

Society for Pediatric Pathology
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Abstracts are listed in presentation order, beginning with Platform Presentations.

Platform Presentations:

1 Stem Cell Therapy for Bronchopulmonary Dysplasia: Not Just Yet.

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Background: Premature infants exposed to mechanical ventilation are at risk for bronchopulmonary dysplasia (BPD), a chronic lung disease associated with high perinatal morbidity. BPD is characterized by disrupted alveolar development, which results, at least in part, from alveolar epithelial cell death. The potential for stem cell-based therapy aimed at restoring the alveolar epithelium in newborn lungs is therefore very attractive. The role of marrow-derived cell therapy in neonatal lung injury is largely unknown. Our aim was to study the fate and effects of adult bone marrow-derived cells in a murine model of neonatal lung injury.

Design: Wild-type mice placed in a normoxic or hyperoxic (95% O₂) environment received bone marrow cells from animals expressing green fluorescent protein (GFP) at postnatal day 5 (P5) via intranasal approach. Controls received vehicle buffer. Lungs were analyzed between post-transplantation (TPX) day 2 and week 8. Population kinetics of donor cells were monitored by GFP-immunostaining combined with stereological volumetry. The phenotype of marrow-derived cells was studied by cell-specific double immunofluorescence and confocal microscopy. The functional effects on lung growth and alveolarization were studied by histomorphometry and proliferation studies. Statistical analysis was by ANOVA.

Results: The volume of GFP-immunoreactive donor cells remained constant between post-TPX weeks 1 and 8 and was similar in normoxic and hyperoxia-exposed recipients. Virtually all marrow-derived cells showed colocalization of GFP and the pan-macrophage marker, F4/80. Epithelial transdifferentiation was not seen. Marrow cell administration had adverse effects on somatic growth and alveolarization in normoxic mice, while no effects were noted in hyperoxic recipients. Reexposure of marrow-treated animals to hyperoxia at P66 resulted in marked expansion of the donor-derived macrophage population.

Conclusion: Intranasal administration of unfractionated bone marrow cells to newborn mice does not achieve epithelial reconstitution but establishes persistent alveolar macrophage chimerism. The predominantly adverse effects of marrow treatment in newborn lungs are likely due to macrophage-associated paracrine effects. While this model and route of cell therapy may not achieve epithelial reconstitution, the role of selected stem cell populations and/or alternate routes of administration for cell-based therapy in injured newborn lungs deserve further investigation.

2 Antibody-Mediated Rejection In Pediatric Cardiac

Allografts: Correlation Between Immuno-histochemistry, Immunofluorescence And Presence Of Anti-HLA Antibodies

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Background: Antibody-mediated rejection (AMR) is believed to be a cause of cardiac graft dysfunction and loss. Detection of complement split product C4d by immunofluorescence (IF) on frozen endomyocardial biopsy specimens (EMBs) has been used as a marker of AMR. However, the value of C4d staining in predicting clinical outcome in children is unclear. Furthermore, IF is limited by the need to procure fresh tissue, introducing more risk. Immunohistochemistry (IHC) can demonstrate C4d and does not require fresh tissue. Previously we have shown in a small series that C4d IHC correlates with IF. This study was designed to compare the two methods in an expanded series and to determine if the histologic, IF and IHC features associated with AMR correlate with serologic evidence of circulating anti-HLA antibodies.

Design: H&E slides from EMBs submitted for histology and IF over a 25-month period were reviewed. Paraffin-embedded tissue was additionally stained for C4d by IHC. The presence of C4d staining was scored on a three-tier scale for IF and IHC. The presence of histologic findings suggestive of AMR was also quantified. Antibody levels were ascertained by measurement of response to a panel of reactive antibodies (PRA I and II) at the time of biopsy.

Results: 185 EMBs from 59 patients had sufficient material for IF and IHC. 6 (3.2%) were positive for C4d by both IHC and IF, 169 (91.3%) were negative by both methods, 6 (3.2%) were IF+/IHC-, and 4 (2.2%) were IF-/IHC+. 41 biopsies (22%) had histologic features suggestive of AMR. Using IF as the "gold standard" IHC had a sensitivity of 50%, and a specificity of 97.7%. Positive predictive value of IHC was 60% and negative predictive value was 96.6%. No statistically significant association was found between IHC or IF staining and grade of cellular rejection, panel reactive antibody levels or histologic features of AMR (p>0.5).

Conclusion: In pediatric cardiac allografts, C4d deposition and histologic features of AMR are uncommon. IHC has a strong negative predictive value if IF is used as a surrogate for AMR, but weak positive predictive value. However, neither IHC nor IF staining for C4d showed statistically significant correlation with circulating antibodies, bringing into question the utility of either methodology to diagnose AMR in the pediatric population.

3 Genetic Testing for Inherited Thrombophilia Risk Factors: Data from a Major Pediatric Institution

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Background: Risk factors for thrombosis are divided into acquired (trauma, surgery, estrogen, age, etc.) and inherited (Factor V Leiden (FVL), Factor II mutation, proteins C, S, AT deficiency and certain polymorphisms of Methyltetrahydrofolate reductase gene (MTHFR)).

The inherited risk factors are more commonly detected in a child with thrombophilia than an adult counterpart - where acquired risk factors play a more significant role.

The leading inherited risk factors (FVL, F II and MTHFR mutations) are caused by point mutations, for which testing is widely available. Genetic testing can be performed on small samples (an advantage in children) and is not influenced by patient's clinical status (unlike coagulation based tests or protein levels). The inheritance of multiple mutations increases the thrombotic tendency exponentially, a fact that justifies testing for multiple risk factors.

While screening for these mutations is neither recommended nor cost effective, testing of certain patient groups is crucial in an attempt to reduce the incidence of thrombosis.

We reviewed and analyzed our testing data over the last 5 years.

Design: Testing for FVL, F II and MTHFR mutations is performed at our laboratory by Real time PCR, using peripheral blood leukocytes' DNA. We limited our data to pediatric age group <21yo.

Results: Between 12-30-02 and 9-19-08, a total of 868 pediatric patients were tested for FVL, 558 for F II mutation, 455 for MTHFR 677 C>T and 58 for MTHFR 1298 A>C. The most common clinical diagnoses listed: 1- Embolism/Thrombosis, 2- Coagulation defect, 3- Convulsions. Testing yielded 9 patients (1 %) homozygous and 124 (14 %) heterozygous for FVL, 27 patients (5%) heterozygous for F II mutation, 52 patients (11 %) and 167 patients (37 %) homozygous and heterozygous for MTHFR 677 C>T polymorphisms and 31 (53%) heterozygous and 7 (12%) homozygous for MTHFR M1298 A>C respectively.

Considering patient location (ER vs outpatient vs inpatient), F II mutation was detected in 10% vs 6.2 % vs 2.8 %, heterozygous FVL in 15.2%, 17.8% and 8.5 % and homozygous FVL mutation in 3% vs 1.4% vs 0.3 % respectively.

Conclusion: 1- FV Leiden testing is ordered most commonly, followed by FII and MTHFR mutations. 2- Tested children are most commonly listed under the diagnoses: Thrombosis/Embolism, Coagulation defects and Convulsions. 3- There is a higher detection rate of a mutation among ER and outpatients compared to inpatients, perhaps reflecting a more prominent role of acquired risk factors in hospitalized patients.

4 HMGA2 Immunoreactivity in Benign Bone Neoplasms

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Background: The differentiation of benign bone lesions is particularly challenging since many present with a similar pattern of mononuclear and giant cells. Cytogenetic analysis can be helpful, but is often not possible in small biopsies. Immunohistochemical markers would be useful, but have not been identified. Aberrant expression of the high mobility group protein A2 (HMGA2) transcription factor on 12q14 is found in

Pathology, Nationwide Children's Hospital, Columbus, OH several mesenchymal tumors, including lipomas, chondromas, and leiomyomas. We examined the utility of HMGA2 immunoreactivity in differentiating histologically similar benign neoplasms of bone.

Design: We performed an HMGA immunohistochemistry (rabbit polyclonal antibody, Biocheck) on formalin-fixed paraffin-embedded sections from six tumors of bone. The study contained 65 cases identified from our departmental files over the last 20 years that included chondroblastoma (CB; n=11), chondromyxoid fibroma (CMF; n=4), giant cell tumor of bone/soft tissue (GCT; n=11/6), giant cell reparative granuloma (GCRG; n=6), aneurysmal bone cyst (ABC; n=17; 8 confirmed by chromosome 17p13 aberrations), and non-ossifying fibroma (NOF; n=10). Appropriate positive and negative controls were applied throughout.

Results: All chondroblastomas demonstrated intense (dark signal filling the nucleus) HMGA2 reactivity in both chondroid and less-differentiated mononuclear cells. In one-third of cases more than 80% of cells were positive, while less than 20% were positive in the others. Four cases of GCT had weak staining (did not fill the nucleus), two involving 5% of cells and two with more than 60% positivity. Several other GCTs, GCRGs, and the CMFs had rare (<1%), weakly positive mononuclear cells. All ABCs and NOFs were negative.

Conclusion: The presence of intense nuclear immunoreactivity for HMGA2 is a useful marker to distinguish chondroblastoma from similar benign neoplasms of bone. Immunoreactivity should exclude ABCs and NOFs. Scattered, weak reactivity is observed in some GCTs. The two GCTs with numerous positive cells had atypical karyotypes with 12q and 8 aberrations. Some CBs have reported aberrations of chromosomes 5 and 8, but not the HMGA2 locus on 12q14. Since HMGA2 expression is associated with mesenchymal differentiation and has been found in more mature chondroid lesions, it is not surprising that it is also expressed in CB. The intense HMGA2 expression in CBs may reflect their commitment to a chondroid phenotype. While some GCTs may utilize similar molecular pathways, ABCs and NOFs are not associated with HMGA2 expression.

5 Thrombotic and Hemorrhagic Complications in Pediatric Extracorporeal Membrane Oxygenation (ECMO)

Nonsurvivors: An Autopsy Series

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Background: Extracorporeal membrane oxygenation (ECMO) is a life saving therapy for infants and children with cardiac and respiratory failure. However, hemorrhage and thrombosis are common complications, arising as a result of anticoagulation and contact with a thrombogenic surface. Few studies have evaluated evidence of disordered hemostasis in ECMO nonsurvivors who come to autopsy, and none in the last 15 years.

Design: Records and slides were reviewed from 25 consecutive autopsies between January 2004 and July 2008 of pediatric patients who received ECMO therapy at our institution. A control autopsy group of patients with congenital cardiac disease not treated with ECMO was compared. Laboratory parameters, including activated clotting time, platelet count and heparin dosing will be correlated with anatomic findings at autopsy.

Results: In the study population, the median age at death was 49 days (range: 1 day to 11 years), and patients spent a median of 7 days on ECMO. 76% of patients had congenital cardiac disease, and 36% were septic at the time of death. Death occurred during or immediately following ECMO therapy in 76% of patients. Duration of ECMO therapy, presence of sepsis at the time of death, and patient age did not predict either hemorrhage or thrombosis at autopsy. Thrombosis and hemorrhage were very common findings among ECMO patients, with one or both seen in 84%. Nearly two-thirds of patients (64%) had intravascular thrombosis at autopsy, with macroscopic thrombi identified in 9 of 25 patients, and microscopic thrombi in an additional 7. Thrombosis was significantly more common in patients with congenital cardiac disease (15 of 19 cardiac patients, compared to 1 of 6 noncardiac). Of the 22 patients not known to have hemorrhage when ECMO was initiated, macroscopic hemorrhages were found in 50% at autopsy, including intracranial hemorrhage in 6 of the 18 patients (33%) for whom examination of the brain was permitted. Incidence of hemorrhage did not differ between patients with and without congenital heart defects. Macroscopic hemorrhage and thrombosis coexisted in 24% of patients.

Conclusion: Thrombosis and hemorrhage are frequent complications in patients who die during or following ECMO therapy, despite progress in management of coagulation parameters. Congenital cardiac disease appears to be a risk factor for thrombosis, but not hemorrhage. Correlation of autopsy findings with laboratory coagulation values may reveal additional factors that predict thrombosis and hemorrhage on ECMO.

6 Significance of Isolated PFA-100 ADP Aggregation Abnormalities in the Identification of Pediatric Patients with von Willebrand Disease (vWD)

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Background: The PFA-100, an instrument that evaluates platelet function by aspiration of whole blood through a membrane aperture coated with collagen and either epinephrine or ADP as an agonist, is often included in the screening tests for pediatric patients evaluated for vWD. Initial PFA-100 testing with epinephrine only is considered the usual screening test but some case series found some vWD cases will be missed unless the ADP screen is also performed. This study was undertaken to clarify whether PFA-100 screening should include both epinephrine and ADP determinations.

Design: A retrospective review of limited data sets abstracted from a laboratory computer system in a pediatric hospital was performed. Data sets were included only if PFA-100 determinations of both epinephrine and ADP aggregation could be linked to factor VIII, ristocetin cofactor and von Willebrand factor antigen levels. Repeat PFA-100 determinations, multimer analysis and DDAVP correction studies were also collected if performed. vWD was diagnosed using NHLBI guidelines.

Results: 39 (5.4%) of 727 PFA-100 tests including both epinephrine and ADP determinations over a 2.5 yr period showed isolated ADP abnormalities. 5 (13%) of these 39 met criteria for vWD; all 5 were consistent with type I vWD

(multimers normal, DDAVP correction). Repeat of PFA-100 determinations in these vWD patients showed persistent isolated ADP abnormality in 4 and both epinephrine and ADP abnormal in 1. In the 34 other patients 24 had a repeat PFA-100 testing. 19 (79%) of these 24 demonstrated normal epinephrine and ADP results upon retesting.

Conclusion: Approximately 10% of pediatric patients with PFA-100 isolated ADP abnormalities will have type I vWD consistent with a recommendation that ADP testing be included in PFA-100 screening. Repeat PFA-100 testing in most patients with isolated ADP abnormality without vWD will show normal results for both epinephrine and ADP when retested whereas abnormality persists in vWD patients.

7 Birth Weights Smaller or Larger than the Placenta Predict Bmi at Age 7 Years in the Collaborative Perinatal Project (Cp)

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Background: Fetal growth is a complex process depending on parental genetics, maternal /uterine environment and placental function. Placental growth measures collected in the CPP (disk shape, larger and smaller diameters, disk thickness, site of cord insertion and cord length) account for ~40% of birth weight variance and have been shown to have independent effects on birth weight (Salafia CM et al. Birth Defects Res A Clin Mol Teratol. 2007;79(4):281-8.) We hypothesized that the altered placental proportions that influence birth weight would also affect childhood body proportions. We further hypothesized that these effects would be independent of birth weight.

Design: Using linear regression with birth weight as the dependent variable, placental variables were entered as predictors. The predicted birth weights based on placental factors were then obtained. The ratio of the actual birth weight to that predicted by placental parameters (observed/expected ratio, OER) was used as the independent variable in analyses of age 7 year body mass index (BMI) in the 15,902 singleton liveborns delivered between 34-42 weeks. Multivariate linear regressions considered $p < 0.05$ significant.

Results: After adjustment for the precise age at which the BMI measures were obtained, the OER had a significant independent effect on BMI; for each decile greater OER ratio, BMI increased 0.16 units ($p < 0.0001$). A significant OER effect on age 7 yr BMI persisted after adjustment for gestational age at delivery, sex, socioeconomic status, and maternal pre-pregnancy height and weight.

Conclusion: Birth size discrepant with one's placental proportions has detectable effects at age 7 yrs that are independent of many known predictors of childhood growth. Being larger or smaller than one's placenta would "predict" affects childhood body composition. The gross placenta should be more fully assessed, as birth weights discordant with placental size and shape measures appear to have lasting impact.

8 Birth Weight Smaller or Larger than the Placenta Would "Predict" Is Associated with Diastolic Blood

Pressure at Age 7 Years: An Analysis of the Collaborative Perinatal Project (CPP).

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Background: Fetal growth is a complex process depending on parental genetics, maternal /uterine environment and placental function. Placental growth measures collected in the CPP (disk shape, larger and smaller diameters, disk thickness, site of cord insertion and cord length) account for ~40% of birthweight variance and have been shown to have independent effects on birth weight. (Salafia CM et al. Birth Defects Res A Clin Mol Teratol. 2007;79(4):281-8.) We hypothesized that altered placental proportions (and different vascular structure) might affect the fetal cardiovascular system, and may be reflected in variation in childhood blood pressure.

Design: Using linear regression, we generated a birth weight predicted by the available placental measures (disk shape, smaller and larger diameters, disk thickness, site of cord insertion, and cord length). The ratio of the actual birth weight to that predicted by placental parameters (observed/expected ratio, OER) was used as the independent variable in analyses of age 7 year diastolic and systolic blood pressures in the 15,902 singleton liveborns delivered between 34-42 weeks.

Results: After adjustment for the precise age at blood pressure measurement, the OER had a significant independent effect on diastolic blood pressure ($\beta=3.4 \pm .63$, $p<0.001$) at 7 years of age. This effect persisted after further adjustment for gestational age, sex, socioeconomic status, and maternal pre-pregnancy height and weight.

Conclusion: The proportionality of fetal and placental weight, proxied here by the observed/expected ratio, is associated with altered hemodynamics at age 7 years. Placental proportions such as shape, larger and smaller diameters and disk thickness, reflect the vascular architecture of the arborizing placenta; different proportions imply different numbers and distributions of vessels at different levels of the villous tree. A small chorionic plate with a thick disk would suggest reduced large caliber chorionic vessel length and increased fetal stems, the principal site of placental vascular resistance. We interpret our findings as consistent with the theory that placentas with different vascular composition may affect fetal cardiac work, and may influence childhood diastolic blood pressure.

9 Advanced Paternal Age Affects Birth Weight and Placental Thickness in Female but Not Male Infants: an Analysis of the Collaborative Perinatal Project (CPP).

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Background: Advanced paternal age (APA) has recently been identified as a risk factor in female offspring for neurodevelopmental and neuropsychiatric outcomes such as autism and schizophrenia. The mechanism has been speculated to involve age-pathology of the paternally-derived X chromosome. We tested the hypothesis that advanced paternal

age would affect the placenta (in which paternal genes are preferentially expressed) of female but not male infants, and that those placentas would yield a smaller birth weight than placentas from pregnancies with a younger father.

Design: 11120 male and 10542 female newborns had complete data for the placental variables (disk shape, larger and smaller diameters, cord insertion site, disk thickness, placental weight), and maternal and paternal age. Univariate and multivariate linear regressions were used with $p<0.05$ significant.

Results: In univariate regression, advanced paternal age was associated with greater birth weight in males ($p<0.001$) and females ($p<0.0001$). In male infants, paternal age effects disappeared after adjustment for maternal age, while in female infants, such adjustment led to a significant negative effect of paternal age ($p<0.0001$). Paternal age also showed an inverse relationship to disk thickness in female ($p<0.0001$) but not in male infants ($p=0.62$). Paternal age was not associated with placental weight or chorionic disk area (calculated from the larger and smaller placental diameters) in either sex.

Conclusion: Advanced paternal age is a risk factor for autism and schizophrenia in females, yet these disorders are far more common in males. Autism and schizophrenia are also disorders of abnormal neuronal connectivity. One speculation has been that the paternally derived X chromosome carries altered/mutated genes and/or abnormal/alterd epigenetic programming. We propose that the observed effect of thinner (less well arborized) placentas of girls with older fathers mirrors fetal branching in other organs, including neurons. Study of the placenta may therefore be a proxy for analysis of the brain proper.

10 Villous Histology Predicts Karyotype in Early Pregnancy Loss

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Background: Historical doctrine states that gestational karyotype is not predictable from villous morphology except in molar gestations. We investigate and challenge that dictum. Specifically, we propose that karyotypically abnormal (KABNL) gestations are predictable by histology in early pregnancy loss.

Design: Files of Brigham and Women's Hospital from 2005-2008 identified 92 pregnancy losses with histologic and karyotypic analysis. All slides from all cases were evaluated by: one current fellow, one recent former fellow, one junior faculty, one senior faculty. Case karyotypes: 15 normal (NL, 46,XY), 14 partial hydatidiform moles (PHM), 13 complete hydatidiform moles (CHM), 50 non-molar aneuploids (AN, primarily trisomies). Histologic features: villous 1) contour complexity 2) trophoblast inclusions 3) enlargement 4) syncytiotrophoblast hyperplasia 5) stromal cytotrophoblast 6) basement membrane calcification 7) myxoid stroma; 8) extravillous cytotrophoblast hyperplasia with/without atypia (CT), 9) implantation site atypia (IS); 10) embryogenesis (nrbc, cord, amnion, somatic tissue) and 11) embryonic demise (karyorrhectic nrbc, villous edema/sclerosis, embryo autolysis). Each case received a gestalt diagnosis of normal, non-molar aneuploid, partial or complete mole.

Results: Percent correct gestalt diagnoses ranged from 49% to 74%; the most senior pathologist scored best. 53% of cases were correctly diagnosed by the majority (3 or 4) of pathologists as 47% NL, 57% PHM, 92% CHM, 33% AN < 13 w, 56% AN > 13 w. Statistically significant histologic features correctly diagnosed by the majority included the following: villous contour complexity (39/41 KABNL, 1/7 NL ($p<0.005$)); villous enlargement (41/41 KABNL, 0/7 NL, $p<0.005$); villous syncytiotrophoblast hyperplasia (12/12 CHM v 3/8 PHM and 0 AN, 0 NL, $p<0.05$); villous myxoid hypercellular stroma (10/12 CHM v 0 PHM, 0 AN, 0 NL, $p<0.005$); extravillous and/or implantation cytotrophoblast hyperplasia +/- atypia (10/12 CHM v 0 PHM, 0 NL, $p<0.005$). Evidence of embryogenesis and/or spontaneous demise did not discriminate among categories, except to exclude CHM ($p<0.005$). Completely missed diagnoses involved karyotypes unassociated with gestational trophoblastic disease (7 trisomies, 3 normals).

Conclusion: 1) Karyotype/histologic phenotype associations in early pregnancy loss are predictable by morphology alone, 2) Predictions gain accuracy with experience, 3) Villous dysmorphology and enlargement are predictive of abnormal karyotype, 4) Embryogenesis with subsequent demise are not predictive of karyotype. 5) However, villous morphology combined with embryogenesis accurately predicts normal vs abnormal karyotype ($p<0.05$).

11 Effects Of Transcutaneous Electrical Nerve Stimulation On Placental Vascularization In Rats With Induced Restriction Of Uterine Flow

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Background: Based on studies using Transcutaneous Electrical Nerve Stimulation (TENS) as a treatment for placental insufficiency, the aim of this study was to evaluate the effect of TENS on fetal and placental development in pregnancies with restricted uteroplacental blood flow experimentally induced by clamping the uterine artery.

Design: Were used 16 Wistar rats: 4 were included in the group of animals with uterine artery clamping and submitted to TENS (CE), 4 with clamping and not stimulated (CS), 4 in the group which a filament was passed around the uterine artery and stimulated (FE) and 4 with filament and not stimulated (FS). The uterine artery clamping surgery was performed on the 15th day of pregnancy. TENS sessions were performed immediately postoperative until the 19th day of pregnancy, with duration of 30 minutes, one session per day. On the 19th day of pregnancy, the animals were euthanased, and the fetuses and placentas were weighted and measured. To analyze the number of placental blood vessels, immunohistochemistry was performed with Factor VIII.

Results: Cases clamped presented a higher number of fetal reabsorptions ($p=0.017$), and smaller values of fetal weight ($p<0.001$), fetal length ($p<0.001$), placental weight ($p<0.001$)

Conclusion: In one of the largest series of its type, we show that there was significant variation both in the genitourinary and anorectal features and in the associated findings in other systems. The male to female ratio was striking and is

and placental volume ($p<0.001$). Cases stimulated presented smaller values of fetal weight ($p=0.01$), fetal length ($p=0.002$), placental weight ($p<0.001$) and placental volume ($p=0.016$). The number of placental blood vessels ($p<0.001$) was smaller in cases stimulated, independent of procedure ($p=0.828$). There was a positive correlation between number of fetuses and number of placental vessels in cases which were stimulated ($p=0.0131$), between number of fetuses and reabsorptions ($p=0.00444$) and between placental and fetal weights ($p<0.001$). There was a negative correlation between number of fetuses and fetal length in cases which were stimulated ($p=0.0150$).

Conclusions: Unlike other works describing beneficial effects of TENS on tissue perfusion, in our study, stimulus did not elevate the number of placental vessels and seems not to be beneficial to fetal and placental development in rats with uterine blood flow restriction. We suggest that TENS should be used cautiously during pregnancy, because within certain parameters of utilization, it may have harmful effects on intra-uterine development.

Key-words: blood flow; placental vascularization; restriction; TENS

12 A Report On The Autopsy Findings In 17 Cases Of Urorectal Septum Malformation Sequence

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Background: The Urorectal Septum Malformation Sequence describes a phenotype in which there are severe genitourinary and anorectal abnormalities together with ambiguous genitalia and absence of urethral, vaginal and anal orifices. The full phenotype is typically fatal due to oligohydramnios and pulmonary hypoplasia. The partial phenotype has a single perineal opening from a common cloaca. Occurrence is generally sporadic.

Design: Cases of Urorectal Septum Malformation Sequence were identified from the perinatal autopsy and surgical pathology records at Mount Sinai Hospital. Cases were identified by manually screening autopsy records and by searching the current laboratory information system for terms likely to identify cases e.g. "imperforate", "ambiguous". Similar conditions e.g. VATER, OEIS were excluded and the remaining confirmed cases were then compared to those previously published.

Results: 15 cases of the full Urorectal Septum Malformation Sequence were identified, representing the largest reported collection of such cases from a single institution. In addition, 2 cases of a partial phenotype were identified. The genitourinary, anorectal and systemic findings in these cases were fully documented. 12 of the cases were male and 5 were female. 5 of the males were reported as having megapenis, a finding that has not been previously highlighted. 1 had congenital heart disease, 2 had structural pulmonary abnormalities, 2 had lower limb pterygia and 3 had central nervous system abnormalities.

previously unreported. Although dysplastic genitalia are a feature of this condition, megapenis has not been described. Based on this study we recommend that autopsy examinations on fetuses with the potential for genital anomaly should include

measurement and photographic documentation of genital structures using standard methods. The lack of attention previously ascribed to this at fetal stage is evidenced by a relative lack of published normative values. More accurate attention to measurements at autopsy will allow correlation with antenatal ultrasound examination. In this series we present a group of cases that further highlight the complexity and poorly understood nature of urorectal development and that highlight the need for detailed documentation of observed anomalies.

13 Morphological Findings In Progressive Familial Cholestasis 2 (PFIC2): Correlation With Genetic And Immunohistochemical Studies

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Background: PFIC2 is a genetic disorder characterized by cholestasis in infancy that may progress to cirrhosis. PFIC2 is caused by mutations in ABCB11, which encodes bile salt export pump (BSEP). Specific mutations in ABCB11 are related to BSEP expression and clinical features. We correlate morphological and ultrastructural features in PFIC2 with genetic and immunohistochemical studies.

Design: Six patients with clinical PFIC had mutations in ABCB11. Each patient had at least one liver biopsy; 2 had liver transplantation. H&E and trichrome stains, and immunostains for BSEP and multidrug resistance-associated protein 2 (MRP2), were performed on at least one liver sample from each patient. Electron microscopy (EM) was performed in 5 of 6 patients.

Results: Age ranged from 5 months to 7 years at presentation. All had pruritus, elevated transaminases, and normal to mildly elevated GGT. Clinical features were intermittent in one patient. The rate of disease progression was highly variable. All biopsies showed cholestasis and hepatocyte pseudorosettes. All had centrilobular fibrosis in at least one biopsy, often accompanied by periportal fibrosis. Mononuclear inflammation and giant cell transformation were highly variable. The 3 patients with multiple biopsies showed progressive fibrosis but little change in hepatitis. EM demonstrated similar features in all cases: lobular cholestasis with chronic canalicular dilatation, partial to complete loss of microvilli, prominent collars of pericanalicular intermediate filaments, and prominent canalicular bile plugs without typical Byler bile. No canalicular staining for BSEP was observed in 5 patients; BSEP expression was present in the sixth patient, the oldest, who had presented with intermittent symptoms. Canalicular expression of MRP2 was present in all patients. Sequencing of ABCB11 revealed previously described or novel but predicted to be deleterious mutations in all patients. Nonsense, missense, and/or splice site mutations were identified in the five patients without demonstrable BSEP; a missense mutation and intron mutation were identified in the sixth patient.

Conclusion: LM and EM findings in PFIC2 support the diagnosis of PFIC in the typical clinical setting, but are variable and non-specific, underscoring the importance of immunohistochemistry and genetics. The startling variability of PFIC2, both clinically and pathologically, may be related to

specific genetic mutations, coexistent genetic or intercurrent infectious disease, varying treatment, or other factors.

14 Characteristic Chromosomal Copy Number Changes Are Associated With Specific Histologic Subtypes Of Hepatoblastoma By Combining Laser Capture Microdissection with Oligo-Array CGH Analysis

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Background: Hepatoblastoma (HB) is the most common liver tumor in childhood. Both staging and histological type are important in determining prognosis in these patients. A number of cytogenetic studies have documented abnormalities in HB, however very limited information is available regarding the genetics of specific histologic components in these tumors, in particular for the small cell (SC), known to be associated with a more aggressive clinical behavior.

Design: Fourteen hepatoblastoma specimens from 10 patients (3 pure fetal, 2 embryonal, 1 small cell (SC), 4 mixed epithelial) were examined by oligo-array based Comparative Genomic Hybridization (aCGH) to screen for genetic losses and gains. DNA from tumor and matched normal liver was extracted from frozen and/or paraffin embedded tissue using the QIAmp DNA extraction system. The different histologic components of six tumors were laser capture microdissected (LCM) to obtain pure cell populations, and subsequently amplified by Taq DNA polymerase based whole genome amplification. Samples were hybridized to Agilent Human Genome CGH Microarrays 244A and data from microarray profiles analyzed using Agilent CGH Analytics Software.

Results: Chromosomal copy number gains and/or losses were identified in all but 1 (9/10) pure fetal HB. The most common abnormality was chromosome 1q gains (5/9 cases), identified in all histological types. In 2 embryonal HBs, 1q copy number changes were associated with 4q35 losses. Abnormalities on chromosome 19 were also identified in all histologic types (8/10) including: 1 fetal (19p loss), 3 embryonals (19p loss, 19p gain and monosomy 19), and all SC lesions studied (3 trisomies and one 19p gain). In addition, chromosome 22 copy number abnormalities, including two trisomies, were exclusively found in the SC component (3/4). Previously reported changes such as chromosome X losses and 1 trisomy 2 were only identified in embryonal HBs.

Conclusions: Our data highlights the relevance of 1q locus abnormalities in HB. Recurrent gains at this locus (and a putative oncogene) have also been identified in a subset of hepatocellular carcinomas, suggesting a potential common oncogenic mechanism. Our results also demonstrate the specificity of chromosome 22 and 19 changes in the small cell subtype, which may represent clonal progression to a more aggressive genotype in these heterogeneous neoplasms.

15 Anthracycline Leiomyopathy: A Suggested Explanation for the Constipation Affecting Cancer Patients Treated with Anthracycline

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Background: Anthracycline has been used for more than three decades in oncologic chemotherapy. One limiting side effect of treatment, well documented in the literature, is a life-threatening cardiomyopathy. Another side effect is chronic constipation. The pathogenesis of this gastrointestinal disturbance is unclear and, to our knowledge, has not been the subject of a morphological investigation. Therefore, we decided to conduct a study in search of morphologic evidence that might explain this side effect.

Design: Autopsies performed in our institution between 2002 and 2007 were reviewed to select cases of children who had received Anthracycline therapy for various neoplasms. A total of seven cases were found, patients whose diagnoses included leukemias, lymphomas, and renal solid tumors. Review of their clinical history revealed that they all suffered from chronic constipation. Tissue from the heart, gastrointestinal tract, uterus, and skeletal muscles were studied by light microscopy, immunohistochemistry, and electron microscopy, using as controls tissues from autopsies of patients who never received Anthracycline, had a neoplasm, or had a history of chronic constipation.

Results: The myocardium of all Anthracycline-treated patients showed loss of myofilaments, fibrosis, mitochondrial proliferation, and accumulation of pools of Z band material. At all levels of the gastrointestinal tract examined in all Anthracycline-treated patients, the muscularis propria and muscularis mucosae displayed disorganization, including loss of myofilaments with increase in their focal densities. Similar lesions were noted in the vascular smooth muscle cells throughout all tissues examined in these patients. No alterations were seen in the controls. Surprisingly, no lesion could be detected in the skeletal muscle of the Anthracycline-treated patients.

Conclusion: In addition to evidence of myocardial injury, this study demonstrates morphologic abnormalities in the smooth muscle of the gastrointestinal tract of patients treated with Anthracycline and who had experienced chronic constipation associated with their chemotherapy. The changes observed strongly suggest that, in addition to injuring the myocardium, the chemotherapy received by those patients also damaged smooth muscle fibers, in particular those of the gastrointestinal tract. This injury most likely causes the constipation from which the patients suffer and, based on the data previously established at the level of the myocardium, the most likely culprit is Anthracycline, although the role of other agents the patients receive can be debated. Absence of detectable damage to skeletal muscle may be related to physiological differences between muscle types.

16 Current Practices in Pediatric Gastrointestinal Pathology: A Survey of the Membership of the Society for Pediatric Pathology

AM. Gomez and V Singh for the Practice Committee, Society for Pediatric Pathology

Background: Pediatric gastrointestinal pathology is a rapidly expanding area which is perceived to account for an increasing proportion of the work load in pediatric pathology practices. We undertook a survey of the members of the Society to Pediatric Pathology to collect information on the current practices of certain aspects of pediatric gastrointestinal

pathology.

Design: A written questionnaire was e-mailed to all the members of the Society for Pediatric Pathology using Member clicks. Questionnaires were returned by e-mail or by fax.

Results: A total of fifty six responses were received, the majority (54) from North America. Sixty-eight percent of respondents reported that gastrointestinal (GI) mucosal biopsies account for more than 20% of the specimens accessioned in their laboratories; further, 42% of respondents reported that GI biopsies account for more than 30% of their accessions. In most institutions, GI biopsies are read by general pediatric pathologist; only in 2 institutions, GI biopsies are read by a fellowship-trained gastrointestinal pathologist. Regarding the examination of suction rectal biopsies for the diagnosis of Hirschsprung's disease, approximately half of the respondents froze one piece for acetylcholinesterase (ACE) staining, while the others fixed all pieces in formalin for H&E staining. Thus, 47% of respondents never use acetylcholinesterase staining for the diagnosis of Hirschsprung's disease, while 12% perform ACE staining in all suction rectal biopsies, and 33% only stain selected cases. The number of sections examined to rule out aganglionosis varies, with the majority of respondents examining between 20 and 100 H&E stained sections. Forty-two percent of respondents use immunohistochemical stains when evaluating suction rectal biopsies for ganglion cells; 13% use Calretinin. There appears to be considerable variation in the criteria for the diagnosis of eosinophilic esophagitis, the reporting of Marsh scores in the evaluation of biopsies for Celiac disease, and in the use of special stains for the diagnosis of *Helicobacter pylori* gastritis as well as many of the other parameters investigated.

Conclusions: Although gastrointestinal mucosal biopsies account for a substantial proportion of the work load of the pediatric pathology practices surveyed, there is significant variation in the way these biopsies are examined and interpreted by the respondents to the survey.

17 Chronic Gastritis in Children: A Histologic Definition and Its Clinical Utility

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Background: There is no reliable histologic criterion for diagnosing chronic gastritis (CGa) in children.

Design: H&E-stained antrum (A) and corpus (C) biopsy slides from 110 disease cases (56M/54F/mean age=14y) containing the diagnosis "chronic gastritis" and from 50 control cases (26M/24F/average age=9yr) without any diagnostic abnormalities were reviewed from the files of Hospital Name collected between 8/06 and 8/08. Every case contained an antrum biopsy and 50-100% of the cases had a corresponding corpus biopsy. The maximum number of mononuclear inflammatory cells (lymphocytes and plasma cells, MIC) per superficial lamina propria intercrypt space (ICS) [MIC per ICS=M/I] and the percentage of ICSs with >20 MIC [%20] were quantified in each biopsy at 40x and 10x, respectively. Groups with n>6 data points were compared using unpaired Student's t tests with $\alpha=0.05$.

Results: See table 1. A diagnosis of CGa, rendered when there were ≥ 15 M/I in an antrum biopsy, occurred in patients with celiac (CeD, n=28), Crohns (CD, n=46) and GE reflux (GER, n=12) disease, H pylori infection (HP, n=10), eosinophilic esophagitis (EE, n=6), and lymphocytic (LC, n=2) and ulcerative (UC, n=6) colitis. Statistically significant differences among the group averages included: each disease's antrum & corpus M/I exceeded those of controls ($p \leq 0.017$); antrum M/I exceeded corpus M/I within all groups ($p \leq 0.006$); antrum %20 exceeded corpus %20 for each disease ($p \leq 0.016$) except HP ($p = 0.15$); HP and CD had the highest antrum M/I ($p \leq 0.016$); and HP had the highest corpus M/I ($p \leq 0.04$).

Table 1: Average M/I and %20 for Groups

Group (#A/#C)	M/I (range)		%20	
	A	C	A	C
Control (50/36)	11 (5-15)	9 (5-13)	0	0
All Disease (includes EE, LC, UC) (110/91)	51 (15-100)	24 (8-65)	68	34
Celiac Disease (28/20)	39 (15-90)	20 (6-35)	57	30
Crohns Disease (46/42)	56 (28-100)	24 (9-60)	77	36
GE Reflux (12/7)	41 (24-65)	18 (11-32)	56	16
H pylori (10/9)	60 (24-88)	36 (20-55)	72	57

Conclusions: 1. Finding >15 M/I in at least 50% of intercrypt spaces within antrum biopsies affords a pragmatic and reliable histologic criterion for diagnosing CGa in children. 2. Antrum biopsies have higher average M/I and usually have greater average %20 values than corpus biopsies. 3. CD and HP have higher average antrum M/I values than other diseases associated with CGa in this study. 4. CGa as quantified above can corroborate known or suggest potential immune-mediated gastrointestinal disorders and therefore provides a useful adjunct to clinicopathologic evaluation of these diseases.

18 Alterations of the Intrahepatic Biliary Tree in Heterotaxy Syndrome: Histologic and Morphometric Analyses.

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Background: We have previously identified intrahepatic biliary tree (IHBT) abnormalities in heterotaxy syndrome (HS) patients, with or without extrahepatic biliary atresia (EHBA). Our aim was to further characterize these abnormalities by morphometric analysis.

Design: Autopsy livers of HS patients were grouped into four IHBT categories: normal, interlobular bile duct paucity (presence of ILBD in $\leq 50\%$ of portal tracts), bile duct proliferation, and bile duct proliferation with ductal plate malformation (DPM). Digital images from 10 portal tracts (PT) per case using H+E slides (22 HS cases, 5 controls) were studied using "Image-Pro Plus version 6.0". An outline of each PT, hepatic artery (HA), portal vein (PV), and bile duct (BD) was drawn. The number of HA, PV, and BD was recorded, and the area of each was calculated in square microns. From this raw data, BD/PT, HA/PT, PV/PT areas ratios and BD/HA were calculated.

Results: Of the 22 HS patients, 17 had polysplenia and 5 had asplenia. IHBT were normal in 6 and abnormal in 16 (BD paucity 6, BD proliferation 3, BD proliferation with DPM 7). Using morphometric analysis, as expected, HS patients with paucity of ILBD had significantly fewer BD/PT than controls (1.1 vs 3.0, $p < 0.05$) and HS patients with bile duct proliferation had significantly greater BD/PT than controls (6.7 vs 3.0, $p < 0.05$). HS with IHBT abnormalities had statistically significantly larger PT areas than controls (160500 vs 85344 square microns, $p < 0.05$) and HS patients with normal ILBT (160500 vs 95353 square microns, $p < 0.05$). Patients with bile duct proliferation with DPM had the largest average portal tract area (205628 square microns). In addition, PV were significantly smaller in HS patients with abnormal IHBT than controls (6221 vs 9553 square microns, $p < 0.05$), and again the smallest average portal vein area was seen in patients with DPM (5941 square microns). HS patients with ILBT abnormalities had smaller BD/PT and PV/PT areas ratios than both HS patients with normal IHBT and normal controls.

Conclusion: Livers from patients with HS and IHBT abnormalities, especially those with DPM, have significantly larger portal tracts and smaller portal veins than control livers. These findings suggests that bile duct abnormalities in heterotaxy syndrome may be related to abnormal portal vascular development associated with increased portal fibrosis. These abnormalities may be hypothetically linked with genetic, and/or developmental vascular processes.

19 Co-localization of Polyductin and Liver Progenitor Cell Markers During the Development of the Human Intrahepatic Biliary System and in the Ductal Plate Malformation of the Liver

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Background: The longest open reading frame of PKHD1 (polycystic kidney and hepatic disease 1), the autosomal

recessive polycystic kidney disease (ARPKD) gene, encodes a single-pass, integral membrane protein named polyductin or fibrocystin. A fusion protein comprising its intracellular C-terminus, FP2, was previously used to raise a polyclonal antiserum shown to detect polyductin in several human tissues, including liver. Oval cells appear and expand in the liver when

Design: In the current study, we performed an immunohistochemical co-localization of polyductin and oval cell markers in liver tissue sections of human fetuses with normal and abnormal development of the biliary system. As abnormal development of the biliary system we studied the DPM of the liver in gestational age-matched human fetuses with Meckel-Gruber syndrome, a genetic syndrome characterized by renal cystic dysplasia, encephalocele, polydactyly and DPM of the liver. In addition we studied Caroli's disease, a biliary disease characterized by dilation of the nonobstructive saccular dilatation of the intrahepatic bile ducts, which usually is segmental. As oval cell markers, we used alpha-feto-protein, cytokeratin 9, OV-1 antigen, $\alpha 6$ integrin, connexin 43, MUC-1. In addition, we used the cell surface markers Ep-CAM, which stains the true progenitor cells capable of repopulating injured rat liver, and CD44, which has also been identified in liver progenitor cells.

Results: We found that polyductin was co-localized in some ductal plate cells together with oval stem cell markers. Oval cell markers were identified in the ductal plate cells and sequentially located in the same cells showing polyductin expression. At the remodeling stage of the intrahepatic biliary system results were, however, variable. Moreover, CD44 was particularly expressed in the DPM of the liver in Meckel-Gruber syndrome. A liver sample with Caroli's disease showed moderate to intense Ep-CAM staining of the biliary epithelium and focal co-staining with FP2 at the limiting plate of the portal tract.

Conclusion: Polyductin and liver progenitor cell markers seem to co-localize during the early development of the human intrahepatic biliary system and in the DPM of the liver possibly indicating that a co-activation may be present at early stages of liver development.

20 Identification of Invasive Streptococcal Disease in a Pediatric and Infant Death Cohort – Iowa, 2007-2008

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Background: In March of 2008, the Iowa Department of Public Health requested assistance in the investigation of 26 unexplained deaths of infants and young children from 12/16/07 to 03/02/08. The age range was from 18 days to 45 months; 22 were infants aged < 1 year. The expected number of deaths in infants for the same time interval last year was 12.

Design: Wet tissues and/or paraffin blocks were submitted for 26 cases. Cases were evaluated by H&E, Lillie-Twort Gram and Warthin-Starry stains. Based on the clinical history, postmortem cultures and histopathology, immunohistochemical and/or molecular tests for influenza A, influenza B, respiratory syncytial virus, *Mycoplasma pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *S. pneumoniae* were performed.

hepatocyte proliferation is compromised. Many different markers have been attributed to these cells, but their nature still is matter of debate. Ductal plate malformation (DPM) is an abnormal development of the primitive intrahepatic biliary system, which is differentiated in three stages: ductal plate, remodeling ductal plate and remodeled bile ducts.

Results: Histopathologic indications of infection with bacterial agents were found in 4 of 26 cases. The presentations included tracheobronchitis, pharyngeal cellulitis, tracheitis, and a necrotizing pneumonia with parenchymal bacterial colonies. All had acute inflammatory infiltrates of varying severity while none had contributory post-mortem cultures. IHC and/or molecular-based assays identified and confirmed streptococcal infections in all four cases. *S. pneumoniae* infection presented as pharyngeal cellulitis in one case, where an inflammatory infiltrate was dissecting through pharyngeal muscle with gram-positive diplococci. The second case presented as pneumonia, showing focally prominent inflammatory infiltrates. A *S. agalactiae* case showed tracheitis with pneumonia. The last case had necrotizing pneumonia and sepsis, with parenchymal *S. pyogenes* colonies in the heart. IHC tests for viral agents were not contributory in this cohort.

Conclusion: One of the *S. pneumoniae* cases was known to have received at least two doses of pneumococcal conjugate vaccine; invasive disease may reflect vaccine failure due to inadequate immunity or a pneumococcal serotype outside pediatric vaccine coverage. *S. agalactiae* infection is an interesting finding given the late onset at one month of age, and its apparent association with breastfeeding in this case. These cases indicate that streptococcal disease should be considered in the differential in cases of unexpected infant death with histologic evidence of infection, and that autopsy can provide an organism-specific diagnosis when special stains, IHC, and molecular methods are utilized.

21 PAX2 and PAX8 are Useful Immunohistochemical Markers for Clear Cell Sarcoma of the Kidney

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Background: PAX2 and PAX8 are nephric lineage-determining transcription factors required for normal renal development during embryogenesis and both have a specific expression pattern in the normal postnatal kidney. Both PAX2 and PAX8 are expressed in renal tumors or related lesions such as renal cell carcinoma, Wilms tumor, and nephrogenic adenoma, and can be used as a marker for lesions of renal origin. However, little is known about their expression in pediatric renal tumors, particularly non-Wilms tumors such as clear cell sarcoma of the kidney (CCSK), a rare pediatric renal tumor with an uncertain histogenesis. In this study, we used immunohistochemistry (IHC) to investigate the expression of PAX2 and PAX8 in CCSK.

Design: IHC for PAX2 and PAX8 was performed on representative slides from 6 CCSK, 5 rhabdoid renal tumors, 2 congenital cellular mesoblastic nephromas, and 1 Wilms tumor using the avidin-biotin peroxidase method after antigen

retrieval. Immunopositivity was interpreted as distinct nuclear staining.

Results: All 6 CCSK were diffusely immunopositive for PAX2 while 5/6 cases were diffusely immunopositive for PAX8. In the non-CCSK group, nuclear positivity for both PAX2 and PAX8 stains was observed only in the Wilms tumor and 1 rhabdoid tumor was focally and weakly positive for PAX8.

Conclusion: PAX2 was diffusely expressed in all CCSK and PAX8 was strongly expressed in nearly all CCSK cases (5/6). Simone T. Sredni¹, Fabrício F. Costa¹, Christopher Hamm¹, Chiang-Ching Huang², Maria de Fátima Bonaldo¹, Jared Bischoff¹, Tanadori Tomita³, Stewart, Goldman³, Veena Rajaram⁴ and Marcelo B. Soares¹, ¹Cancer Biology and Epigenomics Program, Children's Memorial Research Center and ²Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University; Departments of ³Pediatrics and ⁴Pathology, Children's Memorial Hospital. Chicago, IL.

Background: Pediatric brain tumors (PBT) are the second most common malignancies among children. As this is a heterogeneous group of tumors the molecular mechanisms of each tumor type needs to be individually investigated. Most of the studies focus on the more frequent PBT missing the opportunity to understand the biology and improve the treatment of the rare ones. MicroRNAs (miRNAs) are short regulatory RNAs that negatively modulate protein expression at a post-transcriptional level. Their deregulation has been correlated with many cancers indicating that they can function as classical tumor suppressors or oncogenes.

Design: The aim of this study was to investigate the miRNA expression profile of different PBT types in order to uncover markers of diagnosis, prognosis and/or potential therapeutic targets not only for the most frequent PBT types but also for the rarest ones. To achieve this, total RNA was extracted from 21 frozen primary PBT samples and the expression profile 365 different miRNAs was determined by a Fast Real-Time PCR system in 384-well low density arrays. The analysis included 6 Medulloblastomas (MB); 4 Atypical Teratoid-Rhabdoid tumors (ATRT); 4 Juvenile Pilocytic Astrocytomas (JPA); 4 High Grade Gliomas (HGG) and 3 Ependymomas (EP).

Results: The comparison of the miRNA expression among the 5 groups of tumors resulted in promising candidates. MiR-221 and miR-222 were among the top candidates. They showed to be strikingly upregulated in HGG, JPA, ATRT and EP but not in MB (FC 14.2; $p < 0.0001$). As the deregulated expression of miR221/222 was recently demonstrated to have the ability to promote neoplastic growth by inhibiting the expression of p27KIP1, we performed Real time PCR for p27KIP1 in the tumor samples. Our results validated this finding confirming the negative regulation of p27KIP1 by miR-221/222.

Conclusions: MiR-221/222 are known to be highly expressed in Leukemias, Thyroid Papillary Carcinomas, Prostate Carcinomas, Hepatocellular Carcinomas and Glioblastomas and the anti-miR-221 therapy was recently proposed as an alternative treatment for leukemia. In this study we demonstrated that other PBT as ATRT, EP and JPA have the involvement of p27KIP1 regulated by miR-221/222 in their tumorigenesis. The absence of this regulation in MB and the similar regulation between JPA (that is low grade), ATRT and HGG (that are high grade) demonstrate that this is not an event that is common to all malignant tumors and that is not related to the degree of malignancy as it has been previously proposed.

The results suggest that CCSK might be a developmental neoplasm of the fetal kidney as is Wilms tumor. Co-expression of PAX2 and PAX8 may be a useful and sensitive immunohistochemical marker for differentiating CCSK from its mimickers.

22 Regulation of the Cell-cycle Inhibitor P27kip1 by Mir-221 and Mir-222 in Pediatric Brain Tumors.

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23 PLAG1 (Pleomorphic Adenoma Gene 1) Immunohistochemical Expression in Pediatric Soft Tissue and Bone Tumors and Its Role in Diagnosis of Lipoblastoma

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Background: Chromosome 8 rearrangements in pleomorphic adenoma of salivary gland and lipoblastoma involve PLAG1, a developmentally regulated nuclear transcription factor. PLAG1 immunohistochemistry (IHC) with laboratory-derived polyclonal antibodies has been reported in a very small number of tumors positive for translocation of chromosome 8. IHC expression of PLAG1 in a broad spectrum of pediatric tumors has not been studied and its diagnostic value is unknown.

Design: We developed IHC protocols for 2 commercially available anti-PLAG1 antibodies (goat polyclonal, Santa Cruz Biotechnology and mouse monoclonal, clone 3B7, Novus Biologicals) in formalin-fixed paraffin-embedded (FFPE) tissue, using tumors with known chromosome 8 translocations as positive controls. Negative controls were run for each experiment and staining results were validated by Western blot and immunoprecipitation in fresh frozen lipoblastoma samples (n=3). We then studied the expression of PLAG1 in 258 FFPE pediatric soft tissue and bone tumors from our institutions. More than 50% of studied lipomatous tumors had confirmatory karyotypes. Tissue array technology was utilized.

Results: Both antibodies were specific and immunoprecipitated PLAG1 in protein extractions from fresh frozen lipoblastoma samples and both were sensitive and labeled the nuclei of FFPE control tumors by IHC; clone 3B7 was more specific than the polyclonal antibody. IHC showed PLAG1 expression in 13 of 14 lipoblastomas, 0 of 3 lipomas, 0 of 4 liposarcomas, 16 of 33 desmoids fibromatoses, 5 of 18 embryonal rhabdomyosarcomas, 0 of 21 alveolar rhabdomyosarcomas, 1 of 70 Ewing sarcomas, 0 of 40 osteosarcomas and 0 of 55 neuroblastomas.

Conclusion: PLAG1 expression in virtually all lipoblastomas likely reflects its activation as a result of chromosome 8 translocation; IHC with PLAG-1 mouse monoclonal antibody 3B7 may play a role in differentiating lipoblastoma from lipoma and liposarcoma in FFPE tissue. PLAG1, with rare exceptions, is not expressed in neuroblastoma, osteosarcoma, Ewing sarcoma and alveolar rhabdomyosarcoma. Observed PLAG1 immunoreactivity in 48% of desmoid fibromatoses and 28% of embryonal rhabdomyosarcomas might reflect polysomy 8 reported in subsets of these tumors. Further studies may elucidate the prevalence and significance of PLAG1 expression in lipomatous and other tumors.

24 Stromal β -Catenin Expression Does Not Differentiate Giant Forms of Childhood Fibroadenomas. Are Pathways Other Than or Complementary to Wnt5a & Insulin Growth

Background: Although 4% of all Fibroadenomas (FA) occur in childhood, giant forms are rare. FA are 'Giant' when they measure over 5cm, weigh > 500gms or are disproportionately large for breast size. β -Catenin plays a vital role in cell adhesion & transcriptional activation; such transcription is activated by Wnt pathway. Wnt involves inhibition of β -Catenin degradation. Stromal nuclear expression of β -Catenin is noted in fibroepithelial lesions of the breast and especially in Phyllodes tumor (PT). Studies show that the β -Catenin pathway is activated by Insulin Growth Factor (IGF) I; IGF-II causes β -Catenin translocation to the nucleus. We examined 27 pediatric FA including 6 giant forms for variations in β -Catenin staining.

Design: We reviewed the clinical information, pathology, and nuclear stromal cell β -Catenin expression in 27 archival formalin-fixed, paraffin embedded FA. 4 micron, paraffin sections were stained with β -Catenin, clone 17C2 (Leica Microsystems), diluted 1:400. Antigen retrieval was accomplished with EDTA at high temperature, & detection was with the Refine DAB polymer detection system.

Stromal cell nuclear expression of β -Catenin was sought; intensity was recorded on a 0 to 4+ scale (0 being negative, 1+ slight, and 4+: strongest staining).

Results: Cases included 11/27 Juvenile FA with 6 giant forms, 15/27 usual FA, and 1/27 Cellular FA. The FA ranged from 1.5cm-13 cm in size, with a median of 3 cm. The patients ranged in age from 10-17 with a median of 14 years. 16/27 patients were African American, 4/27 Caucasian, 1/27 Hispanic, and the race of 6/27 was not recorded. All cases were unilateral and only 1 case had multiple FA. β -Catenin revealed variable nuclear staining, of occasional stromal spindle cells, in all cases with no difference in 'giant' FA. None of the 27 cases showed stromal atypia, 'leaf-like' pattern or increased stromal mitoses.

Conclusion: There are no distinguishing features in expression of β -Catenin in 'giant' forms of childhood FA. Nuclear β -Catenin in patchy stromal cells has been previously reported. Studies have shown an over expression of β -Catenin in FA, along with IGF-I & II, in fibroepithelial tumors of the breast including PT. Studies have also shown that areas of stromal overgrowth in FA can be monoclonal. When overexpression of β -Catenin is noted, it indicates active Wnt signalling and IGF overexpression. Because no variation in staining was noted; this small study suggests that the stromal overgrowth in 'giant' forms (showing similar β -Catenin staining to non-giant forms) is perhaps regulated by factors other than or complementary to the Wnt5a and IGF I & II pathways. Further study is required to fully understand the biology of giant childhood FA.

25 HMGA2 Immunoreactivity in Pediatric Lipomatous Tumors

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Background: The differentiation of benign lipomatous tumors is particularly challenging, especially in biopsy material. Cytogenetics can be helpful, but is often not available.

Factors I and II, involved in Stromal Overgrowth?

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Translocations at 12q13-15, associated with aberrant expression of the high mobility group protein A2 (HMGA2) embryonic transcription factor, are observed in several mesenchymal tumors, including a majority of lipomas, well-differentiated liposarcomas, and uterine leiomyomas. We examined the utility of HMGA2 immunoreactivity in differentiating lipomatous tumors of childhood.

Design: We performed HMGA2 immunohistochemistry (rabbit polyclonal antibody, Biocheck) on formalin-fixed paraffin embedded sections of 58 lipomatous neoplasms in childhood identified from our departmental files over the last 20 years. These included 12 lipomas and 5 intramuscular lipomas (5 with documented 12q aberrations), 15 lipoblastomas (12 with documented 8q aberrations), 8 cases of lipomatosis, 7 cases of lipofibromatosis, and 11 fibrous hamartomas of infancy. Appropriate positive and negative controls were performed with each assay and 8 cases of benign adipose tissue were also studied.

Results: All lipomas displayed strong nuclear HMGA2 immunoreactivity in both adipocytes and cells in the fibrous septa. Four of five intramuscular lipomas were positive with reactivity in the adipocytes, septal cells, and perimysial stromal cells. The endothelial and skeletal muscle cells were negative in all tumors. All cases of lipomatosis and benign adipose tissue were negative, as were cases of lipofibromatosis and fibrous hamartoma of infancy. Additionally, all lipoblastomas, including mature lipoma-like tumors, were negative.

Conclusion: HMGA2 nuclear immunoreactivity is a useful marker to differentiate true lipomas and intramuscular lipomas from benign adipose tissue and lipomatosis and other lipomatous neoplasms of childhood, including lipoblastoma, lipofibromatosis, and fibrous hamartoma of infancy. Many lipomas are associated with reactivation of the embryonic mesenchymal transcription factor HMGA2 via a 12q translocation. The pathway appears critical for adipocyte proliferation rather than differentiation, perhaps accounting for its association with mature lipomatous neoplasms. That there is expression in adipose and spindled cells suggests both components are lesional and derived from a common progenitor cell. The other lipomatous tumors tested display a range of mesenchymal/adipocyte development and do not appear to involve the HMGA2 pathway, even in areas that are fully mature.

26 Glypican 3 in Pediatric Tumors - an Immunohistochemical Study

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Background: Glypican 3 (Gly3) is a heparan sulfate proteoglycan expressed and demonstrable by immunohistochemistry in hepatoblastomas (HB), yolk sac tumors (YST) and hepatocellular carcinomas (HCC). Its expression in other pediatric tumors has not been extensively studied although a genetic mutation in Gly3 has been implicated in the Simpson-Golabi-Behmel syndrome associated with Wilms tumor. The expression of Gly3 has also been shown

in a single gene array study in rhabdomyosarcomas. We have previously shown expression of Gly3 in YST, HB and rare pediatric HCC and hence undertook this study to evaluate the expression in pediatric tumors especially WT and RMS as well as to study expression in potential HB mimics in the liver.

Design: We studied 81 cases of pediatric tumors: RMS (25), WT (16), EWS/PNET (11), neuroblastoma (NB=11), pancreatic tumors (6), synovial sarcoma (SS=3), malignant rhabdoid tumor

Results: All cases of EWS/PNET, SS, ES, AMFH, CCSK, high grade sarcoma, pancreatic tumors, NB and metastatic carcinoma were mostly negative for Gly3. The staining in MRT, 1 case of NB and pancreatoblastoma was focal 3+ cytoplasmic staining. The WT showed variable staining with again a predominance of focal intense staining in blastema and epithelial components and only 1 case showed a strong and more diffuse staining pattern. No specific pattern stood out and nephrogenic rests were also focally positive. The embryonal RMS (13/14 cases) were strongly positive for Gly3 in spindle cells as well as in cells with cytoplasmic differentiation. The one negative case was a post-treatment tumor. In comparison ARMS (11 cases) showed no staining in small blue cells and focal staining in cells with rhabdomyoblastic differentiation.

Conclusion: Besides HB and YST among pediatric tumors, the only other embryonal tumor that shows strong expression of Gly3 appears to be ERMS. A more consistent and diffuse pattern of staining for Gly3 appears to differentiate ERMS from ARMS. Gly3 therefore has the potential to be a marker to identify areas of ARMS in mixed RMS cases. Staining in WT is more variable with no specific cell pattern showing staining. Gly3 staining is a useful tool for use in liver masses (HB) with the ability to differentiate HB/HCC from other primary/secondary liver tumors except YST and ERMS.

27 Microtubule Associated Protein-2 Is a Sensitive Marker of Primary and Metastatic Neuroblastoma

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Background: Microtubule associated protein-2 (MAP-2) is a protein expressed in high levels in cells derived from the neural crest. It is a very robust marker of neuronal differentiation and has been used most often in evaluating neuronal differentiation in tumors of the central nervous system. To the best of our knowledge, MAP-2 expression has not been evaluated on a large number of paraffin-fixed neuroblastomas. We sought to examine the diagnostic utility of MAP-2 as a marker of neuroblastoma and attempted to better characterize the expression of this protein on other tumors in the differential of neuroblastoma. Additionally, we sought to evaluate the specificity of MAP-2 in the setting of bone marrow metastases, given the presence of non-specific reactivity that can be seen with other markers of neuroblastoma.

Design: We evaluated MAP-2 staining using tissue microarrays, with the goal of comparing its expression in tumors that may be encountered in the differential diagnosis of neuroblastoma. We evaluated: 44 primary neuroblastomas (31 pre-treatment & 13 post-treatment), 19 Ewing sarcoma, 30 rhabdomyosarcoma, 16 neuroblastoma, 6 desmoplastic small round cell tumors, 9 osteosarcomas, 7 hepatoblastomas, 1 embryonal sarcoma, and 6 acute myeloid leukemias (including 4 acute megakaryoblastic leukemias and 2 myeloid sarcomas).

(MRT=2), high grade sarcoma (2), carcinoma (2), CCSK (1), epithelioid sarcoma (ES=1) and angiomatoid fibrous histiocytoma (AMFH=1). We used for comparison cases of dysgerminoma, YST and HB from our prior studies with the YST and HB being strongly positive for Gly3. Immunohistochemistry for Gly3 was done using the Ventana Autostainer with adequate positive and negative controls.

We also evaluated 13 metastatic neuroblastoma cases in the bone marrow and 13 bone marrows from patients with neuroblastoma, but lacking metastatic disease. Significant reactivity was defined as strong cytoplasmic staining in at least 10% of the tumor cells. Weak and diffuse staining, defined as a blush of immunoreactivity requiring high-power evaluation, is not considered significant for the purposes of this study.

Results: MAP-2 showed significant cytoplasmic reactivity in 95% of primary neuroblastomas and 100% of metastatic tumors. Three of 30 cases of Ewing sarcoma demonstrated significant immunoreactivity. MAP-2 did not show significant reactivity in any of the other tumors within the morphologic differential. Additionally, MAP-2 showed comparable sensitivity in staining primary neuroblastomas (pre- and post-treatment samples) as compared to synaptophysin, chromogranin, CD56, and beta-catenin. In contrast to other markers of neuroblastoma, MAP-2 did not show significant cross reactivity to native bone marrow precursors, thus eliminating a potential source of confusion.

Conclusions: MAP-2 is a useful immunohistochemical marker of neuroblastoma, both in primary and metastatic sites. It can be a helpful marker in differentiating neuroblastoma from other primitive tumors in its differential, especially in the setting of small biopsy specimens.

28 Pattern of Aberrant Phenotypes in Pediatric Acute Myeloid Leukemia by Gene Expression Profiling: data from a single institution

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Background: Differentiation antigens (DA) are acquired on normal hematopoietic cells in a lineage- and ordered stage-specific fashion. Some cases of acute myeloid leukemia (AML) express DA physiologically restricted to other stages of differentiation or different lineages (aberrant phenotypes, AP). To determine whether AP associates with prognostically important genotypes of AML subgroups, we examined the pattern of AP in pediatric AMLs by gene expression profiling.

Design: 55 cases of newly diagnosed pediatric AML from 1/1999 to 8/2008 were divided into 8 subgroups according to distinct gene profiles including CBFA/ETO, CBF α -MYH11, PML-RARA, MLL gene rearrangement (GR), RBM15-MKL1, Trisomy 8, Monosomy 7, and others. DA was detected by flow cytometry.

Results:

	CD2+	CD7+	CD19+	CD20+	CD56+	HLA-DR-
CBFA/ETO	0/6 (0%)	0/7 (0%)	4/6 (67%)	0/6 (0%)	1/5 (20%)	0/6 (0%)
CBF α -MYH11	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)
PML-RARA	1/2 (50%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	
MLL GR	1/15 (7%)	2/15 (13%)	1/15 (7%)	0/15 (0%)	7/14 (50%)	4/15
RBM15 -MKL1	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	
Trisomy 8	1/6 (17%)	3/7 (43%)	1/7 (14%)	0/7 (0%)	2/6 (33%)	0/7 (0%)
Monosomy 7	0/3 (0%)	2/3 (67%)	0/3 (0%)	0/3 (0%)	0/1 (0%)	0/3 (0%)
Others	0/17 (0%)	4/18 (22%)	1/17 (6%)	0/18 (0%)	7/17 (41%)	4/18

Conclusions: CD56+ and HLA-DR- are more frequently present in AML with MLL GR; CD19+ appears to be associated with CBFA/ETO group; CD7 is more often expressed in AML with Monosomy 7; and AP is not noted in both CBF α -MYH11 and RBM15 -MKL1 groups. These findings suggest that distinct genotypes may play an important role in dysregulating DA expression in pediatric AML. The large series of study is required to confirm these findings.

Poster Presentations:

29 Pulmonary Dendritic Cells: Neglected Participants in Neonatal Lung Injury?

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Background: Preterm infants exposed to mechanical ventilation are at risk for bronchopulmonary dysplasia (BPD), a severe chronic lung disease characterized by disrupted alveolar remodeling and microvascular dysangiogenesis. Recent evidence suggests that critical immune regulatory cells, such as dendritic cells, NK cells and Treg cells, may have important (anti- or pro-) angiogenic effects. The potential role of this immune vascular axis in the pathogenesis of BPD remains undetermined.

Design: We studied the immunoangiogenic phenotype of postmortem lungs of preterm ventilated infants with BPD born between 23 and 29 weeks gestation. Controls were age-matched infants, with or without infection/inflammation, who had lived less than 24 hours. Dendritic, Treg and NK cells were identified, localized and quantified by immunohistochemical analysis using DC-SIGN, FoxP3 and CD56 antibodies, respectively. Immune cells were localized with respect to endothelial cells (CD31), smooth muscle cells (SMA) and macrophages (CD68) by double immunofluorescence studies. Statistical analysis was by ANOVA.

Results: Lungs of non-ventilated, non-infected preterm infants (23 to 29 wks gestation, saccular stage) contained DC-SIGN-positive dendritic cells in interstitium and perivascular/peribronchial soft tissue (6.97 ± 0.87 cells/HPF, $N = 12$). Lungs of ventilated preterm infants with BPD showed a dramatic increase in dendritic cells, particularly in interstitium, perivascular tissue and interlobar septa (16.59 ± 2.49 cells/HPF, $N = 7$, $P < 0.005$). Interestingly, pulmonary dendritic cell numbers were higher in non-ventilated infants with chorioamnionitis/antenatal infection (12.46 ± 3.21 cells/HPF) compared with infants without infection. The DC-SIGN immunoreactivity was indicative of an immature dendritic cell phenotype. Double labeling revealed a close association between dendritic and endothelial cells and confirmed that DC-SIGN-positive cells were distinct from smooth muscle cells and macrophages. BPD lungs showed a modest increase in NK cells. Treg cells were rare in all groups, but focally prominent in mononuclear cell infiltrates in infants with pneumonia.

Conclusion: Contrary to published reports, we demonstrate that dendritic cells are an integral part of normal postcanalicular lungs. We further determined that antenatal infection and BPD are associated with a robust increase in pulmonary dendritic cells, likely due to recruitment from the systemic circulation. Elucidation of the immunopathology of BPD may lead to novel therapeutic approaches for this disease.

30 Trends in the Society for Pediatric Pathology Workforce Survey from 2003 and 2008

K Eldin, AM Gomez, T Pysher, D Parham, J Black, M Davis., Society for Pediatric Pathology Practice Committee.

Background: A shortage of pediatric pathologists is widely perceived both in North America and abroad. Increasing workload and specimen complexity, in addition to an aging work force, contribute to the perceived deficit of pediatric pathologists.

Design: Voluntary workforce surveys were submitted to members of the Society for Pediatric Pathology in 2003 and 2008. Surveys addressed current and anticipated future workload, practice scope/setting, as well as retirement plans.

Results: 71 full time pediatric pathology responses were received in the 2003 survey, compared to only 29 in the 2008 survey. The majority of respondents were employed at a Children's hospital (51% in 2003 compared to 75% in 2008). The percentage of respondents over age 50 increased from 54% in 2003 to 67% in 2008. Seventy-five percent of 2003 survey respondents planned to retire by 2020, compared to 41% in the 2008 study. A 5-year 16% increased need in workforce was predicted by the 2003 survey and the 2008 survey demonstrated a continued workforce need increase of 16% by 2013 and a 35% projected workforce need increase by 2018. Scope of practice was generally similar between the two surveys, with increases in adult pathology responsibilities between the two surveys (17% adult AP, 5% adult CP, and 10% adult autopsy per 2003 data versus 31% adult AP, 14% adult CP, and 14% adult autopsy per 2008 data). Pediatric pathology board certification increased from 58% in 2003 to 69% of respondents in 2008. 14% of the 2008 survey respondents will require maintenance of certification.

Conclusions: Comparison of 2003 and 2008 data demonstrate that the full-time pediatric pathology workforce continues to age suggesting inadequate recruitment of young pathology trainees into the specialty. A 35% increase in workforce is anticipated in the next 10 years, coincident with an estimated retirement rate of 41% of respondents. More full-time pediatric pathologists also appear to be increasingly participating in adult autopsy and adult clinical/surgical pathology coverage. These survey results suggest a critical shortage of pediatric pathologists with expected worsening within the next decade. Aggressive recruitment of pathologists-in-training, as well as pathologists currently in practice, is imperative to avert this crisis.

31 Immunophenotype Of NF1-Associated Pseudarthrosis Of Tibia With Or Without Genetic Loss Of Heterozygosity Ashrafi S, Zhou H, Stevenson DA, Viskochil DH., University of Utah, Salt Lake City, UT.

Background: Congenital pseudarthrosis of the tibia (CPT) is a rare but serious complication of Neurofibromatosis type 1 (NF1). Usually, tibial anterolateral bowing precedes the development of pseudarthrosis (PA), and it affects ~5% of patients with NF1. We previously showed loss of heterozygosity (LOH) in CPT tissue from patients with NF1-related PA. However, LOH is not seen in all CPT cases. We aimed to compare immunophenotyping of PA tissue in NF1 patients who present with and without LOH.

Design: Seven patients who had developed PA were included in this analysis. One patient has known LOH. Three patients had no LOH, and the other three cases have unknown LOH status. All patients underwent surgical correction for PA and sections were obtained proximal to the PA, distal to the PA, and at the PA site. Samples collected from surgical correction of PA were fixed and stained with SMA, CD31, S100, Osteopontin, p53, and p21.

Results: In PA tissue, the gap in bone was filled with a spindled proliferation of mesenchymal cells lacking normal osteogenic activity with osteoblasts and osteoclasts. There is no significant difference in PA tissue patterns between those with LOH and those without LOH. The SMA staining was seen in the smooth muscle layer of larger vessels and the CD31 staining highlighted smaller vessels. This could represent increased vascularity at the PA site. The specimens were uniformly negative for p53 and p21. This suggests that formation of PA is not a neoplastic process. The results from S100 staining showed no evidence of neurofibroma.

Conclusion: These results indicate that PA formation does not involve p53 or p21 pathways to suggest a desmoid tumor. The proliferating mesenchymal cells in CPT have decreased osteogenic activity but a relatively preserved myofibroblastic phenotype resembling a reactive process rather than neurofibroma or fibromatosis. Although there is more prominent vasculature in the PA site compared to tissues distal or proximal to the PA, no difference is seen between those with or without LOH.

32 Pathogenesis Of Early Renal Involvement In Hemolytic Uremic Syndrome

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Background: Hemolytic uremic syndrome (HUS) is the most common cause of acute renal failure in childhood. Up to 30% of children exposed to Shiga toxin (Stx)-producing *E. coli* develop HUS and up to 5% of these subjects die during the acute phase of the illness. In a primate model, we have previously shown that a single 100ng/kg dose of Stx-1 or four 25ng/kg doses of Stx-1 plus lipopolysaccharide (LPS) resulted in clinical and histologic features of HUS, but that disease did not develop in animals receiving four 25ng/kg doses of Stx without LPS or single or divided doses of LPS without Stx-1; and that LPS upregulated Stx receptors. We report here the results of studies of neutrophil influx, renal cell proliferation, apoptosis, and vascular endothelial growth factor (VEGF) expression in this model.

Design: Stx-1 and/or LPS were administered to juvenile baboons, after which they were monitored closely for 72 hours and then euthanized. Postmortem autopsies were immediately performed. Formalin fixed, paraffin embedded sections of kidney, adrenal gland, lung, and bowel were evaluated by routine histology and immunohistochemical staining with antibodies against myeloperoxidase, MIB-1, caspase-3, and VEGF; and renal tissue was examined by electron microscopy.

Results: The groups that developed HUS showed localization of neutrophils and increased cellular proliferation that was greatest in peritubular capillaries. This group also showed endothelial cell injury in glomerular and peritubular capillaries. A lesser response was seen in the groups that received divided doses of Stx-1. LPS alone did not show a significant increase in cellular proliferation or inflammation. Caspase-3 expression was only seen in glomerular capillaries. Weak VEGF staining was noted in proximal tubules in all groups and in scattered podocytes in the groups that developed HUS, although the staining was very weak.

Conclusion: These studies extend our previous observations that at 72 hours after injection of Stx-1, the inflammatory

response in HUS is concentrated in glomerular and, especially, peritubular capillaries, suggesting that tubular injury may be due, at least in part, to ischemia.

33 Fibroepithelial Lesions in Pediatric Pathology: an Immunohistochemical Analysis

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Background: Fibroepithelial lesions can become a diagnostic challenge not only because of their morphologic variability, but also because their distinction is based on a histologic continuum with overlapping features, rather than on discrete criteria. The differential diagnoses most investigated are those involving fibroadenoma versus phyllodes tumor and benign versus malignant phyllodes. Most of these studies have been performed in the adult population. In pediatric pathology, an additional lesion enters the equation: the cellular (juvenile) fibroadenoma. Few studies have attempted to immunophenotype the cellular fibroadenoma in search of diagnostic and prognostic indicators. Although rare, malignant phyllodes are seen in the pediatric population and the distinction between cellular fibroadenoma and its related counterparts (both phyllodes and adult type fibroadenoma) remains subjective. In a review of fibroepithelial breast lesions diagnosed at our institution between 2003 and 2008, 34 of 79 fibroadenomas were classified as the cellular type. This incidence is much higher than the 8%-10% reported in the literature. EGFR, CD117 and CD10 antibodies have been investigated for diagnostic and prognostic purposes in adult fibroepithelial lesions. The aim of this study was to determine the immunohistochemical phenotype of cellular fibroadenomas, and to explore their relationship with the adult type fibroadenomas, as well as with phyllodes tumors.

Design: Tissue retrieved from case files archived at Children's Medical Center was immunostained with antibody to epidermal growth factor receptor (EGFR), CD117 and CD10. The cases reviewed consisted of 10 adult type fibroadenomas, 10 cellular fibroadenomas, and 2 phyllodes tumors (one borderline and one with malignant features). The patients were all females between the ages of 12 and 17 (mean: 15.5 years). The fibroadenomas ranged in size from 1.0 cm to 6.2 cm (mean: 3.0 cm), the cellular fibroadenomas ranged from 1.5 to 5.5 cm (mean: 3.9 cm), and the phyllodes tumors ranged from 2.3 to 4.2 cm (mean: 3.3 cm).

Results: None of the fibroepithelial lesions, including the two phyllodes tumors, showed reactivity to EGFR. The epithelium in all cases reacted with CD117, while the stroma was generally non-reactive. CD10 highlighted the myoepithelium in all cases. The staining of the stroma varied slightly, with the cellular fibroadenomas staining diffusely in 8 of 10 cases, the adult type fibroadenoma staining diffusely in 4 of 10 cases, and the phyllodes tumors showing only focal stromal staining.

Conclusions: EGFR and CD117 did not differentiate among the fibroepithelial lesions investigated in this study, including the phyllodes tumor with malignant features. CD10 stromal reactivity appears reduced in the phyllodes tumors in comparison to the juvenile (cellular) fibroadenomas. The number of cases is insufficient to tell whether a difference exists between the reactivity of CD10 in the stroma of adult type fibroadenomas and that of the other two groups.

34 Arthrogryposis with Polymicrogyria and Delayed Skeletal Muscle Maturation: A Specific Syndrome?

E Rudzinski, R Hevner., Seattle Children's Hospital, Seattle, **Background:** Lethal Multiple Pterygia Syndrome (LMPS) and related terms describe overlapping phenotypes which result from decreased fetal movement, and often manifest as severe pterygia, limb contractures and scoliosis. The underlying etiologies are diverse and include CNS diseases and primary myopathies. When primary abnormalities of the brain or spinal cord are present, muscle may show secondary neurogenic changes such as fiber type grouping or replacement of fascicles with fibroadipose tissue. Features such as persistent central nuclei or the presence of myotubes are usually regarded as evidence of a primary myopathic etiology. However, the findings in a series of recent autopsies at our institution suggest an under-recognized association of CNS pathology with "myopathic" features exists in a subset of mid-gestation fetuses with LMPS.

Design: We searched our autopsy database between the years 1998 and 2008 to identify fetuses with limb abnormalities. We identified cases at 20-23 weeks estimated gestational age and reviewed the muscle and CNS pathology. This included 9 cases where the cause of death was attributed to LMPS, 10 cases with limb abnormalities but not diagnosed as LMPS, and 9 cases with no limb abnormalities.

Results: We identified changes of delayed skeletal muscle maturation, increased numbers of central nuclei (10-30%) and frequent linear arrangements of central nuclei (myotubes), in 6 cases of LMPS. CNS histology in 4 of the 6 showed hypoxic/ischemic injury with polymicrogyria, mineralized neurons and microinfarcts. Placental or other pathology in 4 of these cases revealed additional signs vascular compromise. One of the 6 cases showed increased central nuclei (10-15%) in skeletal muscle, but the CNS showed no lesions; this was interpreted as a primary myopathy. No CNS histology was available for the remaining case. Another fetus with LMPS had hypoxic/ischemic CNS injury without polymicrogyria or skeletal muscle changes. The remaining cases of LMPS and both control groups showed very few (<1%) central nuclei in the skeletal muscle and no CNS injury.

Conclusion: Polymicrogyria is described in association with LMPS in the literature, but no reports also describe delayed skeletal muscle maturation. We describe 4 cases of this association, in which the skeletal muscle changes are profound and could be interpreted as a primary myopathy. We suggest that this combination of neural and muscular pathology represents a recognizable "syndrome", which probably results from diffuse hypoxic/ischemic injury during early-to-mid-gestation.

35 A 20 Year Retrospective Review of Pediatric Accidental Bathtub Drownings

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Background: Drowning has been demonstrated to be a significant cause of death among the paediatric population.

Design: Asymptomatic women with a transvaginal cervical length *25 mm between 16-24 weeks underwent cervical M. hominis and U. urealyticum cultures and a micro-cervical biopsy at the 4 o'clock position on the ectocervix using The

WA.

Whilst the literature has addressed the issue of drowning as whole, it has given limited attention to the unique nature of bathtub drownings in particular. The objective of this study was to identify specific factors associated with bathtub drownings. **Design:** A retrospective review of all pediatric drownings 1986-2006 was conducted by searching the Office of the Chief Coroner's files for drowning victims under the age of 18. Data obtained included demographics, location and month of the drowning episode. For bathtub drownings, additional data obtained included supervision, cobathing with siblings, bathseat use and the presence of predisposing medical conditions.

Results: Overall, 570 drowning deaths were identified in children less than 18 years of age, with 58 of these being bathtub drownings. Bathtub drownings occurred in significantly younger children ($p < 0.0001$). Whilst all non-bathtub drownings affected significantly more males than females ($p < 0.0001$), bathtub drownings had no significant gender bias ($p = 0.1151$). The majority of bathtub drownings occurred in children aged three years and under (62%), with a smaller percentage occurring in children aged 4 years and over (38%). Within the younger age group, lack of supervision was present in 94% of cases, whereas in the older age group, a predisposing medical factor was present in 84% of cases. Finally, whilst non-bathtub drownings have shown a significant decrease over the 21-year study period, bathtub drownings have shown no such decrease.

Conclusion: Bathtub drownings form a distinct subcategory of pediatric drowning, with significant differences in age, gender bias and predisposing conditions. Furthermore, while preventative campaigns have been successful in reducing the numbers of non-bathtub drownings, bathtub drownings have not been as successfully targeted. This, together with the aforementioned differences, highlights the need to create preventative campaigns that specifically address the unique circumstances (lack of supervision and predisposing conditions) associated with bathtub drownings.

36 A Novel Method Of Assessing Cervical Collagen Integrity Utilizing Image Segmentation Analysis

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Background: Cervical shortening is a strong and early predictor of spontaneous preterm birth, and its pathogenesis and pathophysiology are poorly understood. Collagen histologic staining reflects collagen integrity. We describe a novel method of analyzing cervical collagen integrity using image segmentation analysis.

Bard® Monopty® Instrument (C.R. Bard, Inc., Murray Hill, N.J.). Amniocentesis was performed and amniotic fluid cytokine concentrations were assayed using the Bio-Plex multianalyte detection and quantitation system (Bio-Rad,

Hercules, CA). Cervical biopsies were stained with hematoxylin and eosin (H&E) and examined by a single reviewer blinded to clinical data. One 40X photomicrograph was taken of each biopsy. The R channel (collagen stains red-pink with H&E) histogram was extracted and its mean, standard deviation, skew and kurtosis calculated. A single collagen staining factor score (CFS) was extracted from these data by principal components analysis. Dense collagen is represented by a high CFS and loose collagen by a low CFS. Spearman correlation was used to determine the relationship between cervical culture status and the CFS.

Results: Eighty seven paired cervical cultures and cervical biopsy specimens were available for analysis. The collagen staining score was related to the mycoplasma and ureaplasma cervical culture status with a correlation score of 0.264 ($p=0.014$). Thirty three paired AF and micro-cervical biopsy specimens were available for analysis. Spearman's correlations demonstrated associations of the CFS with AF IL-6, IL-8, Eotaxin, IP-10 and MCP-1 (each $p<0.05$), but not with AF IL-10, G-CSF, INF-gamma, MIP-1a, or PDGF-bb (each $p>0.10$). A trend was seen with levels of MIP-1b ($p=0.07$).

Conclusion: Image segmentation of cervical collagen on the R channel extracts structural features and informs on collagen integrity that is significantly related to cervical M. hominis and U. urealyticum culture status in patients with an ultrasonographic short cervix in the midtrimester. Collagen structural features also were highly correlated with inflammatory cytokines, suggesting that intra-amniotic inflammation and the structural integrity of the cervix are related.

37 Segmental Mediolytic Arteriopathy and the Pediatric Heart, Not Just a Lesion of Infancy.

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Background: Segmental, noninflammatory, necrotic and lytic lesions of the media of coronary arteries (CA) have been described in stillbirths and infants, usually associated with perinatal hypoxia/asphyxia, epicardial petechiae and myocardial necrosis. These vascular lesions, known as segmental mediolytic arteriopathy (SMA), are more commonly seen in older adults, and most often affect intra-abdominal muscular arteries, leading to aneurysms, rupture, hemorrhage and dependent tissue ischemia. Review of the literature identified only one pediatric case of SMA beyond infancy. Inappropriate vasospastic response is the predominant postulated etiology. Resemblance of healed lesions to fibromuscular dysplasia, frequently with segmental medial gaps, suggests that SMA may be a precursor lesion.

Design: Between 2003 – 2008 we identified SMA in the postmortem hearts of 3 children and 2 stillbirths. Three adolescents aged 13-15 years had severe fungal, bacterial, and/or viral infection. The two stillbirths, 37-38 weeks gestation, had fetal thrombotic vasculopathy with definite or probable umbilical cord compression. Hypoxemia, hypotension and/or septic shock were present in all 5 patients. Postmortem examination was complete in 4 cases; the brain was excluded in one. At least one section of each major epicardial coronary artery and two sections from each ventricle were examined from each patient. The conduction system was examined in one child with unexplained arrhythmias.

Results: In stillbirths, SMA was multifocal in epicardial CA. No intramural or extracardiac SMA lesions were seen. Conversely, all 3 adolescents had SMA in multiple intramural CA, with epicardial SMA in two. The patient with arrhythmias had extensive, focally circumferential and transmural, lesions in the sinoatrial nodal artery with focal involvement of the artery to the atrioventricular node. Extracardiac SMA was present in one child involving a serosal branch of a mesenteric artery. All vascular lesions were acute. Myocardial ischemia and multiorgan ischemic necrosis was present in all 5 patients. Direct myocardial infection with bacterial microabscesses was seen in one child.

Conclusions: SMA of the heart has been described, primarily in epicardial CA of fetuses and infants with hypoxia/asphyxia. We describe SMA in the CA of 3 adolescents with infection associated hemodynamic compromise and multiorgan ischemia.

Predominant or exclusive intramural CA lesions in the setting of septic shock and involvement of conduction system CA with associated complex arrhythmias have not been previously reported. The complex cytokine milieu in these patients may contribute to the development of SMA.

38 Occurrence of Bocavirus in Nasopharyngeal Swabs from Children with Respiratory Symptoms in the Southwest Region of the United States

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Background: Human bocavirus (HBoV) was first described in 2005 as a potential lower respiratory pathogen detected primarily in pediatric populations. HBoV is a member of the Parvoviridae family of single-stranded DNA viruses. Phylogenetic analysis suggests HBoV is closely related to the bovine and canine minute parvoviruses; hence its proposed name. There are currently no reports from the Southwest region of the United States.

Design: The analysis was performed on two hundred and seventy four (274) nasopharyngeal swabs submitted to the virology laboratory for screening of respiratory viruses and subsequently stored at -70°C . Real-time PCR was performed on a SmartCycler system utilizing a 76 bp region of the NP-1 gene (GenBank DQ000496). Confirmation of positive results was obtained by sequencing of a larger region of the NP-1 gene. Positive cases were also correlated with age, sex, viral DFA and culture results.

Results: Seventy eight samples (28.5%) had positive DFA results [RSV (54), Parainfluenzae 1 (11), Influenzae A (4), Parainfluenzae 3 (1), Adenovirus (5), Influenzae B (3)]. Twenty one samples (7.6%) had positive cultures [Enterovirus 71 (1), Parainfluenzae 1 (6), Coxsackievirus B1 (1), ECHO 9 (3), CMV (5), Coxsackievirus B4 (1), RSV (1), Adenovirus (2) and Enterovirus-non-polio (1)]. HBoV was detected in 15 cases (5%) by real-time PCR and confirmed by sequencing in all cases. Among the positive HBoV, only three had DFA positive results [Adenovirus (1), Parainfluenzae 1 (1) and RSV (1)] and one had an associated positive culture [Parainfluenzae 1]. The population age ranged from 2-30 month. There was no sex predilection [7 females: 8 males].

Conclusion: HBoV is commonly detected in nasopharyngeal swabs among hospitalized children with respiratory symptoms in the Southwest region of the United States. The real-time PCR protocol reported by Neske et al in 2007 is an adequate method

for routine analysis. The frequent isolated detection suggests that this virus is a potential pathogen.

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Background: Hepatitis C virus (HCV) is a significant cause of chronic liver disease. Of the 3-4 million people infected each year by HCV, at least 80% will develop a chronic infection with 10-20% progressing to cirrhosis, end stage liver disease and/or hepatocellular carcinoma. Viral clearance depends on an early and vigorous multispecific response against HCV by both T4 of Th1 type and their cytokines as well as CD8 cytotoxic lymphocytes. When these mechanisms fail to clear the virus, they can lead to persistent intrahepatic inflammation with resultant injury and fibrosis. It is postulated, therefore, that differences in the chemokine and cytokine responses driving this immune response underlie the heterogeneity of disease presentation.

Objective: To study the plasma cytokine/chemokine profiles of patients with chronic hepatitis C and to correlate these profiles with the clinical course of the disease.

Design: Plasma from patients with chronic hepatitis C was subjected to multiplex analysis of cytokine/chemokine levels (IL-1 β , IL-10, IFN- α , IL-6, IL-12p40, RANTES, Eotaxin, IL-13, IL-15, IL-17, MIP-1 α , GM-CSF, MIP-1 β , MCP-1, IL-5, IFN- γ , TNF- α , IL-1RA, IL-2, IL-7, IP-10, IL-2R, MIG, IL-4 and IL-8) by Luminex technology using the Biosource human multiplex kits. The resulting cytokine/chemokine profiles were evaluated and were correlated with viral load.

Results: Two hundred plasma samples of patients diagnosed with chronic Hepatitis C virus infection were tested. In 83 patients, the viral load was undetectable or below the lowest limits of detection. In the remaining patients, the viral load ranged from "positive" to >500,000 copies. The majority of patients (136 of 200) had no cytokine or chemokine elevations. Thirty-three patients had elevations of a single cytokine. No single cytokine was consistently elevated in this group. Among the remaining thirty-one patients with two or more elevated cytokines, elevations of IL-1 β , IL-1RA, IL-2, IL-5, IL-8, IL-15, RANTES, IFN- α and MIP-1 α were most commonly observed. Viral load was not significantly correlated with elevations of any single or combination of cytokine/chemokines.

Conclusions: The majority of patients with cytokine/chemokine elevations demonstrated increased levels of IL-1 β , IL-1RA, IL-2, IL-5, IL-8, IL-15, RANTES, IFN- α and MIP-1 α . Although these levels did not correlate with viral load, the consistent pattern seen suggests the possibility of a common pathogenetic mechanism. Correlation with other disease indicators may be helpful in explaining this pattern and utilizing the data to develop prognostic tools in the evaluation of chronic HCV.

40 Placental Chorionic Surface Vascularization Potential Is Already Partly Determined By 11-14 Weeks

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39 Multiplex Analysis Of Cytokine/Chemokine Profiles In Patients With Chronic Hepatitis C

Background: Adequate placental growth and vascularization are essential for fetal growth. Better understanding of critical periods of influence on placental growth could allow early detection and potentially intervention to improve fetal outcomes.

Design: 10 placentas from term deliveries with birthweight centiles (BW%) ≤10 were matched with 10 BW% ≥75. The placentas were digitally photographed, and the perimeter and the surface vasculature of the chorionic plate traced. The "mean vascular distance" (MVD) was calculated as the average distance from the cord insertion point to all of the vascular pixels. Stored 3D ultrasound volumes, obtained at 11-14 weeks, were used to isolate the placenta using VOCALII. We measured the distance of the cord insertion to the nearest placental margin (CordDist) every 45 deg. The vascularization index (VI~vessel density); flow index (FI~vascular intensity); and vascularization-flow index (VFI~density and intensity combined) were calculated using 4DVIEW (GE). Spearman's correlations considered $p < 0.05$ significant.

Results: The VFI at 11-14 weeks was significantly correlated with term MVD ($r = 0.54$, $p = 0.015$) and was more highly correlated than either VI ($r = 0.47$, $p = 0.039$) or FI ($r = 0.45$, $p = 0.049$). The mean CordDist and the shortest CordDist at 11-14 weeks were also highly correlated with term MVD ($r = 0.68$, $p = 0.001$, $r = 0.66$, $p = 0.001$). After controlling for mean CordDist, there was no correlation between VFI and MVD ($p = 0.274$), while the correlation between mean CordDist and MVD remained robust after controlling for VFI ($r = 0.66$, $p = 0.002$).

Conclusion: At 11-14 weeks, the extent and density of chorionic surface branches in the term placenta is correlated with VFI, an index of placental vascularity and flow, but is more strongly correlated with cord eccentricity. We speculate that early cord eccentricity may reflect in altered placental development leading to chorionic surface branching density, an important component of the nutrient transport network between fetus and placenta

41 Rectal Biopsy Findings in Down Syndrome Patients with Chronic Constipation: Hirschsprung Disease Vs. Non-hirschsprung Disease

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Background: Hirschsprung disease (HD) occurs in one of every 5,000 live births (0.02%). From 0.6-3% of all individuals with Down syndrome (DS) have HD. Constipation is common in DS. Most cases are due to a combination of low muscle tone, poor motility of unknown and/or hypothyroidism. When constipation is severe, the rectal suction biopsy (RBx) is performed to evaluate for HD. We investigated the patterns of calretinin IHC and acetylcholinesterase (ACE) activity patterns in RBx in constipated patients with DS.

Design: Among 713 patients with DS registered at CCHMC with, 27 patients with severe chronic constipation had RBx. We compared ACE and calretinin IHC in 14 DS patients with HD to 13 DS patients with normal ganglia and to 45 ganglionated control specimens from non-DS patients obtained because of clinical suspicion for HD. Calretinin staining was evaluated for nerves in the lamina propria, muscularis mucosae and submucosa. ACE was evaluated for abnormal distribution and intensity of staining reaction in the lamina propria, muscularis mucosae, excess of small submucosal nerves and large submucosal nerves >35 microns as previously reported (Ped Dev Path 2008; 11: 274-282).

Results: 2% of DS patients in our institution had short segment HD (mean age at Dx 20 days). Average age at rectal biopsy is about same in DS controls (503 days) and in non-DS normal controls (572days) All HD with DS had absent calretinin staining in the lamina propria and muscularis mucosae and a diagnostic abnormal ACE pattern. Large submucosal nerves > 35 microns were less frequent in DS with HD than in non-syndromic HD (38% vs 54%). In RBx with ganglia, many samples had the maximum normal number of small nerve twigs in the muscularis mucosae. Moreover, 5/13 patients with DS had a borderline abnormal ACE pattern with a few normal delicate twigs intermixed with a few coarse ACE positive nerve twigs entering the muscularis mucosae making interpretation of the ACE reaction challenging. All five cases had calretinin staining of rare fine long nerve fibers in the lamina propria, a normal pattern.

Conclusion: Diagnosis of HD in the setting of DS is approached in the same manner as non-syndromic HD. However we found a disproportionate number of patients with DS had an equivocal ACE stain. In this situation, and especially with small sample size, which may limit availability of submucosa for nerves and ganglia, calretinin IHC can provide important information.

42 Cd43 Expression Distinguishes Pediatric Hematolymphoid Small Round Cell Tumors from Other Small Round Cell Tumors

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Background: CD43 has been proposed as a useful marker for hematolymphoid neoplasms in pediatric small round cell tumors (SRCT), reacting with a broad spectrum of immature hematolymphoid neoplasms, as well as mature T-cells. However the specificity of CD43 in the spectrum of SRCT has not been published. We used tissue microarrays (TMA) to study CD43 immunoreactivity in 188 cases of non-hematolymphoid SRCT, and 23 hematolymphoid cases.

Design: Three TMA, and 20 archival whole section slides were immunostained containing the following: neuroblastoma (n=45), rhabdomyosarcoma (n=111), PNET/Ewing sarcoma (n=11), Wilms tumor (n=15) and desmoplastic small round cell tumor (DSRCT, n=6), diffuse large B cell lymphoma (DLBCL, n=3), chronic lymphocytic leukemia/lymphoma (CLL, n=3), mature T-cell lymphoma (n=2), precursor T-cell lymphoblastic leukemia/lymphoma (pre-T ALL, n=2), precursor B-cell lymphoblastic leukemia/lymphoma (pre-B ALL, n=3) and acute myeloid leukemia (AML, n=10). Slides were stained with CD43 (Becton Dickinson, San Jose, CA; 1:2000 dilution) using

standard heat retrieval on a Benchmark immunostainer (Ventana, Tucson, AZ). Each sample was scored for CD43 staining: 0 (no staining); 1 (uninterpretable/core missing); 2 (weak membranous/cytoplasmic); and 3 (strong membranous).

Results: Strong membranous staining was restricted to hematolymphoid neoplasms, and present in nearly all cases of immature hematolymphoid neoplasms (all AML including 4 cases each of M5 and M7, all pre-B ALL, 1 of 2 cases of pre-T ALL). Weak membranous staining was seen in one case of DLBCL and 2 cases of CLL/SLL. One case of pre-T ALL, and 1 case of CLL were negative. The majority of non-hematolymphoid tumors were negative for CD43. Cytoplasmic blush reactivity was seen in a minority of cases of neuroblastoma (9/45, 20%), rhabdomyosarcoma (9/111, 8%) and Wilms tumor (1/15, 7%), typically in foci with cytoplasmic maturation. None of the non-hematolymphoid neoplasms had membranous staining.

Conclusion: Strong membranous CD43 reactivity is a useful marker to distinguish SRCT of hematolymphoid origin from other SRCT in the pediatric setting. Reactivity in AML M5 and M7 cases is particularly useful, as these may present as extramedullary disease lacking CD34 or CD45 reactivity.

43 Ewing Sarcoma (EWS): How Does Percent Necrosis of the Primary Tumor, in Post Chemotherapy Surgical Specimens, Aid in Distinguishing Treatment Failures?

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Background: Although necrosis after chemotherapy is incorporated into osteosarcoma treatment protocols, and treatment-related necrosis has been identified as a prognostic indicator for EWS, it has not been used to direct further treatment in EWS. We systematically evaluated treatment-related necrosis as a potential prognostic and therapeutic indicator in EWS.

Design: 29 patients with non-metastatic EWS, who received standard EWS therapy, were candidates for this retrospective review. Only patients who underwent standard induction chemotherapy followed by surgical resection of the primary tumor were included. Outcome was correlated with sex, age, osseous versus extra-osseous primary and percent necrosis of tumor cells in the post chemotherapy pathologic specimen. Three pathologist reviewed specimens with standardized methods for quantification.

Results: All patients with surgical resection specimens, with at least 90% necrosis, are alive to date. Three patients are dead of disease, all of whom had an osseous primary. The patients, aged 15 to 18 years, had 80% necrosis (n = 2) and 20 % necrosis (n =1) in their surgical resection specimens.

Table 1.

Necrosis	Sample #	Average Age in years	Average outcome in months
100%	n = 13	11	90
95%	n = 3	10	86
90%	n = 4	11	36
80%	n = 3	13	104, 2 DOD
75%	n = 2	4	61
70%	n = 1	7	174
50%	n = 1	14	72
40%	n = 1	6	79

20% n = 1 15 DOD

Conclusion: Our data corroborates other's reports that percent necrosis may predict who is more likely to fail therapy and possibly can be incorporated into treatment protocols to direct therapy. In our study, cases with 90% necrosis or greater

44 Spontaneous Abortion In Fetuses Less Than 500 Grams – Can Complete Autopsy Provide Answers?

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Background: The value of complete autopsy in the spontaneously aborted fetus less than 500 grams is not well reported. The paucity of studies in this category contrasts with the therapeutic abortion category. The aim of the study was to review the diagnostic efficacy of post mortem examination in the spontaneously aborted fetus less than 500 grams.

Design: Recorded data from 200 consecutive cases from 2003 to 2008 were obtained from the Institution's post mortem database. Post mortem reports and placenta histology reports were reviewed. Birth weight, placental diagnosis, histology findings, cytogenetic results and final anatomical diagnoses were documented.

Results: The weight range of the fetuses was 1.69 to 499 grams. The incidence of final anatomical diagnoses were as follows: malformation/aneuploidy (16.5%), infection (30%); retroplacental hemorrhage (14%); umbilical cord accidents (10%); other (e.g. rhesus isoimmunization, cervical incompetence)(8%) and unexplained (21.5%). Histology findings were present in 150 cases and fell into three categories. Hypoxia effects-myocardial contraction bands (52 cases); two-tone fatty change in liver (31 cases); central nervous system hemorrhage (4 cases); Evidence of infection - intra-alveolar neutrophils (39 cases); presence of neutrophils in lumen of gastrointestinal tract (28 cases); Stress effects - growth arrests in ribs (46 cases); stress effects in thymus (36 cases); Oil Red O stain, routinely performed on adrenal glands showed a range of stress related fatty change in 95 cases. Maceration was documented in 73% of cases. Karyotyping was possible in 121 of 178 cases tested. There was higher yield from the placenta than the skin in macerated cases (68% vs. 12% of cases tested)

Conclusions: Placental examination provided the lead diagnosis in the majority (58%) of cases with autopsy histology of individual organs providing support for this diagnosis. Histology of individual organs can usefully demonstrate a cause and effect profile necessary for the overall understanding of the case. These histological results were obtained despite the expected presence of maceration in 73% of cases. Cytogenetic studies provide useful information and unsurprisingly there is a higher yield of results from placenta compared with fetal skin in macerated cases. The diagnostic yield for this scope of examinations is significant in leaving only 21.5% of cases unexplained. This compares favorably with reported series in larger fetuses. In contemporary clinical practice more questions are asked about miscarriage and complete autopsy can provide answers in a large number of cases.

45 Androgenetic Biparental Mosaicism Unmasks an Autosomal Recessive Disorder

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and/or extra-osseous EWS survived, and those with less necrosis and/or age greater than 15 years were more likely to fail.

Background: Androgenetic biparental mosaicism (ABM) denotes an embryo in which a subset of cells contains no maternal chromosomes and instead the cells possess a diploid complement of paternal chromosomes. Such embryos are at risk for placental mesenchymal dysplasia (PMD), certain imprinting disorders, and neoplasms. Although a theoretical risk exists for conditions caused by recessive alleles in the androgenetic cell population, no such example has been reported. We present a case of PMD associated with fetal Ivemark (renal-hepatic-pancreatic dysplasia) syndrome, an autosomal recessive disorder in which some cases harbor mutations in the Nephrocystin-3 (NPHP3) gene.

Design: Gross and microscopic pathology findings established the diagnoses of PMD and Ivemark syndrome (multicystic dysplastic kidneys, pancreatic cysts, and hepatic ductal plate malformation) in a cytogenetically normal 20-week female fetus. DNA extracted from paraffin-embedded placenta, liver, lung, pancreas, and kidney was used to assess the relative amounts of paternal and maternal alleles using: 1) quantitative PCR amplification of the differentially methylated Prader-Willi/Angelman syndrome locus and 2) short tandem-repeat (STR), microsatellite DNA analysis on multiple chromosomes. In addition, NPHP3 exons and their flanking intron regions were sequenced. Ploidy was assessed by flow cytometry.

Results: Flow cytometry confirmed that cells in fetal and placenta tissues were diploid. Differential methylation studies showed a marked excess of paternally imprinted chromosomes ($p < 0.0001$) and STR analysis revealed marked skewing of allelic ratios on multiple chromosomes, indicative of 58-66% androgenetic cells in the tissues studied. These ratios were statistically significant when compared with normal diploid controls. At present, approximately half of the 27 exons in NPHP3 have been sequenced and no mutations have been identified.

Conclusion: The molecular data in this case indicate ABM in fetal tissues, as well as in the placenta. The result is consistent with the hypothesis that Ivemark syndrome arose in this patient because a paternally acquired recessive mutation was "unmasked" in androgenetic cells, which lack maternal chromosomes. The identity of the putative mutant allele is unknown and no mutation has been detected by partial sequence analysis of NPHP3. This case suggests that ABM may play a role in the pathogenesis of a variety of pediatric lesions, including autosomal recessive disorders.

46 Pediatric Epithelial Salivary Gland Tumors: Spectrum of Histologies and Cytogenetics at a Children's Hospital

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Background: In children, epithelial salivary gland tumors (ESGT) are near equally split between benign (pleomorphic adenomas-[PA]) and malignant (mucoepidermoid[ME] most common). Most occur in the parotid. In series of mixed tumors in children and adults, cytogenetic abnormalities occur in

~70%, with abnormalities in 8q12 (PLAG1) in 39%, 12q13 (HMGA2) in 8%, and sporadic clonal changes in 23%. No studies have evaluated the spectrum of cytogenetic abnormalities found only in children.

Design: The histology, cytogenetics, and follow-up of all

Results: There were 13 epithelial salivary gland tumors, 12 were PAs, 1 acinic cell carcinoma(ACC).

Age	Race	Location	Dx	Cytogenetics	Follow-up
13F	AA	L Parotid	PA	46XX,t(3;8)(p21;q12)	IE, 5 yrs, NED
12M	C	Palate	PA	46XY,t(3;8)(p21;q12)	IE, 10 yrs, NED
13M	C	R Subm	PA	46XY,t(3;8)(p21.3;q12)	IE, Recurred 5 yrs, reexcised, 1 yr NED
17F	C	Palate	PA	46XX,ins(18;8)(q21.1;q12q22.2)	CE, 3 yrs, NED
11M	AA	R Subm	PA	46,XY,t(8;9)(q12;p22)	CE, 6mo,NED
14F	C	Palate	PA	45XX,der(6)t(6;12)(q13;q13)-12	IE, 1 yr, NED
14F	AA	L Pharynx	PA	46XX	CE, 10 yrs, NED
17F	AA	L Parotid	PA	46XX	IE, 8 yrs, NED
13M	C	L Parotid	PA	46XY	CE, 9 yrs, NED
10F	C	L Parotid	PA	46XX	CE, 6mo,NED
17F	C	L Parotid	PA	NA	IE, 13 yrs, NED
16F	C	R Parotid	PA	NA	IE, 14 yrs, NED
11F	C	R Parotid	ACC	46XX,t(1;12)(q32;q13-15)	CE, 10 yr, NED

PA-plaemorphic adenoma, ACC-acinic cell carcinoma, CE-complete excision, IE-incomplete excision

Of the PAs, 8 were female, 4 male. 8 were Caucasian, 4 AA (hospital population 58% AA, 36% Caucasian). 3 PAs were initially diagnosed by FNA. None degenerated into a malignant tumor. One 13yo AA F had Klippel-Trenaunay-Weber syndrome, all other patients were normal. 10 PAs had cytogenetic studies. 4 were normal. 5 involved 8q12, 1 involved 12q13. The ACC also had an abnormality involving 12q13.

Conclusion: The 8q12 site associated with the PLAG1 abnormality was identified in 50% of our PAs, and the 12q13 associated with HMGA2 was identified in 10%, similar to that reported in mixed adult and child studies. No sporadic clonal abnormalities were identified, in contrast to the 23% reported in mixed studies. MEs are absent from our series. Most tumors were benign, more like that reported in adults rather than children. Most tumors did not recur, although 7/13 were incompletely excised. EGSTs were near evenly split between the parotid (7) and other sites (2 submandibular, 4 minor), unlike other series in children where 90% arose in the parotid. M:F ratio was 1:3, similar to other studies. Most (12/13) children had no underlying abnormality.

47 Coexistence of Eosinophilic Esophagitis (Ee) and Celiac Disease (CD)

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Background: EE, characterized by dense infiltration of esophageal mucosa by eosinophils, is often associated with food allergy/hypersensitivity. CD is a distinct food-induced, immune-mediated gastrointestinal disease. We report children who have both EE and CD (Group 1) and compare them to children with CD only (Group 2).

Design: Patients were identified in the pathology database; medical records and available slides were reviewed. Inclusion criterion for EE was peak intraepithelial eosinophil number ≥ 24 per high-power field (HPF) in esophageal biopsies. Marsh classification was used to type CD. Quantitative histology yielded peak number of intraepithelial lymphocytes (IEL)/100 epithelial cells in duodenal biopsies; peak IEL/HPF in esophageal epithelium; peak eosinophil number (PEN)/HPF in esophageal and duodenal biopsies. Student t-test was performed and significance set at $P \leq 0.05$.

epithelial salivary gland tumors encountered at a children's hospital from 1994-2008 were reviewed.

Results: There were 6 patients (M/F: 4/2; mean age 8 years, range: 4-14 years) in Group 1, and 9 (M/F: 3/6; mean age 7 years, range: 3-14 years) in Group 2. All 15 patients had type 3 CD with flattened villi, elongated crypts and ≥ 40 IEL/100 enterocytes. Follow-up of 5/6 in Group 1 documented clinical and histologic response to gluten-free diet (GFD); 5/5 in Group 2 were clinically improved, but follow-up biopsies were not available. Prediet tissue transglutaminase was elevated in 3/3 in Group 1, and in 8/9 in Group 2. Prior to GFD, PEN in esophageal biopsies was 70 ± 24 in Group 1 vs 0 in Group 2. PEN in duodenal biopsies did not differ between the groups: 62 ± 14 vs 64 ± 14 ($P \geq 0.05$). IEL was elevated in duodenal biopsies in both groups: 69 ± 32 vs 79 ± 44 ($P \geq 0.05$), and IEL was elevated in esophageal biopsies in Group 1: 35 ± 15 vs 6 ± 2 ($P \leq 0.05$). Compared to pre-GFD values, biopsies after GFD in 3 Group 1 patients showed that PEN was not significantly reduced in esophageal biopsies: 71 ± 16 vs 89 ± 88 ($P \geq 0.05$), but was reduced in duodenal biopsies: 70 ± 5 vs 41 ± 12 ($P \leq 0.05$). IEL was significantly reduced in duodenal biopsies: 77 ± 42 vs 7.7 ± 2.5 ($P \leq 0.05$), but not in esophageal biopsies: 43 ± 15 vs 36 ± 1 ($P \geq 0.05$).

Conclusion: The coexistence of EE and CD suggests that these diseases may share pathogenetic mechanisms, requiring further study. However, although GFD is sufficient to treat CD, it is not sufficient to treat EE in patients who have both diseases: GFD significantly reduces eosinophilic and lymphocytic inflammation in the duodenum but not esophagus.

48 The Placental Signature of Twin-to-Twin Transfusion Syndrome

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Background: Twin-to-twin transfusion syndrome (TTTS) is a multifactorial disorder with placental, fetal and possibly maternal contributions that develops in 10-30% of diamniotic-monochorionic (DiMo) twin gestations. The relative importance of candidate placental markers of TTTS, and the degree of imbalance of deep arteriovenous (AV) anastomoses in particular, remains largely undetermined

Design: We performed a prospective morphometric analysis of 281 consecutive monochorionic twin placentas, studied by vascular injection. Following exclusion of monoamniotic, multiple, TRAP and laser-ablated placentas, as well as placentas associated with intrauterine demise or $>20\%$ birth-weight discordance, 191 placentas were considered for this study (24 TTTS and 167 non-TTTS with birth weight discordance $<20\%$, "DiMo control"). In addition to routine twin placental parameters, including vascular distribution patterns, we recorded the number, type and size of intertwin anastomoses (AA, VV and AV) and calculated the net cross-sectional area of AV anastomoses. Data were compared by chi-square, univariate

and multivariate analysis.

Results: Univariate analysis identified five independent post-hoc predictors of TTTS: velamentous cord insertion (risk ratio (RR) 4.5; $P < 0.001$), magistral vascular distribution (RR 4.9; $P < 0.001$), >25% difference in placental share (RR 7.7; $P < 0.002$), absence of AA anastomoses (RR 4.4; $P < 0.001$) and presence of VV anastomoses (RR 2.8; $P < 0.001$). In stepwise multivariate modeling, absence of AA, vascular distribution pattern and cord insertion were most important, although all **Conclusion:** TTTS has distinct placental associations, warranting their routine inclusion in the DiMo placental pathology report. These critical placental variables can usually be assessed without vascular injection, suggesting this technique may be reserved for research purposes and laser-treated placentas. Finally, the association of TTTS with absent AA anastomoses and mainly magistral vascular distribution patterns supports the recent notion that TTTS may be an anti-angiogenic condition.

49 Thymolipoma: Evidence Supporting a Clonal Neoplastic Process

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Background: Thymolipomas are rare benign thymic tumors comprised of often abundant mature adipose tissue admixed with thymic tissue lacking a normal lobular organization. They have been considered a hamartomatous process. Except for one reported case, they have not been examined for clonal cytogenetic aberrations.

Design: Thymolipomas with conventional cytogenetic karyotypic analysis were identified in the departmental files of Children's Hospital Boston. Clinical data, imaging, and pathologic material were reviewed, including gross photographs, histopathology, immunohistochemistry, flow cytometry, and cytogenetic reports.

Results: Two cases were identified with cytogenetic data, both with clonal abnormalities. The tumors were 2700 g and 195 g masses incidentally discovered in a 12 year-old female and 14 year-old male, respectively. Both tumors were solid with significant fat attenuation on CT. They were well-circumscribed and lobulated with a yellow and white cut surface. Histologic examination revealed abundant mature adipose tissue admixed with large areas of thymus containing lymphocytic and epithelial components but lacking a normal lobular organization. The total amount of thymic parenchyma was considerably increased compared to expected values for age. Both lesions contained a rim of normal residual thymus. Immunohistochemical and flow cytometric analyses were consistent with normal thymus. Cytogenetic analysis documented a supernumerary ring chromosome in the first tumor and a t(10;12)(q22;q14) translocation in the second tumor.

Conclusion: We document clonal cytogenetic abnormalities in 2 thymolipomas. Translocations involving 12q13-15 are found in two-thirds of lipomas and in the only reported thymolipoma. The translocation results in expression of the high mobility group antigen protein A2 (HMGA2), an embryonic mesenchymal transcription factor normally silent in mature tissues. The 10q22 breakpoint, common in uterine leiomyomas, has also been documented in several lipomas. Supernumerary

five variables provided independent risk. The total number of anastomoses was similar in both groups (median 6 (range 2-23) in TTTS versus 7 (0-23) in DiMo control). Interestingly, the net cross-sectional area of deep AV anastomoses tended to be lower in TTTS than in DiMo control placentas (0.39 mm² (0-6.67 mm²) versus 0.79 mm² (0-42.78 mm²)) and net AV imbalance was not identified as independent risk factor for TTTS.

ring chromosomes, often involving 12q, are the primary aberration in a small subset of lipomas. Since aberrant expression of HMGA2 results in adipocyte proliferation and expression of a related homologue, HMGA1, is critical for lymphoid development and neoplasia, dysregulated expression of HMGA2 in the thymus might account for the unique phenotype of a lipomatous tumor with associated lymphoid hyperplasia found in thymolipoma.

50 Immunohistochemical Expression of Claudin 3 and 4 in Duodenal Biopsies from Patients with Gluten Sensitive Enteropathy

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Background: The diagnosis of gluten sensitive enteropathy (GSE) is a clinicopathologic one based on the presence of increased intraepithelial lymphocytes, villous blunting and lamina propria plasmacytosis on duodenal biopsies (DuoBx) in the context of supporting clinical and serologic data. Immunohistochemical (IHC) expression of the enterocyte junction proteins claudin 3 and 4 have not been characterized in cases of GSE. In this pilot study, we sought to compare IHC expression of these claudins in DuoBx from patients fulfilling criteria for a diagnosis of GSE, biopsies with increased intraepithelial lymphocytes (IELs) but normal villous morphology and normal biopsies.

Design: Nineteen DuoBx from 14 patients with histologic features of GSE (range 4-17 years, median age 11 years), 7 with IELs and normal villous morphology (range 2-16 years, median 10 years) and 10 normal biopsies (range 4-18 years, median 9 years) were retrieved from our files. IHC staining was performed using commercially available rabbit polyclonal antibodies to claudins 3 and 4 at a dilution of 1:2000. The pattern of villous and crypt enterocyte membrane staining were blindly assessed and subsequently correlated with clinical, endoscopic and serologic data.

Results: There was diffuse enterocyte staining within villi and crypts with claudin 3 in all normal biopsies, without any discernible change in pattern of expression in the GSE and IELs categories. However, claudin 4 was expressed in enterocytes restricted to the upper third of villi from all normal biopsies. In all 19 biopsies from 14 patients histologically compatible with GSE, there was diffuse contiguous expression of claudin 4 in surface enterocytes as well as expression on enterocytes within crypts. Clinical and serological data supported the diagnosis of GSE in these cases.

Claudin 4 expression in 2 of 7 biopsies in the IELs category was similar to that of the normal biopsies while the remaining 5 cases demonstrated increased villous staining as well as crypt expression. Two of the five had biopsies from the bulb consistent with GSE along with supporting clinical and serologic data. Another 2 had elevated tissue transglutaminase

(tTG) levels, one of whom had a strong family history of GSE and the other presented with failure to thrive. All 4 of these 5 patients were placed on gluten-free diet with good response. The last patient (1/5) had a normal tTG level and was kept on a normal diet, but continued to have symptoms.

Conclusion: Confirmation of this aberrant claudin 4 expression

in DuoBx of GSE and in cases with IIELs in a large series would suggest potential for application into clinical practice in identifying cases of latent or early GSE.