after Batts and Ludwig.

**Results:** All granulomas were of epithelioid type without central necrosis composed of epithelioid cells, giant cells surrounded by different inflammatory cells. Granulomas were always present in the portal tracts and less frequently in lobules. However in three cases, (patients with intralobular cholestasis) numerous granulomas were present also in the lobules. In the case of fungal coinfection in a patient with Burkitt type Lymphoma, portal granulomas were surrounded by eosinophils and lymphocytes. Most of portal tracts were enlarged, fibrotic and contained numerous inflammatory cells with the predominance of purulent inflammation

**Conclusion:** Hepatic granulomas are a rare finding in pediatric liver biopsies and occur in 1,7% of all biopsies in the pediatric group of patients. Usually they form epithelioid, non caseating nodules situated in portal tracts. They represent a cell-mediated immune response and occur in immunocompromised patients. Most of them accompany other morphological changes in autoimmune hepatitis and immune deficiency/proliferative syndromes.

## **26-Diagnostic Use of the Calretinin Immunostain in Rectal Biopsies to Rule Out Hirschsprung's Disease: An 18 Month Review of Practice, and a Comparison of Local vs Referred Patients.**

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**Background:** A fine neural plexus in the lamina propria of normal intestine can be marked with an immunostain to calretinin. This plexus is absent in aganglionic bowel in Hirschsprung's Disease (HD). The diagnostic utility of the calretinin stain has been studied retrospectively, but live use in diagnostics has not been reported to our knowledge. The following report is an analysis of diagnostic use over an 18 month period with comparison of results of biopsies done locally vs referred.

**Design:** Biopsy reports and medical records were studied in 35 patients biopsied in house (IH) and 44 patients biopsied at other facilities that sent the tissue to us for analysis (OC, outside

cases). Data collected included the age at biopsy (and re-biopsy, if done), the age at resection (for HD), the case pathologist, the type of biopsy, and the findings of the H and E stain, acetylcholinesterase stain (ACHE), and the calretinin stain (if done).

**Results:** A calretinin stain was ordered in 13 of 79 cases (16%). Eight of these cases showed no ganglion cells (GC) on initial H-E stained serial sections, four showed rare or single GC, and one showed a reactive acetylcholinesterase stain. A calretinin stain was not ordered on the 5 cases that were aganglionic and had light microscopic and ACHE features of HD (all 5 proven to be HD on pull thru). Of the thirteen cases studied with calretinin, all had a mucosal plexus, and none had an ACHE stain regarded as consistent with HD. Of these 8 cases with no GC on primary review, 3 were found to have GC on re-biopsy, 4 were found to have GC on additional studies, and 1 received no additional studies. When the IH and OC cases were considered separately, 1 of 7 OC cases initially read as having no GC proved to be HD while 4 of 8 IH cases proved to be HD. Comparison of the age distribution of the IH and OC patients at the time of biopsy showed identical populations ( $p=0.99$ ): IH – mean 81, sd 125 weeks; OC – mean 81, sd 150 weeks. HD patients' ages at biopsy were 5-9 days for IH and 17 days for OC.

**Conclusion:** 1. Demonstration of a calretinin plexus rules out HD and obviates the need for re-biopsy when GC are not demonstrated. 2. Establishment of an age threshold for biopsy appears indicated. 3. Biopsies in non infants need not include muscularis propria. 4. IH and OC practices equally biopsy older patients. 5. Absence of GC was more likely to be HD in our IH vs OC population.

## **27-Underlying diseases and other gastrointestinal abnormalities in patients with celiac disease**

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**Background:** Celiac disease (CD) is an immune-mediated disorder that occurs most often in a setting of HLA DQ2 or DQ8 genotype individuals. Patients with autoimmune endocrine and chromosomal disorders have a higher risk for CD. While the pathological changes of CD are characteristic in distal duodenum and jejunum, other regions of the gastrointestinal tract may have concomitant abnormalities. We sought to study in our CD patient population the epidemiological trends of underlying diseases and other concomitant gastrointestinal changes.

**Design:** We performed a retrospective study by identifying all tissue transglutaminase antibody (tTG) positive individuals from our laboratory's database between 09/2004 to 09/2008. Many of the tTG positive individuals also underwent gastrointestinal endoscopy with duodenal biopsies and/or serological testing for endomysial antibody (EMA). A review of the patients' charts was done to collect data on the test results of tTG, EMA, biopsy diagnoses, and underlying disorders if any. The diagnosis of CD was rendered in individuals who had positive tTG antibody level and characteristic histological changes in duodenum or a combined positive tTG and EMA, if no biopsy was performed.

**Results:** Analysis of the data showed that there were 108 CD patients, of which 78 were biopsy proven. Out of the 78 biopsy proven CD patients, 68 (87.2%) had concomitant chronic gastritis. We also found 4 patients with eosinophilic esophagitis and 1 patient each with eosinophilic gastritis, eosinophilic colitis and IgA dermatosis. Unexpectedly, 42 (39%) of 108 patients had type 1 diabetes mellitus (T1DM) as an underlying condition. There were 4 patients with Trisomy 21, and 3 patients with autoimmune thyroiditis. There was a strong correlation between higher tTG levels and higher Marsh grade, although occasional cases with higher tTG levels had a lower Marsh grade.

**Conclusions:** Our study found a surprising trend in the newly diagnosed CD patients in that a high proportion of them have T1DM as an underlying disorder. This may reflect aggressive screening of T1DM patients. The presence of chronic gastritis in many CD patients suggests a common pathogenetic mechanism; however this speculation needs to be substantiated by systematic studies. The presence of concomitant mucosal eosinophilia in a small minority of the patients is an interesting finding and probably pathogenetically unrelated to CD.

### **28-Elevated Fecal Calprotectin in Childhood Inflammatory Bowel Disease: Correlating Biopsy Findings with Clinical and Endoscopic Features, a Review of 17 Cases**

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**Background:** Fecal calprotectin (FC) is a calcium binding protein with antibacterial & antifungal activity. FC inhibits metalloproteinase and induces apoptosis in malignant and nonmalignant cell cultures. FC constitutes 60% of neutrophil cytosolic protein, is resistant to bacterial degradation in the gut and is stable in stool for one week at room temperature. FC is more likely to be raised in inflammatory bowel disease. Recent studies have shown that inflammatory bowel disease in remission show normal FC levels. The threshold for invasive procedures is higher in children, therefore fecal markers that may indicate significant gastrointestinal disease, make the clinical use of FC appealing. Studies have shown that FC concentration reflects migration of neutrophils through inflamed mucosa. We examined the clinical history, laboratory data, endoscopic findings and biopsies in 17 children with inflammatory bowel disease, showing elevated FC.

**Design:** The clinical features, laboratory data, endoscopic features and biopsies of 17 children with elevated fecal calprotectin were reviewed. Patient ages ranged from 2-17years (median: 9), 12 were female and 5 male. All cases were previously diagnosed with inflammatory bowel disease and presented with symptoms while they were being monitored.

**Results:** The commonest symptoms were abdominal pain (7/17), rectal bleeding (7/17), and weight loss or poor weight gain (6/17). All patients had elevated fecal calprotectin ranging from 142-2500 microg/g. Only 4/17 had concomitantly elevated neutrophils on CBC. On scoping, 9/17 had severe pan colitis, 7/17 had colonic erythema and friability, 2/17 had aphthous ulcers, and 1 had proctitis. Only 1/17 had a superimposed infection (rotavirus). All cases were negative for giardiasis, adenovirus, enterovirus, ova and cysts. 13/17 were ulcerative colitis and 4/17 Crohn's disease. 4/17 had duodenitis and 4/17 had ileitis. Additionally, 8/17 had chronic active non helicobacter gastritis and 3/17 had active esophagitis. One patient each had Celiac disease, primary sclerosing cholangitis and hepatic fibrosis.

**Conclusion:** Fecal calprotectin is elevated in active pediatric inflammatory bowel disease and is a useful marker in monitoring disease activity. The level of elevation was proportional to severity of histological active inflammation. Four cases with no evidence of active inflammation on biopsy, showed modestly elevated FC, indicating alternate mechanisms, probably macrophage related FC production. Our small study validates the measurement of FC as a measure of presence and degree of inflammation. Further detailed study is required to fully understand the utility of FC in inhibiting epithelial dysplasia and bacterial growth in affected children.

### **29-Persistent Hyperinsulinemic Hypoglycemia of Infancy: Constitutive Activation of the mTOR Pathway in the Exocrine Pancreas with Histogenetic and Therapeutic Implications**

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**Background:** Persistent hyperinsulinemic hypoglycemia of infancy (PHHI) is manifested histopathologically by a focal form with well defined regions of islet cell proliferation, and a diffuse form, with global disorganization of islets architecture, also called “nesidiodyplasia”. Metabolically, amino acids stimulate the mammalian target of rapamycin (mTOR) pathway and specifically mTORC1 (raptor + mTOR), and the mTOR pathway integrates amino acid- and energy-sensing pathways, including glutamate dehydrogenase, in beta cells. Moreover, rapamycin

inhibits beta cell function. We examined the mTOR pathway in the exocrine pancreas in the diffuse form of PHHI and its possible association with neoislet formation.

**Design:** Pancreatic tissue from three patients was analyzed. One with normal histology served as a control. The other two are newborns (term pregnancies of non-diabetic mothers) with refractory hypoglycemia, one with SUR gene mutation consistent with PHHI and the other with a family history of hypoglycemia of infancy. A phosphospecific probe for mTOR phosphorylated on serine 2448 and an immunohistochemical probe for insulin were applied. Representative sections were processed for transmission electron microscopy (TEM).

**Results:** Microscopic examination of the hematoxylin-eosin slides showed neof ormation of the islets from ductal elements. Islet cells could also be seen within acini in a diffuse pattern and outside any well-defined islets. Phosphorylated (p)-mTOR (Ser 2448) on the plasmalemmal (P) and cytoplasmic but not nuclear compartment, consistent with mTORC1, was strongly expressed on acinar and ductal elements. Mild expression was noted in the islets, per se. Double immunostaining revealed occasional acinar cells expressing mTOR that also secrete insulin, a result enhanced by multispectral imaging. No such co-expressions were seen in the control. TEM showed that such acinar cells contain zymogen granules and hormone secreting granules.

**Conclusion:** Constitutive activation of mTOR in the exocrine pancreas associated with insulin-expressing neof ormed islet cells were identified in the diffuse form of hypoglycemia of infancy. Rapamycin, which inhibits mTORC1 and has been shown to inhibit both the function of beta cells and the neof ormation of islet-precursor ductal cells, may be a therapeutic option in the diffuse form of PHHI.

### **30-Molecular Abnormalities In Paediatric Barrett’s Oesophagus: Can We Test For Neoplastic Progression?**

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**Background:** Barrett’s oesophagus (BO) is a preneoplastic condition that predisposes to oesophageal adenocarcinoma and is a consequence of prolonged gastro-oesophageal reflux. BO displays specialised intestinal metaplasia (IM) which is visible endoscopically above the gastro-oesophageal junction and can be confirmed histologically. The aim of our research was to identify genetic biomarkers of disease progression in paediatric BO.

**Design:** We performed Fluorescence In Situ Hybridisation (FISH) with probes from Abbott

Vysis Corporation on sections taken from 49 paraffin embedded sequential biopsies (bp) of 10 cases (C) of BO. The probes were specific for HER2 at 17q-/17 centromere/4 centromere; p16 at 9p21/9 centromere; TP53/17 centromere/6 centromere and CCND1 at 11q-/11 centromere. Each case had the four probe sets applied, these had been validated at the same time in 10 cases of adult Barrett's adenocarcinoma.

**Results:** Molecular abnormalities (MA) were present in 5 (4 males, 1 female) cases with mean age at first biopsy of 9.2 (range 3-15) years. The following MA developed in successive biopsies done over 5 years: C2: deletion of p16 (2nd bp); C3: borderline amplification of HER2 (1st bp), deletion of p16 (2nd bp) and aneuploidy of chromosomes 4, 17 and 11 (3rd bp); C5: gain of chromosome 9 (3rd bp) and deletion of p16 (5th bp); C8: borderline amplification HER2 (1st bp) and C9: gain of chromosome 11 (1st bp). The corresponding histology of these cases featured: columnar lined oesophagus (CLO) with IM (goblet cells) in those cases with deletion of p16, amplification of HER2 and gain of chromosome 9; severely inflamed oesophageal mucosa without BO in the 1st biopsy of C3 which had borderline amplification of HER2 and villiform mucosa with IM in the 3rd bp of the same case which showed aneuploidy of chromosomes 4, 17 and 11.

**Conclusion:** This is the first time MA are demonstrated in paediatric BO. The MA identified in 50% of our cases were also present in those cases with adult Barrett's adenocarcinoma. We propose that the panel tested in this study could be used to identify those children with BO at risk of disease progression.

### **31-Sudden Unexplained Death in Childhood: an Epidemiological Profile.**

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**Background:** In Ireland the number and proportion of deaths certified as sudden unexplained death in children >52wks (SUDC) has increased significantly in recent years. The reasons for this are unknown and due to the rarity of SUDC there is little information available on these deaths in the literature. Establishing an epidemiological profile of SUDC cases in isolation from classic SIDS may provide valuable information, particularly any factors which distinguish them from SIDS, which might aid in future diagnosis of these cases.

**Design:** A ten year population based case control study including all cases of SIDS and SUDC registered with the Irish National SIDS Register from 1994-2003 and controls matched for date of birth and geographical location. Data was extracted from questionnaires completed by families, post mortem reports and hospital records. Differences in the distribution of proportional variables were examined using chi square analysis in STATA 8.

**Results:** 319 cases of SIDS and 26 cases of SUDC were available for inclusion in the study. The most significant finding was that of a higher incidence of children being found prone in the SUDC group (70% cases vs 11% of controls,  $P < 0.001$ ) as compared with SIDS (23% cases vs 7% controls,  $p < 0.01$ ). Furthermore, significantly more SUDC than controls had visited their GP because of illness in the week preceding death; 35% vs 7%,  $P < 0.01$ , an effect which was not apparent for SIDS cases, while in both groups, significantly more cases than controls had a history of illness during their lifetime; 75% vs 33% (SUDC) and 61% vs 26% (SIDS). While low birth weight and prematurity were more prevalent among SUDC (29% and 17% respectively) than SIDS (16% and 11%), levels of infant-parent co-sleeping and maternal

smoking were lower among SUDC families (15% and 38% respectively) than SIDS (49% and 72% respectively). The classic male predominance of SIDS (60%) was not as apparent in the SUDC group (53%).

**Conclusion:** This audit aims to establish the epidemiological profile of SUDC in an Irish population and to add to the sparse data available. The study indicates that while SUDC shares some characteristics with SIDS, there are also some important differences. Further data collection from this ongoing case control study will help determine whether SIDS and SUDC represent the same pathophysiological entity or not.

### **32-Hepatic and Renal Histopathology in Cpt1a P479L Homozygous Sudden Death Cases**

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**Background:** Carnitine palmitoyltransferases (CPT) are key enzymes in the carnitine dependent transport of long chain fatty acids across the mitochondrial membranes. CPT1 has three tissue specific isoforms: liver (CPT1a), muscle (CPT1b) and brain (CPT1c). The clinical presentation of CPT1a deficiency consists of recurrent attacks of fasting hypoketotic hypoglycemia. A variant of this gene, CPT1a p.P479L (c.1436C>T) was first described in individuals of First Nations (Native American) descent in British Columbia.

**Design:** All cases of First Nations with unexpected death in infancy and childhood from January 2001 to August 2008 who had autopsy examinations were identified. Acylcarnitine profile and mutation analysis to detect the presence of the CPT1a P479L variant were performed in all cases. The histology of the organs (including special stains and Oil Red-O (ORO) in cases shown to be homozygous for the CPT1a P479L variant was compared that with age-matched controls with normal acylcarnitine profiles who are either P479L heterozygous or those in whom the P479L variant was not detected (wild type).

**Results:** Of 24 cases of unexpected death in children of First Nations origin, 14 cases were shown to be homozygous for CPT1a P479L, 4 cases were heterozygous P479L, and 6 cases had wild type P479L. None of the cases was diagnosed with a fatty acid oxidation defect. The microscopy of liver in homozygous cases showed: microvesicular steatosis in all 14, ranging from mild to moderate by H&E and ORO stains, glycogen depletion in 11, neonatal hepatitis and giant cell transformation 3 cases, and extramedullary hematopoiesis 3 cases. The kidneys showed variable numbers of microvesicles in the tubules in 11 cases; ORO was performed in 3 cases and confirmed the presence of fatty vesicles in renal tubules. Of the 4 heterozygous, mild microvesicular steatosis was present in 1, glycogen depletion in 3 cases, and extramedullary hematopoiesis in 1 case. Liver examination in the 6 wild type cases showed microvesicular steatosis in 3, glycogen depletion in 3, and extramedullary hematopoiesis in 3. ORO was performed in 1 case and showed microvesicular steatosis but was negative in the kidney. No microvesicles in renal tubules were seen in heterozygous and wild type cases on H&E sections.

**Conclusions:** Microvesicular steatosis of the liver does not reliably distinguish between the homozygotes and the others in this population but the addition of microvesicular fatty change in the kidneys may serve to heighten consideration of this disorder.

### **33-The Mesomorphic Method for Determining Duration of Post Fetal Demise Retention**

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**Background:** Many fetal deaths are not witnessed clinically despite wide use of electronic fetal

heart monitoring. Histomorphic methods applicable at autopsy are often arbitrary but the method described in Amer.J.Obst.Gyn. 68:213-229, 1964 included detailed parallel experimental work in rabbits for direct tissue comparison. We here revisit and update the work originally based on histological changes in just four organs: lung, liver, skin, and kidney.

**Design:** The 1964 method was applied seriatim to 550 stillborns over the 25 year period 1983-2007 at gestational age 20-43 weeks. Correlated clinical information included FHT and sonographic findings. The 1964 method was refined over time with emphasis on the aspects useful during particular time frames and in correlation with changes commonly attributed to fetal hypoxia.

**Results:** The basic system was confirmed as highly accurate in the first 12 hours post demise, acceptably applicable for the intervals 18-24 up to 72-96 hours, and 7-10 days, the latter when changes in the thymus were examined in addition to the original four tissues. In the original correlative study of 53 stillborns (1964) 50% were delivered by 9 hours post demise; this midpoint was not reached in the current study until 27 hours, the difference attributed to a greater proportion of very immature fetuses in the UT study. The maximum retention time was 10 days in both.

**Conclusion:** Detailed examination of five organs of the stillborn fetus, lung, liver, skin, kidney, and thymus for autolytic changes in middle range structures ("mesomorphic") yielded information derived from simple observation on routine H&E sections pertinent to the estimate of the length of time a fetus is retained post demise. This has special value in judging when a fetus dies after trauma such as occurs in automobile accidents and other forms of physical impact on the pregnant uterus.

### **34-Elevated Brain Weight/ Liver Weight Ratio in Normal Bodyweight Centile Term Perinatal Deaths: an Indicator of Terminal Intrauterine Malnourishment?**

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**Background:** In recent years we have noted that a proportion of antepartum/intrapartum stillbirths (IUD), with a normal or elevated body weight centile also show an elevated brain weight : liver weight ratio. We postulate that this is an indication of intrauterine malnourishment/incipient intrauterine growth restriction (IUGR) which may have a bearing on the cause of death.

**Design:** This is a retrospective study, based on our departmental post mortem MS access database. All cases with a consented post mortem examination are entered in the database (more than 3000 entries in the last four years). For the purposes of this study we have identified 331 cases, 37/40 weeks gestation and over of IUD/intrapartum death (254; 77%) or early neonatal death (77; 23%) respectively. A brain weight/ liver weight ratio (BLR) of >4.0 was regarded as abnormal. The customised body weight centile was calculated using the Gestation Network centile calculator ([www. http://www.gestation.net/birthweight\\_centile/birthweight\\_centiles.htm](http://www.gestation.net/birthweight_centile/birthweight_centiles.htm)).

**Result:** Of the 331 cases, the BLR was >4.0 in 74 (22.4%). 19 (25.7%) of these 74 cases had a body weight above the 25th centile and these were all IUD's. Of these, 6 had elevated brain weight: thymus weight ratio (>60) as an indication of thymic atrophy/stress. Where data were available, 10 out of the 16 (62.5%) mothers were overweight and obese (BMI>25) and 7 were obese (BMI>30) (43.8%).

**Conclusion:** Our data show that in approximately one quarter of unselected cases of perinatal death with a brain: liver weight ratio of >4, the body weight is above the 25th customized centile. One third of these show thymic atrophy. We suggest that this indicates intrauterine malnourishment/incipient IUGR, which would be missed if weight centile is the only criterion used to assess IUGR/fetal nutrition. Nearly two-thirds of the cases had an increased maternal BMI. It is possible that in this group the altered carbohydrate metabolism of the mother resulted in a macrosomic baby, who suffered a terminal decline in placental nutrient supply.

### **35-Spare The Heart; Spoil The Lungs: Pulmonary Arterial Injury Following Sano Shunt For Hypoplastic Left Heart Syndrome**

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**Background:** The Norwood procedure for hypoplastic left heart syndrome (HLHS) palliation initially used a Blalock-Taussig (BT; subclavian to pulmonary artery) shunt for pulmonary perfusion. The Sano shunt, connecting right ventricle to pulmonary artery via Gortex shunt to reduce cardiac ischemia from diastolic runoff, began to be used in the mid 1990s. We noted damage resembling thrombotic microangiopathy (TMA) in small pulmonary arteries of patients autopsied after Sano procedures.

**Design:** We reviewed lung tissue slides from 40 congenital heart disease patients autopsied between 1992 and 2008 to assess the frequency of pulmonary vascular injury in congenital heart diseases with differing blood flow profiles. Cases selected by diagnosis from the autopsy files were distributed among 4 groups: HLHS with a Sano shunt (n=10); HLHS with BT shunt (n=9); tetralogy of Fallot (TOF; n=11); and atrioventricular canal (AVC; n=10). In each group, the most recent cases were selected. BT shunt cases were older than Sano cases because the latter replaced the former. All lung slides from each case (1-6 slides) were reviewed for evidence of TMA including intramural red blood cells/rbc fragments, medial necrosis, and thrombi within small pulmonary arteries. TMA-like injury was diagnosed when at least 2 features were found in at least 2 vessels in a case. The frequency of injury was compared between the Sano-palliation HLHS group and the other three groups by Fisher's exact test.

**Results:** TMA-like vascular injury was detected in 5 of 10 cases of HLHS with Sano, 2 of 9 HLHS with BT, 1 of 11 TOF, and 3 of 10 AVC defect. The Sano shunt was significantly associated with TMA-like vascular injury ( $p=0.047$ ) when compared with BT shunt, TOF, and AVC.

**Conclusions:** The Sano shunt is associated with TMA-like injury. Pulsatile flow associated with Sano shunt appears unlikely to be the mechanism of TMA, since a similar degree of TMA was not observed in cases of TOF, which have more pulsatile flow after repair. The mechanism of vascular damage following Sano procedure for HLHS is unclear and requires further study.

### **36-The Broward County Pediatric Autopsy Registry: Results of a Four-Year Study**

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**Background:** In 2003, Broward County, Florida, implemented a four-year study of fetal and infant mortality. In Broward County, nonwhite infants die at approximately twice the rate of white infants.

**Design:** The program provided autopsies at no cost to any Broward County mother who lost a

fetus or infant between 20 weeks gestation and the first birthday. Medical examiner's cases were also included in the study population. Autopsy causes of death were grouped into 13 categories (e.g., infection, SIDS, accidents, congenital anomalies, maternal medical issues, perinatal asphyxia, etc.).

**Results:** The program achieved autopsy rates of 31% for infant deaths and 35% for fetal deaths. Causes of fetal death varied considerably by gestational age, with chorioamnionitis occurring more frequently in younger fetuses and maternal medical issues playing a more prominent role in older fetuses. The cause of death was unknown in 17% of the fetuses. Infections were the leading cause of infant deaths, followed by SIDS, accidents, congenital anomalies, and complications of prematurity. Among infant deaths, in no case was the cause of death unknown. There was less racial disparity in infant deaths occurring on the first day of life than in the remainder of the neonatal and postnatal periods. Among neonates, excess nonwhite deaths were due to complications of prematurity, congenital anomalies, and accidents. The largest absolute racial disparity in the postneonatal period was noted in the medical examiner series, where there were substantial excess deaths due to SIDS, infection, homicide and accidents. The study also looked at information on death certificates compared with previous autopsy findings. Less than half of fetal death certificates listed a cause of death in the same group as the autopsy diagnosis. Overall, there was better agreement between death certificates and autopsy findings at older ages.

**Conclusion:** The study concluded that over 40% of all fetal and infant deaths in Broward County were preventable. Reduction of preventable deaths due to infection, SIDS, maternal medical causes, accidents and homicides would have the largest impact on overall perinatal mortality. Within Broward County, there are substantial differences in the pattern of fetal and infant mortality by race, which should be considered in the development of program efforts to target this disparity.

## **POSTERS**

### **37 - Review Of Autopsies With Placental Abruption**

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**Background:** Some autopsies of stillborn infants do not show an anatomic mechanism of death, but do show intrathoracic petechiae. This study tests the hypothesis that those petechiae are a marker of acute asphyxia. A large retroplacental hemorrhage (RPH) is an anatomically identifiable cause of intrauterine asphyxia, which if the hypothesis is correct, should demonstrate intrathoracic petechiae. In addition, the development of overlying infarction in the placenta should be approximately the same as the duration of intrauterine postmortem retention. This study correlates histological placental infarction features with intrauterine duration of the fetal death.

**Design:** A database of personally performed autopsies was searched for infants greater than 26 weeks of gestation with RPH which had available a gross description of the chest and placenta, photographs of the chest and placenta, and/or available microscope slides. Eleven controls from other mechanisms of death were randomly selected that had a photograph of the open chest. Petechiae were evaluated using the microscope slides and/or photograph. Placental histological features were recorded from the microscope slides.

**Results:** Intrathoracic petechiae were present in all 14 infants with RPH > 50% of the placental

area, in 2 of 7 infants with < 50% area RPH and in 3 of 11 infants with other diagnoses (1 of 2 with infection, 2 of 5 with chronic utero-placental ischemia, and 0 of 3 with hydrops). The placental pathology demonstrated basal plate neutrophils in all cases of RPH that had slides (N=11). Early coagulation necrosis in the villi overlying the hemorrhage was present in 2 of 10 cases in the 4 to 24 hours retention interval, and complete coagulation necrosis was present in 1 of 2 cases in the 48 to 96 hours of retention interval.

**Conclusion:** This study supports the hypothesis that intrathoracic petechiae are a marker of intrauterine asphyxia in stillborn infants. Basal plate neutrophils are a useful early marker of retroplacental hemorrhage. The infrequency of early coagulation necrosis suggests that it begins in the later part of the 4 to 24 hour interval, and that it may not be complete at 48 hours.

### **38-Both Membrane and Chorionic Disc Placental Microscopic Chorionic Pseudocysts Are Associated with Increased Amount of Extravillous Trophoblasts, Non-inflammatory Patterns of Placental Injury and Perinatal Mortality**

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**Background:** Placental membrane microscopic chorionic pseudocysts (MCP) are associated with clinical conditions and placental features of in-utero hypoxia and with increased number of migratory trophoblastic cells in placental membranes. So far, microscopic chorionic pseudocysts occurring in the cell islands, chorionic plate, placental septa and maternal floor of the chorionic disc (DCP) have been thought to have degenerative/liquefactive and/or secretory etiology and no clinical significance.

**Design:** To study the clinical and placental associations of microscopic chorionic pseudocysts, 31 clinical (maternal and fetal) and 62 gross and microscopic placental features were statistically compared between 219 consecutive cases with microscopic chorionic pseudocysts (Study Group, SG) and of 656 consecutive placentas without microscopic chorionic pseudocysts (Control Group, GG). SG was further subdivided into: A: 139 placentas with DCP (at least three cysts per one placental parenchyma section), B: 93 cases with MCP (at least three chorionic lakes per membrane roll), and C: 34 cases with both MCP and DCP in same placenta.

**Results:** Average gestational age in SG and CG was  $35.6 \pm 4.5$  and  $34.3 \pm 5.9$  weeks, respectively. SG contained statistically significantly ( $p \leq 0.05$ , Chi-square) more cases than CG of diabetes mellitus (7 vs 2%), less cases with preterm premature ruptures of membranes (12 vs 18%), more multiple pregnancies (12 vs 8%), less chorioamnionitis (27 vs 38%), more cases of massive perivillous fibrin deposition (11 vs 6%), and more cases of excessive amount of chorionic disc extravillous trophoblasts, determined by the number of placental septa/cell islands per chorionic disc section (18 vs. 6%), respectively. Within SG, statistically significant differences among SGA, SGB and SGC were found in maternal diabetes mellitus (3, 12 and 12%), total perinatal mortality (19, 3 and 12%), neonatal mortality (6, 0, and 6%), cesarean deliveries (37, 53, and 59%), chorioamnionitis (34, 17, and 21%), histological meconium staining (51, 34, and 32%), chorangioma (8, 10, and 26%), and excessive amount of chorionic disc extravillous trophoblast (19, 7, and 38%), respectively.

**Conclusion:** Associations and probably etiopathogenesis of DCP and MCP may be similar as both are common in DM and are associated with increased amount of extravillous trophoblasts in general and massive perivillous fibrin deposition in particular. Seen also in normal placentas and more common than MCP, DCP are more strongly related to perinatal mortality, particularly

postnatal, and their joint occurrence with MCP in same placentas is more likely to be seen in placentas with chorangiosis, i.e. low grade preuterine hypoxia.

### **39-Dizygotic Twin Pregnancy with a Normal Fetus and a Nodular Embryo Associated with a Partial Hydatidiform Mole**

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**Background:** While twin pregnancies complicated by co-existing complete mole are uncommon, those with partial mole (PM) are exceedingly rare, only several well documented cases diagnosed antenatally. Well documented (triploid) PM, albeit rarely, can be complicated by persistent gestational trophoblastic disease (<3%), intraplacental (.0001%) and even metastatic choriocarcinoma (one case). Detection rates of PM by sonography even in singleton pregnancies are more than three times lower than that for complete moles.

**Design:** A 29 year old female, G3P3, with chronic renal impairment secondary to reflux nephropathy, had a dichorionic twin pregnancy with viable embryos diagnosed at 8 weeks gestation. At 12 weeks, sonography showed death of one embryo with an "essentially empty" gestational sac. A healthy fetus was delivered by caesarean section at 28 weeks because of worsening maternal renal function.

**Results:** The dichorionic diamniotic fused placenta contained a normal 156g part and a flattened fibrotic 39g part with an intact chorionic sac, absent umbilical cord, calcified yolk sac remnant, and a 0.4cm embryo fused with the chorionic plate, the latter studded with up to .8cm vesicles. Microscopically, the embryonal tissue contained disorganized skin and cartilage, and the placenta a double population of chorionic villi with features suggestive of PM. p57 was positive in the molar and normal placental parts. FISH of molar and embryonal tissue showed trisomy 13, 18, 21, and X therefore a presumed 69,XXX karyotype, and a 46,XY karyotype of the normal placenta. Retrospective evaluation of the 8 weeks prenatal ultrasounds revealed a dichorionic pregnancy with two viable embryos and cystic structures in one placenta, both disappearing on the 12 weeks ultrasounds which showed a twin pregnancy with vanished embryo. This is a case of a twin pregnancy with a normal diploid fetus and placenta and a vanishing triploid embryo with PM that eluded antenatal diagnosis due to apparent lack of clinical, ultrasonographic or biochemical ( $\beta$ hCG) indicators of molar pregnancy. Diagnosis was made by gross and microscopic placental examination and fluorescence in-situ hybridization (FISH). Follow up with maternal blood  $\beta$ hCH showed its level <2.0 IU/L a year after delivery.

**Conclusion:** To our knowledge, this is the first case of a triploid vanishing embryo with PM diagnosed on placental examination. Careful gross and histological examination of vanishing twins and their placentas may disclose an unexpected PM which rarely can be associated with a trophoblastic tumor. Retrospective studying of sonography images in such cases may help to refine diagnostic criteria for very early partial moles.

### **40-Remodeling Defect Leads to a Novel Malformation Variant of the Cardinal Venous System.**

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**Background:** The cardinal veins are the first largest systemic veins to form in the infant and are composed of three precursor veins that remodel to give rise to the inferior and superior vena cava and their tributaries. Developmental anomalies of this system are uncommon and usually asymptomatic, but if unidentified they can lead to life-threatening complications during surgeries or catheterization. We report a detailed anatomical description of a case with a novel malformation variant of the cardinal venous system.

**Design:** We performed a detailed autopsy with careful in situ dissection of heart and large vessels in a 3-1/2 week old full term infant. The patient was diagnosed with complex congenital heart disease at birth; and systemic venous malformations were suspected by imaging studies. The patient died of heart failure after an attempt to repair the cardiac malformation. A correlation between pre-mortem imaging data and post-mortem anatomical findings was performed.

**Results:** Both superior and inferior left venous systems developed abnormally. There was a persistent left superior vena cava (PLSVC) that drained into the right atrium via the coronary sinus. The left innominate vein was absent. There was a persistent left inferior vena cava (PLIVC) in continuation with the hemi-azygos vein which drained into the PLSVC. The left renal vein was abnormally connected to the hemi-azygos vein. Two common iliac veins were identified. The left drained into the PLIVC and the right drained into the right inferior vena cava. The right superior and inferior caval veins were connected to the right atrium in the usual fashion and the azygous vein was normally sized. Echocardiographic imaging during fetal and postnatal period identified only the dilated hemiazygous vein draining to an LSVC and a small IVC. The complex congenital cardiac malformations including hypoplastic left ventricle with hypoplastic aortic arch and subaortic stenosis were diagnosed by fetal ultrasound and confirmed by perinatal images.

**Conclusion:** Remodelling of certain components of the cardinal system takes place in different time points during development and the mechanisms that guide this process is unknown. Defects in this development process can lead to variable malformations as demonstrated by this case. To our knowledge the combination of complex malformations of both superior and inferior vena cava system that extends to the common iliac veins has not been reported. Knowledge of these malformations is invaluable when performing perinatal autopsy as these malformations are complex and can be difficult to identify especially when imaging is equivocal.

#### **41-CPR-related Rib Fractures in Infancy: Change in Technique Associated with Increased Incidence**

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**Background:** Since the implementation of revised cardio-pulmonary resuscitation (CPR) practices for infants, there has been an apparent increase in the number of CPR-related rib fractures identified at autopsy in this population. A study was therefore undertaken to document the annual frequency of CPR-attributed rib fractures found in infants at autopsy; and to determine

whether the perceived rise correlates with recent changes in paediatric CPR techniques.

**Design:** All postmortem files of the DPLM at HSC from 1997-2007 were reviewed to determine the frequency, number and location of CPR-associated rib fractures and to correlate them with patient demographics; length and nature of CPR; and the underlying cause of death.

**Results:** During the study period, 515 infants aged 6 months or less had resuscitative efforts performed prior to pronouncement of death. Rib fractures ascribed to CPR were identified in 15 decedents, 11 females and 4 males, with ages ranging from 0 to 142 days (mean, 75 days). An anatomical cause of death was established in 7 infants; in 8 a definitive cause was not found. Ten infants became unresponsive at home whereas 5 were hospital in-patients. CPR involved EMS personnel in 10 cases; and lay rescuers in 3 cases. The average duration of CPR was 49 minutes (range: 31 to 147 minutes). The number of fractures varied from 1 to 11 and did not correlate with the length of CPR. All were anterior to lateral in distribution and most frequently involved the fourth and fifth ribs. From 1997 to 2005, there were 5 CPR-related rib fracture cases. In 2006 (when CPR practices were revised), there were 3 cases of CPR related rib fractures; and in 2007 there were 7. ('old' CPR vs. 'new' CPR,  $p = 0.0002$ , Fisher exact test)

**Conclusion:** These findings confirm that there has been a significant increase in the incidence of resuscitation-associated rib fractures in infants since the implementation of revisions in paediatric CPR practices in 2006. It is important for pathologists to consider these findings when investigating sudden unexpected deaths in infants as such fractures ought not to be misinterpreted as evidence of non-accidental / inflicted injury.

#### **42-Fetal and Neonatal Postmortem Series with Stippled Epiphyses**

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Division of Human Genetics

**Background :** Stippled epiphyses are well demonstrated on babygrams of fetuses and neonates. Infants who survive will have a short stature with or without spinal deformity depending on the sites of stippling.

**Design:** All cases of epiphyseal stippling seen on fullbody Xrays over a 15 year period from the files of the Division of Anatomical Pathology, University of Cape Town Medical School were retrieved, reviewed and analysed.

**Results:** Thirty cases were found. There were 15 males and 15 females. Fetal age ranged from 17 weeks to 2 day old term infant. Maternal age ranged from 17-43years. Maternal Warfarin ingestion was present in three cases. Aneuploidy appeared likely in 7 cases, and was confirmed in four. Hydrops fetalis was found in 4 cases and included one with the multiple pterygium syndrome. Facial clefting was found in 4 cases: one case had aneuploidy; one case had severe facial clefting and features suggestive of a deficiency of sonic hedgehog gene; one had a cleft lip and Trisomy 18; and one had a repaired cleft lip in utero and macrocephaly due to intracerebral haemorrhage. Congenital heart disease was present in 11 cases including the four with proven aneuploidy. There was one case with cardiomyopathy. Cerebral pathology was a frequent finding. There were three cases with neural tube defect ranging from craniorachischisis to spina bifida occulta with spinal lipoma. Three cases had polymicrogyria and one with holoprosencephaly. There were three cases with brain haemorrhage: one causing hydrocephalus; one with intraventricular haemorrhage and white matter necrosis; and one with associated asymmetric intrauterine growth restriction. Single cases of congenital infection, CHARGE syndrome, & familial mental retardation with dysmorphism & congenital malformation were found. Stippling of only the calcanei was the most frequent finding in 15/30 cases. Three cases had stippling of the talus only. Single cases of stippling of the sacrum only and the tibia were also found. Cases with multiple sites of stippling included three cases with 4 involved sites [2 of

which had Warfarin exposure]; 3 cases with 3 sites; and 4 cases with two sites. Abnormalities of multiple organ systems was present in 10 cases excluding the cases of aneuploidy IUGR and stippling were present in 4 cases and were the only findings in 1 case.

**Conclusion:** Analysis of a postmortem series of 30 cases with epiphyseal stippling seen on babygrams showed a minority of cases in which a definite cause for the stippling was identified. These included aneuploidy and Warfarin exposure. In a large number of cases no definitive cause was found. The performance of fibroblast culture for possible metabolic cases including both lysosomal and peroxisomal disorders, and sterol analysis on plasma samples or mutation analysis of Xp11-11p.23 for Emopamil-binding protein mutations may assist in making a definitive diagnosis.

### **43-Reliability of Automated Neutrophil Quantitation in Digitized H&e Stained Slides: Pilot Analysis of Correlation with Amniotic Fluid Proteomics Score**

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**Background:** The diagnosis of intraamniotic infection is considered clinically relevant to maternal, fetal, neonatal and childhood morbidity and mortality. However, intraobserver variability, even among expert pathologists, remains problematic, apart from 0.96 agreement on “present v absent” (Pediatr Dev Pathol. 2003;6(5):435-48), with “consensus” (rather than valid biomarkers) being the gold standard. An automated and reliable method for quantitation of neutrophils would be a useful diagnostic tool.

**Design:** We sampled 10 cases with amniotic fluid proteomics (AFMR) scores, 2 each of scores 0-4 as per Buhimschi et al, BJOG. 2009 Jan;116(2):257-67. Two images were taken at random of extraplacental membranes; each image was analyzed at both 10x and 20x magnification. The image analytic technique begins with scanned slides in the form of color (RGB) images. We first segment the image to separate tissue from cells of interest by using the difference in color that results from staining. After this initial segmentation, we then must filter out image features that are the correct color but fail to fit the expected shape of a neutrophil, such as [carrie insert name of elongated cells here]. This requires the computation of the area, perimeter, and eccentricity (a measure of how circular a shape is) of each connected component identified by the segmenter. Three segmentations were done: 1. based on color threshold alone; 2. based on color and then rejecting anything bigger or smaller than the area threshold interval; 3. based on color, rejecting groups based on area threshold as well as rejection based on eccentricity (rejecting cells such as fibroblasts that have cigar shaped nuclei).

**Results:** Using Method 1, only the number of pixel groups was associated with AF MR ( $r=0.494$ ), with Method 2, AF MR was associated with both positive pixel count ( $r=0.544$ ), and percent positive pixels ( $r=0.544$ ). With Method 3, positive pixel count ( $r=-.546$ ), percent positive pixels ( $r=0.546$ ), and the number of pixel groups ( $r=0.505$ ) were high correlated with AF MR. These counts also significantly correlated with histologic grading of neutrophils in amnion, chorionic and decidua, and in umbilical cord. Magnification at analysis did not modify the strength of the associations.

**Conclusion:** Pilot data suggests that reproducible and reliable automated segmentation and quantitation of neutrophils can be performed, with strong correlations with amniotic fluid proteomic markers of infection and inflammation. We anticipate that a larger image sample per

tissue will result in improved correlation with AFMR score.

#### **44-Human Pulmonary Lymphatic Vessel Development and its Alteration in Neonatal Lung Disorders**

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**Background:** The temporo-spatial development of pulmonary lymphatic vessels (PLVs) in the growing human fetus is not well established and it is unknown if this process is affected in neonatal lung disorders (NLD). We aimed to establish normal pattern of distribution and morphology of PLVs through gestation and compare to those of selected NLD.

**Design:** Human fetal autopsy lung tissue representing all three trimesters (12, 13, 16, 18, 34, 39 and 40 weeks of gestational age WGA), as well as from 1 and 10 years of age with no significant lung pathology were used. In addition lung sections of patients with congenital diaphragmatic hernia (CDH, n=5) and bronchopulmonary dysplasia (BPD, n=5) were selected. The sections were stained with HE, trichrome, and with antibodies against smooth muscle actin (SMA) and D2-40.

**Results:** The usual anatomical distribution (septal, pleural, and bronchovascular) of D2-40 positive PLVs was established as early as 12 WGA, and the PLVs between these compartments became more prominent as lung maturation evolved. PVLs were observed as distal as the terminal bronchiole level in perinatal and postnatal lungs, but none was seen in the alveolar walls. PVLs did not show the presence of smooth muscle at any age or in any anatomical compartments. In NLD, PLVs were generally dilated, especially in the septae, without smooth muscle. Frequent PVLs with small caliber were observed in the alveolar walls of all cases of both CDH and BPD.

**Conclusion:** The usual temporo-spatial distribution of PVLs observed in mature lung is established by 12 WGA in the human fetus. The lymphatic endothelial specific marker D2-40 is excellent in highlighting maturing PLVs. In NLD such as CDH and BPD there is excess of PVLs in the alveolar septa with no smooth muscle accumulation. This likely represents disordered vascular development rather than a reactive phenomenon because both CDH and BPD are known to manifest vascular developmental aberrations.

#### **45-Allopurinol To Prevent Neurological Damage In Fetal Asphyxia.**

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**Background:** Asphyxia during pregnancy and labour can result in severe acidosis and hypoxic-ischemic encephalopathy, predictable for the development of motor and cognitive behavioural disturbances later in life. Animal models and human data have demonstrated that damage not only occurs during the hypoxic / ischemic event but also in the period of reperfusion and reoxygenation. This damage is caused by the formation of free radicals during the post hypoxic-ischemic reperfusion. In an animal model we studied the effect of Allopurinol during a period of ischemia-reperfusion in the fetal lamb. We tested the hypothesis if fetuses suffering from a severe hypoxic-ischemic-reperfusion insult caused by repeated occlusion of the umbilical cord will suffer from less severe brain damage after antenatal treatment with Allopurinol in comparison with a non-Allopurinol treated control group.

**Design:** Twelve pregnant ewes were prepared surgically at 127 days of gestation (term normally occurring at 147 days) under general anesthesia. The lambs were partially instrumented and an inflatable cord occluder was placed around the proximal end of the umbilical cord. The lambs were exposed to ischemia-reperfusion protocol consisting of five cycles of umbilical cord compression of 10 minutes followed by 10 minutes of reperfusion. Half of the treated lambs received Allopurinol after the third umbilical cord compression at a mean pH of 7.1 and the other half received a buffered saline solution. Forty-eight hours after completion of the experiment fetal brains were removed and fixed in 4% formalin. A few weeks after fixation samples of cortex, hippocampus, cerebellum, brainstem, thalamus and basal ganglia were embedded in paraffin. All samples were stained with H and E, acid-fuchsin-theonin and stained with antibodies against CD68, activated caspase 3 and GFAP. The severity of damage was scored semi quantitative.

**Results:** In all areas studied the allopurinol treated animals demonstrated less severe neuronal damage. There was a significantly less intense staining of neurons with the acid-fuchsin-theonin stain and in the several immunohistological stains less damage was observed after Allopurinol treatment.

**Conclusion:** It is expected that antenatal Allopurinol treatment of mothers during an episode of intra-uterine asphyxia can have a neuroprotective effect on the fetal brain.

#### **46-The Innervation of the Fetal Human Pancreas under the Lens**

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**Background:** The delineation of pancreatic nerve innervation during fetal life may contribute to our understanding of pancreatic pain modalities after birth. The aim of this study was to characterize the distribution of nerve structures in the human pancreas throughout gestation in a 2D and 3D platform as well as to define the peripheral sensory and sympathetic fibers involved in transmitting and modulating pancreatic pain.

**Design:** Computer-based image morphometry was performed to quantify nervous structures in the head, body and tail of the pancreas following immunohistochemistry using a polyclonal antibody against two S-100 proteins that reacts strongly with human S100A and B that are detected in Schwann cells. Immunoreactivity was found in the parenchyma of head, body and tail of the pancreas with the relative density being head > body > tail. In addition to this extensive set of nerve fibers terminating in the pancreas there were large bundles of en passant nerve fibers in the dorsal region of the pancreas that were 3D reconstructed and were associated with the superior mesenteric plexus. Myelinated sensory fibers were labeled with an antibody raised against neurofilament (NF) and post-ganglionic sympathetic fibers were labeled with an antibody raised against tyrosine hydroxylase (TH). Choline acetylase (ChAT) at cholinergic synapses was labeled with a conventional antibody.

**Results:** If at first glance, the perimeter and the width of the nerve fibers seem to increase at a continuous rate up to term in all three regions of the pancreas, spatial and temporal co-analysis identified that the head of the pancreas shows a 2-peak growth increase at 14 and 22 weeks of gestation with regard to the area, perimeter and width of the nerve structures, while the body and tail regions show a unique peak at 20 weeks. A developmental deceleration was found between the 22nd and the 36th week of gestation for the head region only. NF, TH, and ChAT

immunoreactive fibers were present in the parenchyma of head, body and tail of the pancreas at variable density, but the relative density of both NF and ChAT expressing fibers seemed to be increasing head>body>tail, whereas for TH, a relatively even distribution was observed.

**Conclusion:** The developmental dynamics of the pancreas nerve innervation corresponds approximately to the remodeling of the intrahepatic biliary system. These data suggest that the pancreas receives a significant sensory and sympathetic innervation during fetal life.

Understanding the factors and disease states that may alter the distribution of nerve structures can be of significance for the development of therapies in pancreatic disorders of child- and adulthood.

#### **47-Identical Twins with Lethal Congenital Pulmonary Airway Malformation Type 0 (Acinar Dysplasia): Further Evidence of Familial Tendency**

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**Background:** Congenital pulmonary airway malformation (CPAM), also referred to as congenital cystic adenomatoid malformation (CCAM), is a rare maldevelopment of the lung with a wide range of presentations. CCAM was first classified by JT Stocker in 1975 as types 1-3 and later was reclassified into CPAM types 0-4. Type 0 is the least frequent type and is incompatible with life. The gross and histological appearance of type 0 consists of pulmonary hypoplasia with severe maturation arrest of lung tissue and small cysts <0.5 cm in diameter. The genetic cause of CPAM remains unknown.

**Design:** We report the first case of identical twins with CPAM type 0. The female twins were born to Hispanic parents without consanguinity. Both females developed respiratory distress after delivery, and they were treated with high ventilator support. The first twin had severe hypoxemia and acidosis, and she died after 6 hours of life. ECMO support kept the second twin alive; however, care was withdrawn after the autopsy diagnosed the first twin with CPAM type 0. The family had a previous male with pulmonary hypoplasia and respiratory failure who also died shortly after birth; however, no autopsy was done to confirm the diagnosis.

**Results:** For both females, autopsy findings included severe hypoplasia of the lungs. Sections from all lobes of both twins demonstrated severe maturation arrest with lung tissue composed primarily of terminal bronchioles and rare alveoli present at the subpleural area consistent with CPAM type 0. Chromosomal analysis was normal.

**Conclusion:** Including the twins in this report, nine of the ten documented cases of CPAM type 0 have been females. Two families have previously been reported with multiple children afflicted with this malformation. From all reported cases, this malformation has a tendency to recur in families up to 40%. We believe that this is the first description of CPAM type 0 in identical twins. The combination of both affected female twins and likely a male suggests an autosomal recessive inheritance pattern. Further chromosomal analysis and molecular genetic testing of families with CPAM is needed to better understand the pathogenesis of CPAM type 0 and the relationships between subtypes.

#### **48-Placental Pathology in Congenital Bronchopulmonary Malformations**

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Children's Hospital of Philadelphia, Philadelphia, PA.

**Background:** Recent studies have shown that many examples of congenital bronchopulmonary malformations of the lung are associated with bronchial atresia (BA) and may arise as a secondary change related to bronchial obstruction. Although the etiology of bronchial atresia is unknown, it is a biologically plausible hypothesis to consider that a vascular disruptive event in the embryologic period leads to bronchial atresia and its sequelae. The aim of our study is to explore the possible etiology of pulmonary lesions by determining if the placentas from patients with congenital bronchopulmonary malformations have evidence of remote fetal vascular thrombosis.

**Design:** Search of files at the Children's Hospital of Philadelphia from 1998-2008 identified 10 cases of congenital pulmonary malformations in which histological examination of the placenta was also performed. Age matched controls (n=16) from late third trimester or term placentas were identified at NYU; and included only normal spontaneous vaginal deliveries or routine Cesarean sections with no history of prenatal complications. Hematoxylin-eosin-stained slides of the pulmonary lesions were reviewed, and lesions were categorized as having evidence of bronchial atresia, regional hyperexpansion, microcystic maldevelopment, or fetal cystic adenomatoid malformation (CCAM) changes. Hematoxylin-eosin-stained slides of the placenta were reviewed for the presence and location of fetal vascular thrombosis, avascular villi, and/or infarction. Results were compared with Fischer-exact test.

**Results:** Of the ten cases identified in the files, histological examination of the lungs revealed 2 cases of extralobar sequestration, which were excluded from subsequent analysis. Of the remaining 8 cases, we identified 5 cases of fetal CCAM, 1 definitive case of BA; 1 case of probable BA, and 1 case with microcystic maldevelopment. Histological examination of the placentas revealed the presence of avascular villi in 4/8 cases with lung lesions and one control (1/16),  $p = 0.028$ . Avascular villi were present in 2/2 cases of BA and one control (1/16),  $p = 0.020$ .

**Conclusions:** Although the data set is small, the findings suggest that congenital bronchopulmonary malformations are associated with avascular villi in the placenta. Since avascular villi likely represent evidence of remote placental fetal vascular thromboses, these findings provide further support for a vascular disruptive pathogenesis for these pulmonary lesions.

#### **49-Paneth Cell Metaplasia In Children With Allergic Proctocolitis**

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**Background:** Paneth cells (PC) in humans are found in the duodenum, jejunum and ileum. In normal adults they occur sparsely in the proximal colon and appendix. Paneth cell metaplasia (PCM) is a variable feature of diverse diseases of the colon and rectum including chronic ulcerative colitis (CUC), Crohn's disease, juvenile polyps, villous adenomas and colonic adenocarcinoma. In rectal biopsies of CUC patients the presence of PCM denotes long-standing disease, and it is this group of patients who have the highest rate of malignant disease. The English language literature only mentions anecdotal cases of PCM in rectal biopsies of children with severe gastroenteritis or "food allergy". This study reports the unexpectedly high incidence of PCM among children with diagnosis of allergic proctocolitis (APC).

**Design:** All cases of allergic proctitis/proctocolitis in children 0-18 years of age diagnosed at Connecticut Children's Medical Center between 2003 and 2007 were retrieved from the files of

Hartford Hospital Pathology Department. Histological features were reviewed and patient's demographics were obtained. Rectal biopsies from children with chronic inflammatory bowel disease (IBD) and histologically normal rectal biopsies (NL) taken from children with upper gastrointestinal diseases were used as controls.

**Results:** Sixty-six (66) cases of APC were found in our files. From them, 31 (47%) showed histological evidence of PCM. In this group, 18 patients were males and 13 were females (M:F ratio 1.3). The patient's age ranged from 7 weeks to 6 years (mean age: 12 months). The most common histological findings associated with PMC were increased lamina propria eosinophils, intraepithelial eosinophils (IEE) and rare intraepithelial neutrophils (IEN). None of the cases showed distortion of the crypt architecture (DCA) or granulomas (GR). In the IBD group (n=14), 50% of the cases showed PCM. Associated histological findings in this group included DCA, increase in lamina propria mononuclear cells, prominent lymphoid aggregates, neutrophilic cryptitis and GR. IEE were not seen. In the normal group (n=13), PCM was not seen in any biopsy, and no significant histopathological abnormalities were identified in this group.

**Conclusion:** PCM is identified in a high number of rectal biopsies in children with APC, with percentages similar to the ones seen in cases of IBD. The PCM in APC was not associated with histological features indicative of long-standing chronic disease as in the IBD group. In addition, PCM was seen in patients as young as 7 weeks old. These observations suggest that PCM in children with APC may represent histological evidence of regeneration/repair rather than a sign of chronicity.

## **50-Clinical, Pathological And Epidemiological Features Of Eosinophilic Oesophagitis in the North of England**

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**Background:** Eosinophilic oesophagitis (EO) is a clinico-pathological entity usually presenting in young adults, with male predominance, frequent dysphagia and normal esophoscopy and pH monitoring, in absence of gastro-oesophageal reflux disease (GORD). The aim of our study is to describe the clinical, endoscopic and histopathological features of our cases of EO presenting during the period comprised between 2007 and 2008. The incidence of paediatric EO in our region and the identification of a subgroup of patients with signs of both GORD and EO (so called "overlap" syndrome) is also analysed.

**Design:** All oesophageal biopsies with  $\geq 15$  eosinophils/HPF received during the study period were retrospectively reviewed. Eosinophil count was performed on the HPF with highest concentration of eosinophils. Other histological features of EO were also described. Clinical and endoscopic features were obtained from the clinical notes.

**Results:** 24 cases (13 M, 11F) with an average of 6 years (range: 6 months-15 years) were identified. The endoscopy was described as furrowing or trachealisation in 10/24, normal in 9, Candida in 2; oesophagitis in 2 and unknown in 1. Presenting symptoms were feeding problems (50% patients); other gastrointestinal symptoms (42%) and some kind of allergy (including cow milk protein allergy) in 46 % of cases. In 5 (21%) children the EO was associated with GORD ("overlap syndrome"). This diagnosis was later confirmed with pH studies.

The average number of eosinophils was 32 (range: 16-57)/HPF. The number of eosinophils/biopsy site was: 24.5 (4-55)/HPF in proximal biopsies; 37.5 (range: 22-55)/HPF in middle; 38 (range: 20-57)/HPF in distal biopsies and 32 (range: 16-45)/HPF in "unknown" site samples.

Other features seen were various degrees of dilated intercellular spaces; basal cell hyperplasia; papillary elongation and vacuolation of the epithelial cells. Microabscesses in the superficial mucosa were identified in 4 cases.

**Conclusion:** EO is a rising diagnosis that reflects a growing incidence. During the 2 years of the study period, 1046 patients had an upper gastro-intestinal endoscopy and 15% of them had features of oesophagitis on histology. This reflects that the incidence of EO was 2.1 % of all oesophageal biopsies and 15% of those with oesophagitis of any kind.

### **51-Flowcytometric Immunophenotyping In The Diagnosis Of Pediatric Hematological Malignancy – How Reliable Is It And How Can We Optimize Its Use?**

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**Background:** Flowcytometric Immunophenotyping (FCI) is a very useful investigation in hematopathology, commonly employed to diagnose and classify leukemias, myeloproliferative disorders and autoimmune lymphoproliferative disorders. Bone marrow aspirates and peripheral blood are routinely used for analysis. Solid tissues have been used in a few centers with success but such reports from exclusive pediatric histopathology units are infrequent. The aim of this study was to evaluate the role of FCI in tissues as an adjunct to histological examination in pediatric hematological malignancies. We also propose an algorithm to triage cases that could benefit most from FCI in routine pediatric histopathology practice.

**Design:** FCI was performed on fresh tissue samples submitted to the histopathology laboratory with a clinical suspicion of malignancy during a 4-year period. A specially devised local protocol was used. The FCI results were correlated with histology and immunohistochemical findings. To assess if imprint cytology could be used to triage specimens for FCI, imprints on 4 cases were assessed within one hour of receipt of specimen, independent of FCI and histology.

**Results:** 42 samples were included in the study comprising equal numbers of benign and malignant entities. The overall correlation of FCI with histology was 69% and when Hodgkin lymphoma was excluded, the correlation was 87.8%. The sensitivities for Non-Hodgkin lymphoma, neuroblastoma and other non-hematological malignancies were 75%, 50% and 50% respectively. The reasons for discordant results were low numbers of malignant cells, presence of necrotic debris and insufficient material in the FCI samples. Correlation of imprints with FCI and histology was 75%.

**Conclusions:** FCI produced rapid same-day results and had a high sensitivity for benign lesions and Non-Hodgkin lymphoma but was not helpful for Hodgkin lymphoma. Use of CD81, GD2, CD9 and CD56 increased the sensitivity for detecting neuroblastoma by FCI. Imprint cytology could be used reliably to categorize tissues into major diagnostic groups and to identify high-grade lymphomas. Incorporating the utility of imprint cytology, considering the sensitivity of FCI for non-Hodgkin lymphoma and the cost of the FCI analysis, we believe that the algorithm proposed by us to triage specimens can optimize the use of FCI in routine practice.

### **52-Could It Be Avoided? - Central Venous Catheter Related Cardiac Tamponade Leads to Sudden Death of Very Low Birthweight Neonates: Series of Five Autopsies Including Cases with Appropriate Tip Positions**

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**Background:** Total parenteral nutrition (TPN) via central venous catheter (CVC) is widely used to provide adequate nutrition especially for very low birth weight neonates (VLBWN). Pericardial effusion/cardiac tamponade (PE/CT), one of the most serious CVC related-complications with high mortality, has been reported since the 1970s. The majority of the earlier cases were associated with the suboptimal positioning of the tip of the CVCs. In the 1990s, the concerns lead to an FDA (U.S. Food and drug Administration) recommendation that the tip of the CVC should be placed proximal to the right atrium (RA). However, a significant number of cases of lipid pericardial effusion/tamponade continue to be reported even after this recommendation, including cases where the tips were placed in the optimal positions. Only a few reports mentioned the cardiac histopathologic findings. Most of these reports indicated that the myocardium had been mechanically damaged by the CVCs. There is no histopathologic description in the cases with optimal tip positioning. This is the first series of 5 autopsies that focuses on myocardial histology and includes the cases with the tips in the recommended positions.

**Design:** This report presents the autopsy findings of 5 neonates receiving continuous TPN via CVCs, who suddenly died from PE/CT.

**Results:** The five neonates range in age from 4 to 29 days old, and include 3 males and 2 females. Birth weights range from 580 to 3,142 grams; four of these neonates were VLBWN of 22-26 week gestational ages and one was a term neonate. The tips of the CVCs were proximal to the RA in 2 cases, and in the RA in 3 cases. The tip was tightly lodged into the RA of the term neonate. All cases revealed milky-white effusions distending pericardiac sac. Effusion analysis performed in 3 cases showed high triglyceride levels (717-714 mg/dl), consistent with intralipid component of TPN. Dye infusion into the RA revealed no leakage. Gross and microscopic cardiac examination of four VLBWN showed mildly dilated lymphatics and edema (n=4), a minute atrial thrombus (n=1), and focal fibrinous epicarditis (n=1). No necrosis, perforation or rupture was found. In contrast, findings of the RA of the term neonate revealed focal coagulative necrosis, acute and organizing hemorrhages, foci of collagen deposits and myocyte hypertrophy and endocardial thickening, which suggests direct myocardial damage by the CVC.

**Conclusions:** The RA of four VLBWN with fetal PE/CT showed only mild interstitial edema and dilated lymphatics without direct damage of the myocardium. In two of those cases, the tips were at the recommended positions. These findings suggest that there might be a significant risk even when the tip is appropriately positioned. Expedient recognition of pericardiac effusion may avoid a fatal outcome.

### **53-Maxillary Lesions in Children: a Retrospective Study of 70 Cases**

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**Background and Design:** This study describes pathological features of maxillary lesions in a cohort of 70 children, 37 males and 33 females, aged 5 weeks to 17 years, followed in Trousseau hospital between 1987 and 2005 (22 years). Lesions are classified in two groups: non odontogenic lesions (n=40) consisting of tumours (n=26) and pseudotumours (n=14) and odontogenic lesions (n=30) consisting of tumours (n=12) and cysts (n=18).

**Results:** Non odontogenic tumours represent 68% of all maxillary tumours. They are more often benign (81 %) than malignant (19 %). Malignant tumours are represented by conventional osteosarcoma (n=2), Burkitt lymphoma (n=2) and Ewing sarcoma (n=1). Benign tumours are represented by fibrous dysplasia (n=5, 2 family cases), myxoma (n=2), desmoid fibroma (n=2), ossified fibroma (n=2), fibroma (n=2), melanotic neuroectodermal tumour (n=2), Langerhans cell histiocytosis (n=2), congenital hemangioma (n=2), infantile myofibromatosis (n=1) and schwannoma (n=1). Pseudotumours are represented by giant cell granuloma (n=4), anevrismal bone cyst (n=4), bone cyst (n=2) and reactive inflammatory lesions (n=4).

All odontogenic tumors (32 %) are benign. They are represented by odontoma (n=4), keratocystic odontogenic tumour (n=4), ameloblastoma (n=2), adenomatoid odontogenic tumour (n=1) and ameloblastic fibroma (n=1). Odontogenic cysts are represented by radicular cyst (n=17) and corono-dental cysts (n=1).

**Conclusion:** There is a wide spectrum of maxillary tumours and pseudotumours in children. Pathological characterization and differentiation between true tumours and reactive processes is difficult. The pathological characterization of these lesions should help to progress in the management of children and to better understand the molecular mechanisms involved in their development.

#### **54-Antibody-Mediated Rejection And Necrotizing Coronary Artery Vasculitis 9 Years After Orthotopic Heart Transplantation In A Child**

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**Background:** Cardiac allograft vasculitis is rarely reported in the transplanted heart and is usually described in association with acute cellular rejection. Late onset antibody mediated rejection is very rare in cardiac transplants especially in children and necrotizing vasculitis of large coronary arteries has not been previously noted in this form of rejection.

**Design:** We report the first pediatric case of a 9 year-old boy, which underwent cardiac transplantation at two months of age for congenital heart disease. He had only one single episode of clinically diagnosed rejection during the initial transplant hospitalization but subsequently he had no other episodes of rejection. Nine years later, he presented with abdominal discomfort, vomiting, and reduced appetite, and was found to have exam findings of severely impaired cardiac output. The clinical picture was believed most compatible with acute cellular rejection, so he was treated emergently. Multiple anti-HLA antibodies of class I and II were found at that time. The patient had a gradual improvement in cardiac systolic function and clinical status but he died unexpectedly 1 month later following complaints of chest pain.

**Results:** The autopsy showed severe myocardial ischemic changes of variable age without significant neutrophilic or lymphocytic infiltrate. C4d deposition in association with endothelial swelling and intravascular macrophages consistent with antibody mediated rejection were present. The epicardial coronary arteries showed necrosis, segmental thrombosis, transmural lymphocytic infiltrate and many stained positive with C4d. Only mild chronic changes were present but no significant atherosclerotic changes or foamy macrophages as described in chronic

transplant vasculopathy were seen.

**Conclusion:** This case demonstrates several unique features. To our knowledge it is the first documented pediatric case with the longest posttransplant interval for development of late onset humoral rejection. It is also the first case that shows the association between coronary artery vasculitis and humoral rejection in a pediatric cardiac transplant patient. Our case suggests that necrotizing vasculitis of the coronary arteries may occur in humoral rejection, however, we cannot entirely exclude the possibility that the vasculitis occurred in association with acute cellular rejection that has been previously treated or was stimulated by infection.

### **55-Renal Cell Carcinoma as an Early Secondary Malignancy Post-Embryonal Rhabdomyosarcoma - Implications for the Role of TP53**

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**Background:** The most recent WHO classification of renal tumors includes the new entity 'neuroblastoma-associated renal cell carcinoma'. These typically occur with long latency and usually after treatment for neuroblastoma, although occurrence synchronously, and in treatment-naïve patients are reported. Isolated cases of renal cell carcinoma as secondary malignancies in pediatric oncology are also reported following other primary malignant tumors, again generally after many years. No genetic link between these entities is known at this time.

**Design:** Our patient is a 5 year-old girl, who initially presented to the oncology service at age 2 years with a left psoas mass, diagnosed as embryonal rhabdomyosarcoma [ERMS]. Within two years of completing standard cytotoxic treatment, she developed a mass within the contralateral kidney, which had not been within the radiation field. This was examined histologically and cytogenetically.

**Results:** The initial malignancy was embryonal rhabdomyosarcoma with anaplasia. Treatment response was excellent with ~99% tumor necrosis documented at resection. The secondary malignancy was a renal cell carcinoma with features entirely in keeping with the newly-recognised oncocytoid, neuroblastoma-associated renal cell carcinoma.

Immunohistochemical staining for TFE3, TFEB and MiTF were all negative, while karyotypes of the biopsy and subsequently resected renal tumor showed different and inconsistent clonal aberrations from each culture, but no evidence of any of the characteristic translocations. In light of the anaplastic features in the ERMS, TP53 immunostain was applied to sections of both primary and secondary tumors. This produced remarkably strong nuclear staining, diffusely throughout both tumors. The latter findings raised concern for constitutional TP53 aberration, the results of which are pending. There was no family history to suggest Li-Fraumeni syndrome.

**Conclusion:** This is a first report of renal cell carcinoma arising post-ERMS and indeed after a remarkably short interval. We hypothesise a constitutional predisposition to genomic instability in this patient, based on the various abnormal karyotypes, the early second malignancy, and that this may be related to deficient TP53 function. Abnormal TP53 staining may be seen in tumors of patients with 'true' Li-Fraumeni syndrome, bearing constitutional TP53 mutations, and also in tumors of Li-Fraumeni-like syndrome patients having no constitutional mutation of TP53.

Wider analysis of the role of TP53 in secondary renal cell carcinomas in childhood is warranted.

### **56-CD21 Expression Distinguishes Quilty B from Acute Cellular Rejection in Cardiac Allograft Biopsies in Children**

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**Background:** Differentiating between aggressive Quilty (Quilty B) and rejection in cardiac allograft biopsies can be a difficult diagnostic challenge in children. Morphologically, Quilty B lesions are typically defined as an endocardial lymphocytic infiltrate with a lymphoid-tissue like organization of B and T cells extending into the myocardium with or without associated myocyte damage. Quilty B lesions can lead to the over diagnosis of acute cellular rejection in the absence of direct continuation with the surface endocardium despite leveling of the tissue. Satter et al, found CD21 (follicular dendritic cell network) expression as a reliable diagnostic tool with 96% sensitivity and 100% specificity in greater than 0.3 mm (300 microns) cardiac allograft Quilty B lesions in adults. We extended the use of CD21 in distinguishing acute cellular rejection from Quilty B to cardiac allograft biopsies in children.

**Design:** Seventy-four cases of cardiac transplant biopsies were identified in our pathology archives originally diagnosed as Quilty B (23 cases), mild rejection (14 cases), moderate rejection (16 Grade 2 and 10 Grade 3a). In addition, eleven cases demonstrated lesions suspicious for Quilty B; however, rejection could not be ruled out (QBvsR). Quilty A and severe rejection cases were excluded from the study. All seventy-four biopsies were classified on H&E according to the International Society for Heart and Lung Transplantation. Formalin-fixed tissue blocks were retrieved from all 74 cardiac transplant biopsies, and immunohistochemical stains were performed using antibodies against CD21 (2G9 clone of CD21, Nova Castra Labs, Norwell, MA), CD3 (T cell lymphocytic marker), CD20 (B cell lymphocytic marker), and CD68 (histiocytic marker).

**Results:** CD21 highlighted follicular dendritic cells in the center of lymphoid infiltrates of which nine of the eleven cases that had been QBvsR, and in seventeen of twenty-three unequivocal Quilty B lesions. CD21 reactivity was absent in all 40 cases of acute cellular rejection. CD20 highlighted clusters of lymphocytes in the center of ten of the eleven QBvsR and all Quilty B lesions thereby mimicking a germinal center in lymphoid follicles. The thirty-three cases of Quilty B, including 10 originally diagnosed QBvsR, ranged in size from 102-629 microns (mean = 328 microns/0.33 mm).

**Conclusion:** Irrespective of the size the lesion, the combination of CD20 and CD21 immunostains can be of diagnostic utility for distinguishing Quilty B lesions from acute cellular rejection thus preventing over treatment.

### **57-A Clinicopathologic Review of Esophageal Candidiasis In Pediatric Patients**

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**Background:** Esophageal candidiasis (EsoCan) is rarely seen in upper endoscopic biopsies of the gastrointestinal tract in pediatric patients and there is the perception that patients are either immunocompromised or predisposed as a result of administered medication. However, we have recently seen cases in our practice in which patients had no apparent predisposition. As far as we are aware, there has been no systemic study of EsoCan in the pediatric population. We therefore sought to establish the clinicopathologic characteristics of pediatric patients with EsoCan and to determine the extent of T-lymphocyte participation in the inflammatory process.

**Design:** We reviewed the clinical, endoscopic and histopathologic features of EsoCan cases in patients twenty-one years and younger retrieved from our surgical pathology files for the period

November 2000-October 2008. Cases of EsoCan were defined by clinicopathologic criteria in which endoscopic findings of white plaques, patches or furrows were present along with the histologic demonstration of fungal pseudohyphae consistent with *Candida* species involving the intact mucosa and associated with an inflammatory response or supported by a positive esophageal brushing. CD3 immunohistochemical staining of residual paraffin embedded tissue from cases of EsoCan and normal controls were performed to assess the T-lymphocyte participation in the inflammatory process.

**Results:** Seventeen patients were diagnosed with EsoCan (age range 7-20 years, median age 13 years). There were 3 males and 14 females. Two patients (2/17) had been treated with antibiotics, 1/17 had combined antibiotic and immunosuppressive therapy for cystic fibrosis and Crohn's disease (CD), 3/17 had immunosuppressive therapy for collagen vascular disease (2) and CD (1), 4/17 had distortion of esophageal anatomy and 7/17 had no apparent predisposition. Of the latter 7 patients, 6 were females (age range 7-17 years, median 14 years). CD3 staining showed foci with > 20 intraepithelial lymphocytes (IELs) per high power field (HPF) in 14/17 cases. Two of the seventeen with < 20 IELs/HPF had no apparent predisposition while one was the patient with cystic fibrosis and Crohn's disease. All normal controls had no focus > 10 IELs/HPF

**Conclusion:** Esophageal candidiasis is a rare finding in the pediatric population in which up to 40% of cases had no apparent predisposition. There is a prominent lymphocytic infiltrate in the majority of cases and female patients predominate. The latter finding raises the possibility of hormonal imbalance being a contributory factor, but more in depth study is required to exclude the presence of subtle immune dysfunction.

### **58-A Case Of Classical Hodgkin Lymphoma With Aberrant T-cell Expression, Radiologically Simulating Pleuropulmonary Blastoma**

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**Background:** Hodgkin's and Reed-Sternberg cells of classical Hodgkin Lymphoma (CHL) are largely of B-cell origin, but expression of one or more T-cell markers has been reported (Tzankov et al. *Mod Pathol* 2005;18:1542-49). Micro-dissected lesional cells demonstrated gamma T-cell gene rearrangement in a subset of the reported cases. This can clearly be problematic since the main morphologic and immunohistochemical (IHC) differential diagnosis is anaplastic large cell lymphoma (ALCL).

**Design:** A 10 year-old boy was referred to our institution by his primary pediatrician after he presented with a week long history of fever and cough and found to have decreased breath sounds on auscultation of the left chest. Complete opacification of the left hemithorax was seen on chest x-ray. CT scan of the chest demonstrated a large heterogeneous mass, with areas of necrosis and cystic change, involving over 85% of the left hemithorax and extending across the midline into the right superior mediastinum. The images were compared to an archival case of pleuropulmonary blastoma and thought to be identical. Frozen section at the time of open biopsy was interpreted as a pleomorphic and spindle cell neoplasm with sarcomatoid features.

**Results:** On the permanent routine sections, sheets of pleomorphic and multinucleated cells in a background of a polymorphic inflammatory infiltrate with foci of eosinophils were noted. On IHC stains, the lesional cells were found to be positive with antibodies to CD30, CD15, MUM-1, and the T-cell marker CD4; weakly positive with PAX-5; and negative with CD45, CD43, CD3, CD5, CD7, CD20, and ALK-1. A T-cell gamma clonal population was isolated by polymerase chain reaction (PCR) of paraffin embedded tissue. A diagnosis of CHL with aberrant T-cell

expression was rendered with agreement on external consultation at the National Cancer Institute, Bethesda, MD. Bone marrow biopsies and aspirates were negative for CHL. PET scan after the diagnosis of CHL, demonstrated restriction of disease related FDG avidity to the chest. The patient is on a chemotherapy regimen for intermediate risk stage IIB CHL. Repeat CT and PET scans after 3 cycles of chemotherapy has shown an interval decrease in the chest mass but presence of persistent FDG avidity.

**Conclusion:** CHL is the most common malignant neoplasm of the thorax in the pediatric population and must always be considered even when the radiologic appearance is atypical, as in this case. Furthermore, recognition that CHL can show aberrant T-cell markers on IHC and can even be accompanied by a clonal T-cell population by PCR is essential to prevent a misdiagnosis of non-Hodgkin lymphoma, in particular ALCL, and consequent inappropriate therapy.

### **59-KBA.62, A Novel Melanocytic Marker is Superior to HMB 45 in Evaluating Spitz Nevi in Childhood**

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**Background:** Spitz Nevi (SN) occur frequently in prepubertal children and at birth, with a predilection for the face and neck. SN may be junctional, compound or intradermal. There is an inherent tendency to pleomorphism and an often alarming intra-epidermal growth pattern.

KBA.62 is a novel marker and reacts with a melanocyte associated antigen and can be detected on routinely fixed paraffin embedded tissues. KBA.62 has not been studied in childhood melanocytic lesions. KBA.62 has been shown to be superior to HMB45 in adult melanocytic lesions. We examined the utility of KBA.62 and compared it with HMB45 in 12 childhood SN.

**Design:** The history, clinical features, and skin biopsies of 12 children with SN were reviewed from the archives of a tertiary care children's hospital. Hematoxylin and eosin stained slides were reviewed. The SN were also stained with KBA.62 (Immunotech) and compared with HMB45 (Biocare Medical, EDTA antigen retrieval). Detection was with the Refine DAB polymer detection system, with all staining performed on BondMax (Leica Microsystems).

**Results:** A total of 12 SN were examined. 7 were female & 5 male. The ages ranged from 1 year to 14 years, with a median of 5 years. The size of the nevi ranged from 2 mm to 6 mm. with a mean of 4 mm. The face was involved in 10/12 patients with the right cheek being the commonest site (5/12). 2/12 were from extremities, right foot & left shoulder. All cases showed melanocytic lesions with confluent distribution of spindled & epithelioid nevus cell nests, ganglion-like giant cells and Kamino bodies. 2/12 were atypical (architectural disorder, cytological atypia, fibrosis, pigment incontinence and prominent lymphocytic response). All cases showed epidermal attenuation. Aberrant displacement of nevocytes in the epidermis was noted in 4/12 cases. KBA.62 showed uniform, strong and diffuse crisp membranous staining of melanocytes in all cases. HMB45 showed variable cytoplasmic, granular staining of melanocytes in all cases with a 'dirty' background. However KBA.62 was a cleaner, crisper stain, and highlighted lesional cells within the epidermis in a clearer fashion. KBA.62 was also superior in evaluating margins.

**Conclusion:** Spitz Nevi can be troublesome lesions when pagetoid spread and features of atypia are present. KBA.62 is a novel melanocytic marker, and is superior to HMB45 in evaluating SN, with crisper membranous cytoplasmic staining, higher resolution and cleaner background. We believe this is the first, although small, study of KBA.62 in pediatric SN, and staining patterns

that we noted are similar to recent studies comparing KBA.62 to HMB45 in primary & metastatic melanomas. KBA.62 is a sensitive, and superior stain compared to HMB45.

### **60-Candida Albicans-Associated Necrotizing Vasculitis - Uncovering a Novel Pathogenetic Mechanism**

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**Background:** Candidal infection produces vascular pathology either through direct vascular wall invasion producing mycotic aneurysm, or occlusion of the vessel lumen by fungal organisms [septic embolization]. A majority of patients have documented fungal infection prior to the development of candidal vasculitis. Established risk factors for fungal infections include steroid therapy and prolonged neutropenia. In children undergoing induction chemotherapy for acute leukemia, fungal colonization rates with *Candida albicans* are as high as 82.4 % during the neutropenic phase. Disseminated or life-threatening candidal infections are much less frequently observed and there is a lack of evidence to support routine antifungal prophylaxis in these patients.

**Design:** Our patient is a child, who while undergoing treatment for acute lymphoblastic leukemia, developed life-threatening gastrointestinal tract hemorrhage. Multiple samples were taken for culture, and pathologic examination of resected small intestine was conducted.

**Results:** *Candida albicans* was cultured from the oral cavity, urine and liver abscess. Endoscopy showed no source for the brisk gastrointestinal tract hemorrhage, resulting in emergent exploratory laparotomy with segmental jejunal resection. Examination of the resected jejunum showed deeply penetrating, cleanly punched-out ulcers scattered throughout. Histological examination showed multifocal deep ulcerations with underlying necrotizing vasculitis. The pattern of vascular pathology here was unusual and highly reminiscent of that seen in polyarteritis nodosa, with extensive fibrinoid necrosis of medium calibre vessels within the intestinal submucosa, but no evidence of fungal organisms on special stain or culture. Review of the literature revealed no reports of similar pathology in humans, however striking similarity with the fibrinoid vascular necrosis observed in mice experimentally injected intra-peritoneally with candida-associated wall substance [CAWS, a water-soluble extracellular polysaccharide fraction from the fungal wall obtained from culture supernatant of *Candida albicans*] was noted. It emerges that this pathological response in the mouse model is strain-specific, with one strain [DBA/2] showing necrotizing vasculitis in 100% injected animals, while other strains were relatively protected [CBA/JN]. Linkage to inflammatory cytokine loci on chromosome 1 was demonstrated in the mouse strain with maximum susceptibility to arteritis.

**Conclusion:** Current understanding of patient susceptibility to, and severity of resultant fungal infections is limited. For the unusual pathology seen in this case, we hypothesize a genotype-specific susceptibility in humans, similar to that in mouse models.

### **61-Pediatric Collagenous Gastroenterocolitis: Importance Of Early Recognition For Timely And Successful Patient Management**

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**Background:** Collagenous gastroenterocolitis (GEC) is a recently described rare cause of chronic diarrhea with only few case reports in the literature, all affecting older adults. Collagenous colitis and gastritis have been reported individually in the pediatric population. We report the first case of collagenous GEC in a child. The etiology of collagenous gastrointestinal disease is uncertain, however, autoimmunity and *Aeromonas hydrophila* infection have been reported as the underlying etiologies in collagenous colitis.

**Design:** The hematoxylin and eosin stained slides supplemented by trichrome stain were examined from upper and lower endoscopic biopsies before and after patient treatment to determine effectiveness of therapy.

**Results:** A 15-month-old previously healthy boy with congenital bilateral microcorneas was admitted with a one month history of explosive diarrhea and anasarca. The review of other systems was unremarkable. Differential diagnoses included protein losing enteropathy, nephrotic syndrome, and congenital lymphedema. The esophagogastroduodenoscopy and colonoscopy revealed gastritis, and diffuse areas of colonic edema and inflammation. Biopsies of duodenum, gastric antrum and body, sigmoid colon, and rectum showed variable foci of irregular thickening of the subepithelial collagen table with epithelial layer separation, surface epithelial degenerative changes, and lamina propria fibrosis. No increased intraepithelial lymphocytes were identified. The rectum was the most affected site, with lesser involvement in upper gastrointestinal biopsies. Trichrome stain confirmed these findings, which were consistent with collagenous GEC. Based on this diagnosis, the patient was started on oral budesonide 3 mg once daily for 30 days, and also tapering doses of prednisolone solution (Orapred) 3 mg/ml solution twice a day; 6 mg for 7 days, and then 3 mg for 14 days. He did remarkably well, improving clinically by the time of discharge at 28 days post admission. His prednisolone was tapered and budesonide was discontinued on an outpatient basis. He subsequently suffered a relapse that required reinstitution of both budesonide and also increasing his prednisolone dosage for few days. The repeat upper and lower endoscopic biopsies 3 1/2 months after initial biopsies revealed unremarkable upper gastrointestinal tract. However, collagenous colitis was still present in all colonic biopsies performed at six different levels. He remains on tapering doses of prednisolone. At the latest outpatient follow-up, he showed continued clinical improvement, was asymptomatic, and had no intercurrent illnesses.

**Conclusion:** Collagenous gastroenterocolitis can cause protein-losing enteropathy and anasarca in the pediatric population. This case demonstrates that both clinicians and pathologists working in concert need to consider the possibility of this entity in children for an earlier diagnosis and timely patient management.

## **62-Abnormal Expression of RAB8 in an Infant with Microvillus Inclusion Disease**

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**Background:** Microvillus Inclusion Disease (MVID) is extremely rare fatal autosomal recessive enteropathy of early onset characterized by the lack of microvilli on the surface of enterocytes and intracellular vacuolae containing unexposed microvilli. Mutation of several genes known to be involved with apical/baso-lateral transport of proteins in polarized epithelial cells have been described in association with both early and late-onset MVID, including MYO5B and RAB8. The latter is a small GTP-binding protein localized in the peri-Golgi area and regulates cellular protein traffic through recycling endosomes/RE to the plasma membrane, including primary

ciliary membrane biogenesis.

**Design:** A 3-month-old girl was presented to us with intractable watery diarrhea from birth. Duodenal biopsy showed microscopic and ultrastructural features of MVID. Additional investigations undertaken during subsequent hospitalization revealed very broad spectrum of features, including hypopigmented hair, dysplastic and hypocellular bone marrow (requiring frequent blood transfusions), increased platelet count (in absence of inflammation and low CRP), episode of acute renal failure requiring peritoneal dialysis before spontaneous resolution. This unusual set of clinical features initiated a request for investigating the possibility of abnormal RAB8 gene expression/function in our patient.

**Results:** Histology of a duodenal biopsy showed subtotal villous atrophy, markedly deficient microvillus layer in all enterocytes with spherical inclusions (positive on PAS, CEA, CD10 stains). EM revealed short and scanty microvilli on surface of enterocytes with round inclusions in the apical pole of cytoplasm which contained numerous abortive microvilli pointing towards the centre. Anti-RAB8 antibody was applied (by T. Sato, Gumma University, Japan) and confirmed severely reduced cytoplasmic reaction in all enterocytes, compared to the control.

**Conclusion:** RAB8 is linked to MVID and Bardet-Biedl syndrome by its ability to promote ciliary membrane growth through a complex biosynthetic mechanism. Sato's experiment with RAB8 knockout mice showed that: a) the phenotype of the knockout mice closely resembled the phenotype of the patient with MVID, b) RAB8 is responsible for the localization of apical proteins in intestinal epithelial cells, and c) RAB8 is necessary for normal absorption and digestion of various nutrients in small intestine. Taken together, these data suggest that defects in the transcriptional regulation of RAB8 may be linked in some instances to the pathogenesis of MVID, confirmed by this case. The broad spectrum of clinical symptoms in our patient suggests possible involvement of different RAB or other genes, too.

### **63-Burkitt Leukemia with an Atypical Immunophenotype: Report of a Case and Review of Literature**

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**Background:** Burkitt Leukemia constitutes a small fraction of Acute Leukemias in children. Its recognition and proper designation is important as treatment differs from those of Precursor B-Acute Lymphoblastic Leukemia (ALL). Burkitt Leukemia is separated by its typical morphologic features (Blasts with FAB: L3 morphology compared to FAB: L-1/L-2 in Precursor B-ALL), a classic immunophenotype (blast positivity for CD45 [bright] CD20 [bright], CD10, CD19, SIg, Ig light chain restriction and negative TdT) compared to Precursor B-ALL blasts (which are positive for CD45 [dim], CD10, CD19 and TdT with negative CD20 and SIg). The diagnosis is confirmed by the characteristic cytogenetics findings. The combination of Burkitt morphology with Precursor B-cell immunophenotype may present a diagnostic pitfall, with resulting delay in proper management. We describe a case with morphologic and cytogenetic findings that are typical for Burkitt Leukemia, but an atypical immunophenotype.

**Design:** Case report and literature review.

**Results:** The patient, a 12 year old girl, presented with renal failure. She was found to be anemic, thrombocytopenic with rapidly rising WBC and peripheral blood blasts of FAB: L-3 morphology. Immunophenotyping study favored Pre B-cell immunophenotype (blast positivity for CD10, CD19, CD20, and CD45 with negative SIg), but TdT was negative. With these

overlapping findings, molecular testing was expedited, revealing the t(8,14)(q24;q32) and confirming Burkitt leukemia diagnosis. A review of our files of Burkitt Leukemia/ Lymphomas in the last 10 years showed no similar cases. A review of literature showed only rare reports of such cases. One report described 5 similar cases in a review of 5280 ALL patients from POG files. Of note is that 4 out of these 5 cases were initially misclassified and treated as Precursor B-ALL, with change of treatment only following availability of cytogenetics findings.

**Conclusion:**

1- Cases with Burkitt Leukemia morphology and atypical immunophenotype (i.e. resembling precursor B-cell immunophenotype) are rare, but important to separate and treat on Burkitt Leukemia protocols.

2- Awareness of these cases and the timely incorporation of morphologic, immunophenotypic and molecular genetics findings are crucial steps in ensuring correct classification.

3- Making the diagnosis prospectively starts with recognizing the typical Burkitt morphology, and also noting that the bright staining for CD45, CD20 and negative TdT should raise doubts about Precursor B-cell ALL (despite the negative SIg).

4- Molecular genetics testing for Burkitt translocations should be expedited for confirmation in these cases.

**64-Ultrastructural Investigations of the Upper Gastrointestinal Tract in a Child with Congenital Plasminogen Deficiency**

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**Background:** Congenital plasminogen deficiency is a rare autosomal recessive disorder, which is characterized clinically by chronic mucosal pseudomembranous lesions consisting of subepithelial fibrin deposition and inflammation. This condition may be considered a life-threatening systemic disease in which patients develop pseudomembranous lesions of mucosal surfaces exposed to minor trauma. It is most commonly clinically encountered as ligneous conjunctivitis because the most common clinical manifestation is ligneous ('wood-like') conjunctivitis, a redness and subsequent formation of pseudomembranes mostly on the palpebral surfaces of the eye that progress to white, yellow-white, or red thick masses with a wood-like consistency that replace the normal mucosa. Pseudomembranous lesions of other mucous membranes often occur in the mouth, nasopharynx, trachea, and female genital tract. It has been observed that some affected children may present with congenital occlusive hydrocephalus.

**Design:** The patient is a nine-year-old male child presenting with vomiting, gastro-esophageal reflux and failure to thrive. He underwent upper gastrointestinal endoscopy, which showed a subacute duodenitis with fibrinoid deposits within the lamina propria, chronic and subacute ulcerative gastritis with fibrinoid deposits within the lamina propria, and chronic esophagitis consistent with reflux with fibrinoid deposits present in the papillae. An ultrastructural study of the gastrointestinal specimens was performed.

**Results:** Light microscopically, the tissues showed innumerable deposits of paucicellular hyaline material with adjacent inflammation. Histochemical and electron microscopic analyses revealed the amorphous material to be fibrin and collagen. In particular, there was no increase of the lysosomal or mitochondrial compartment of the surface epithelial cells. The basal membrane, however, showed a variable thickness. The material was also very close to the micro-vessels.

**Conclusions:** To the best of our knowledge, this is the first case of congenital plasminogen deficiency with detailed electron microscopic investigation of the upper gastrointestinal tract. We speculate that micro-traumas of the vascular compartment of the lamina propria are associated with the characteristic deposits.

### **65-Clear Cell Sarcoma of Kidney: Morphoproteomic Analysis Reveals Genomic Correlates and Therapeutic Options**

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**Background:** Clear cell sarcomas of the kidney (CCSK) are aggressive tumors with current treatment involving radical nephrectomy, aggressive chemotherapy and irradiation. Increasing knowledge of molecular pathways of tumors has led to therapeutic strategies, providing individualized therapy to a patient. Morphoproteomics helps to assess the activation of proteins, understand the biology of a tumor and to identify possible therapeutic targets.

**Design:** Three cases of CCSK were retrieved, each containing viable tumor and normal kidney. Sections were stained immunohistochemically using antibodies against proteins activated in candidate molecular pathways, grouped as upstream signal transducers (PKC $\alpha$ , phospholipase D1,  $\beta$ -catenin), downstream effectors (pERK, pAkt, p-mTOR, p-p70S6K, pSTAT3), cell cycle related analytes (Ki67, Skp2, cyclin D1), stem cell markers (nestin, CD133, CD44, CD56), and tumorigenic/antiapoptotic markers (Hsp90, Bcl2, pNF- $\kappa$ Bp65, INI1/BAF 47). The mitotic index was noted. Immunostain intensity was scored (0-3), with respect to localization of signal in cytoplasmic, membranous and nuclear compartments, percentage of positive tumor, and protein activation in the form of compartmental translocation and phosphorylation. Markers of the cell cycle were quantified using an automated imaging system.

**Results:** All cases showed strong nuclear expression of cyclin D1, Ki67 and Skp2, indicating cell cycle progression. There was nuclear expression of mammalian target of rapamycin (p-mTOR) and downstream effectors pAkt and p70S6K suggesting activation of mTOR complex 2. All cases showed expression of phospholipase D1, Hsp90, Bcl2 and pNF- $\kappa$ Bp65. The neural stem cell markers CD56, nestin and CD133 were positive in all cases. Expression of CD44, PKC $\alpha$ , pSTAT3 and  $\beta$ -catenin was not observed.

**Conclusion:** Morphoproteomic analysis of CCSK led us think that cyclin D1, upregulated mainly by the Sonic hedgehog and the NF- $\kappa$ B pathways and in part by the mTORC2/PI3K/Akt pathway, HSP90 and phospholipase D1, could play the central role in the pathogenesis of CCSK. Positivity for neural stem cell markers CD56 and CD133 points towards the theory of neural stem cell origin of this tumor. We also noted cell cycle progression, involving both the G1/S and the G2/M phases. Based on this study, and considering the age group of the patients, inclusion of less toxic therapies in the form of statins, 13-cis retinoic acid, curcumin and 17-AAG in the treatment strategies may be beneficial.

### **66-A Case Of Undifferentiated (Sarcomatoid) Wilms Tumor With An Aggressive Clinical Course Occurring In A Patient Previously Treated For Stage 4 Neuroblastoma**

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**Background:** A diagnosis of Wilms tumor (WT) is largely made on H&E sections when there is a primary resection. However, a combination of immunohistochemical (IHC) stains with antibodies to WT-1 and bcl-2 has been reported to be useful in cases of primitive undifferentiated stromal WT (Shao et al. *Pediatr Dev Pathol* 2004;7:577-82). We report a case of undifferentiated (sarcomatoid) WT in which the diagnosis rested on such IHC staining.

**Design:** A boy without stigmata of Beckwith-Wiedemann syndrome was diagnosed with a left retroperitoneal neuroblastoma (NB) with metastatic disease to multiple bones at the age of 3 years. He was treated with a combination of chemotherapy, resection, peripheral blood stem cell transplantation and radiation therapy at multiple outside institutions. He had been in remission for 3 years when his family relocated, necessitating follow-up at our institution. Of note, there is a family history of a first cousin who died from NB. At the age of 9 years he was found to have a right-sided kidney tumor. A biopsy with frozen section and primary resection demonstrated a spindle and round cell tumor with focal hemangiopericytoma-like vascular pattern prompting a working diagnosis of synovial sarcoma. Tumor involved the perirenal fat, renal vein and renal sinus with positive renal sinus margin. Hilar lymph nodes were negative for metastatic tumor, but one node was involved by treated NB. Bone marrow biopsies were negative for both metastatic WT and NB. Initial IHC stains demonstrated positive staining with antibodies to vimentin, bcl-2, CD99, and focally cytokeratin. Desmin, chromogranin and synaptophysin were negative. As a result tissue was submitted for FISH analysis for EWS and SYT gene rearrangements, both of which were absent. In the interim, an IHC stain performed for WT-1 was found to be diffusely positive. Although morphologic criteria for anaplasia were absent, there was focal positive staining with p53. Sections were submitted for central review. A repeat WT-1 stain and BAF47 stain both demonstrated positive nuclear staining, excluding malignant rhabdoid tumor and confirming the diagnosis of WT. The patient underwent chemoradiation therapy for stage III favorable WT. A year after diagnosis he was found to have metastatic disease to the right lung which was resected. However, he had relapse to the lung and brain, succumbing to his disease 18 months after the diagnosis of WT.

**Conclusion:** This is an example of an aggressive undifferentiated (sarcomatoid) WT in which the diagnosis rested on positive WT-1 staining. As far as we are aware, outside testing for gene mutations associated with familial NB have been negative. Perhaps testing for p53 mutation is indicated.

### **67-Myogenin and INI-1 Immunostains Help to Differentiate Rhabdomyosarcoma and Rhabdoid Tumor, with Caveats**

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**Background:** Rhabdomyosarcoma and rhabdoid tumor have overlapping morphologic features, leading to potential diagnostic difficulty. Rhabdomyosarcomas with rhabdoid features lack the marked propensity for occurrence in infants and dire clinical prognosis seen with true rhabdoid tumors, which do not respond to rhabdomyosarcoma therapy. Deletion and lack of expression of INI1 typifies rhabdoid tumor and myogenin staining typifies rhabdomyosarcoma, but rigorous testing of these patterns has not been reported.

**Design:** Immunostaining for INI1 and myogenin was first performed on tissue microarrays constructed at the Human Cooperative Tissue Network and containing sections of 23 rhabdoid tumors, 33 alveolar rhabdomyosarcomas, and 32 embryonal rhabdomyosarcomas. INI1 immunostains were then performed using a standard avidin-biotin peroxidase method and monoclonal antibody BAF47 on a series of 12 soft tissue sarcomas with rhabdoid features. These had been submitted for COG pathology review between 2000 and 2009, and cases were selected based on availability of unstained sections. Myogenin stains were available on all 12 cases from the previous pathology review and had been similarly stained. Only nuclear staining was considered a positive result.

**Results:** As expected, all 65 rhabdomyosarcomas from the tissue arrays stained positively for myogenin and INI1. Similarly, all 23 rhabdoid tumors from the array sections stained negatively for INI1 and myogenin; native tissues on the same array stained positively for INI1. Of the 12 sarcomas with rhabdoid features, two stained negatively for INI1, with reactivity seen in native tissue. Retrospective review of the COG reviewers' report after evaluation of stains revealed that both of these lesions had a COG review diagnosis of rhabdoid tumor, not rhabdomyosarcoma, although both stained focally for myogenin. One myogenin-positive rhabdomyosarcoma lacked INI1 expression in neoplastic and native tissues, possibly due to archival section storage. All other cases showed INI-1 expression, although 2 were myogenin-negative. All of the INI1 positive sarcomas except the myogenin-negative ones had received a review diagnosis of rhabdomyosarcoma.

**Conclusions:** INI1 negativity can be used to confirm the diagnosis of rhabdoid tumor when rhabdomyosarcoma is being considered. Native tissues should stain positively for confirmation. Focal myogenin staining may be present in occasional cases of rhabdoid tumor, although most cases are negative.

## **68-The Pathologist's Contribution to Diagnosing Cancer Predisposition Syndromes in Children.**

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**Background:** Cancer predisposition syndromes (CPSs) are characterized by defects at the germ line level in tumor suppressor genes, oncogenes, or DNA repair genes, resulting in increased risk for cancer development. In most cases, the presence of suggestive clinical features usually allows a prompt diagnosis. However, many children lack these features, especially if a full phenotype has not yet developed, resulting in a delayed diagnosis. Distinctive pathological features can be found in many cases, allowing the pathologist to raise suspicion about a CPS and thus play an important role in its primary diagnosis and appropriate management.

**Design:** Our goal is to raise awareness by graphically illustrating distinctive lesions occurring in children with CPSs, stressing the need for pediatric pathologists to recognize them in order to contribute to the establishment of the clinical diagnosis. We selected examples of lesions studied in an academic pediatric pathology setting, that due to their unusual nature or low frequency, sometimes despite their seemingly trivial appearance, should trigger the suspicion for a CPS. This suspicion should be stated in the pathology report, and clinicians should be alerted about CPS unsuspected cases.

**Results:** The following lesions diagnosed in pediatric patients with CPS, were selected: basal cell carcinoma, cardiac fibroma and odontogenic keratocyst (Gorlin syndrome); focal and segmental glomerulosclerosis and gonadoblastoma (Frasier synd.); diffuse mesangial sclerosis (Denys-Drash synd.); Gardner-associated fibroma, gastric adenoma (familial adenomatous polyposis); microscopic intestinal ganglioneuroma, thyroid medullary carcinoma (MEN 2); hamartomatous polyps (Peutz Jeghers synd.); multiple neurilemmomas (NF3/schwannomatosis); pancreatic endocrine adenomatosis, renal medullary dysplasia (Beckwith-Wiedemann synd.). In addition, we selected cases in which common pediatric lesions occurred in uncommon associations, such as rhabdomyosarcoma, chondrosarcoma and leiomyosarcoma (Li-Fraumeni synd.); or Wilms tumor and myelodysplasia (Bloom synd.). On many of the selected cases, a CPS diagnosis was established only after it was suggested in the pathology report.

**Conclusions:** A wide spectrum of lesions may foretell syndromes that predispose to the development of cancer and manifest during childhood. Pediatric pathologists must be aware of these lesions and their significance, in order to detect a suspicious/diagnostic finding, and then take steps towards a confirmation, appropriate treatment, and follow-up with genetic counseling. The developing organism has highly variable ways of phenotypic expression with which pediatric pathologists should be intimately familiarized.

### **69-Congenital Neoplasms - a Clinicopathological and Perinatal Autopsy Study**

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**Background:** Distribution, incidence and behaviour of congenital neoplasms have not been defined in perinatal autopsy series in this part of the world where foetal autopsies are not done routinely. Analysis of 1526 consecutive perinatal autopsies was conducted to evaluate congenital neoplasms

**Design:** A uniform autopsy examination protocol was employed. Foetuses of 20 weeks gestation and neonates upto 7days after birth were included in the study. Data from 1526 cases from January 1984 to December 2008 and clinical manifestations and pathological data from 22 neonates with congenital tumours, registered at the Children's Hospital during the same period were analysed.

**Results:** In the audit of 1526 perinatal autopsies, eight congenital tumours were identified with 0.5% incidence. 1328 (87%) cases were still births and 198 (13%) immediate neonatal deaths. The sex incidence was almost equal. 1082 (72%) were preterm fetuses and 775 (50.8%) were of very low birth weight (<1500gms). Foetal hydrops was seen in 10%, polyhydramnios in 2%, oligohydramnios in 5% and congenital anomalies in 6.5%. The congenital tumours included 3 teratomas (37.5%), 3 neuro blastomas (37.5%), one mesoblastic nephroma and one bilateral intralobar nephroblastomatosis. Two large mediastinal teratomas were associated with polyhydramnios. One teratoma was intracranial. Two neuroblastomas were intraadrenal and one large with liver metastasis. The mesoblastic nephroma was large and nephroblastomatosis was associated with hydrocephalus. 22 congenital tumours were identified from the surgical pathology material of neonates operated during the first week of life. These included 15 (68.5%) sacrococcygeal teratomas, 2 (9.0%) gastric teratomas, one case each of hepatic haemangioendothelioma, mesoblastic nephroma, intracranial teratoma, extrarenal malignant rhabdoid tumour and yolk sac tumour of oronasal region. The sacrococcygeal tumours were

benign and gastric teratomas immature. The newborn with malignant rhabdoid tumour had intracranial PNET, developed wide spread metastasis and died.

**Conclusions:** Congenital tumours are rare in occurrence and the incidence in the present series is comparable with previous reports in Caucasian population. Germ cell tumours are the most common and associated with polyhydramnios. Neuroblastomas may be incidental lesions. Sacrococcygeal and gastric teratomas have better survival rates than mediastinal and intracranial teratomas.

### **70-Distribution of Mucosal Eosinophils in the Non-inflamed Pediatric Colon: a Clinico-pathologic Correlation of 50 Children**

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**Background:** The presence of prominent or increased eosinophils in gastrointestinal biopsy material is often encountered causing confusion to the pathologist and clinician. The precise classification of eosinophil associated gastrointestinal disorders is hampered by the paucity of information regarding the normal numbers and distribution of eosinophils in the non-inflamed lower gastrointestinal tract in children. The purpose of this study was to document mucosal eosinophil distribution in non-inflamed colonic biopsies.

**Design:** We reviewed colonic and other available gastrointestinal biopsies from 50 children, between September and December 2008, from a tertiary care pediatric teaching hospital. Only patients without colitis were selected. The findings were correlated with clinical and laboratory information. All biopsies were reviewed by a pathologist blinded to clinical and other information and recorded. Each biopsy was examined for the presence of lamina propria eosinophils. Three areas with the highest concentration of eosinophils were noted and eosinophils were counted per high power field (40X). The average of the 3 counts was recorded. Eosinophils were not recorded if identified in the vicinity of lymphoid aggregates. We also noted the age, gender, race, atopic history (asthma, seasonal or drug allergy, eczema) and medication history (classified as anticholinergic, antiepileptic, antacid including H2 blockers & proton pump inhibitors, and "others". The presenting complaint and final diagnosis were also recorded.

**Results:** The patients ranged in age from 1 to 18. The average age of the patients studied was 9.94 years, with a median of 11 years. Of 50 patients, 44(88%) were Caucasian, 2 (4%) were black, 1 (2%) Hispanic, and 3 (6%) were of "another" race. 18/50 patients had an atopic history (seasonal allergy, rhinitis, eczema, or asthma). 25 (50%) presented with abdominal pain, 12 (24%) with rectal bleeding, 5 (10%) with vomiting, 12 (24%) with diarrhea, and 4 (8%) with failure to thrive. 4 (8%) patients were ultimately diagnosed with gastritis, 4 (8%) with hemorrhoid or polyps, 4 (8%) with irritable bowel syndrome, 3 (6%) with eosinophilic esophagitis, and 1 with celiac disease. Our eosinophil counts per high power field were gastric antrum: 9, duodenum: 30, Terminal Ileum: 40, ascending colon: 20, transverse colon: 32, descending colon: 29 and rectum: 7.

**Conclusion:** The non-inflamed ascending colon had 20/hpf (range:16-51), transverse colon: 32 (16-41), and descending colon 29/hpf (14-38). There was no consistent correlation between eosinophil counts and medications. Colonic eosinophil counts are extremely variable. Larger, detailed studies are important in determining acceptable "normal" values.

### **71-Parenchymal Calcifications in Third Trimester Placentas Contain Little or No Detectable Lead As Determined by Atomic Absorption Spectrophotometry (AAS) and X-ray Spectrophotometry (XRS)**

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**Background:** We previously have shown that intrauterine growth restriction is not associated with low maternal serum lead levels (<4 µg/dl). Now we hypothesize that placental calcifications may sequester lead in the third trimester when there is the greatest risk for resorption from maternal bone.

**Design:** Five 3rd trimester placentas with large numbers of parenchymal calcifications and five 3rd trimester placenta controls were chosen to undergo analysis of lead content by both AAS and XRS. For both assays, multiple cores were extracted from formalin-fixed paraffin-embedded tissue blocks. In test cases, areas with the most calcifications were sampled and for controls, random cores were taken. For AAS, nitric acid digests were prepared from the tissue cores and analyzed for lead with a Model AA600 Perkins-Elmer graphite furnace atomic absorption spectrophotometer. Lead levels in µg/g dry weight were calculated for test cases and controls then compared using an unpaired t test. For XRS (EDAX system), 2 cases were analyzed and underwent deparaffinization, rehydration, fixation in glutaraldehyde, osmium tetroxide treatment, dehydration, and infiltration with epoxy resin and analyzed using both transmission and scanning electron microscopy.

**Results:** By AAS there was no significant difference in lead levels between cases (range 0.30-0.73 µg/g) and controls (range 0.32-1.21 µg/g)(P=0.46). No lead was detected in 2 cases using the XRS method although calcium was detected.

**Conclusion:** Minimal to no lead was detectable in third trimester placental calcifications using 2 different methods. The presence of calcifications, therefore, does not appear to be a marker for sequestration of lead from bone during the 3rd trimester of pregnancy.

### **72-Congenital Hyperinsulinism in Brazilian Neonates: a Study of Histology, Proliferation of β-cells, and K-ATP Channel Genes**

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**Background:** Congenital hyperinsulinism (CHI) is a rare pancreatic endocrine cell disease, and the pancreatic histology alone seems not to be able to explain its dramatic clinical features. The most severe cases of CHI, which are unresponsive to diazoxide, are found to be associated with genetic defects in the β-cell K-ATP channels genes in about half of the patients. The aim of this study was to examine pancreatic histology, β-cell proliferation and β-cell K-ATP channels genes mutations in blood samples from Brazilian patients with severe medically unresponsive CHI who underwent a pancreatectomy.

**Design:** Pancreatic histology and β-cell proliferation (double immunostaining for Ki-67/insulin, quantified by image analysis) of 11 CHI patients were compared to pancreatic samples from 19 age-matched controls, died of diseases not related to the pancreas. Genomic DNA of 4 patients

was extracted from peripheral blood. Mutational screening was performed throughout PCR amplification for the entire single exon of the KCNJ11 gene and for exons 33 to 37 of the ABCC8 gene, which codes for the NBF-2 (Nucleotide Binding Fold 2), one of the two ATP molecule binding sites of the SUR 1 protein that is essential for channel function.

**Results:** Ten cases were classified, according to histology, as diffuse form (D-CHI) and one, as focal form (F-CHI).  $\beta$ -cell nucleomegaly and abundant cytoplasm were absent in controls and were observed only in patients with D-CHI. Ki-67 Labeling Index (Ki-67-LI) was used to differentiate the adenomatous areas of the F-CHI case (10.15%) from "loose clusters of islets" found in two D-CHI (2.29% and 2.43%), and one control (1.54%) samples. D-CHI patients also had significantly higher Ki-67-LI (2.41%) than age-matched control group (1.87%) ( $P = 0.009$ ). No mutations or new polymorphisms were found in the 33-37 exons of the ABCC8 gene (SUR 1) or in the entire exon of the KCNJ11 gene (Kir 6.2) in 4/4 patients evaluated.

**Conclusion:** Enhanced  $\beta$ -cell proliferation seems to be a constant feature in CHI patients, both in diffuse and focal forms. A new histological finding was identified, called "loose clusters of islets", in which Ki-67-LI of  $\beta$ -cells was crucial to differentiate it from adenomatous areas of F-CHI. In this first genetic study of the K-ATP channels genes in Brazilian children no mutations or new polymorphisms were found compared to other populations ([www.ensembl.org](http://www.ensembl.org)). This is interesting if considered the mixed ethnical population of our country. Although the number of patients is small, we hope these results can be the starting point for future prospective studies of Brazilian children.

### **73-The Broward County Pediatric Autopsy Registry: A Successful Model of a Community-Based Fetal and Infant Mortality Surveillance Program**

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**Background:** In 2003, Broward County, Florida, implemented a four-year study of fetal and infant mortality uniquely designed as a community-based autopsy surveillance program, unlike similar programs based in universities, government agencies, or managed care organizations. In the early 1990s, community activists in Broward County envisioned a county-wide pediatric autopsy registry to study racial disparities in fetal and infant mortality. Over the next decade, several community and not-for-profit groups, such as the League of Women Voters and Healthy Mothers, Healthy Babies of Broward County (HMHB), as well as the local medical examiner, embraced the idea and approached various county agencies to solicit financial support.

**Design:** In the early 2000s, the North Broward Hospital District agreed to administer the program, which was jointly funded by Broward County and the Hospital District. Broward General Medical Center hired a pediatric pathologist and provided pathology services for the program. HMHB, the custodians of the national Fetal and Infant Mortality Review (FIMR) program for the county, provided data management and one support person. HMHB also provided education about the program to nursing staff in participating hospitals and a trifold, color brochure for patients. The program provided autopsies at no cost to any Broward County mother who lost a fetus or infant between 20 weeks gestation and the first birthday. Data from eligible infants autopsied by the medical examiner were also included in the registry. The medical examiner's office funded and provided logistics of body transport from the referring hospitals. Approximately two years into the program, a multidisciplinary committee convened to review its progress, report on interim findings, and compile a final report at the program's end.

**Results:** The program was well received by the community, achieving autopsy rates of 31% for infant deaths and 35% for fetal deaths. The program ended in 2007 due to funding cuts by Broward County after sharply falling property tax revenues and a major hurricane in 2005. The county is currently using the registry results to develop strategies and programs addressing fetal and infant mortality.

**Conclusion:** This successful program provides a useful template for community-based autopsy surveillance programs.

#### **74-Fryns Syndrome with Bilateral Pulmonary Agenesis, Bilateral Rocker Bottom Feet and Antero-lateral Type Congenital Diaphragmatic Hernia. Ceaselessly Incredulous?**

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**Background:** Fryns syndrome (FS) is an autosomal recessive multiple congenital anomaly syndrome characterized by congenital diaphragmatic hernia (CDH), craniofacial dysmorphism, brachytelephalangy, pulmonary hypoplasia and internal malformations. The purpose of the study was to describe bilateral pulmonary agenesis and bilateral rocker bottom feet as associations of FS, never earlier described in the FS literature in the 112 reported cases and to further provide credence to the proposed inheritance as autosomal recessive.

**Design:** A retrospective review of records and slides of 67 consecutive fetal autopsies between May 2005 and May 2009 from The Apollo Hospitals and The Randers Regional Hospital, Denmark along with an exhaustive review of literature were performed.

**Results:** Two cases, both from the same parents, were identified. The radiological findings of the first showed polyhydramnios, diaphragmatic hernia and pulmonary hypoplasia while the second presented with ventriculomegaly, non-visualization of the lungs, diaphragmatic hernia and bilateral renal cysts. There was parental consanguinity and the amniocentesis was normal in both the instances. There was no history of medications during the pregnancy and all hematology and biochemical parameters were within normal limits. At autopsy, the first fetus showed craniofacial dysmorphism, brachytelephalangy, nail hypoplasia, Bochdalek hernia, pulmonary hypoplasia and ventricular septal defect while the second in addition to craniofacial dysmorphism, brachytelephalangy and nail hypoplasia demonstrated short neck, clinodactyly bilateral rocker bottom feet, anterolateral type congenital diaphragmatic hernia, complete bilateral agenesis of lungs, atretic trachea, transversely positioned heart with an atrial septal defect and renal multicystic dysplasia.

**Conclusion:** Bilateral pulmonary agenesis and bilateral rocker bottom feet are remarkable findings hitherto never ascribed to FS literature. Parental consanguinity and a similarly affected sibling provide further credence to the proposed inheritance of FS as autosomal recessive. Important differentials excluded were recurrent cytogenetic aberrations, including trisomy 22 and Pallister–Killian syndrome, Simpson–Golabi–Behmel syndrome, Matthew–Wood syndrome, an extreme form of Poland anomaly and prenatal exposure to Mycophenolate Mofetil (MMF), an immunosuppressive drug which can produce a phenocopy of FS, apart from others.

#### **75-Intracranial Haemorrhages Are an Important Diagnostic Feature of Placental Abruptio When Associated with Intrathoracic Haemorrhages**

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**Background:** Placental abruption is a devastating event characterised by the presence of massive haemorrhage into the maternal decidua with separation and compression of the placenta by a blood clot. It is associated with a high mortality rate due to asphyxia. The classical presentation in the fetus or neonate is the presence of numerous petechiae and small haemorrhages in the lungs, along the coronary arteries in the heart, and in the thymus. The aims of our study were: 1) to describe the frequency of intracranial haemorrhages seen in cases of placental abruption and 2) to propose a pathogenic mechanism to explain the occurrence of intradural, subdural and other intracranial haemorrhages in these cases.

**Design:** We describe 16 fetuses of > 24 weeks gestation in whom a clinical diagnosis of placental abruption was made. As part of our routine practice we sample the dura matter (falx and tentorium) in all fetuses over 24 weeks gestation who are non or minimally macerated (<24h) and in neonates and infants.

**Results:** All 16 cases had significant gross intradural haemorrhage (IDH) and 6 also had a thin film of subdural haemorrhage associated with the IDH. Other types of intracranial haemorrhages were present in 12/16 cases: 5 cases had parenchymal haemorrhages, 5 cases had subarachnoid haemorrhage, 1 case had both and 1 case had intraventricular haemorrhage. As previously described in the literature, many cases had petechiae in the lungs (11), thymus (12), and heart (10). All cases had generalised visceral congestion and variable degrees of extramedullary haematopoiesis. Presence of features of early or moderate hypoxia in the brain was seen in 16 cases and was related to the survival period.

**Conclusion:** Macroscopic significant intracranial haemorrhage is an important diagnostic feature of placental abruption and is more constantly present than lung, thymic or heart haemorrhages. We believe the mechanism of the haemorrhage in this setting is the overload of the fetal circulation with consequent raised central venous pressure. This, in turn, is responsible not only for the intrathoracic haemorrhages but also for the intracranial haemorrhages, especially IDH. If severe and diffuse, IDH gives rise to subdural haemorrhage, which in all cases presents as a thin film on the posterior fossa and over the cerebral hemispheres.

## **76-Duodenal Intraepithelial Lymphocytes Are Increased in Childhood Primary Glomerulonephritis: Is There an Oral Tolerance Breakdown with an Intestinal Role in the Pathophysiology of Certain Glomerulonephritides?**

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**Background:** Patients with primary glomerulonephritides and other autoimmune disorders are thought to have a possible breakdown in oral tolerance. Studies in adults have shown a significant increase in intraepithelial T lymphocytes in the intestinal biopsies of patients with primary glomerulonephritides and celiac disease. We studied the clinical features, laboratory data and gastrointestinal biopsies of a 15 year old child with membranoproliferative glomerulonephritis (MPGN) and a history of chronic abdominal pain, without Celiac disease.

**Design:** We reviewed the clinical features, laboratory data and biopsies of a 15 year old child with MPGN and chronic abdominal pain. She had biopsy proven (routine, electron microscopy and immunofluorescence) MPGN Type-2. She had undergone cholecystectomy for biliary dyskinesia and an incidental appendectomy. She continued to have abdominal pain for months,

and was biopsied and the gastrointestinal biopsies were carefully studied. The duodenal biopsies were further examined with immunohistochemical stains CD3, CD4, CD8, CD45, CD20, CD43, CD56, CD68, CD79a, CD138, CDX2, PAX5 and Perforin.

**Results:** The gastric, duodenal and colonic biopsies showed an increase in lamina propria lymphocytes. Increased intraepithelial lymphocytes (IEL) were noted in the gastric (30 IEL/100 epithelial cells) and duodenal (150-160 IEL/100) biopsies. In the duodenal biopsies, the predominant IEL were highlighted by CD8 and CD3. A smaller subset of lamina propria lymphocytes were highlighted by CD4, and CD43. CD 79a and CD20 highlighted scattered B lymphocytes in the lamina propria, while CD138 highlighted scattered plasma cells. PAX5 and Perforin were negative. CDX-2 highlighted epithelial cell nuclei, as expected. No active inflammation was noted. Cytolytic protein molecules (found in CD8 T lymphocytes) were notably absent (Perforin negative). There were no B cell lineage specific lymphocytes or pre-B lymphocytes among the IEL (PAX5 negative). She was tested for HLA-DQ2 and -DQ8 signatures and they were not detected.

**Conclusions:** We identified profoundly elevated IEL counts in duodenal biopsies of a patient with MPGN type II and chronic abdominal pain. The intraepithelial lymphocytes were exclusively CD3 & CD8 positive T lymphocytes. The lamina propria was packed with lymphocytes without any active inflammation. It is known that therapy with antiCD3 antibodies and diet modification restore self tolerance. Our findings highlight the first documented childhood case of increased IEL with MPGN. Detailed larger studies are required to fully understand the link between increased intestinal epithelial lymphocytes, possible breakdown in tolerance and immune injury to the kidneys.

### **77-Hobnail Hemangioma: a Mimic of Patch-stage Kaposi's Sarcoma**

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**Background:** Hobnail hemangioma (HH) is a rare, benign lymphatic skin lesion of children and young adults that shows a striking similarity to early Kaposi's sarcoma (KS). Like KS, HH presents on the extremities or trunk and histologically, each show ramifying, jagged, slit-like capillaries in the dermis. Unlike KS, HH has no association with HHV-8 or HIV. We review in this abstract 2 cases of HH each arising in healthy male HIV-negative patients, age 10 (case 1) and 17 (case 2), with emphasis on the characteristics that distinguish HH from KS.

**Design:** Paraffin-embedded sections from each case were stained with H&E, Prussian blue, and the immunohistochemical stains CD31 and D2-40.

**Results:** Biopsies of the 2 patients each show proliferating lymphovascular spaces lined by a single layer of bland endothelial cells extending down to the upper third of the reticular dermis. There are ectatic lymphovascular spaces within the papillary dermis that diminish in size as they descend into the reticular dermis, becoming a ramifying network of jagged vessels in between collagen bundles. The endothelial cells demonstrate focal hobnailing into the vascular lumen. In case 1, but not case 2, a small vessel protrudes into an ectatic vascular space, a so-called "promontory sign." The endothelial cells are positive for CD31 and D2-40 in each case. Scattered hemosiderin-laden macrophages are highlighted by Prussian blue. Mild superficial perivascular lymphohistiocytic infiltrates are present in each case, but plasma cells are absent. No hyaline globules or cellular atypia are present in either case.

**Conclusion:** Histologic features that distinguish HH from KS include absence of plasma cells and hyaline globules, but an accurate clinical history is probably of greatest importance in

establishing the diagnosis. HIV-positivity or immunosuppression would favor KS, especially if the patient has lymphadenopathy. IHC for HHV-8 will be positive in KS, and negative in HH. The promontory sign is a well-known feature of KS but is not specific and can be seen in other vascular lesions, both benign and malignant. Little has been reported about this in the pediatric pathology literature. We present these cases to increase awareness that HH can mimic KS, which can help avert misdiagnosis and unnecessary emotional distress for the patient.

### **78-Wilms Tumor In A Child with L-2-Hydroxyglutaric Aciduria**

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**Background:** L-2-hydroxyglutaric aciduria (OMIM #236792) is a rare autosomal recessive metabolic disorder characterized by a variable degree of progressive encephalopathy and no known treatment. It is caused by mutations in the gene encoding the enzyme L-2-hydroxyglutarate dehydrogenase. In addition to degenerative brain disease, L-2-hydroxyglutaric aciduria has been associated with development of brain tumors, with at least nine brain tumors reported from patients ranging in age from three to 26 years. However, no tumors outside of the central nervous system have been reported.

**Design:** A 14-month old boy, evaluated for developmental delay, was found to have abnormal signaling in subcortical white matter and globus pallidi suggestive of a metabolic disorder. A semi-quantitative urine organic acid analysis by GC/MS revealed an isolated large peak of 2-hydroxyglutaric acid. At the age of 21 months, the child was evaluated for abdominal pain and a right renal mass was detected on radiologic examination. A stage II Wilms tumor was diagnosed after pathologic examination of surgical resection. The child is free of tumor recurrence 3 years after the surgery.

**Results:** The urine organic acid analysis, by GC/MS, was consistent with the diagnosis of 2-hydroxyglutaric aciduria. However, this method does not distinguish between L-2-hydroxyglutaric acid and D-2-hydroxyglutaric acid. Subsequently, a cell line derived from the Wilms tumor was analyzed by LC/MS-MS, which characterized the accumulated 2-hydroxyglutaric acid as L- 2-hydroxyglutaric acid, establishing the diagnosis of L-2-hydroxyglutaric aciduria.

**Conclusion:** We report the first case of Wilms tumor occurring in a child with L-2-hydroxyglutaric aciduria. While this single report does not establish L-2-hydroxyglutaric aciduria as a Wilms tumor predisposing condition, the known occurrence of brain tumor in this disorder makes this an intriguing proposition. The establishment of the cell line derived from the Wilms tumor in this case provides an opportunity for exploring mechanistic hypotheses.

### **79-Primary congenital infantile fibrosarcoma arising in the heart of a 7-month-old male**

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**Background:** Congenital infantile fibrosarcoma (CIFS) is a rare mesenchymal tumor that primarily presents in the distal extremities in young infants and newborns (approximately 40% at birth). However, a few unusual locations for this tumor have been reported, including lungs (primary bronchopulmonary fibrosarcoma), retroperitoneum, ovary, bowel, and kidney (cellular mesoblastic nephroma). CIFS shows slight predominance towards males, and over 80% of the

cases occur in the first year of life. Despite its large and ominous appearance, CIFS is considered to be of low malignant potential with an excellent prognosis. The diagnosis is usually confirmed by molecular analysis which shows t(12;15)(p13;q25) translocation and the related fusion gene *ETV6-NRTRK3*.

**Design:** The authors investigated the clinical and pathological findings in a 7-month-old patient. The child presented with lethargy and loose bloody stool. A CT scan revealed evidence of pericardial effusion, and an echocardiogram confirmed the presence of a large pericardial effusion with early signs of tamponade. Additionally, a large mass was seen in the upper part of the left ventricular wall protruding beneath the mitral valve and extending through the full thickness of the left ventricular wall and showing as a finger-like projection into the pericardial sac. Urgent pericardiocentesis was performed, and an open biopsy of the mass was obtained.

**Results:** Pathological examination of the biopsy showed a spindle cell neoplasm with hemangiopericytoma-like vasculature consistent with congenital infantile fibrosarcoma. This diagnosis was confirmed by molecular analysis which identified t(12;15)(p13;q25) translocation and the associated fusion gene *ETV6-NRTRK3*. The patient began chemotherapy soon after.

**Conclusion:** To our knowledge, this is the first reported case of primary CIFS arising in the heart.

### **80-Sudden Unexpected Death in Childhood: an Audit of Autopsy Reporting.**

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**Background:** Sudden unexplained death in childhood (SUDC) over 1 year of age is not widely documented or studied. Much of our knowledge on the subject relates to infants less than 1 year of age (Sudden Infant Death Syndrome, SIDS). In recent years with the advent of the 'back to sleep' campaign and educational awareness about risk factors for SIDS, as the numbers of SIDS have reduced, however the numbers of SUDC have relatively increased. In Ireland all SIDS and SUDC cases are investigated and reviewed by the National Sudden Infant Death Register (NSIDR). However, not all paediatric autopsies are carried out in specialist centres by paediatric pathologists often resulting in sub-optimal reporting.

**Design:** The database of the National Sudden Infant Death Register in Ireland was interrogated and cases of SUDC over a thirteen-year period (1994-2007) were compared to SIDS cases whose autopsies were conducted in the same hospital in the same year as the index SUDC case. The autopsy reports for each case were examined and Rushton scores<sup>1</sup> calculated. The groups were compared in terms of autopsy findings, Rushton scores, regional versus non-regional centres and date of autopsy.

**Results:** Over the thirteen-year period there were 43 SIDS cases and 37 SUDC cases. Full autopsy reports were available in 37(86%) of the SIDS cases and 34(91%) of the SUDC cases. Overall Rushton scores were higher in the SIDS cases, with 26(60%) cases obtaining the arbitrary minimum score of >300 compared to 18(49%) of SUDC cases. Paediatric pathologists in specialist centres carried out more of the SIDS autopsies than SUDC autopsies (53% SIDS 38% SUDC). Autopsies carried out by paediatric pathologists had higher Rushton scores in both groups. Following publication of the protocol for care and investigation of sudden unexpected death in infancy by the Royal College of Pathologists in 2004<sup>2</sup> Rushton scores for post-mortems improved in Ireland (SIDS 88%, SUDC 66% >300), with more cases being referred to regional centres.

**Conclusion:** Comparison between SIDS and SUDC autopsy reporting is difficult due to incomplete reporting of SUDC cases which had low Rushton scores. Autopsy reporting improved in both regional and non-regional centres following issue of the most recent guidelines. However, centralisation of the paediatric autopsy service promotes optimal post-mortem examination and investigation.

References:

1. Rushton DI. West Midlands perinatal mortality survey, 1987. An audit of 300 perinatal autopsies. BJOG 1991 Jul;98(7):624-7.
2. Royal College of Pathologists, Royal College of Paediatrics and Child Health. Sudden Unexpected Death in Infancy. A multi-agency protocol for care and investigation. London:2004.

### **81-Diffuse Neonatal Hemangiomas with Gastrointestinal Involvement in a 4-month-old Infant**

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**Background:** Diffuse neonatal hemangiomas (DNH) is a rare disease characterized by multiple cutaneous and visceral hemangiomas. Most patients have skin and liver involvement; less frequently involved organs include pleura, lungs, intestines, central nervous system (CNS), and eyes. Children with DNH, particularly with extensive visceral involvement, have mortality rates between 50% and 95%. The leading cause of death is usually high-output cardiac failure. Other complications include coagulopathy, and hemorrhage, particularly involving the skin, gastrointestinal (GI) tract, and CNS.

**Design:** We report a case of diffuse neonatal hemangiomas in a 4-month-old female presenting with gastrointestinal bleeding and anemia.

**Results:** The patient is a 4-month-old female with unremarkable prenatal and birth history, who presented with melena, diarrhea and a hemoglobin of 8.5 G/DL. She underwent esophagogastroduodenoscopy, colonoscopy and upper GI series, all of which were normal. Due to clinical suspicion for Meckel's diverticulum, she underwent exploratory laparotomy. During surgery a portion of the distal ileum was noted to have multiple, irregular, patchy foci of serosal erythema. Upon opening, multiple nodular areas of erythema were noted on the mucosal surface. Sectioning revealed nodular areas of expansion and hemorrhagic tissue within the bowel wall, suspicious for vascular lesions grossly. Approximately 20.0 cm of the affected small bowel was resected. Microscopy revealed multiple hemangiomatous lesions involving the lamina propria and submucosa, as well as focal involvement of muscularis propria and serosa. The lesions consisted of numerous lobular proliferations of thin-walled vascular spaces, with rare larger caliber vessels, intervening with areas of unremarkable intestine. The patient did not experience any subsequent episodes of bleeding postoperatively. Further workup was negative for any other organ involvement by DNH at this time.

**Conclusion:** Although DNH is typically a diffuse process that can involve multiple organs, our patient had limited GI involvement with multiple hemangiomatous lesions involving a 20.0 cm segment of small intestine.

Although rare, DNH should be considered in the differential diagnosis of any patient with refractory gastrointestinal bleeding with negative imaging and endoscopy studies as this is a potentially life threatening disorder.

## **82-A Novel TCIRG1 Gene Mutation Leads to a Severe Form of Osteopetrosis with Altered Content of Monocytes/macrophages in Several Organs**

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**Background:** Osteopetrosis (OP) is a rare, clinically heterogeneous disorder characterized by osteoclastic dysfunction leading to failure of bone remodelling. The autosomal recessive forms that are found primarily in neonates and children are the most severe. Defects of the TCIRG1 gene (regulating the H<sup>+</sup>ATPase proton pump) are most frequently implicated in autosomal recessive OP and result in a severe form of osteoclast rich OP. Osteoclast precursors are of monocyte lineage. It is unclear how the content and/or function of monocytes/macrophages (MoMØ) of other organs rich in MoMØ are affected.

**Design:** We report a 5-month-old boy who presented with macrocephaly and a bulging fontanelle. A skeletal survey revealed abnormal appendicular mineralization with cupping and fraying of the long bones and sutural diastasis of the lateral ribs, suggestive of osteopetrosis. Bone marrow biopsy was abnormal with decreased trilineage hematopoiesis and atypical bone formation. On morphological examination, the osteoclasts lacked ruffled edges on the bone, a finding associated with the osteoclast rich form of OP. At autopsy, IHC for CD163, CD14 and CD68 in liver, spleen, lung, testes and lymph nodes was performed and the MoMØ content was compared to that of age-matched organs.

**Results:** At autopsy there was massive remodeling defect of bones with total replacement of BM space by woven bone and abnormal enchondral ossification at the epiphyseal plate. Molecular studies identified a homozygous IVS 12+5G>C transversion in the TCIRG1 gene region. The patient underwent matched unrelated BM transplantation and died of disseminated adenovirus infection 30 days after the transplant. There was no hematopoietic recovery or engraftment. The bone marrow showed numerous osteoclasts but no other hematopoietic elements or stroma. The IHC of MoMØ showed a high phagocytic content in the LN, testes and more epithelioid forms in the liver sinusoids. The MoMØ content of the spleen was depleted and the lung was devoid of alveolar macrophages.

**Conclusion:** We report a case with a novel TCIRG1 gene mutation that led to an osteoclast rich OP that failed BM transplantation. Osteopetrosis patients respond poorly to bone marrow transplantation. The altered monocyte-macrophage contents in many organs argues for a more systemic process in which monocyte derived hematopoietic elements are affected. This could aggravate the already existing poor immune status of patients with severe forms of OP and at least be partially responsible for poor engraftment.