

**Society for Pediatric Pathology
Annual Meeting, San Diego, California
March 24-25, 2007**

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

Platform Presentations:

1 PAX5 Immunoreactivity In Pediatric Small Blue Cell Tumors

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Background: PAX5 is member of the family of paired box transcription factors involved in development. Its expression has been well characterized amongst hematopoietic malignancies where it is a specific marker for the B-cell lineage. Its expression has also been reported in medulloblastomas, neuroendocrine carcinomas and urothelial carcinomas. Recently, neuroblastoma cell lines have been shown to express PAX5. We investigated the use of PAX5 immunohistochemistry in the evaluation of pediatric small blue cell tumors.

Design: A tissue microarray was constructed from 85 small blue cell tumors consisting of 22 neuroblastomas, 18 Wilms tumors, 16 rhabdomyosarcomas (11 embryonal and 5 alveolar), 11 Ewing family tumors, 8 lymphoblastic lymphomas, 6 hepatoblastomas, and 3 granulocytic sarcomas. Each tumor was represented by two 1.0 mm cores. A 4.0 um section was immunostained using an antibody directed toward PAX5 (BD Biosciences) at a dilution of 1:250. The percentage of nuclear staining was scored semiquantitatively by all three investigators.

Results: All 4 of the B-cell lymphoblastic lymphomas stained strongly. Additionally, all 18 Wilms tumors showed diffuse nuclear staining of variable intensity (strong staining in the epithelial component, weak staining in blastema, no staining of the stromal component). Four of the 5 alveolar rhabdomyosarcomas (ARMS) were immunoreactive while none of the 11 embryonal rhabdomyosarcomas (ERMS) stained. The ARMS case that did not stain was morphologically diagnosed as the solid variant although neither the t(2;13) nor t(1;13) was identified by karyotype. No other tumor type represented on the array was immunoreactive with the antibody to PAX5.

Conclusion: PAX5 immunoreactivity was limited to B-cell lymphomas, Wilms tumors and ARMS. Although PAX5 expression has been reported in hematopoietic and neural development as well as spermatogenesis it has not been seen in nephrogenic or muscle embryogenesis. It is possible that the immunoreactivity seen in Wilms tumors and ARMS is secondary to cross-reactivity as PAX2 and PAX8 are expressed in kidney development and PAX3 and PAX7 fusion genes characterize the majority of ARMS. As this immunoreactivity may be useful for the immunohistochemical distinction between categories of RMS, further study of this antibody in a larger series of RMS is warranted.

2 Immature Interstitial Mesenchymal Tumor (IIMT): A Distinctive New Entity Of The Infant Lung

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Background: In the course of our review of cystic or tumefactive lesions of the lung in infants with concern for possible pleuropulmonary blastoma (PPB), we have identified 4 similar cases with unusual morphology distinct from PPB or any other previously described pathologic entity. These 4 cases are presented as examples of a new entity which we have designated "immature interstitial mesenchymal tumor (IIMT).

Design: 4 cases of a distinctive congenital lung lesion from 4 different institutions were referred to the authors in a 12-month interval. Gross descriptions, radiographic studies and histologic sections were examined in each case. Special studies included immunohistochemical (IHC) staining (n=3) electron microscopy (EM) and cytogenetics (n=1 each).

Results: Radiographically, 3 of the lung lesions were solid and involved the right lung; 1 was cystic and involved the left upper lobe. 3 infants were male and 1 was female. One case was detected in utero and the others were noted at birth, 4 and 12 days old. Each infant had a surgical resection within 5 months of diagnosis. One infant had adjuvant chemotherapy. No recurrences have been observed to date (follow-up range 1.3 to 2 years). Microscopically, the tumors are composed of a dense network of thickened septa resembling immature lung and containing a uniform population of "immature" appearing round cells occupying the entire interstitium without rhabdomyoblastic or chondroid differentiation. These mesenchymal cells show minimal, if any, differentiation, but the nuclei are not hyperchromatic. Atypical mitotic figures are not seen. A low cuboidal, non-ciliated epithelium lines the intervening spaces. The interstitial cells stain for vimentin and are generally non-reactive for muscle markers (n=3). Rare entrapped normal appearing small airways are present within the lesion. The interstitial cells have no specific differentiating features by EM. One karyotype was normal.

Conclusion: The IIMT is a lobar, tumefactive process of the lung that presents at birth and mimics developing lung architecturally. This lesion appears to be distinct from PPB, but like the latter, appears to result from a proliferation of uncommitted interstitial mesenchymal cells. It is unclear whether the IIMT is a manifestation of anomalous lobar development or a true neoplasm.

3 Composite Fibrous/Myofibroblastic Tumors: A Subset Of Fibrous Tumors Of Childhood?

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Background: Fibroblastic/myofibroblastic tumors comprise 10-15% of all pediatric soft tissue tumors, and most have a distinctive microscopic appearance. However, we have encountered cases with more than one histologic pattern and for these we have resorted to the designation of composite fibrous/myofibroblastic tumor (CFMT). These latter tumors have been analyzed and compared in order to

ascertain the distribution of the component morphologies, and whether clinically significant differences existed between them.

Design: We electronically searched departmental archives and personal consult files (LPD) for cases with a diagnosis containing "myofibromatosis (MYOF)," "lipofibromatosis (LF)," "fibromatosis (FIB)," "desmoid (DES)," "congenital infantile fibrosarcoma (CIF)," "infantile digital fibromatosis (IDF)," "hemangiopericytoma (HPC)," "fibrous hamartoma of infancy (FHI)," "giant cell fibroblastoma (GCF)," "calcifying aponeurotic fibroma (CAF)," and/or "composite fibrous-myofibroblastic tumor (CFMT)." Patients over 18 years of age were excluded. Cases were compared with regard to age, sex, anatomic site, constituent morphologies, and clinical outcome data.

Results: We identified 271 fibrous/myofibroblastic tumors from 252 patients (male:female = 1.6), who ranged in age from 3 days to 18 years (mean: 5 years, median: 1.7 years). The majority of tumors presented in the extremities (n=102 cases), head & neck (n=80), trunk (n=50). Other sites included pelvis (n=18), visceral organs (n=12), paraspinous region (n=5), abdomen (n=2), and mediastinum (n=2). The distribution of subtypes is as follows: DES (104 cases), MYOF (90), LF (30), CIF (15), IDF (12), HPC (10), CAF (9), FHI (6), GCF (2) and CFMT (47). The 47 composite tumors, as defined by two or more distinctive patterns, occurred in younger patients (mean: 7 months) than tumors comprised in exclusively of MYOF (2.3 years), LF (3 years), CAF (6.1 years) and DES (10 years). There were no incidences of metastasis or malignant transformation in the composite tumors, although local recurrences were reported.

Conclusion: Composite fibrous-myofibroblastic tumors exist as a subset of fibrous tumors of childhood which presents at a younger age on average than the other tumor types. One of the central issues at the time of diagnosis has been the appropriate designation for these tumors. These tumors with multiple patterns raise the interesting question as to the relationship of these several tumor types to each other especially in light of recent molecular genetic studies.

4 Curcumin Induces Apoptosis And Suppresses Growth In The Anaplastic Wilms Tumor Cell Line SK-NEP-1

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Background: Curcumin or diferuloylmethane (C₂₁H₂₀O₆) is a yellow polyphenol extracted from the rhizomes of turmeric (*Curcuma longa*), a food additive used in Indian cooking and a therapeutic agent in traditional Indian medicine. Along with other plant polyphenols such as catechins, gallocatechin, and quercetin, curcumin is being actively investigated as a "natural" cancer chemotherapeutic and chemopreventive agent and has been shown to cause cell cycle arrest and induce apoptosis in many adult cancers; but curcumin has not been studied in any detail in pediatric cancers.

Design: We studied the effect of curcumin exposure on the anaplastic Wilms tumor cell line SK-NEP-1. After exposure of the cell line to varying concentrations of curcumin (0-12 microM) for 24-48 hours, we performed MTS assay for cell viability; flow cytometry for cell cycle analysis and for annexin V staining; Western blot for Akt and phospho-Akt.

For assessing the effect of curcumin on anchorage independent growth of Wilms tumor cells, we studied colony formation in soft agar at 14 days.

Results: Curcumin caused downregulation of phospho-Akt, producing apoptosis in Wilms tumor cells at an IC₅₀ of 4 microM. Curcumin partially prevented progression of cell cycle beyond G₀/G₁ phase leading to a 10% reduction in the S phase fraction. A marked reduction in colony formation was seen in cells exposed to curcumin (p=0.0027).

Conclusion: Curcumin induces cell cycle arrest, induces apoptosis, and suppresses colony formation in the anaplastic Wilms tumor cell line SK-NEP-1. This is significant since anaplastic Wilms tumors are resistant to currently available chemotherapeutic drugs. Despite successes of modern therapeutic approaches in favorable histology Wilms tumors, the outlook for anaplastic Wilms tumors remains dismal, especially at higher stages. Our findings indicate a need for further evaluation of curcumin in chemotherapy as well as chemoprevention of Wilms tumors.

5 Medullary Renal Cell Carcinoma (MRCC) And Malignant Rhabdoid Tumor (MRT) Share Loss Of INI1 Expression And May Be Related Entities

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Background: MRCC, an uncommon, highly malignant neoplasm seen almost exclusively in the setting of sickle cell hemoglobinopathy (SCH), presents with disseminated disease and survival measured in weeks. To date, fewer than 50 cases have been reported, primarily in teenagers and young adults with a two-fold male predominance. Its pathogenetic relation to SCH remains a biologic enigma. MRCC has an infiltrative growth and several patterns, but the tumor cells often have a "rhabdoid" appearance similar to MRT. Some regard MRCC as a variant of collecting duct carcinoma (CDC). Because of the resemblance to MRT, similar clinical behavior, and a single report of MRCC with a 22q deletion, we investigated the similarity of these tumors with respect to INI1 protein expression and genetic status.

Design: The archives were searched for cases with diagnoses containing "kidney" in combination with "medullary carcinoma," "MRT," or "CDC" and identified 7, 12 and 20 cases of each, respectively. Microscopic examination confirmed the diagnosis in each case. A single representative slide from each case was immunohistochemically stained with the BAF47 (INI1) antibody; adjacent normal renal parenchyma and lymphocytes present in each section served as internal controls. Fluorescent in situ hybridization (FISH) was performed on corresponding tissue sections of INI1 (-) tumors using a BCR probe (22q11.2) in close proximity to the hSNF5/INI1 locus along with a more telomeric NF2 probe (22q12).

Results: All cases of medullary carcinoma and MRT demonstrated loss of nuclear INI1 protein expression by immunohistochemistry. Nuclear INI1 expression was retained in 5 of 7 CDCs. Intriguingly, one of the two INI1 (-) CDCs was displayed homozygous BCR and hemizygous NF2 deletions, consistent with biallelic hSNF5; this tumor occurred in a 46 year old male.

Conclusion: MRCCs and MRTs share important characteristics including loss of nuclear INI1 protein expression, chromosomal 22q abnormalities involving the hSNF5/INI1 locus, and similar morphologic features. We postulate that MRCCs and MRTs are related neoplasms and may, in fact, represent members of a common family.

6 Ovarian Surface Epithelial Neoplasms In The Pediatric Population: Incidence, Histologic Subtype, And Natural History

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Background: Surface epithelial neoplasms (SEN) account for a small, but significant proportion of pediatric ovarian tumors. However, the histologic subtype, natural history, and optimal treatment have not been sufficiently examined.

Design: Relative incidence of pediatric SEN was evaluated based on a complete search of the pathology archives for all ovarian mass lesions from pediatric patients (birth to 18 years) in a ten-year period (August 1996 to August 2006). Subsequently, all pediatric ovarian SEN evaluated at Stanford University over the past 32 years were retrospectively reviewed.

Results: In a ten-year period, 79 pediatric ovarian masses removed for pathologic examination were identified: 28 physiologic cysts (39%) and 51 tumors (61%). The relative proportion of each tumor subtype mirrored that reported in the pediatric literature, although with a distinct age distribution (Table 1). Expanding the retrospective search to 1974 identified 45 SEN: 16 benign (9 serous, 6 mucinous, 1 mixed), 24 low malignant potential (14 serous, 8 mucinous, 2 mixed), and 5 malignant (4 low-grade serous, 1 mucinous). Two tumors of low malignant potential were initially misclassified as carcinoma. Recurrences occurred in 3 of 16 patients with cystadenomas (one patient had two recurrences), 1 of 24 with tumors of low malignant potential, and 1 of 5 with carcinomas. The single patient with recurrent low-grade serous carcinoma died of disease at 23 months.

Table 1. Ovarian Masses 1996–2006

	# Cases	%	Age, mean (year)
Germ cell neoplasms	31/51	61%	10.3
Surface epithelial neoplasms	15/51	29%	12.6
Benign	7/15	47%	10.0
Low malignant potential	6/15	40%	15.8
Malignant	2/15	13%	12.0
Sex cord stromal neoplasms	5/51	10%	15.2

Conclusion: Surface epithelial neoplasms are not uncommon in pediatric patients and typically arise in children ≥ 10 years of age. Virtually all pediatric SEN are serous or mucinous; SEN with endometrioid, clear cell, or Brenner histology are exceedingly rare. Although most are benign or of low malignant potential, low-grade carcinomas do occur (11% in this series). High-grade surface epithelial carcinomas, however, are not seen. Recurrence does not necessarily reflect aggressive clinical behavior, since patients with benign and low malignant potential tumors may develop recurrent disease and are successfully treated by limited surgical excision. Given the risk of misclassification, the use of chemotherapy in these patients should be considered with caution and only after expert review.

7 Neonatal Outcome: Predictive Value Of Placental Examination

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Background: Placental evaluation is often neglected or poorly performed. As a diary of fetal life, the placenta is a

meaningful predictor of neonatal outcome for level 3 NICU admissions.

Design: During a three month period, placentae from consecutive admissions to the level 3 NICU were compared to neonates referred to the observation nursery (ON). Criteria for level 3 admission include: assisted ventilation, CNS depression, Apgar scores less than 3, seizures, hemodynamic instability, etc. Less affected infants are monitored in the intermediate care, observation nursery (ON). Pathology reports, glass slides and clinical records were reviewed. Two ON consecutive controls per level 3 admission were studied. In total, 133 patients were analysed, NICU $n=48$ vs. ON $n=89$. The mean birth of weight and gestational age for NICU patients was 2046 grams and 32.8 weeks vs. 3176 grams and 37.8 weeks for ON. Placental observations were correlated with birth weight, Apgar score, length of stay, co-morbidities, etc. Histopathologic findings were classified according to six main categories: infection, hemorrhage and vascular disorders, hypoperfusion and ischemia, genetic abnormalities and normal for gestational age. Infection was stratified as maternal (acute chorioamnionitis), fetal inflammatory response (stem vessel vasculitis and funisitis) or both. $P < 0.05$ was set for statistically significant difference between groups. Data were analyzed using the Epi-Info software (version 3.3.2, CDC, Atlanta, GA).

Results: Abnormal histology was present in 95% of level 3 neonates as compared to 21% admissions to the ON ($P < 0.001$). Level 3 neonates had infection, (36.4%) followed by hypoperfusion-ischemia (34.1%) and retroplacental hemorrhage (18.2%). In the ON group, 79% had a normal placenta and 17% had infection, ($P < 0.001$). As expected, placental pathology correlates with low 1 minute Apgar, ($P < 0.001$) and low five minute Apgar score ($P < 0.005$). Significant differences were found in infants with stem vessel vasculitis (SVV), funisitis and acute chorioamnionitis (CA) as compared to CA alone. 15 level 3 patients had SVV as compared with 2 in the ON. Infected infants (SVV) had a longer length of stay, median 23.5 days vs. 2 days in CA only, and lower one minute apgar scores (6.5 vs. 8.8) ($P < 0.001$).

Conclusion: Pathological findings in the placenta predict neonatal outcome. Distinction between maternal inflammatory response (CA only) versus fetal inflammatory response (SVV, funisitis, with or without CA) should be recognized and promptly reported as a critical predictive value to the neonatologist.

8 Evaluation Of Criteria For Diagnosis Of Non-Acute Umbilical Cord Compression As Cause Of Third Trimester Stillbirth

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Background: We previously performed a study of placentas from a series of third trimester stillbirths and proposed a combination of clinical, gross, and histologic findings as criteria specific to the diagnosis of "cord accident." We now test these parameters on an independent series of cases to further assess their validity, and to establish sensitivity and specificity.

Design: We reviewed placental slides from 42 cases of third trimester stillbirths from 1988 to 2005 at Brigham and Women's Hospital, blinded to autopsy and placenta reports. Half the cases were deemed cord accidents and half had a cause of death other than cord accident, based on autopsy reports. Recorded for each case were 1) presence and location of fetal vascular ectasia and thrombosis, 2) presence and distribution of avascular villi or villous stromal

karyorrhexis, and 3) number of slides containing chorionic plate and placental parenchyma. Additionally, 4) clinical or gross findings predisposing to cord compression, such as true knots and nuchal cords, were noted from autopsy and placenta reports.

Results: As previously determined, histologic criteria for non-acute cord accident were defined by vascular ectasia and thrombosis in the fetal vasculature (specifically, in the chorionic plate and stem villus vessels) as well as avascular villi or villi with villous stromal karyorrhexis in a regional distribution. Clinical/gross criteria included the presence of a nuchal cord or a gross cord abnormality predisposing to cord compression. While the histologic criteria alone excluded non-cord accident cases with a high specificity (95%), they identified only 9 of 21 cord accident cases (sensitivity=43%). This was due primarily to poor sampling of the placental parenchyma, specifically the chorionic plate (average # slides with chorionic plate was 1 per case in cases not identified as cord accident, and 2 per case in cases identified as cord accident). When clinical or gross umbilical cord abnormalities were combined with one of two but not both downstream histologic criteria (chorionic plate/stem villus or terminal villus findings), sensitivity increased to 67%, while specificity remained high at 85%.

Conclusion: Histologic diagnosis of cord accident can be made with confidence using well-defined criteria. Proper sampling of the placenta, to include at least two and ideally four or more sections of chorionic plate, is required when obstructive umbilical blood flow is considered a potential cause of intrauterine demise.

9 Umbilical Cord Compromise Is Not Associated With Specific Placental Pathology

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Background: Recent reports sound optimistic regarding high sensitivity of detecting so-called "placental stasis induced thrombotic vasculopathy" for identification of umbilical cord (uc) compromise in placentas from stillbirths. Many years' experience in placental examination leads me to opposite conclusions. In addition, not all uc accidents end in stillbirths; hence this case-controlled analysis of clinically proven/suspected uc compromise.

Design: Frequencies of 18 clinical and 34 placental parameters were retrospectively statistically compared between 175 consecutive clinically proven/suspected cases of uc compromise (study group, sg) and 175 clinically matched cases without uc compromise (control group, cg). Sg comprised 33 cases with variable decelerations, 96 with uc entanglement and 20 true knots found at delivery, and 26 cases of uc prolapse.

Results: The average gestational age in both groups was 34.1 weeks. Clinically, sg and cg differed (Chi-square, $p < 0.05$) only in rates of abnormal fetal rate tracings, 31 vs 13%, induction of labor 12 vs 6%, and antepartum hemorrhage, 5 vs 12%, but the differences in frequencies of oligohydramnios, 8 vs 7%, and perinatal deaths, 17 vs 11%, were statistically not significant in sg and cg, respectively. There were no statistically significant differences in any placental gross or microscopic findings between sg and cg either. Specifically, the frequencies of focal fetal thrombotic vasculopathy was 4 vs 5%, recent thrombi in fetal vessels, 7 vs 8%, and diffuse luminal abnormalities of chorionic villi with secondary villous fibrosis (lvaf), 10 vs 10%, in sg and cg, resp. Recent fibrin thrombi in uc and fetal vessels seen in placentas from macerated stillbirths in both sg and cg were morphologically of shorter duration than the estimated period of in-utero retention of dead fetus. Similarly, lvaf correlated

with macerated stillbirths in both sg and cg. Other types of uc pathology (abnormal uc insertion, meconium toxicity, chorda, candida funisitis, focal coagulative necrosis or segmental absence of Wharton's jelly, edema, hemorrhage, long uc, ulcers, aneurysms, and amniotic bands) were not significantly more common in sg than in cg, 22 vs 18% respectively.

Conclusion: As results of placental examination do not correlate with clinically evident/suspected uc compromise, they are unlikely to be diagnostic in clinically occult/unsuspected cases. This may be due to short duration of uc compromise which may have been readily identified and managed clinically. In cases of macerated fetuses with uc compromise, potential "placental stasis-induced thrombotic vasculopathy" may have been obscured by lvaf which develops with time irrespectively of the cause of intrauterine fetal death.

10 The Placenta In Trisomy 13: Overexpression Of Soluble VEGFR1 Correlates With Clinical Pre-Eclampsia And Villous Hypermaturity

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Background: Overexpression of soluble VEGF receptor-1 (sVEGFR1 or sFlt1) has been linked to pre-eclampsia (PE) and is thought to precede the onset of clinical signs and symptoms. Women pregnant with a Trisomy 13 fetus have long been known to be at increased risk for PE; recently, it has been proposed that this is due to overexpression of sVEGFR1 from its gene locus on 13q12. This study was performed to evaluate expression of sVEGFR1 in Trisomy 13 placentas in relation to diagnosis of PE.

Design: Eighteen Trisomy 13 placentas were selected from the archives of Brigham and Women's Hospital based on fetal or placental karyotype. Fourteen of these cases were second trimester specimens, with 12 being terminations for malformation, one a spontaneous loss, and one with a post-partum diagnosis of PE; the remaining 4 were third trimester specimens, all of which were inductions for PE. Twenty-one second trimester non-Trisomy 13 placentas without history of PE were used as controls: these included 7 Trisomy 21, 2 Trisomy 18, and 12 placentas with normal karyotypes. In addition, 4 (2 second and 2 third trimester) placentas with history of PE were used as positive control. One representative section of placental parenchyma from each case was stained with an antibody to sVEGFR1 (Abcam, 1:400 dilution), and scored as 1+ (<5%), 2+ (5-25%), 3+ (25-50%), or 4+ (>50%), based on percent syncytiotrophoblastic staining, blinded to history and karyotype.

Results: All 9 placentas with diagnosis of PE, including the 5 Trisomy 13 placentas, showed terminal villous hypermaturity characteristic of PE, as well as strong (3+ or greater) staining for sVEGFR1. In addition, while only 1 of the 21 second-trimester control placentas had any sVEGFR1 staining, 4 of the 14 second-trimester trisomy 13 placentas showed strong (3+ or greater) staining. Of these four cases, one had a post-partum diagnosis of PE, confirmed by histologic changes of hypermaturity in the placenta; the remaining 3 cases had foci of hypermaturity, which correlated with areas of sVEGFR1 overexpression.

Conclusion: sVEGFR1 overexpression strongly correlates with the clinical diagnosis of PE in both Trisomy 13 and non-Trisomy 13 cases. In a small but significant percentage of Trisomy 13 placentas, sVEGFR1 is overexpressed by hypermature villous syncytiotrophoblasts in the absence of clinical PE, suggesting this to be a precursor to disease onset.

11 Impaired Placentation In Fetal Alcohol Syndrome

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Background: Intrauterine growth restriction (IUGR) is one of the key features of fetal alcohol syndrome (FAS), and IUGR can be mediated by impaired placentation. One of the important events during placentation is expansion of maternal circulation into the placenta through vascular transformation of maternal spiral arteries, which is mediated by extravillous trophoblasts (EVT) that are motile and invasive. Previously we have demonstrated that EVT express high levels of aspartyl-(asparaginyl) beta-hydroxylase (AAH), which has a critical role in cell motility and invasion. The present study examines the hypothesis that ethanol impaired placentation is mediated by inhibition of AAH expression in trophoblasts.

Design: Pregnant Long Evans rats were fed isocaloric liquid diets containing 0% or 37% ethanol by caloric content. Placentas harvested on gestation day 16 were used for histopathological, mRNA, and protein studies to examine AAH expression in relation to vascular transformation of maternal spiral arteries and ethanol exposure.

Results: Chronic ethanol feeding prevented or impaired the vascular transformation of maternal spiral arteries required for adequate placentation. This finding was associated with significantly reduced levels of AAH expression by Western blot analysis and immunohistochemistry. Comparative analysis of AAH expression in the mesometrial triangle and labyrinthine region of placenta by real-time quantitative RT-PCR demonstrated significantly reduced levels of AAH mRNA levels in ethanol-exposed placentas, particularly within the mesometrial triangle which corresponds to deep placental bed. Finally, ELISA studies confirmed the results obtained by Western blot analysis and demonstrated significantly reduced levels of AAH immunoreactivity in both the mesometrial triangle and labyrinthine regions of ethanol-exposed relative to control placentas.

Conclusion: Ethanol-impaired placentation is associated with inhibition of AAH expression in trophoblasts. This effect of chronic gestational exposure to ethanol may contribute to IUGR in FAS.

12 Placental Pathology In Mouse Model Of Thrombophilia Due To Factor V Leiden And High Levels Of Factor IX

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Background: Inherited thrombophilia has been associated with pregnancy loss and other adverse pregnancy outcomes, but the effects of genetic thrombophilia on placental histology remain controversial. Both Factor V Leiden (FVL) and elevated levels of factor IX affect ~5% of the general population and ~20% of thrombophilic patients. Crossing heterozygous FVL mice with mice transgenic for Factor IX (tFIX) results in a model of thrombophilia where the most affected genotype (FVL(+)/tFIX) results in fetal death. Since few clinical studies have correlated maternal, paternal and fetal genotypes with placental findings, we chose to characterize placental histology in this animal model.

Design: All mice were on C57Bl/6 background for at least 6 generations. Parents for all breedings were heterozygous FVL and in addition, one parent was tFIX expressing factor IX at 100-200% above normal levels. Pregnancies were interrupted at E16.5, and placentas collected. Fetal genotype was obtained from PCR performed on the yolk sac. Paraffin

embedded placental tissue was examined using H&E and fibrin stains. The maternal tissues, fetal vasculature of the chorionic plate, and labyrinth were examined for evidence of thrombosis, fibrin deposition or other circulatory pathology. Lesions within the labyrinth were recorded on a semiquantitative scale: 0 = none, 1 = <5 foci, 2 = 5-10 foci, 3 = >10 foci. Statistical analyses were performed using SPSS.

Results: 52 placentas were examined from 13 litters. The litter size ranged from 2 to 10 pups (mean 4.28). There were 12 fetuses with the most severely affected genotype (FVL(+)/tFIX), and all of their placentas showed numerous fetal vaso-obliterative lesions in the labyrinth. The lesions consisted of occlusion of the fetal circulation associated with karyorrhectic debris, red cell fragmentation, and mineralization of the surrounding stroma. These changes were also seen in placentas from fetuses with other genotypes (N=40), but significantly less frequently (p<0.0001). Mural fibrin deposition in maternal vascular walls was seen more frequently in association with maternal FIX overexpression than paternal (16/25 vs 9/27, p<0.03). Occlusive maternal vascular thromboses were seen in 6 placentas, 3 each associated with maternal or paternal FIX overexpression.

Conclusion: These data suggest that both parental and fetal genotypes for FVL and FIX contribute to the overall placental histology associated with genetic thrombophilia. This model may provide opportunities to better understand thrombophilia-related complications in humans, and test possible therapeutic interventions.

13 Fetal Gonadoblastoid Testicular Dysplasia: A Focal Failure Of Testicular Development

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Background: Fetal gonadoblastoid testicular dysplasia (FGTD) is a rare type of testicular malformation, described originally in association with hydrops fetalis and other malformations. FGTD has been identified in patients with Walker-Warburg syndrome (WWS), and it was suggested that it could even be a marker of this disorder. FGTD's phenotype strongly resembles gonadoblastoma, with large, malformed cords or nodules containing Call-Exner-like bodies.

Design: We describe two perinatal autopsy patients with both FGTD and hydrops fetalis, providing a detailed topographical, histological and immunophenotypical analysis. A review of all five previously described cases is conducted.

Results: In both cases, histology of the testicular parenchyma revealed a crescent-like zone between the albuginea and the normal testicular cords, with several epithelial solid/compact nodules containing Call-Exner-like bodies. The cells within them predominantly resembled Sertoli cells and germ cells, expressing inhibin and placental alkaline phosphatase (PLAP), respectively. A third cell type with immature characteristics, lacking the expression of both immunohistochemical markers, was also present. In one case, FGTD was associated with non-specific brain lesions and a myopathy that did not fit the diagnosis of WWS; on the other, the muscle phenotype was completely unremarkable.

Conclusion: FGTD is a rare and peculiar lesion of the fetal and newborn testis that should be recognized due to its resemblance to gonadoblastoma, and its association with other abnormalities, mainly WWS. Distinction from gonadoblastoma must be based on the lack of association

with intersexual states or gonadal dysgenesis, which usually accompany the latter, and also based on the focal pattern of FGTD. Although the association of FGTD and WWS was present in 4 out of the 5 cases studied in literature, we have not confirmed this observation, although a non-specific muscle disorder was found in one of our examples. The features of this lesion support a focal defect in testicular developmental as its most likely pathogenesis. For its mechanism of formation, we favor the notion that groups of immature celomic cells, represented by the undifferentiated cell type described above, have failed to differentiate normally into sex cords after they have invaded the testicular blastema.

14 The Expression Of cFLIP In Human Fetal Testis

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Background: The fetal and neonatal development of male germ cells is a dynamic and complex process. Testicular germ cell proliferation and differentiation must be tightly regulated and balanced by apoptosis during development. Cellular caspase-8 (FLICE)-like inhibitory protein (cFLIP) was originally identified as an inhibitor of death-receptor signaling through competition with caspase-8 for recruitment to Fas-associated via death domain (FADD). cFLIP is expressed in a variety of normal cells. Recently, it has been shown in mice that cFLIP is expressed in fetal testis and it plays a role in protecting gonocytes from Fas-dependent apoptosis. In the present study we evaluated the expression of cFLIP in human fetal testis.

Design: Approval of the Institutional Research Committee was obtained for this study. Formalin-fixed, paraffin-embedded fetal testis tissue blocks (40 cases) were retrieved from pediatric autopsy archives. The gestational age ranges from 15 to 38 weeks. Cases with chromosomal abnormalities or urogenital malformations were excluded from the study. cFLIP expression was analyzed by immunohistochemistry.

Results: cFLIP expression was detected in Leydig cells and germ cells. The expression was mainly in the cytoplasm. In Leydig cells, the expression of cFLIP was detected as early as 15 weeks and remained constant up to 38 weeks. In contrast, in germ cells, cFLIP expression was not detected until 22 weeks. The relative number of cFLIP-positive germ cells and the staining intensity showed an increasing trend with advancing gestational age. At 36 weeks, when cFLIP expression was the highest, nuclear staining was also observed in germ cells. Sertoli cells were negative for cFLIP at all gestational ages.

Conclusion: The gestational age-dependent cFLIP expression in testicular cells in the current study is comparable to findings in the mouse. In humans, testicular apoptosis starts as early as the first trimester of pregnancy. It occurs in all cell types and continues throughout gestation. Apoptotic cell death is highest in the Leydig cells, followed by germ cells and is very low in Sertoli cells. There seems to be a positive correlation between cFLIP expression and apoptosis in terms of cell distribution and developmental age. The results of the present study suggest a possible role for the anti-apoptotic gene cFLIP in regulation of programmed cell death in testis development.

15 Expression Of Polyductin, The PKHD1 Gene Product, During Normal And Abnormal Fetal Development Of The Intrahepatic Biliary System, Biliary Atresia, Paucity Of The Intrahepatic Biliary System And Liver Tumors

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Background: PKHD1, the ARPKD gene, encodes multiple alternatively spliced transcripts predicted to generate membrane-bound and secreted proteins. The longest open reading frame encodes polyductin (fibrocystin), a putative 4074 amino acid protein with a single transmembrane domain and an intracellular C-terminus. FP2, fusion protein 2, has been used to detect polyductin (FP2pAb). The aim of this study is to investigate polyductin expression in the normal (ductal plate, remodeling ductal plate, remodeled bile ducts) and abnormal development of the intrahepatic biliary system, biliary atresia, paucity of the intrahepatic biliary system and liver tumors.

Design: 19 fetal samples including normal human liver tissue and tissues with ductal plate malformations in Meckel-Gruber-Syndrome, Caroli syndrome and chromosomal anomalies were collected. 5 Neonatal and 56 infantile samples were also studied including 20 cases of paucity of intrahepatic bile ducts of different etiologies, 8 cases of biliary atresia, 9 cases of neonatal hepatitis and 2 cases of congenital retardation of bile duct development. Additional cases included metabolic diseases, drug-induced liver disease cases and cardiovascular disease with hepatic involvement. 11 hepatocellular (HCC) and 5 cholangiocellular carcinomas (CCC) were also studied. Institutional review board approval was granted.

Results: We found polyductin expression in the early ductal plate stage and ductal plate malformation. Remodeling ductal plate was inconsistently positive, but there was no apparent expression in the stage of remodeled bile ducts. FP2 staining was observed in neonatal hepatitis, biliary atresia and also in paucity of intrahepatic bile ducts. Another interesting finding was the weak expression of polyductin in hepatocytes. FP2 staining was constantly positive in CCC, but was absent in HCC.

Conclusion: Polyductin expression is a regular feature of bile duct epithelium in the early stages of human development. Later, polyductin presence in such cells is found in pathological conditions, showing fibrous and cystic changes as well as cholestasis, and affecting bile ducts and hepatocytes. Additionally, polyductin could be a marker to differentiate cholangiocellular from hepatocellular carcinomas.

16 The Number Of NeuN Positive Ganglion Cells Is Increased In Pediatric Colon Diseases

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Background: NeuN is present in most neuronal nuclei of the central nervous system and some enteric ganglion cells (GC). In our first study, we quantified NeuN positive GC in normal colon specimens of pediatric patients. We identified a subset of GC and constant NeuN expression throughout postnatal colon development. Some have shown chemical coding shifts in GC of patients with inflamed bowel. We hypothesize that numbers of NeuN positive GC change in pathologic (P) conditions.

Design: Pediatric patients with colonic abnormalities were grouped as follows: IBD (n=5): inflammatory bowel disease, DFC (n=5): defunctionalized colon, NEC (n=6): necrotizing enterocolitis, PO (n=5): pseudoobstruction. GC were identified by cytologic features and double-immunostaining with anti-NeuN and S-100 antibodies. Controls were

adequate. NeuN+ and total number of GC were counted in submucosal (SM) and myenteric (MP) plexi. Density was calculated as mean NeuN+ GC/cm, and proportion as mean NeuN+ GC/NeuN- GC. Statistical analyses between P group and normal (NL) and aged-matched GC counts and within P were done using paired t-test and ANOVA.

Results:

Table 1: Overall mean, density and proportion of GC in P vs NL (An asterisk indicates a statistically significant result at a p-value less than 0.05).

	Myenteric Plexus		Submucosal Plexus	
	Normal	P group	Normal	P group
NeuN+ / cm	12	15	19*	37*
NeuN- / cm	94	125	55	62
NeuN+ / NeuN-	0.11	0.14	0.27*	0.56*

Table 2: Mean, density and proportion of GC by specific abnormality vs NL

	NeuN+/cm		NeuN-/cm		NeuN+/NeuN-	
	MP	SM	MP	SM	MP	SM
IBD/ NL	7/3*	13/8	40/23	48/29	0.19/0.18	0.28/0.30
DFC/NL	16/8	56/8*	128/57	80/34*	0.14/0.13	0.72/0.30*
NEC/NL	30/33	52/31	246/186	84/73	0.16/0.18	0.63/0.42
PO/ NL	6/9	20/13	56/63	37/40	0.12/0.13	0.53/0.29

Twenty-one percent (2456/11609) of GC were NeuN+ in P compared to 17% in NL (p<.001). MP and SM NeuN+ GC were significantly less than NeuN- GC in P and NL. Proportion and density of NeuN+GC in SM were significantly different in NL and P (Table 1). While the proportion of NeuN+/NeuN- GC in MP was similar in P, this was increased in SM of DFC, NEC and PO compared to NL. Conversely, IBD NeuN+ GC were significantly increased in MP compared to NL (Table 2).

Conclusion: Similarly to our previous findings, the number of NeuN+ GC was significantly lower than NeuN- GC. However, the number of NeuN+ GC was increased in P compared to NL, particularly in the SM compartment of DFC, NEC, and PO, while IBD showed increases in NeuN+GC in MP. DFC, NEC and PO are characterized by decreased motility functions versus IBD, thus we speculate the observed phenotypic shift may signify a role for NeuN in regulation of colonic motility.

17 Topographic Variability Of Acetylcholine Esterase (ACE) Histochemistry In Distal Rectal Mucosal Biopsies From Short Segment (SS) And Total Colonic (TC) Hirschsprung Disease (HD) And Controls.

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Background: Topographic variability of ACE histochemistry in rectal suction biopsies for diagnosis of HD has not been systematically studied.

Design: We retrospectively examined the ACE stained slides from distal rectal mucosal specimens in 36 consecutive cases of short segment HD (age 2d-2m), 8 cases of total colonic aganglionosis (age 1d-72m) and 45 normal controls (33/45<12 mo). Aggregate specimen length evaluated in SSHD, TCHD, and control cases was 115mm, 42mm, and 136mm respectively. A representative section from each specimen was evaluated in 1 mm segments for abnormal patterns in the lamina propria (LP), muscularis mucosa (MM), multiple small submucosal nerves (MSSN) and prominent submucosal nerves (PSN) defined as > 35µm in diameter. The fraction of 1mm segments showing each pattern, and the most common aggregate patterns were tabulated and compared.

Results: Mean length was 3 mm in each group (range 1mm-10mm). In SSHD 100/115mm from 33 patients had diagnostic nerve twigs in the MM, PSN or both. A diagnostic MM pattern was seen in at least one 1mm segment in 27/36 patients. Prominent aganglionic LP nerves alone were seen in 31/115 with SSHD compared to 11/136 controls (p<.001). MSSN occurred in 89/115 segments, including 8/15 nondiagnostic segments, vs 54/136 (p<.001) controls. In both forms of HD, subtle abnormalities in the ACE staining in the MM of otherwise nondiagnostic segments were helpful in difficult cases. Specimens 2mm or less were over-represented in problem cases. One segment had a transition zone pattern. Rebiopsy was requested in 3 patients to confirm HD. In TCHD, the diagnostic pattern in the MM occurred in 31/42 mm, similar to SSHD, but PSN were less common (8/42 vs 75/115), p<.001. Prominent LP nerves were more common in TCHD, 19/42 vs 31/115, perhaps due to greater mean age at biopsy. ACE was inconclusive in 4/45 controls with normal ganglia.

Conclusion: ACE histochemistry on frozen sections of distal rectal mucosa is a reliable indicator of HD but topographic variability may be challenging, especially in specimens equal or <2mm in length. Non-diagnostic and/or transition zone pattern was observed in 15/27 1mm segments from 7 patients with SSHD, and in 6/42 segments from 2 patients with TCHD.

18 Tissue Transglutaminase And Endomysial Antibody Correlation With Degree Of Intestinal Damage In Pediatric Celiac Disease At Diagnosis

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Background: Children with diabetes mellitus (DM) have a higher frequency of celiac disease (CD). Tissue transglutaminase (TTG) and endomysial antibody (EMA) tests boast high sensitivity and specificity in screening for CD. Also used to monitor compliance with a gluten-free diet, TTG and EMA have been shown to be less reliable in adults with mild enteropathy but no such studies exist in children. The aim of this study was to evaluate the correlation between values of TTG and EMA with different degrees of intestinal damage in CD children with and without DM.

Design: Retrospective review of patients seen at The Children's Hospital of Philadelphia between 1/1/2002 and 6/15/2006 revealed 60 children (46 girls and 14 boys; mean age 9.8 years; range, 1.6-18.2 years) who had TTG and EMA performed at time of histologic CD diagnosis from endoscopically obtained small intestinal biopsies. 21 of 60 children had DM. All children were stratified for histologic damage according to Marsh classification and villous-to-crypt ratio (VCR).

Results: Marsh (M) I lesions were present in 2 children (3.3%), MII in 2 (3.3%), MIIa in 14(23.3%), MIIb in 15(25%), and MIIc in 27(45%). Biopsies of 15/60 (25%) children showed variable Marsh (M) histology. TTG testing was positive in all and EMA testing was positive in all but one child with CD. Villous blunting, defined as VCR < 2.5, was seen in 57 children (95%). Among all children with CD, mean TTG values were 96 U in MIIa, 277 U in MIIb, and 415 U in MIIc. Mean TTG values were lower in MIIa versus MIIc ($P<0.001$). Mean dilutions of +EMA were 197 in MIIa, 302 in MIIb, and 466 in MIIc. Mean dilutions of +EMA were lower in MIIa versus MIIc ($p<0.001$). Among children with CD but no DM, mean TTG values were 79 U in MIIa, 295 U in MIIb, and 385 U in MIIc. Mean TTG values were lower in MIIa versus MIIc ($p<0.01$). Mean dilutions of +EMA were 173 in MIIa, 318 in MIIb, and 464 in MIIc. Mean dilutions of +EMA were lower in MIIa versus MIIc ($p<0.01$). Among children with CD and DM, mean TTG values were 120 U in MIIa, 204 U in MIIb, and 477 U in MIIc. A trend toward lower mean TTG values was seen in MIIa versus MIIc ($p=0.06$). Mean dilutions of +EMA were 230 in MIIa, 240 in MIIb, and 469 in MIIc. A trend toward lower mean dilutions of +EMA was seen in MIIa versus MIIc ($p=0.10$).

Conclusion: TTG and EMA mean serum values are higher in CD children with severe enteropathy (MIIc) than in those with mild enteropathy (MIIa). Diabetic CD children did not appear to have as close correlation of histology with serology as did non-diabetic CD children but this may be due to sample size.

19 Validation Of A Novel Interphase FISH Method For Detection Of "Triple Trisomy" In Pediatric Precursor B-Cell ALL

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Background: Cytogenetic results in precursor B-cell acute lymphoblastic leukemia (pre-B ALL) are critical for prognosis and risk-adjusted therapy. Nonetheless, these analyses remain challenging due to difficulties in obtaining sufficient numbers of high quality metaphase cells. We routinely use an interphase FISH panel to detect clinically significant chromosome abnormalities in pre-B ALL, that includes probes to identify rearrangements of MLL, BCR-ABL, ETV6-RUNX1 and 9p21. About 1/3 of pediatric pre-B ALLs exhibit high-hyperdiploidy (>53 chromosomes), which is generally linked to a good prognosis. A subset with trisomies of chromosomes 4, 10, and 17 ("Triple Trisomy", TT) has an even more favorable prognosis, requiring less intensive treatment (Leukemia 19:734, 2005). We have validated a tricolor probe mixture to simultaneously detect TT to add to our current pre-B ALL FISH panel.

Design: The TT probe mixture consists of 3 centromeric probes (Vysis, Inc) labeled in different colors as CEP 4 (Orange), CEP 10 (Green) and CEP 17 (Aqua), and mixed in a ratio of 1:1:3, respectively, to optimize signal intensity. Deidentified test slides from 25 pre-B ALL patients, previously analyzed by karyotype and the original ALL FISH panel, were hybridized with the TT probe mixture using a standard FISH procedure. Two observers scored a minimum of 50 nuclei for each case.

Results: Successful hybridization was achieved for all patients' samples with good agreement between the two scorers. Careful reading was required because of a tendency for the centromeric probes to show diffuse patterns, and the sometimes dim chromosome 17 signals (Aqua). Of the 25 patients, 10 were diagnosed as high-hyperdiploid; 7 showed a TT hybridization pattern and 3 showed trisomy for only two of the three chromosomes. Two

cases, diagnosed with near-tetraploidy (>85 chromosomes), had corresponding, complex, hybridization patterns, usually with four signals per probe. The remaining 13 cases displayed primarily diploid patterns for the probes. All of the TT FISH results matched the prior karyotype findings.

Conclusion: The simultaneous detection of "Triple Trisomy" can be performed by interphase FISH using a tricolor probe mixture. Scoring of nuclei must be done with caution and for this reason we recommend that two observers score each case. The lower limit of sensitivity remains to be determined before its application to the detection of minimal residual disease. We are implementing its use on diagnostic specimens as an addition to our current pre-B ALL FISH panel where it is a useful screen to stratify patients for COG protocols.

20 Application Of An RT-PCR Assay For Tyrosinase To Sentinel Lymph Node Biopsies In Pediatric Melanoma Patients

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Background: Sentinel lymph node biopsy (SLNB) has become an integral part of the staging of patients with melanoma. A positive SLNB confers a poor prognosis and requires additional surgical procedures such as complete lymph node dissection. Current protocols use histology and immunohistochemistry to detect metastases, but do not typically include more sensitive techniques such as RT-PCR for melanocyte transcripts. The current study applied such a molecular technique to paraffin-embedded tissue (PET) in a series of SLNB from pediatric patients with melanoma, and the results were compared to those using standard morphological techniques.

Design: Patients with a confirmed diagnosis of melanoma and subsequent SLNB were included in the study. SLNB were examined histologically and immunohistochemically for S100, tyrosinase and MART1 at three levels using standard techniques. Total RNA was extracted from paraffin embedded tissue using a modified version of the TRIzol method. RNA was reverse transcribed and then amplified by PCR using nested primer sets that span exon 2 and 3 of the TYR gene (tyrosinase).

Results: Thirty-six sentinel lymph nodes from eight patients were included in the study. The study group included 4 males and 4 females with an age range of 2y10m to 14y2m. Sites of involvement included head and neck (n=3), trunk (n=3) and extremities (n=2). Seven patients had between 1 and 4 histologically and/or immunohistochemically-positive sentinel nodes and one patient had a negative SLNB (HISTO+; 12/36 [33%] nodes in total). Three nodes were excluded from molecular analysis due to inadequate RNA, leaving 33 for molecular analysis. Of the 33, 29 were positive (MOL+; 88%). In total, 12 were HISTO+/MOL+; 17 were HISTO-/MOL+; 4 were HISTO-/MOL-; and none were HISTO+/MOL-.

Conclusion: The current study is the first to apply a molecular assay for tyrosinase to SLNB from pediatric melanoma patients. The application of this technique to PET significantly increases the detection rate of metastases and changed the status of one patient from negative to positive. Furthermore, the use of PET avoids the need to sacrifice tissue for freezing prior to histological assessment. The clinical impact of adding such a sensitive molecular assay to the protocol of SLNB deserves further study.

21 Childhood Primary Focal Segmental Glomerulosclerosis Variants

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Background: The recently proposed Columbia classification scheme for FSGS variants has not been applied to pediatric patients.

Design: Results from a 12 year retrospective review of patients with primary FSGS at presenting 18 years or younger were separated into the 5 proposed categories. A global chronicity score (CS) was calculated based on arteriosclerosis, glomerular fibrosis, interstitial fibrosis, and tubular atrophy (max=16). Blood pressure, serum albumin, proteinuria, and GFR were compared.

Results: 50 children, 80% African American (AA) 20% Caucasian, were categorized into NOS (21, 42%, CS 4.3), cellular (13, 26%, CS 4.5), and collapsing (16, 32%, CS 6.4). CS was significantly higher in collapsing vs. cellular or NOS ($p < 0.02$). 41 children (80%AA) had initial and follow-up (1-17 yrs, mean 3.9 yrs) clinical data.

Category	n	SBP	SBP %	DBP	DBP %	GFR	SBP	SBP %	DBP	DBP %	GFR
Collapsing	10	¹ 133	96	79*	90*	² 89*	130	90	77	82	65*
Cellular	13	122	81	75	80	108	133	93*	80	84	90
NOS	18	¹ 117*	83	67*	70*	² 116*	120	77*	72	73	109

¹ initial ; ² last follow-up; * significance ($p < 0.04$)

SBP = systolic BP; SBP% = SBP percentile; DBP = diastolic BP; DBP% = DBP percentile.

SBP and DBP were significantly higher initially for collapsing vs. NOS, GFR was significantly lower for collapsing and NOS initially and at last follow-up, and DPB %tile was significantly higher in the cellular variant vs. NOS at last follow-up. No significant differences were found in initial or follow-up serum albumin levels. Racial variant distributions were similar. At last follow-up, SBP, SBP%, DBP, DBP% and urine protein trended higher and the GFR trended lower in AA, but differences were not significant (data not shown).

Conclusion: NOS lesions are the most common FSGS variant in children, as reported in adults (42% vs. 42%) but collapsing and cellular lesions were more frequent than in adults (32% vs. 11%, 26% vs. 3%). No tip or purely hilar lesions were present in our population. Lack of tip lesions may be due to either the young age, or AA predominance in our population, in which reportedly tip lesions are unusual. Collapsing variant reportedly is more frequent in AA and in younger patients, and this may explain the prevalence in our study. CS scores were significantly higher in the collapsing group. Biopsies of children with FSGS may have a different spectrum of lesions than adults. Race seemed not to have significantly affected the type of variant or clinical condition at last observation. Different variants may have clinical significance.

22 Localized Islet Cell Nuclear Enlargement In Congenital Hyperinsulinism: A Distinct Clinicopathologic Entity

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Background: Congenital Hyperinsulinism (HI) typically presents in early infancy with hypoglycemia. Histologic and genetic evaluation of pancreatic tissue has led to the classification of two forms of HI: focal and diffuse.

Design: All pancreatectomy specimens from HI patients who underwent surgery between 1998-2006 were reviewed. The presence of adenomatous hyperplasia of endocrine tissue was classified as the focal form of HI while enlargement of islet cell nuclei throughout the pancreas was classified as the diffuse form. Results were correlated with mutation and/or loss of heterozygosity analysis in the ABCC8 and KCNJ11 genes.

Results: Most patients were easily classified into one of these two categories. However, six patients showed a third histologic pattern consisting of localized islet cell nuclear enlargement (LINE). Their pancreata showed fairly well defined area(s), confined regionally within the pancreas, where otherwise normal islets contained cells with abnormally large nuclei. Multiple samples elsewhere in the pancreas showed no islet cell nucleomegaly or adenomatous hyperplasia. This patient group was also clinically distinct. The median age at presentation was older for patients with LINE (150 days) than patients with classic diffuse (1 day) or focal (1 day) disease. In addition, 5/6 patients with LINE had cured or controlled disease postoperatively, despite only partial pancreatectomy in 3 of these patients. One patient still requires further follow-up to determine postoperative status. Molecular analysis to date has failed to reveal any of the known mutations associated with focal or diffuse disease in these patients, and preliminary studies for loss of heterozygosity are negative.

Conclusion: We present a third clinically, histologically and potentially genetically distinct group of patients with HI. We suggest the term: Localized Islet cell Nuclear Enlargement (LINE) to differentiate it from the focal and diffuse forms of HI. Although histologically this lesion resembles the diffuse form of HI, these pancreata are distinguished by the regional confinement of nucleomegaly and lack of nuclear enlargement throughout the non-lesional pancreas. Further studies, including laser capture microdissection of the abnormal islet cell nuclei, are needed to define the genetic basis of this lesion.

23 Classification Of Diffuse Lung Disease In Older Children And Adolescents: A Multi-Institutional Study Of The Children's Interstitial Lung Disease (Child) Network

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Children's Interstitial Lung Disease (chILD) Network

Background: The Children's Interstitial Lung Disease Network has recently developed a clinicopathologic classification of pediatric diffuse lung disease and consensus terminology to describe the clinical presentation, spectrum of disease, and outcome for infants (age < 2 years) who came to lung biopsy. Using the same multi-institutional collaborative approach, this classification has been applied to biopsies from older children (2-18 years).

Design: Clinical data and histologic slides were reviewed for all patients in this age group with wedge biopsies for diffuse lung disease from 12 institutions over a 4 year period.

Results: Of 205 total, 101 biopsies (49.3%) were from immunocompetent patients, and data are reported for this group only. The major diagnostic category was immune-mediated disease (n=34) including 14 capillaritis, 2 Wegener granulomatosis, 16 collagen vascular disease, and 2 acquired pulmonary alveolar proteinosis. Non-immune mediated vascular disease was frequent and included congestive vasculopathy secondary to cardiac disease (5),

veno-occlusive disease (4), lymphangiomatosis (2), and plexogenic arteriopathy (1). Acute infectious processes (10) included 2 bacterial, 1 viral, and 7 granulomatous. Other conditions included post-infectious constrictive and obliterative bronchiolitis, acute and organizing diffuse alveolar damage (3), eosinophilic pneumonia (3), hypersensitivity pneumonitis (2), aspiration (2), and idiopathic pulmonary hemosiderosis (2). The diseases prevalent in infancy were uncommon in the older children, including abnormal lung growth due to hypoplasia, prematurity, or chromosomal syndromes (4), neuroendocrine cell hyperplasia of infancy (3), and genetic disorders of surfactant metabolism (1). Other uncommon entities included storage disease (1), sarcoidosis (1), EBV-associated lymphoproliferative disorder (1), myofibromatosis (1), cystic fibrosis (1), and Marfan syndrome-associated emphysema (1). Onset of symptoms was after 12 months of age in the majority of patients (75%); biopsy was usually within 2 years of symptom onset. Morbidity and mortality were variable with 43 asymptomatic at follow-up, 1 with lung transplant, and 15 deaths.

Conclusion: Immunocompetent children (aged 2-18) with diffuse lung disease have a distribution of histologic patterns which is distinct from both infants and adults. Funded by: Rare Lung Disease Consortium, NIH RR019498.

24 Detection And Evaluation Of Retinal Hemorrhages In Children By Postmortem Monocular Indirect Ophthalmoscopy

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Background: Retinal hemorrhages (RHs) are inextricably linked to the shaken baby/shaking-impact syndrome. RHs associated with accidental head injuries and other causes are described as rare, and if present, few in number and confined to the posterior pole of the retina. Previous autopsy studies have relied on ocular enucleation to demonstrate retinal hemorrhages; however, while this procedure is done in almost all fatal suspected child abuse cases it is infrequently undertaken in non-abuse childhood deaths. Postmortem monocular indirect ophthalmoscopy (PMIO) readily detects RHs permitting subsequent evaluation.

Design: From June 2004 through July 2006, an observational study using PMIO was conducted to detect and evaluate retinal hemorrhages in deceased individuals. Examined decedents were consented full and restricted hospital autopsies and medical examiner cases (complete autopsy or external examination) at Wake Forest University Baptist Medical Center in Winston-Salem, North Carolina.

Results: Fundi of 180 children less than 15 years of age were examined with PMIO. Deaths were partitioned into age groups corresponding to fatality categories of the National Center for Injury Prevention and Control. In 9 children, fundi were not visualized; however, of the 171 childhood fatalities in which PMIO was successful 37 or 21.6% had retinal hemorrhages (RHs). No documented clinical fundal examinations were present in children with RHs who died in the hospital unless child abuse was suspected or confirmed. Four children, 10-14 years of age, had RHs detected by PMIO - none due to child abuse. Five children, 5-9 years of age, had RHs detected by PMIO. All deaths with RHs were associated with accidental traumatic brain injury (TBI). Five children, 1-4 years of age, had RHs. Causes of death were TBI (4) and obstructed airway/VACTERL association (1). Three of the head injuries were from child abuse and one was accidental. Ten neonates had RHs. The causes of death were TBI - child abuse (3), congenital heart disease (1), in-utero SDH/ICH (1), GBS meningitis (1), disseminated HSV-2 (1), pulmonary hemorrhage/trisomy 11q (1), trisomy

13 (1) and undetermined/liveborn delivery (1). Fifteen infants had RHs. Causes of death were TBI - child abuse (5), SIDS (5), suffocation (1), asphyxia (1), overlying (1), sepsis/prematurity (1) and undetermined (1).

Conclusion: Non-abuse RHs in children are more frequent than previously reported in autopsy series. Documented clinical fundal examinations are rare in children who die with RHs unless child abuse is suspected. Future investigations with PMIO are needed for an expanded evaluation of conditions associated with RHs in non-abuse childhood deaths.

POSTER PRESENTATIONS

25 Recurrent Pregnancy Loss Evaluation: The Role Of The Community Pathologist In Initial Placental And Fetal Examination, The Urgent Need For Standardized Protocols

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Background: The evaluation of recurrent pregnancy loss involves a multidisciplinary approach with the obstetrician, endocrinologist, pediatric pathologist and genetic counselor each playing an important role. Many pregnancies are managed in smaller community hospitals. Thus, the products of conception, placentas and/or fetus are examined at the community hospital by adult pathologists. While performing an excellent job, these pathologists are handicapped by the lack of a universal standardized format to assist the examination. This lack of a standardized format leads to several deficiencies in the assessment of the placenta and/or fetus. Important information that is not recorded may cause difficulty when pregnancy loss is being evaluated at a later time.

Design: We examined the pathology reports, charts and laboratory results of 18 patients with recurrent pregnancy loss, focusing on the placental and/or fetal examination. All cases had at least one pregnancy loss initially evaluated at a community hospital without on-site access to a pediatric or perinatal pathologist. We looked at whether gross photographs had been obtained, whether tissue was saved for further examination in appropriate media and whether a detailed format for placental examination was used. We also noted any significant pathologic finding.

Results: Of 18 patients with recurrent pregnancy loss, four had stillbirths with fetuses available for pathology examination. Three (3/18) were late third trimester losses while one (1/18) was a second trimester loss. Photographs of the fetuses were not available in any of these 4 cases. None of the placentas reviewed recorded a "trimmed" weight. There was no protocol followed for placental examination. Tissue was not saved frozen, in EM fixative or otherwise in any of the fetal autopsies. A pediatric pathologist expert opinion was not sought at the time, in any of the cases. Of the significant changes, one placenta had hemorrhagic endovasculitis and an associated single umbilical artery, five placentas showed acute membrane inflammation, two showed chorangiosis, one had hypertrophic decidua vasculopathy and one showed diffuse villous mineralization. Many patients had more than one pregnancy loss (products of conception and placentas) evaluated.

Conclusions: Many pregnancies are followed and delivered at smaller community hospitals. Non pediatric pathologists often do not take photographs (external or internal) of

fetuses or follow a specific protocol for examination of placentas. It is imperative that we have a standardized protocol including gross placental and fetal examination that can be disseminated to community pathologists to ensure an acceptable level of examination of placentas and fetuses. This should result in a clearer understanding of any pathology that may have contributed to pregnancy loss, and give the reviewing pediatric pathologist and the obstetrician an opportunity to appropriately manage the patient with recurrent pregnancy loss to eventual successful pregnancy and childbirth.

26 What Is Adequate Sampling Of Placental Membranes? A Prospective Randomized Analysis.

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Background: It is the generally accepted practice to submit one or two membrane rolls when examining placentas. We queried whether obtaining additional sections would increase diagnostic yield and if so, to what degree.

Design: In this prospective study, a membrane roll section was taken from each quadrant of its respective singleton placenta and examined histologically. These placentas were submitted for routine pathologic examination, the process of which was entirely separate from this study. After accrual of four membrane rolls from each of 38 placentas, the sections were randomized and assigned new numbers, thereby blinding the pathologist as to the placenta of origin. After review, the sections and their accompanying diagnoses were re-assembled with their respective placentas. We evaluated the incidence of acute chorionitis/chorioamnionitis (ACC) and hypertensive arteriopathy (HA) when only one, two, three or all four slides were examined. The diagnostic yield from all possible combinations of single slides, pairs and triplets of slides was tabulated. When the second, third or fourth slide identified a more extensive pattern of membrane acute inflammation than what was demonstrated on the initial slide, the ACC diagnosis was considered upstaged.

Results: The gestational ages of 38 placentas ranged from 24 to 41 weeks. When one membrane section was examined, 6-8 placentas had ACC. When two slides were examined, 7-12 placentas had ACC. With three slides, ACC was found in 11-14 placentas. All four slides identified ACC in 15 cases. The range in number of placentas with ACC is due to the plurality of combinations possible when reviewing single, paired and triplet slides. Additional sections upstaged the ACC diagnosis infrequently. Examination of three more slides upstaged ACC in 2/15 cases. Four of 38 placentas had HA, based on review of all four slides. Examining just one slide identified 1-2 cases of HA. With the addition of a second and third slide, the number of HA diagnoses increased to 2-4 and 3-4 respectively.

Conclusion: Review of a single membrane roll identified, at most, 53 % of cases of acute chorio(amnio)nititis and 50% of cases of hypertensive arteriopathy. Submitting additional sections increased the yield for both diagnoses in a roughly linear manner. This study does not answer the question of what is the optimal number of membrane sections to submit, but it supports the contention that a single section is inadequate. Whether more than four slides increases diagnostic sensitivity remains untested.

27 Frequency Of Syncytial Knots In The Placenta At Different Gestational Ages

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Background: Syncytiotrophoblastic knots or syncytial knots (SK) are aggregates of syncytial nuclei at the surface of terminal villi. Most SKs are thought to be artifacts from tangential sectioning while the minority are syncytial sprouts or apoptotic knots. However their presence is used to evaluate villous maturity as they increase with increasing gestational age (GA). Furthermore, increased SKs are associated with uteroplacental malperfusion and thus are important in evaluation of placentas in this setting. However, no specific reference values have been developed for the percentage of villi with SKs at various GAs.

Design: From surgical pathology files we obtained 190 normal placentas ranging in GA from 20 to 40 weeks (wks). Those placentas which were 19 4/7 to 20 3/7 were considered 20 wks and so on for each GA. Cases with a history or pathologic evidence of hypertensive disease, malperfusion, intrauterine growth restriction, infection, fetal demise or diabetes were excluded. Using H&E stained sections two pathologists, blinded to gestational age, recorded the number of SK in 100 villi. Values from both pathologists were then averaged.

Results: The average percentage of SKs at each gestational age is given in the following chart:

GA	% SK	GA	% SK	GA	% SK	GA	% SK
20.0	6.9	26.0	10.8	32.0	13.1	38.0	27.7
21.0	5.2	27.0	13.0	33.0	14.7	39.0	28.5
22.0	7.1	28.0	14.3	34.0	18.1	40.0	28.1
23.0	8.0	29.0	13.5	35.0	21.4		
24.0	9.1	30.0	14.7	36.0	22.5		
25.0	7.8	31.0	13.2	37.0	28.6		

As expected, there was a significant positive correlation with GA and %SK (Spearman correlation $r = 0.962$, $p < 0.001$). Term placentas (37-40 weeks) all showed approximately 28% of villi with SK. Interestingly, a drop off to approximately 22.5% was noted at 36 wks, and at 26 to 33 wks SKs varied slightly from 10.8 to 14.7%. Between 20 to 25 wks % SK ranged between 5.2 and 9.1%.

Conclusion: We present a study providing normal reference data for the average % of SKs at GAs ranging from 20 to 40 wks that will facilitate histologic evaluation of malperfusion.

28 Complement Expression In Villitis Of Unknown Etiology

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Background: Villitis of unknown etiology (VUE) is a placental lesion which may be associated with adverse outcome including fetal growth restriction and demise. The etiology is thought to be immune mediated rather than infectious and thus immune complex formation is a proposed mechanism for VUE. A previous study has shown increased deposition of complement in VUE by immunofluorescence. In this study, we evaluate placentas with VUE and normal controls for expression of complement using immunohistochemical methods.

Design: The surgical pathology files were searched for placentas from term deliveries showing moderate to severe VUE. Cases were chosen to exclude those with either a

clinical history or pathologic evidence of infection or growth restriction. 67 cases were identified and compared to 23 normal controls. Immunohistochemical analyses using antibodies to the complement product C3b, a split product formed in the common pathway, was performed on paraffin embedded tissue sections. Intensity of immunoreactivity (0 to 3+) and percentage of cells staining were recorded and an H-score was calculated for each cell type-villous trophoblast (VT) cytoplasm which included cytotrophoblast and syncytiotrophoblast and extravillous trophoblast (EVT) cytoplasm as well as trophoblast basement membrane (BM).

Results: We found evidence of increased complement expression for C3b in the VT cytoplasm (H score: VUE = 101.3 +/- 17, control = 36.3 +/- 28, $p < 0.0001$). Increased expression was also seen in the BM and EVT cytoplasm compared to normal controls but did not reach statistical significance ($p = 0.10$, $p = 0.09$).

Conclusion: This study suggests that complement expression and therefore activation is increased in VUE versus controls. These findings may be important in understanding the mechanism by which VUE leads to villous damage and clinical sequelae.

29 Recurrent Chronic Villitis: Clinicopathologic Implications

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Background: The reported incidence of chronic villitis (CV) ranges from 3 to 20% of placentas examined, with an estimated risk of recurrence as high as 30% for diffuse villitis. While the literature indicates that recurrent CV, when severe, is associated with increased incidence of perinatal morbidity, the perinatal impact of recurrent CV of less severity has not been evaluated in a large group of patients. The aim of our study was to compare perinatal outcomes from pregnancies associated with recurrent and non-recurrent CV ascertained from greater than 10,000 placentas submitted for routine evaluation at a single institution.

Design: This is a retrospective review of placental diagnoses rendered at a county hospital Parkland Memorial Hospital over a seven-year period. Placentas were submitted commensurate with clinical conditions requiring the pediatric resuscitation team to be present at delivery. Patients selected for the study fulfilled two criteria: there were at least 2 pregnancies, and CV was diagnosed in at least one of the placentas from each patient. All placentas had a minimum of two full sections of parenchyma away from the periphery, and each was evaluated histologically by a pediatric pathologist from Children's Medical Center Dallas. Pregnancies were identified as having recurrent or non-recurrent CV. Perinatal outcome measures included prematurity, fetal growth restriction (FGR), Apgars, and meconium staining. Groups were compared using chi-square analysis for differences, with a p-value of < 0.05 defining significance.

Results: There were 10,942 deliveries; 308 women had 2 to 5 repeated pregnancies for a total of 626 analyzed placentas. Sixty-seven of 626 placentas (11%) were diagnosed with CV; 11 of the 308 women (3.6%) had recurrent CV (22 placentas), and 45 of 308 women (14.6%) had non-recurrent CV; 23 diagnosed in the first pregnancy. There was no significant difference between the recurrent and non-recurrent CV groups for the following outcomes: gestational age (38.5 vs. 37 wks, respectively), prematurity (22% vs. 31%, respectively), FGR (4.5% vs. 6.6%, respectively), meconium staining (26.6% vs. 18.8%, respectively), and Apgars (7.1 vs. 7.2 at 1 minute and 8.3 vs. 8.5 at 5 minutes, respectively). The number of placentas

noted to be small for gestational age was similar between the groups.

Conclusion: Our study indicates that, in the setting of routine placental examination in the pathology laboratory, there is no significant difference in perinatal outcome when comparing pregnancies associated with recurrent vs. non-recurrent chronic villitis.

30 Increased Risk Of Pre-Eclampsia In HIV-Infected Pregnant Women On Antiretroviral Therapy Is Not Explained By Altered Fatty Acid Oxidation

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Background: Pre-eclampsia is a multifactorial pregnancy-specific disease. In some cases, severe pre-eclampsia and related diseases, acute fatty liver of pregnancy (AFLP) and hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome have been associated with inherited defects in fatty acid oxidation, especially a deficiency of long-chain 3-hydroxyacyl coenzyme A dehydrogenase (LCHAD). Recently, an unexplained increase in the incidence of pre-eclampsia has been demonstrated in HIV-infected pregnant women on highly active antiretroviral therapy (HAART). We performed this study to determine if the common antiretroviral drugs affect fatty acid oxidation in vitro.

Design: Two normal, one heterozygous LCHAD deficient, and one homozygous LCHAD deficient (positive control) cell lines were exposed to therapeutic concentrations of the antiretroviral drugs: nevirapine, didanosine, lamivudine, and a combination of nelfinavir, zidovudine, and lamivudine (triple therapy). After exposing the fibroblasts to these drugs for varying time periods ranging from 2 to 10 days, we measured the accumulation of 3-hydroxy fatty acids (3-OH-C6 to 3-OH-C18) in the culture media. The fatty acids were measured by stable-isotope dilution gas chromatography/mass spectrometry.

Results: We did not see any significant alterations in concentrations of 3-hydroxy fatty acids in the culture media of normal or heterozygous LCHAD deficient fibroblasts exposed to antiretroviral drugs, in comparison to the corresponding unexposed fibroblasts.

Conclusion: Our results suggest that the commonly used antiretroviral drugs, nevirapine, didanosine, lamivudine, nelfinavir, and zidovudine do not affect fatty acid oxidation. Therefore, an altered fatty acid oxidation is unlikely to be the reason for the reported increased risk of pre-eclampsia in HIV-infected pregnant women on HAART.

31 Two Cases Of Caudal Regression Syndrome In Association With Laterality Defects

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Background: Caudal regression syndrome (CRS) represents a heterogeneous spectrum of congenital caudal anomalies, which include varying degrees of agenesis of the spinal column, anorectal and genitourinary anomalies, and pulmonary hypoplasia, in the context of Potter's sequence. Sirenomelia, characterized by fusion of the lower limbs, could represent the most severe end of this spectrum. The

two main competing etiologic hypotheses are that of an aberrant vascular supply versus axial mesoderm defects, representing an underlying genetic abnormality.

Design: Case report.

Results: We present here the autopsy findings in two fetuses of non-diabetic mothers with sirenomelia and CRS associated with laterality defects, which support the second pathogenesis hypothesis. The karyotype was normal in both cases. In case 1, following ultrasound evaluation, pregnancy was interrupted after 19 weeks of gestation. Sirenomelia was identified, together with agenesis of the external genitalia and bladder. The colon ended blindly at the rectum. Potter's oligohydramnios sequence was secondary to unilateral renal agenesis with contralateral obstructive renal dysplasia with blind-ending duplicated ureters. Atresia of the aqueduct of Sylvius resulted in a secondary obstructive hydrocephalus. A rare additional finding was situs inversus totalis, representing a defect in global situs orientation (complete mirror image of situs solitus). Radiological findings included sacral agenesis, bilateral iliac wing malposition and presence of a single lower limb with fused femoral bones and absent distal bones. In case 2, pregnancy was also interrupted at the 19th gestational week and the autopsy showed CRS, with lumbosacral agenesis, imperforate anus, bilateral lower limb pterygia and intra-uterine growth retardation. Situs inversus totalis was observed. Radiology confirmed the absence of the lumbar vertebrae and sacrum, and showed a fusion of the iliac wings with secondary malposition of the lower limbs. Situs inversus has previously been associated with sirenomelia in only one case report and with CRS in two instances.

Conclusion: Since axis and midline formation, as well as laterality determination occur during blastogenesis, the report of these two cases further supports the hypothesis that some cases of sirenomelia and CRS represent developmental field defects of blastogenesis involving the caudal mesoderm, rather than being related to vascular insufficiency.

32 Expanding The Clinical Spectrum Of Frasier Syndrome

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Background: Frasier syndrome (FS) is a rare disorder defined by glomerulopathy and male pseudohermaphroditism, caused by constitutional mutations in intron 9 of the WT1 gene resulting in excess protein product of a splice variant (KTS+). The renal lesion is characterized as focal and segmental glomerulosclerosis (FSGS) causing nephrotic syndrome with slow progression to terminal renal failure (TRF). Overexpression of KTS+ also leads to sex reversal in XY patients, persistence of müllerian remnants, female genitalia, and streak gonads (gonadal dysgenesis) with proliferation of Leydig-like cells; in XX patients, the phenotype is less severe, with normal gonads. In XY patients, streak gonads (SG) frequently develop gonadoblastomas (GBs), which can evolve into dysgerminomas (DGs).

Design: 4 phenotypically female patients with proteinuria and primary amenorrhea in 3 of them were clinicopathologically studied. Sequencing of WT1 gene was performed in one, an infant with early onset RF, based on renal histology findings.

Results: 3 cases showed FSGS, and 1 TRF. Karyotypes were 46 XY in all cases. WT gene sequencing (case 3) showed a G>A mutation of codon 15 in intron 9. Clinicopathological data are summarized in the table.

Case	Age	Kidney	Gonads	Genitalia
1	12 years	FSGS	SG/ GB	SU/ FT/ EC
2	15 years	FSGS	SG/ GB/DG	SU/ FT/ EC
3	6 months	FSGS	SG/ GB	SU/ FT
4	15 years	TRF/CA	SG/ GB	SU/ FT

CA: Cortical Atrophy; SU: small uterus; FT: Fallopian tubes; EC: enlarged clitoris.

Conclusion: FS is rare but recognizable by its characteristic clinical picture and kidney histology. FS merits special attention due to the high incidence of GB, risk of DG, and the need of early renal transplant. The unusually early onset in case 3 at 6 months (usual age of presentation is 2nd and 3rd decades) is noteworthy and deserves distinction from the closely related entity Denys-Drash syndrome (DDS). However, intron 9 mutations of WT1 are characteristic of FS and not of DDS. Given the classic renal histology and the absence of Wilms tumor (characteristic of DDS) the diagnosis of FS is well supported. The spectrum of FS should be expanded to include cases with early presentation of proteinuria associated with FSGS. Molecular biology in those cases may be confirmatory.

33 Fetal Vein Of Galen Anomalies

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Background: Vein of Galen aneurysms are occasionally identified by antepartum imaging, but pathological documentation is rare.

Design: We reviewed the autopsy files of our institution over the past 5 years and retrieved three cases of Vein of Galen vascular anomalies identified at fetal post mortem, and reviewed clinical, ultrasonographic and pathological data on all three.

Results: The post mortem specimens ranged from gestational age 22 to 24 weeks. In two of the three cases the superior sagittal sinus and straight sinus were massively dilated, and the vein of Galen only minimally dilated. One of these displayed a left frontal pial arteriovenous fistula and one a partially thrombosed left dural arteriovenous fistula. In the case of the pial arteriovenous fistula, a dilated draining vein could be identified emptying into the superior sagittal sinus. In the third case, the dilated straight sinus and vein of Galen contained extensive thrombosis, and there was evidence of prior haemorrhage in the area anterior to the dilated venous structure.

Conclusion: In this small series, midgestational fetal vein of Galen malformations differ in venous angioarchitecture from those diagnosed later in life by their prominent involvement of the straight sinus. In addition, two of the three cases of aneurysmal sinus dilation were associated with a distant, lateral arteriovenous fistula, as opposed to the more frequently reported midline arteriovenous malformation seen later in life. We suggest that a terminology centered on the vein of Galen is misleading.

34 Immunohistochemical Characterization Of Fatal Epstein-Barr Virus Infection With Atypical Lymphoid Infiltration Associated With Clonal T/NK-T Cell Expansion

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Background: Epstein-Barr Virus Infection (EBV) preferentially infects B-cells and cytotoxic T lymphocytes target the viral antigen inducing a variety of systemic symptoms. The cellular immune response is responsible for control of this viral infection. EBV is associated with a number of malignancies, arising from emergence of EBV-antigen driven clones, particularly where there is a failure of the normal cellular immune response. In addition, some children (both immunocompromised and immunocompetent) have been described with a rapidly progressive and fatal EBV syndrome, perhaps resulting from an atypical immune response to EBV infection. We characterize 4 cases of fatal EBV infection in children with immunophenotypic and molecular evidence of T/NK-T cell clonal expansion.

Design: We analyzed a series of four patients with fatal EBV infection associated with prominent atypical lymphoid infiltrates using immunostains and gamma T-cell receptor (TCR) gene rearrangement by PCR. EBV infection was confirmed by EBV serology, EBV in-situ hybridization, and/or EBV polymerase chain reaction. Immunostains panel performed on all cases included CD20, CD2, CD3, CD4, CD8, CD56, CD57, and TIA-1.

Results: Patients' ages ranged from 22 months to 4 years (3 males and one female). Hemophagocytosis was detected in 2 patients and family history of possible X-linked lymphoproliferative disorder was reported in one. All cases demonstrated prominent atypical lymphoid infiltrates with geographic necrosis in 3 of the 4 cases. The atypical lymphocytes were positive for CD3 (cytoplasmic), CD2, CD8, TIA-1 and CD57 in all cases. CD4 was negative with a few weakly stained cells in only 2 cases. CD56 was negative in all cases and CD20 stained only rare scattered large cells. TCR studies were performed on 3/4 cases with sufficient tissue, and all showed reproducible positive clonal peaks, with one case demonstrating a large clonal band while the other cases showed somewhat smaller peaks in a polyclonal background.

Conclusion: We report 4 cases with fatal EBV infection characterized morphologically by atypical lymphoid infiltrates with prominent necrosis that strongly resembles NK-T cell lymphoma by both morphology and immunophenotype. Positive TCR gene rearrangement studies in 3/3 patients suggest a clonal T/ NK-T expansion process most likely driven by EBV infection.

35 Does Bone Marrow Morphology Predict The Presence Of Serum Neutrophil Antibodies In Pediatric Neutropenia?

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Background: The differential diagnosis of neutropenia in children includes genetic and acquired etiologies. A crucial discriminating finding in immune neutropenia is the presence of serum neutrophil antibodies (Neu-Ab), a rarely available test. We sought to determine if the bone marrow examination, which is required to exclude other disorders, could discriminate patients with an acquired autoimmune basis for their neutropenia.

Design: We hypothesized that bone marrow aspirate pathology, particularly the abundance of segmented neutrophils and/or plasma cells, might predict serum Neu-Ab negativity or positivity. We performed retrospective analysis of 25 patients who had concurrent bone marrow aspirates and Neu-Ab flow cytometry studies performed since 2002. Ten cases with positive Neu-Ab results and 15 cases with negative Neu-Ab results were evaluated blindly and segregated into probable negative or positive Neu-Ab categories, using the presence of easily identifiable segmented neutrophils and lack of plasmacytosis as criteria for likely Neu-Ab negativity. Cases with no or only rare mature neutrophils and/or >5% plasma cells were categorized as "likely positive Neu-Ab" cases.

Results: Using our criteria, we were unable to accurately segregate cases with positive versus negative Neu-Ab results. In three of the negative Neu-Ab cases, a morphologic etiology was apparent (two acute leukemias and one myelodysplastic syndrome). The remaining 22 cases revealed the following: Five of ten positive Neu-Ab cases and 10 of 12 negative Neu-Ab cases exhibited easily-identified segmented neutrophils in the marrow aspirates (Fisher exact test, $p=0.2$, two-tailed). Only two of the 22 cases had a mild reactive plasmacytosis that comprised 5-10% of the marrow cellularity, though interestingly, both cases had positive Neu-Ab.

Conclusion: In the laboratory workup of primary immune neutropenia, bone marrow morphology alone does not predict the presence of serum neutrophil antibodies. Therefore, a serum Neu-Ab assay must be performed to definitively establish a diagnosis of autoimmune neutropenia. In the ten positive Neu-Ab cases we reviewed, the marrow aspirate did not provide any additional diagnostic information.

36 Characterization Of Langerhans Cell Histiocytosis-Like Murine Model

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Background: Langerhans cell histiocytosis (LCH) is a rare disease, which occurs typically in young children and has several different clinical phenotypes. Langerhans cell histiocytes are unique dendritic cells that express S100 protein, CD1a, and CD207 (Langerin) and possess pentilaminar structures (Birbeck granules) on ultrastructural examination. Animal models have been difficult to create but development of such a model might provide information regarding pathogenesis and potential therapeutic avenues.

Design: Using the CD11c promoter to obtain dendritic cell-specific expression of the viral oncogene SV40 T oncogene, transgenic mice on a C57BL/6 background were created ($n=5$). From the resultant transgenic mice, two strains were selected for study - Multisystem Histiocytosis 1 (MuSHi-1) and Multisystem Histiocytosis 2 (MuSHi-2), as determined by PCR and flow cytometry for dendritic cell-specific expression. Immunocytochemistry was performed for S100 protein, CD68, fascin, factor XIIIa, CD1a, and CD207. Electron microscopy was also performed. Comparison was made with human LCH (low-risk $n=5$; high-risk $n=5$).

Results: The median age for disease development was 4 months for MuSHi-1 (high transgene expression) and 13 months for MuSHi-2 (low transgene expression). In both groups nodular lesions were detected in the spleens and livers with eventual parenchyma replacement in terminal disease. Bone marrow and thymus were also involved. Lymph nodes were not enlarged. Histopathologic examination of liver and spleen showed proliferating

histiocytic cells with eosinophilic cytoplasm with some demonstrating reniform nuclei. There was a high mitotic rate without atypical or bizarre mitotic figures. Immunocytochemistry demonstrated: diffuse strong CD207, diffuse weak S100 protein, focal CD68 and negative fascin, factor XIIIa and CD1a (not expressed in murine LC cells). Electron microscopy showed rare pentilaminar structures. Human LCH (low and high risk) demonstrated characteristic findings (Langerhans cell histiocytes with reniform nuclei, abundant eosinophilic cytoplasm, low mitotic rate; diffuse CD1a, CD207, S100 protein staining; focal CD68; negative fascin, factor XIIIa; pentilaminar structures).

Conclusion: A transgenic murine model utilizing SV40 T antigen expression of oncogenes in dendritic cells mimics multisystem LCH to a certain extent. The disease process tends to be more aggressive with histiocytic cells which have less cytoplasm, greater nuclear irregularity and higher mitotic activity than is noted in human LCH (low-risk and high-risk). The expression of a similar immunocytochemical and ultrastructural phenotype in this murine model may allow for further investigation of the pathogenesis and therapeutic effect.

37 Primary Pediatric Intrathoracic Primitive Sarcomas: Critical Reappraisal Of The Spectrum Of Pleuropulmonary Blastoma Based On Review Of 63 Patients

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Background: Since the recognition of the entity pleuropulmonary blastoma (PPB), many pathologists have come to regard virtually any primary rhabdomyosarcoma or primitive sarcoma of the lung as PPB. We sought to reappraise the clinicopathologic spectrum of PPB, considering clinical, histomorphologic, immunohistochemical, and genetic parameters.

Design: We identified 63 patients with primitive intrathoracic neoplasms, identified during the COG/IRSG central review process. H&E slides and available clinical data were reviewed. In a subset of cases, immunohistochemical staining (polyclonal desmin, myogenin, and MyoD1) and cytogenetic/molecular analysis were performed.

Results: 42 tumors showed features we considered distinctive of PPB, including a mixture of loosely packed blastema-like cells with organoid darker islands of more closely packed cells often forming central areas of primitive cartilage, frequently accompanied by areas of rhabdomyosarcomatous and fibrosarcoma-like differentiation; anaplasia was a frequent finding, seen in 76% of cases. 12 tumors showed purely rhabdomyosarcomatous differentiation, without other elements. 9 tumors were best designated "sarcoma NOS." PPB arose at a significantly younger age than did pure rhabdomyosarcoma (mean age at diagnosis, 3 vs. 9 years; $p=0.002$). Embryonal phenotype was most common in both PPB and primary rhabdomyosarcoma; an alveolar pattern was identified within 2 PPB and in 5 rhabdomyosarcomas. In PPB, rhabdomyosarcomatous differentiation was patchy, associated with positivity for polyclonal desmin (97% of cases), myogenin (81%), and myoD1 (72%). Associated tumors (including 3 cystic renal lesions, 1 bladder botryoid rhabdomyosarcoma, and 1 unique intranasal cystic tumor in PPB patients and 3 lung tumors in siblings) occurred in 8 patients with PPB and 1 patient with rhabdomyosarcoma (who had a concurrent mediastinal teratoma).

Conclusion: We confirm the existence of a group of intrathoracic neoplasms with distinctive histologic features warranting the diagnosis of PPB and broaden the spectrum

of associated tumors. The distinct clinicopathologic features seen in pure rhabdomyosarcoma suggest a pathobiology different from PPB. A previously unreported finding is the presence of the alveolar pattern of rhabdomyosarcoma in PPB as well as in primary pulmonary rhabdomyosarcoma.

38 Histologic Features Of Type I Pleuropulmonary Blastoma (PPB)

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Background: Pleuropulmonary Blastoma (PPB) is a rare primitive tumor of the lung. In its earliest form, PPB is a multilocular cyst with mesenchymal tumor cells proliferating beneath a benign epithelium (Type I PPB). In later stages mesenchymal components overgrow the cysts to form malignant cystic and solid (Type II PPB) or purely solid (Type III PPB) neoplasms. PPB Type I is strongly correlated with age ($p<0.05$). The diagnosis of Type I PPB in its fully expressed form is straightforward. In some cases, however, the diagnostic features can be subtle and focal. The histologic characterization of Type I PPB forms the basis of this study.

Design: Slides and clinical information from 41 cases of PPB from the International PPB Registry files were reviewed (average 10 slides/case; range 2-32). Pathologic features including atypical primitive cell population, cambium layer, spindle cell or cartilage nodules, blastema, skeletal muscle differentiation, anaplasia, necrosis, dystrophic calcification and hemorrhage/hemosiderin were documented as were clinical attributes age at diagnosis, gender, year of diagnosis, multifocality and outcome.

Results: The study group included 19 girls and 22 boys with age at diagnosis ranging from 31 weeks gestation to 114 months (median 8 months). 19 cases were diagnosed between 1982 and 2001; 21 cases were diagnosed since 2001. 40/41 cases showed atypical primitive cells; 36 of these showed a definitive cambium layer. Primitive spindle cell nodules, cartilage and skeletal muscle were seen in 34, 16 and 20 cases respectively. Regressive features including necrosis, hemorrhage and dystrophic calcification/bone were common. Anaplasia was seen in 2 cases. Tumors in newborns were more uniform in composition and cellularity compared with tumors in older infants. Features of regression did not appear to correlate with age. 30 children are alive, 4 are deceased and 7 have an unknown status. Small numbers precluded outcome analysis.

Conclusion: Type I PPB is characterized by a unique combination of cellular components, cyst wall vasculature and regressive features. De novo regressive changes such as cyst wall necrosis are common and may be responsible for the histologic variability. The factors that control the balance between continued proliferation and regression are poorly understood but may be important in predicting which tumors will progress and/or require adjuvant chemotherapy.

39 Diagnostic Features Of Pleuropulmonary Blastoma (PPB) Are Absent In Congenital Cystic Adenomatoid Malformation

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Background: Cystic lesions of the lung in infants and children are problematic enough, and more so today with the recognition of cystic Type I PPB. In the differential diagnosis

of Type I PPB is congenital cystic adenomatoid malformation (CCAM). Although distinction is generally achievable, both Type I PPB and CCAM can show benign epithelium-lined cysts and mesenchymal proliferations. Further complicating the job of the pathologist, it is clear based on family studies that some PPBs have the capability of near complete or complete regression which may mask the typical diagnostic features. Recently, the histologic characteristics of Type I PPB were defined. This study evaluates these diagnostic features in a retrospective set of CCAMs and other lung cysts from our institution.

Design: The files from 1989 - October 2006 at Washington University School of Medicine were investigated using search terms "lung" and "cyst" or "bullae" or "emphysema," and excluding "pneumocystis" and "cystic fibrosis". Consult cases and patients 21 and older were excluded. The search identified 27 CCAMs and 24 cystic lesions with non-specific descriptive diagnoses. Four reviewers were "trained" to the diagnostic criteria of Type I PPB on a set of 20 Type I PPB cases. Each reviewer then independently used these criteria to evaluate 13 CCAMs and 24 non-specific cysts in which slides were available. Each case was evaluated for the presence of primitive cells with cytologic atypia, cambium layer, primitive spindle cell nodules, cartilage or skeletal muscle, a mesenchymal capillary network, necrosis, hemorrhage and dystrophic calcification.

Results: None of the cases diagnosed previously as CCAM showed features diagnostic of Type I PPB. There was minor morphologic overlap. The two features that were seen in both CCAMs and Type I PPB were mesenchymal capillary network and small cartilage nodules. However, in the other group of cystic lesions all 4 reviewers identified features suggestive of PPB with complete regression in four cases. The original diagnoses of these cases were descriptive and generic: bulla, peripheral cyst, cyst with interstitial emphysema, cyst with fibrosis and multilocular cyst. The clinical characteristics of these 4 cases are unknown.

Conclusion: The diagnostic criteria proposed for Type I PPB are useful in distinguishing Type I PPB from CCAM. In addition, this study suggests the possibility that rare examples of pulmonary cysts, not otherwise classifiable, show features that overlap with regressed PPB. Without a gene-based diagnostic marker, this theory will continue to be speculative. Follow-up with clinical features of these patients including a detailed family history may be additionally informative.

40 Hepatoblastoma With Heterologous Differentiation (Teratoid Hepatoblastoma).

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Background: Since the original description of teratoid hepatoblastoma by Manivel in 1986, few series have described the divergence of heterologous elements present in teratoid hepatoblastoma.

Design: Cases of hepatoblastoma with heterologous differentiation were identified from central review of Pediatric Oncology Group protocols 8696/97, 8945, 9345, and 9645 as well as the consultation files of MJF.

Results: Representative H&E sections of fifteen cases were identified, including four cases with pre- and post-therapy histology. All cases demonstrated a mixed embryonal and fetal histology. 10/15 showed at least focal microscopic small

cell undifferentiated histology; however, no cases contained greater than 10% small cell undifferentiated histology. In 13/15 cases, the heterologous elements resembled other primitive tumors of childhood: immature endoderm, reticular, and papillary patterns of yolk sac tumor (9/15), immature mesenchyme of immature teratoma (8/15), immature neuroepithelium resembling neuroblastoma or primitive neuroectodermal tumor in (2/15), blastema with primitive tubule formation resembling Wilms tumor (1/15). These immature elements were present in both pre-treatment and post-treatment specimens. Other heterologous elements included osteoid (13/15), squamous differentiation (12/15), melanocytes (9/15), cartilage (5/15), mature endodermal glands (5/15), skeletal muscle (3/15), and renal glomeruli (1/15). One case demonstrated carcinoma in situ development within the mature endodermal component.

Conclusion: Teratoid hepatoblastoma exhibits a broad range of divergent differentiation, commonly resembling other primitive tumors of childhood including yolk sac tumor, immature teratoma, neuroblastoma, primitive neuroectodermal tumor and Wilms tumor. The clinical significance of these elements remains to be discerned.

41 Variable Expression Of WT1 In Desmoplastic Small Round Cell Tumour

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Background: Desmoplastic small round cell tumor (DSRCT) is a rare and aggressive neoplasm, which often presents as an intra-abdominal tumour in young adult males. While the clinical, pathologic and immunohistochemical features of classical DSRCT are well described, considerable variation in site of origin, morphology and immunophenotype has been reported. The defining feature is a reciprocal chromosomal translocation, t(11;22)(p13;q12), which fuses the N-terminal of EWS (Ewing sarcoma) on chromosome 22 to the C-terminal of WT1 (Wilms tumour) on chromosome 11. WT1 immunohistochemistry has been reported as being useful in distinguishing DSRCT from other tumours. Positive WT1 immunohistochemistry is considered to reflect expression of the fusion transcript, rather than wild-type WT1. In this study, we describe variable expression of WT1 associated with variant translocations.

Design: Immunohistochemistry for the N-terminal of WT1 was performed in seven cases of DSRCT. The presence of the EWS-WT1 fusion transcript was confirmed in all cases by RT-PCR. Sequencing of fusion transcript and RT-PCR for wild type WT1 was performed in 4 cases with variant transcripts.

Results: Two cases of DSRCT showed no staining for WT1 and two cases showed focal weak cytoplasmic staining. Two cases showed diffuse strong cytoplasmic staining. Unusually, both of these cases were keratin negative and one showed weak expression of the full-length WT1 transcript. One case showed strong nuclear positivity for WT1 and strongly expressed the full length WT1 transcript. This case showed an unusual translocation with two variant fusion transcripts, both of which lacked WT1 exons 9 and 10.

Conclusion: Some cases of DSRCT can express the full length WT1 transcript in addition to the characteristic fusion transcript. Care must therefore be taken in interpretation of WT1 immunostaining if the antibody used is directed against the C-terminal region since both these transcripts can yield positive nuclear immunostaining. The significance of cytoplasmic staining is unclear given that only one case expressed the full length WT1 transcript. WT1 is normally a nuclear protein and the antibody used in this study would not

detect the fusion transcript. Variable expression of WT1, combined with variation in clinical presentation, morphology and immunophenotype of this tumour, emphasizes the importance of molecular testing in establishing a diagnosis of DSRCT. We believe that confirmation of the t(11;22) translocation or the EWS-WT1 fusion transcript is currently the only reliable way to diagnose this entity.

42 Immunohistochemical Reactivity For CD99 Helps Distinguish Clear Cell Sarcoma Of The Kidney From Other Pediatric Renal Neoplasms

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Background: Clear cell sarcoma of the kidney (CCSK), a malignant kidney tumor with a propensity for early metastasis, accounts for about 5% of pediatric renal tumors and typically presents in the first two years of life. The "classic" histology of CCSK - clear, uniform epithelioid cells arranged in nests separated by fibrovascular septa - is present in the majority of these tumors, but often constitutes just a small component within a diverse array of other histologic appearances. Many of these patterns can mimic other pediatric tumors under consideration in the differential diagnosis such as nephroblastoma (Wilms tumor), congenital mesoblastic nephroma, synovial sarcoma, and rhabdoid tumor. To date, CCSK has no known specific associated chromosomal translocations, genetic alterations, or syndromic manifestations. Furthermore, there is no distinct immunohistochemical profile which distinguishes CCSK from these other entities. Therefore, identification of a specific marker to identify CCSK would provide a useful companion to morphology in the accurate diagnosis of this tumor.

Design: Eleven cases of CCSK were identified; five from WU departmental archives and personal consult files (LPD), and six from the National Wilms Tumor Study archive. H&E-stained slides were reviewed to confirm the morphologic diagnosis. Representative slides from each CCSK case, as well as a set of control cases, were immunohistochemically stained using the CD99 (013) monoclonal antibody on an automated Ventana system per the manufacturer's recommended protocol.

Results: All eleven cases of CCSK were immunohistochemically positive for CD99 expression. Unlike the crisp membranous reactivity seen in Ewing sarcoma/PNET, the cells of CCSK demonstrated strong cytoplasmic staining with coarse granularity. No CD99 staining was observed with nephroblastoma, congenital mesoblastic nephroma, or rhabdoid tumor.

Conclusion: In our hands, CCSKs demonstrate granular cytoplasmic staining for CD99, unlike PNET (membranous pattern) or other pediatric renal neoplasms (negative). This may reflect shared biologic features of CCSK and PNET, as has been recently postulated. Based on our small study sample, CD99 immunohistochemical staining may serve as a useful adjunct to morphologic appearance in the diagnosis of CCSK.

43 Anaplasia In Select Pediatric Tumors Is Associated With P53 Overexpression

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Background: P53 overexpression has been shown to correlate with anaplasia and poor prognosis in certain pediatric tumors, including Wilms' tumor, where overexpression has also been positively correlated with anaplasia. The aim of this study was to evaluate whether

P53 overexpression is associated with anaplasia in select pediatric tumors.

Design: 20 tumors with focal or diffuse anaplasia (group 1) and 21 tumors without anaplasia (group 2) were collected from the archives of the Department of Pathology at Children's Hospital Boston. Anaplastic and nonanaplastic Wilms' tumors were included to serve as internal positive controls. A representative block of each tumor was immunostained with P53. P53 was scored as the percentage of positive tumor cell in the area showing the highest P53 labeling (hot spot). P53 labeling was further subdivided according to intensity as follows: 0: negative, 1+: faint, 2+: moderate, and 3+: as intense as the positive control.

Results: Group 1 (7 males, 13 females) consisted of 7 Wilms' tumors, 6 neuroblastomas, 3 rhabdomyosarcomas, 1 rhabdoid tumor, 1 hepatoblastoma, 1 adrenal cortical tumor and 1 desmoplastic infantile ganglioglioma. Mean age was 4.9 years (range: 0.06-17.8 years). Group 2 (10 males, 11 females) consisted of 14 Wilms' tumors and 7 neuroblastomas. Mean age was 3.8 years (range: 0.4-13.4 years). In group 1, nuclear P53 expression was observed in a mean 46.3 % of cells (range: 2-95%). In group 2, nuclear P53 expression was present in a mean of 18.9 % of cells (range: 0-40%). This difference was statistically significant ($P < 0.01$). More P53-negative tumor cells were present in group 2 than in group 1, but did not achieve statistical significance ($P > 0.01$). The number of P53-positive tumor cells in group 1 with 3+ staining (mean: 14%) was more than in group 2 (mean: 2%) ($P < 0.01$); the number of P53-positive tumor cells in group 1 with 2+ staining (mean: 19%) was also more than in group 2 (mean: 5.5%) ($P < 0.01$). No statistical difference in the number of 1+ labeled tumor cells existed between groups ($P > 0.01$).

Conclusion: Our data demonstrate, as previously reported in Wilms' tumors, that anaplasia in select pediatric tumors is statistically associated with P53 overexpression. In particular, neuroblastomas with foci of anaplasia showed more P53-positive tumor cells ($P < 0.01$) than those without anaplasia and they showed more 2+ and 3+ staining intensity when compared to neuroblastomas without anaplasia. We postulate that anaplasia may represent a surrogate marker for P53 overexpression in several pediatric tumors, and that, as previously suggested, anaplasia may be a surrogate marker for cell cycle dysregulation through P53 dependent pathway(s).

44 Nestin Expression In Neuroblastoma Does Not Correlate With MYCN Amplification Or Proliferation Index

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Background: Nestin, an intermediate filament protein, is highly expressed in neuroectodermal stem cells but is downregulated during terminal differentiation into neurons or glial cells. In primitive neuroectodermal tumors and several brain tumors, nestin levels correlate with aggressive behavior and poor prognosis. However, in neuroblastoma the role of nestin is less clear because contradictory reports exist. One group, using neuroblastic cell lines, demonstrated a direct correlation between nestin expression and MYCN amplification (Thomas et al. 2004). Another group, using tissue sections from primary tumors and different methodology, found little nestin expression in tumors with amplified MYCN (Korja et al. 2005).

Design: Twelve poorly differentiated or undifferentiated neuroblastoma cases, with available formalin-fixed, paraffin-embedded tissue and for which MYCN status had previously been determined, were obtained from the University of

Michigan departmental archives. Immunostaining with antibodies to nestin and MIB-1 was performed on an automated Dako system using the manufacturer's recommended protocols, and semiquantitatively assessed using a categorical classification (0-3+). Statistical analysis of potential correlations was performed.

Results: The examined neuroblastomas showed variable nestin positivity, with reactive cases demonstrating both nuclear and cytoplasmic staining. The number of cells positive for nestin was not significantly correlated with MYCN amplification of the tumor ($r^2=0.202$). Interestingly, nestin showed a weak correlation with MIB-1 ($r^2=0.401$), suggesting that nestin expression may be related to rapid cell cycling, whether due to MYCN or some other cause. MIB-1 reactivity was not significantly correlated with MYCN status.

Conclusion: We found no correlation between nestin expression and MYCN amplification, unlike a previous study using neuroblastoma cell lines. However, nuclear expression of nestin was seen, and no correlation between MIB-1 expression and MYCN amplification was identified, in contrast to findings of another study. Whether and how MYCN overexpression modulates nestin expression remains uncertain.

45 A Tissue Micro-Array Based Analysis Of The Staining Frequency Of Beta-Catenin And Cyclin-Dependent Kinase-6 In Medulloblastoma And Other Primitive Neural Tumors

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Background: Medulloblastoma is the most common malignant central nervous system tumor in children. Stratification of patients for optimal use of therapeutic modalities has been a continuous and, often, troublesome task. Recently, Ellison et al. and Mendrzyk et al. have reported the prognostic utility of two immunohistochemical markers: β -catenin and cyclin-dependent kinase 6, in evaluating the outcomes of medulloblastoma on paraffin-embedded tissue. In this study, we attempt to further study the frequency of nuclear expression of these markers in medulloblastomas and other "primitive neural tumors" from cases resected at our institution.

Design: 62 cases were identified by searching the archives of Stanford University Department of Pathology. These include 43 cases of medulloblastoma as well as 19 other "primitive neural tumors" (neuroblastoma, astroblastoma, anaplastic ependymoma, Ewing sarcoma, supratentorial primitive neuroectodermal tumor (sPNET) and atypical teratoid/rhabdoid tumor). A tissue microarray (TMA) was constructed by extracting 1.5 mm diameter cores of histologically confirmed areas of interest and re-embedding these cores into a gridded paraffin block. Controls included: colonic tubular adenoma, colonic adenocarcinoma, pilomatricoma, and non-neoplastic fetal cerebellum. The completed TMAs were immunohistochemically stained using commercially available antibodies to beta-catenin and CDK-6.

Results: Overall, 9/43(23.3%) medulloblastoma cases exhibited nuclear staining with beta-catenin antibodies. Sixteen of 43 (37.2%) medulloblastoma cases exhibited diffuse nuclear staining with CDK6. Interestingly, two-thirds of the beta-catenin nucleopositive tumors also showed diffuse nuclear staining with CDK6. Among the "primitive neural tumors": 2/4 sPNETs, 1/6 neuroblastomas, 1/1 medullomyoblastoma and 1/1 astroblastoma displayed nucleopositive beta-catenin. Diffuse nucleopositivity with CDK6 was seen in 1/4 sPNETs, 3/6 neuroblastomas, 1/1

medullomyoblastoma, 1/1 astroblastoma and 1/1 anaplastic ependymoma.

Conclusion: In our series of 43 medulloblastomas, we note similar frequencies of nuclear staining with beta-catenin and CDK6, to that previously reported by Ellison et al and Mendrzyk et al. It is unclear the net effect that dual nuclear expression of beta-catenin and CDK-6 has on predicting prognosis. To our knowledge, this is the first description of dual nucleopositivity of beta-catenin and CDK-6 in medulloblastomas. In addition, we demonstrate the presence of nuclear beta-catenin and CDK-6 within a variety of other primitive neural tumors. It remains to be seen how analysis of outcome data from this cohort will influence the prognostic value of these two markers.

46 Beta-Catenin Expression In Fibrous Hamartoma Of Infancy

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Background: Fibrous hamartoma of infancy (FHI) is an uncommon soft tissue tumor that occurs primarily in children under 2 years of age. FHI is a benign lesion with a low recurrence rate with or without complete surgical excision. Histologically, an intimate admixture of three elements characterizes FHI: fascicles of fibroblasts/myofibroblasts in a varying amount of collagenous stroma, nests and whorls of primitive oval or stellate cells in a basophilic mucoid stroma, and mature adipose tissue. The presence of all three elements is diagnostic for FHI; however, occasionally, especially in needle biopsies, the fibrous spindle cell component is the dominant or the only element present. In such cases, other spindle cell lesions, especially desmoid fibromatoses are a diagnostic consideration. Aberrant nuclear localization of beta-catenin is a characteristic feature of desmoid fibromatosis, but has not been studied in FHI.

Design: We retrieved 18 cases of FHI diagnosed in the last 10 years from the pathology archives of Children's Medical Center, Dallas. We reviewed the cases and selected a section each for immunohistochemical staining with antibody to beta-catenin; appropriate positive and negative controls were used.

Results: Of the 18 cases of FHI, 5 occurred in females and 13 in males; the age at surgery ranged from 6 months to 31 months (mean 12.5 months, median 10.5 months); 6 tumors occurred in the arm/axilla, 4 in the thigh, 3 in the abdominal wall, 2 in the chest wall, 2 in the back, and 1 in the scrotum. Focal, cytoplasmic staining for beta-catenin was seen both in the fibrous spindle cells and in the primitive mesenchymal cells. None of the tumors showed nuclear staining.

Conclusion: Immunohistochemical staining for beta-catenin is commonly used as a marker for the integrity of the Wnt signaling and *f*-catenin degradation pathways, as well as a diagnostic tool for tumors like desmoid fibromatoses, in which abnormal nuclear localization of beta-catenin is consistently observed. Our results indicate that FHI does not demonstrate aberrant nuclear immunostaining for beta-catenin. While of diagnostic utility in distinguishing FHI from desmoid fibromatosis, our results also confirm that FHI is pathophysiologically distinct from desmoid fibromatosis.

47 Whole Genome Profiling In Infantile Hemangioma And Placental Vessels

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Background: Limited information is present on gene expression in infantile hemangioma. Two studies using a microarray detecting expression of 10,000 genes have drawn attention to increased mRNA expression of insulin-like growth factor 2 as a potential regulator of hemangioma growth. New technology allows detection of a substantially larger gene set selectively in lesional tissue. The Affymetrix GeneChip Human X3P Array is specifically designed for RNA isolated from paraffin-embedded tissue and permits detection of 47,000 transcripts.

Design: Using the Veritas Microdissection Instrument (Arcturus), we captured lesional tissue from formalin fixed and paraffin-embedded sections of proliferative phase hemangioma (PH) (n=3), involuting phase hemangioma (IH) (n=4), and placental vessels (PL) (n=3). Amplified and labeled RNAs were hybridized to the X3P array and analysis was conducted with the GeneSifter software (vizXlabs). Statistical unpaired t-test analysis was performed with a comparison of PH with PL and PH with IH. Data were normalized to the mean and subjected to the Bonferroni correction coefficient. Results were stringently filtered imposing a 3-fold threshold and a quality call of 1 (P) in all replicates. Gene expression was validated by immunohistochemistry or real time PCR for a selected number of proteins.

Results: In the PH and PL comparison, there was differential expression of 843 genes (459 up-regulated and 384 down-regulated). PH was characterized by expression of genes involved in neural and vascular patterning such as neuropilin-2, plexinC1, and EPHB3; endothelial-pericytic interactions such as ANGPT2, JAG1 and NOTCH4; and development such as homeobox genes MEOX1, SIX1 and HOXB9. By contrast, IGFBP-3 was down-regulated. PH and IH showed a differential expression of 289 genes (116 up-regulated and 173 down-regulated). Relative to PH, IH was characterized by expression of chronic inflammatory mediators such as the chemokines stromal cell-derived growth factor 1, C-X-C ligand 15 and C-X3-C ligand 1, in addition to an apoptosis-related gene, clusterin.

Conclusion: Preliminary evidence yields ample differences in gene expression between PH and PL as well as PH and IH. Identification of differentially expressed genes will contribute to our understanding of this important vascular lesion in infants.

48 Adenoviral Appendicitis Presenting Clinically As Acute Appendicitis

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Background: The vermiform appendix may react as part of a generalized viral infection but histologic documentation of appendiceal viral infection is very rare. Adenovirus has been described in association with ileal and ileocecal intussusception but to our knowledge there are no documented cases of adenovirus appendiceal infection presenting clinically as acute appendicitis without intussusception.

Design: We reviewed the pathology records of all appendectomies performed at our institution from 2001-2005. All incidental appendectomies and appendices with acute appendicitis or other pathologic findings were excluded. We selected all negative appendices with lymphoid hyperplasia and reviewed hematoxylin-eosin stained slides. Representative sections of each of these cases were immunostained with adenovirus antibody.

Results: 877 appendectomies were performed during the study period. Of these there were 94 cases which had a clinical diagnosis of appendicitis and were pathologically

negative. 63 of the 94 cases had lymphoid hyperplasia and were stained for adenovirus. We identified 2 positive cases, which also showed epithelial proliferation and viral inclusions. One was a 5 year old female and the other was a 6 year old male. Both had no evidence of intussusception during surgery.

Conclusion: Adenovirus can infect the appendix and clinically mimic acute appendicitis without intussusception. We recommend that all negative appendices be evaluated for lymphoid hyperplasia and epithelial viral changes and possibly stained with immunoperoxidase staining if indicated. We speculate that adenovirus may play a role in the pathogenesis of acute appendicitis.

49 Polymorphonuclear Leukocyte Infiltrate In Diagnostic Biopsies From Patients With Gluten Sensitive Enteropathy. A Clinicopathologic Review

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Background: Gluten sensitive enteropathy (GSE) is a clinicopathologic diagnosis in which the histopathologic criteria for the diagnosis on small bowel biopsy (SBBx) are the presence of increased intraepithelial lymphocytes (IELs), villous blunting (VB), crypt hyperplasia (CHyp) and plasmacytosis of the lamina propria (LPP). While polymorphonuclear leukocytes (PMNs) may be noted in the infiltrate, the incidence and extent have not been well documented.

Design: Archival SBBx from patients 18 years or younger with VB were retrieved from the surgical pathology files of Rhode Island Hospital for the period January 2000-December 2005. Hematoxylin and eosin stained sections were reviewed. In addition to IELs, VB, CHyp and LPP, the PMN infiltrate was scored using a semiquantitative scale: 0 absent/rare, 1+ mild, 2+ moderate with absence of surface epithelial and/or crypt involvement, 3+ moderate with surface epithelial and/or crypt involvement, 4+ severe/erosive. Patient charts were reviewed for confirmation of diagnosis based on clinical, serologic, endoscopic and follow-up data.

Results: Twenty-nine cases fulfilled clinicopathologic criteria for a diagnosis of GSE. The median age was 9 years (range 1-17). There were 17 males and 12 females. The most common presentations were screening for insulin dependent diabetes mellitus (IDDM) with poor glycemic control (8 cases), failure to thrive (FTT, 6 cases), abdominal pain and diarrhea (4 cases), abdominal pain (3 cases) and diarrhea (3 cases). A total of 43 SBBx were done (1 site in 15 patients and 2 sites in 14 patients). The score was 3+ in 16 SBBx from 12 patients (37%), 2+ in 8 from 8 patients (19%), 1+ in 10 from 8 patients (23%) and 0 in 9 from 6 patients (21%). No biopsy demonstrated severe/erosive features. Of the 12 patients with a 3+ score, 8 were distal and 4 proximal SBBx, while 4 were from a single site. The median age of patients with a 3+ score was 9.5 years (range 5-16), 2+ score was 9.5 years (range 2-13), 1+ score was 5.5 (range 1-17) and 0 score was 4 (range 2-16). Four of the 12 patients with a 3+ score were patients with IDDM.

Conclusion: A moderate PMN infiltrate was present in over half of the SBBx specimens, more commonly from older patients, and over one-third had surface or crypt involvement. Interestingly half of the patients with IDDM (4/8 patients) had the latter finding. Additional studies are required for confirmation of these findings or to determine whether regional variation may exist.

50 A Histological Scoring System For Ileitis In Children With Inflammatory Bowel Disease

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Background: Atypical presentations of ulcerative colitis (UC) in children are not rare. In contrast to adults, the majority of children with UC present with pancolitis and only 35% have isolated proctitis or left sided colitis. Patients with pancolitis may have concurrent "backwash ileitis". Furthermore, up to 15% of children with Crohn disease (CD) present with an isolated continuous colitis with or without mild ileal inflammation, creating a diagnostic dilemma. Proctocolectomy and ileal pouch-anal anastomosis are curative in severe UC but are contra-indicated in CD. In order to exclude CD in IBD patients presenting with ileitis, we developed a histological scoring system for ileal inflammation.

Design: Patients were selected from a pediatric IBD database. Ileal biopsies from treated and untreated patients were included in equivalent proportions. Acute and chronic inflammation, location of acute inflammation, erosions, ulcers and lamina propria (LP) expansion, were scored in a semi-quantitative fashion on H&E slides by two pathologists blinded to the clinical diagnosis. The latter was established by chart review after a mean follow-up time 4.7 years. Inter-rater variability was evaluated by kappa statistics. The difference between the mean total score values and the correlation between raters were evaluated by t test and Spearman correlation coefficients respectively. Using the cases of CD with granulomas (in the ileum or elsewhere) as the gold standard, we estimated by logistic regression, the total score value that yielded the highest sensitivity and specificity to diagnose CD. A P value of < 0.05 was considered statistically significant.

Results: 1) 98 biopsies were scored including 47 from untreated and 51 from treated patients. Inter-rater variability and agreement are presented in the following table:

Item	Untreated Biopsies <i>kappa statistic</i>	Treated Biopsies <i>kappa statistic</i>
Crypt distortion	0.54*	0.32*
Villus blunting	0.77*	0.70*
Pyloric metaplasia	0.66	1.00
Acute inflammation in LP	0.74*	0.54*
Acute inflammation in epithelium	0.76*	0.42*
Erosions/ Ulcers	0.66	0.67
LP expansion	0.64*	0.41*
Granulomas	0.73*	0.78*
Signed rank	$\rho=0.43$	$\rho < 0.0001$
Spearman	0.92	0.85

* = weighted kappa statistic

2) A total score of ≥ 5 had a sensitivity of 68% and a specificity of 98% to diagnose CD. 3) The inter-rater agreement for each histological item and the correlation between the scores given by the 2 pathologists were

excellent for untreated biopsies. For treated biopsies, agreement was not as strong and there was a significant difference in the mean total scores reported by the 2 pathologists.

Conclusion: We present a histological scoring system useful in excluding CD in pediatric patients with ileitis at diagnosis.

51 Gastrointestinal Histology In IPEX And IPEX-Like Syndromes

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Background: Chronic diarrhea and failure to thrive in infancy are common manifestations of congenital immunodeficiencies. Among these, a syndrome characterized by Immune dysfunction, Polyendocrinopathy, Enteropathy and X-Linked inheritance (IPEX) is associated with mutations in the FOXP3 gene. The purpose of this study is to compare gastrointestinal biopsy features in IPEX syndrome with those seen with other causes of severe chronic diarrhea in infancy.

Design: Patients with severe chronic diarrhea of infancy were selected from the files of the immunology division database. IPEX patients were identified by the presence of FOXP3 mutations. Patients without demonstrable FOXP3 mutation presenting during the same time period became the comparison (IPEX-like) group. All gastrointestinal mucosal biopsies were reviewed and morphologic features such as villous morphology, villous to crypt ratio, intra-epithelial lymphocytes and lamina propria inflammatory infiltrates were recorded. In addition, the results of immunohistochemical staining with CD3, CD4, CD8, CD20, IgA, IgG, IgM and FOXP3 as performed on the initial small bowel biopsy were compared to age matched controls found to have normal small bowel histology.

Results: Gastrointestinal biopsies from 3 patients with IPEX syndrome and 5 patients with an IPEX-like presentation were reviewed. Abnormal villous morphology at presentation was identified in all but one patient. Villous atrophy was milder in IPEX patients. Intraepithelial lymphocytes were not increased in either group. Inflammation in the lamina propria was comparable in both groups although neutrophils were present less frequently in IPEX patients (1/3) than IPEX-like patients (4/5). Immunostains for both groups revealed one case with absent staining for IgA in the lamina propria in the IPEX-like group. Otherwise, there was no significant difference compared to the normal controls. All IPEX-like patients and normal controls showed positive nuclear staining with FOXP3. Anti-enterocyte antibodies were positive in the one IPEX patient tested and positive in one of four IPEX-like patients tested.

Conclusion: There is extensive histologic and immunohistochemical overlap between gastrointestinal biopsies in patients with IPEX syndrome and other disorders leading to severe chronic diarrhea of infancy. IPEX patients may not present with severe crypt destructive inflammation. Anti-enterocyte antibodies may be present in both groups. Testing for FOXP3 mutation and immunohistochemical analysis for FOXP3 may be useful to distinguish these entities.

52 C4d, A Novel Marker In Autoimmune Hepatitis In A Pediatric Population

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Background: Autoimmune hepatitis (AIH) is a liver disorder with characteristic clinical and histological features, and serum biochemical profile. Histology usually shows an inflammatory interface lymphoplasmocytic infiltrate (periportal and/or periseptal); nevertheless, this interface hepatitis is not pathognomonic of AIH. In AIH, the ANA, SMA and anti-LKM1 auto-antibody titers are characteristically increased. However, in up to 20% of cases none of these antibodies is detected. In such cases, the distinction from other causes of chronic hepatitis remains difficult. The aim of this study was to compare the deposition of C4d in the liver of patients with clinical and serological features of AIH with that of patients with chronic hepatitis B and C, in a pediatric population. C4d is a marker of the activated complement cascade assessed to determine the humoral component of rejection in kidney and in liver. It has also been used to distinguish acute rejection from hepatitis C relapse in a transplanted liver. In the literature, chronic hepatitis C specimens have been shown to stain with C4d in almost 12% of cases. In addition, deposition of C4d has been found in almost 30% of cases of chronic hepatitis B infection or reinfection after transplantation. We present the first study conducted on a pediatric population with AIH.

Design: Immunohistochemical analysis was performed on 54 liver biopsies.

Results: C4d deposit was observed in 37 of the 44 AIH liver biopsies examined (84.9%), in 1 of 6 hepatitis C liver biopsies, and in 2 of 4 hepatitis B liver biopsies. No expression of C4d was observed in 4 non-inflammatory liver specimens, used as negative controls. Although the cohort of hepatitis B and C was small, our observations do not differ from previously published data on the deposition of C4d in liver biopsies from chronic hepatitis B and C patients.

Conclusion: Results reported in this abstract confirm a role for a humoral auto-immune response in a majority of cases of AIH. Consequently, C4d could represent an additional marker in the distinction of AIH from other causes of chronic hepatitis, especially those induced by hepatitis C virus.

53 Plasma Cell Distribution In Autoimmune Hepatitis And Primary Sclerosing Cholangitis

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Background: Autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC) are common causes of pediatric liver disease which may show similar histologic features, and may even occur together as an overlap or sequential syndrome. We characterized the distribution of plasma cells in liver biopsies of both AIH and PSC in an effort to clarify the histologic features of these two processes.

Design: The surgical pathology database was searched for pediatric liver biopsies diagnosed as AIH or PSC between 1986 and 2006. Identified cases were then stained for CD138 (Syndecan) and the distribution of plasma cells in the portal tracts, lobules, central vein and interface was evaluated. The extent of the portal and interface plasma cell infiltrate was also estimated, and scored from 0 (no plasma cells) to 3+ (marked plasma cell infiltrate). Lobular and pericentral plasma cells were scored as 1 (positive) or 0 (negative). These results were correlated with H&E and Masson trichrome stained sections of the liver biopsy, as

well as with the available clinical information including the AIH score.

Results: The search identified 29 biopsies diagnosed as AIH from 22 different patients, 24 biopsies diagnosed as PSC from 8 different patients, and 4 biopsies of sequential AIH and PSC from 2 different patients. In cases of AIH, including 1 case of sequential AIH followed by PSC, CD138 staining showed an intense (2-3+), band-like interface plasma cell infiltrate in 14 biopsies, 13 of 22 patients. The corresponding portal infiltrate was of lesser intensity in these cases. In contrast, only 1 biopsy diagnosed as PSC showed a similarly intense (2+) primarily interface plasma cell infiltrate, present in 1 of 8 patients ($p=0.04$, Fishers Exact Probability Test). Interestingly, in both PSC and AIH, a plasma cell component often persisted in subsequent biopsies, despite treatment. The presence of plasma cells in the lobule did not appear to discriminate between cases of AIH (20/22 patients) and PSC (8/8 patients). Plasma cells were slightly more numerous around the central vein in AIH (8/29 biopsies, 8/22 patients) than in PSC (3/24 biopsies, 3/8 patients).

Conclusion: A prominent, predominantly interface band-like plasma cell infiltrate is seen in AIH more commonly than in PSC, and this finding can be highlighted with a plasma cell marker such as CD138. The presence of plasma cells within the lobule or around the central vein did not appear to be statistically significant in discriminating between AIH and PSC.

54 Delayed Homicides Due To Infant Head Injury Initially Reported As Natural (Cerebral Palsy) Deaths

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Background: There is a spectrum of neuropathology seen in infants who sustain non-accidental head injury. Forensic pathologists and neuropathologists typically see the lethal end of the spectrum, while pediatricians more often see those instances that are not fatal, or at least not immediately fatal. There is, however, an area of overlap, namely, the delayed death due to head injury. Due to a prolonged interval of survival, there is a risk that these deaths initially may not be recognized as a sequel of trauma. Some of these children are given the imprecise sobriquet of "cerebral palsy".

Design: We reviewed the clinical course and autopsy and neuropathologic reports of 4 delayed fatalities due to non-accidental infant head injury in New York City with survival intervals ranging from 2.5 to 17 years. Initially, all four were reported as "natural" deaths.

Results: The initial head injuries occurred at 2-3 months of age and the deaths occurred at 2.5 to 17 years of age. Initially, they were reported as natural deaths by the treating physicians, families, and/or police. In one instance, the treating physician attempted to certify the death as natural. In the other 3 instances, the families initially did not report the remote history of head injury and simply reported a diagnosis of "cerebral palsy". All 4 infants had unexplained or poorly explained remote traumatic head injury that included subdural hematomas. At autopsy, the neuropathologic exam demonstrated remote subdural hemorrhages and lesions related to chronic anoxic-ischemic injury including brain atrophy, arterial infarcts, border-zone infarcts, and cystic encephalomalacia. Each child survived the initial injury but later succumbed to the delayed effects of secondary hypoxic-ischemic encephalopathy.

Conclusion: These four deaths highlight the need to investigate independently the medical history of any child (or adult) who dies with a clinical diagnosis of "cerebral palsy". One should not blindly accept the diagnosis of "cerebral

palsy" due to natural causes without an independent medical investigation of the initial diagnosis and circumstances. The term cerebral palsy often is used as a catchall to label any patient who has had neurologic impairment since infancy or childhood. Cerebral palsy is an etiologically non-specific

term and should not be used as the cause of death. If there is a direct link between the initial injury and the death, even if the injury occurred many years before death, then the injury is the proximate cause of death and dictates the manner of death. All four deaths were certified as homicides.