1. Novel *PDGFRB* Mutations Identified in Infantile Myofibromatosis, Methodology Matters

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Background: Infantile myofibromatosis (IM) affects children under the age of one year with tumors arising in the skin, muscle, bone, or viscera and may be classified into solitary, multiple, or generalized forms. IM can occur as a sporadic disease or can be familial, most often with an autosomal dominant inheritance pattern. Mutations in *PDGFRB* have been previously identified in both familial and sporadic cases of infantile myofibromatosis. To date, approximately half of myofibroma(tosis) cases have demonstrated point mutations in exons 12 or 14 most frequently, followed by exons 11 or 18. This is important as patients with multiple or generalized forms of IM may benefit from targeted therapies (sunitinib).

Studies have described Sanger and targeted sequencing methodologies focusing on these specific codons of *PDGFRB*. However, Sanger sequencing and many of the targeted sequencing platforms described in the literature may be unable to detect large insertion/deletion or structural variants. We wanted to better characterize the mutational landscape in IM, since many cases in the literature have lacked *PDGFRB* mutations by Sanger or targeted sequencing methods.

Methods: We searched the local institutional pathology database for diagnoses of myofibromatosis. Clinical next generation sequencing (NGS) was performed in a local CLIA-certified, CAP-accredited laboratory and is a DNA-based test targeting 262 cancer-related genes, including *PDGFRB*. This test detects single base changes, insertions and deletions, structural rearrangements, copy number variants, and microsatellite instability.

Results: Six cases of myofibromatosis were diagnosed at our institution between 2003-2018, all occurring in infants (<1 year of age). NGS yielded the following results: 3 internal tandem duplications beginning at exon 13, one 33 base pair (bp) insertion in exon 13, one 27 bp deletion in exon 13-intron13, and one case with 2 hot spot mutations (W566G and Y589C, cis), both in exon 12.

Conclusion: We confirmed the presence of *PDGFRB* mutations in our entire cohort of infantile myofibromatosis cases. Notably, 5/6 of these cases involved large insertion/deletion mutations that would be missed by Sanger sequencing or exon-restricted targeted sequencing assays. This suggests that NGS tests interrogating *PDGFRB* for the diagnosis of myofibromatosis should not be restricted to "hotspot" sequencing, and molecular methodologies designed to detect these larger mutation events are required.

2. NGS Testing with the OncoKids^{SM} Panel Identifies Clinically Significant Findings in the Majority of Pediatric Sarcomas/Soft Tissue Tumors

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Background: The OncoKids^{SM} panel is an amplification-based next-generation sequencing assay designed to detect diagnostic, prognostic, and therapeutic markers across the full spectrum of pediatric malignancies. This assay uses low input amounts of DNA and RNA and is compatible with FFPE.

Methods: Sequencing was performed using the Ion S5^{TM} platform. This study was a retrospective analysis of non-CNS solid tumors (including sarcomas and soft tissue tumors) assayed with OncoKids^{SM} between June 20, 2017 and June 20, 2018.

Results: Of over 300 pediatric malignancies assayed, there were 115 non-CNS solid tumors including 15 neuroblastomas, 14 osteosarcomas, 13 rhabdomyosarcomas, 11 sarcoma NOS specimens, six Ewing sarcomas, six sex cord stromal tumors, and a variety of less common tumors.

62 samples (54%) demonstrated at least one variant of strong clinical significance while 10 samples had at least one variant of potential clinical significance (9%). Gene fusions of strong clinical significance were observed in 25 samples (22%). Nine of 25 fusion-positive samples demonstrated a fusion that would be undetectable with standard FISH testing at our institution. This group included therapeutically actionable fusions with targeted therapies such as larotrectinib (TPM3-NTRK1 and LMNA1-NTRK1) and crizotinib (TFG-ROS1) as well as diagnostic fusions for a variety of tumor types (e.g., CIC-DUX4 in Ewing-like sarcoma, MYB-NFIB in adenoid cystic carcinoma, and CCDC6-RET in papillary thyroid carcinoma). Notably, three of 10 fusion-positive samples by OncoKids^{SM} NGS testing were negative with the corresponding FISH probe; this may be due to sample quality or other limiting factors.

Testing of tumor tissue revealed potential underlying germline mutations in cancer predisposition genes in 29 of 115 patients (25%). A recommendation for targeted germline testing resulted in screening 16 patients. Six of the 16 patients had a germline pathogenic or likely pathogenic variant, including three children with DICER1 mutations, and one patient each with mutations in TP53, CBL, and WT1.

Conclusion: Routine molecular profiling of pediatric malignancies in the front-line setting by OncoKids^{SM} provided a significant improvement in the diagnosis and the opportunity for targeted therapy in children with sarcomas and other solid tumors and prompted screening and counselling in patients and families with cancer predisposition.

3. A Somatic Activating *NRAS* Mutation Associated with Kaposiform Lymphangiomatosis

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Background: Kaposiform lymphangiomatosis (KLA) is a rare, frequently aggressive, systemic disorder of the lymphatic vascular system, occurring primarily in children. Even with multimodal treatments, KLA has a poor prognosis and high mortality rate secondary to coagulopathy, effusions and systemic involvement. We hypothesized that, as has recently been found for other vascular anomalies, KLA may be caused by somatic mosaic mutations during vascular development.

Methods: We performed exome sequencing of tumor samples from five individuals with KLA, along with samples from uninvolved control tissue in three of the five. We used digital PCR (dPCR) to validate the exome findings and to screen KLA samples from four other individuals. We also screened six tumor samples from individuals with kaposiform hemangioendothelioma (KHE).

Results: We identified a somatic activating *NRAS* mutation (c.182A>G, p.Q61R) in lesional tissue from 8/9 individuals with KLA, at levels ranging from 1-28%. The mutation was absent from the tested control tissues as well as from tissues of KHE.

Conclusion: The activating *NRAS* p.Q61R variant is a known 'hotspot' variant, frequently identified in several types of human cancer, especially melanoma. It results in a constitutively active NRAS protein, leading to unchecked cellular growth and proliferation. KLA, therefore, joins a growing group of vascular malformations and tumors caused by somatic activating variants in the RAS/PI3K/mTOR signalling pathways. This discovery will expand treatment options for these high risk patients as there is potential for use of targeted RAS pathway inhibitors.

4. DOG1 Expression in *NTRK*-Rearranged Mesenchymal Tumors

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Background: Gastrointestinal stromal tumors (GISTs), the most common mesenchymal neoplasms of the gastrointestinal (GI) tract, are tyrosine kinase driven tumors with common genetic alterations in KIT/PDGFRA and less frequently alterations involving SDH and the RAS signaling pathway. In a small fraction of GISTs, no distinct oncogenic driver mutation has been identified, dubbed the "quadruple wild type (WT)" GIST. Immunohistochemical (IHC) staining with DOG1, CD117, and/or CD34 are used to make this diagnosis, and based on this IHC profile a variety of genetic aberrations have been identified in quadruple WT GISTs, including 3 cases of *ETV6-NTRK3* fusions. The *ETV6-NTRK3* gene fusion was first identified in infantile fibrosarcoma, followed by congenital mesoblastic nephroma (CMN); recently other variant *NTRK* fusions have been described in soft tissue tumors. Therefore, we sought to evaluate the rate DOG1, CD117 and CD34 expression in *NTRK*-rearranged tumors to determine if this IHC staining profile was specific for GIST.

Methods: Eleven molecularly confirmed NTRK-rearranged tumors from 2 institutions were retrieved from archival pathology databases from 2005-2017. Clinicopathologic data was recorded. A representative block from each case was selected for immunohistochemical staining with DOG1, CD117 (GIST titration), and CD34 by standard methods. Immunohistochemistry was scored as follows: 0, no staining; 1+, <5%; 2+, 5-25%; 3+, 26-50%; 4+, 51-75%; 5+, 76-100% of cells; staining intensity was also recorded as weak, moderate, or strong. An overall assignment of "positive" staining required a minimum of 2+ (moderate) staining.

Results: Patient age at presentation ranged from birth-18 months (mean 4 months, median 2 months) with no gender predilection; cases were soft tissue based tumors (4 extremity, 4 trunk, 1 intra-abdominal, 1 head & neck) and 1 case was kidney based (congenital mesoblastic nephroma). The tumor fusions included: *ETV6-NTRK3* (n=5), *TPM3-NTRK1* (n=4), *LMNA-NTRK1* (n=1), and *EML4-NTRK3* (n=1). Seven of 11 cases were immunoreactive for DOG1 with 2 cases demonstrating 5+ staining (1 *ETV6-NTRK3* renal tumor, 1 *TPM3-NTRK1* extremity tumor). CD34 staining was positive in 5 of 11 cases; 3 of which had concurrent DOG1 expression. All cases were negative for CD117.

Conclusion: Cases in this study would not typically be included in the differential diagnosis of GIST (infantile and non-GI based), but several cases showed DOG1 and/or CD34 expression. Including a small subset demonstrated strong diffuse staining analogous to that seen commonly in WT GIST. These findings raise the question as to whether quadruple WT GIST are truly "GIST" or, if they simply should be defined by molecular alteration.

5. Loss of BRG1 (SMARCA4) Expression in a Non-CNS, Non-Renal Tumor Cohort

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Background: Malignant rhabdoid tumors (MRTs) and atypical teratoid/rhabdoid tumors (AT/RTs) of the central nervous system (CNS) are primitive malignancies associated with a poor prognosis. These tumors have previously been characterized by inactivation of the SWI/SNF chromatin remodelling complex protein INI1 (*SMARCB1*). In the last decade, sporadic publications have shown that a different SWI/SNF protein, BRG1 (*SMARCA4*), is associated with a similar rhabdoid phenotype and possible germline mutation (rhabdoid tumor predisposition syndrome type 2). We sought to determine the role of BRG1 expression in pediatric embryonal tumors.

Methods: A search of the pathology database for the terms “rhabdoid”, “undifferentiated”, or “polyphenotypic” resulted in a total of 28 cases after reviewing and excluding those cases with either a diagnostic immunohistologic or cytogenetic profile. A tissue microarray (TMA) was constructed, and where insufficient tissue was available for core removal, unstained slides were utilized. Immunohistochemistry was performed including INI1 and BRG1. The BRG1 TMA was independently scored by two pathologists blinded to the clinical history or immunophenotypic profile.

Results: Of the 28 cases, four (14.3%) showed loss of INI1 staining consistent with MRT (1 liver, 1 renal, 2 soft tissue). Of the remaining 24 cases, three (12.5%) showed complete loss of BRG1 expression. One was an ovarian mass in a 15 year old female consistent with a small cell carcinoma of the ovary, hypercalcemic type (SCCOHT). One was an abdominal mass in a 5-month old boy who died of disease at 9 months. The last was a 3-month male with a right scapular soft tissue mass who was alive and lost to follow-up after 9 years. Conventional karyotype was normal for both of these latter two patients and both showed perinuclear cytoplasmic whorls of intermediate filaments. Vimentin was strongly positive in all three tumors with accentuation of the eccentric cytoplasm. EMA was moderately positive in the shoulder lesion, while negative in the abdominal and ovarian lesions.

Conclusion: This study shows that a subset of pediatric MRTs may be better classified when both INI1 and BRG1 antibodies are employed. Although one case is consistent with a SCCOHT (MRT of the ovary), the two other cases show features consistent with extra-renal MRTs. Interestingly, one case showed a precipitous progression of disease while another showed prolonged survival. Future studies including AT/RTs may further suggest that BRG1 is a reflex second tier stain for INI1 retained tumors.

6. The Impact of Molecular Testing on the Histologic Diagnosis of Pediatric CNS Tumors

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Background: With the institution of the 2016 edition of the WHO Classification of Central Nervous System Tumors, the diagnosis of brain and spinal cord tumors incorporates molecular pathway testing in addition to morphologic assessment of tumors. In our practice, we have occasionally encountered cases in which molecular studies contributed to a change in final diagnosis. We therefore sought to examine diagnostic correlation between initial histopathologic diagnosis of CNS tumors and final diagnosis of those tumors following expert consultation and molecular pathway testing.

Methods: We searched the pediatric pathology case records between 06/2013 – 06/2018 and identified 170 brain and spinal cord tumor biopsies (52) and resections (118). During this period 88 cases were sent out for molecular pathway testing and/or expert consultation. Data collected included patient age, sex, initial morphologic tumor classification, molecular testing performed, and diagnosis following molecular pathway testing.

Results: Initial classification as glial or embryonal was correct in all but one case (87/88, 98.9%) as compared to the final diagnosis based on molecular studies and expert consultation. Additionally, minor diagnostic discrepancies were seen in 4 cases (4.5%), including 3 cases of glial versus glioneuronal tumor and one case initially deemed nondiagnostic but called a pilocytic astrocytoma after discovery of a *BRAF* rearrangement. Molecular testing was most useful in refining classification of the following tumors: 23/30 *KIAA1549-BRAF* fusion assays were positive and used to confirm pilocytic astrocytomas, histone H3.3K27M mutations were detected in 2/16 tested gliomas, 1/4 ependymomas examined had a *RELA* fusion, and 16 medulloblastomas were categorized by molecular pathway analysis. Mutational testing for the BRAF V600E variant was performed in 32 cases for both classification and potential therapeutic application. Of the 61 cases that could be assigned a WHO grade, tumor grade assigned at initial diagnosis correlated with that of the final molecular/expert grading in 86.9% of cases (53/61). Overall, only 3 cases (4.9%) had significant changes in grade – 2 low-grade ependymomas were reclassified as high-grade anaplastic ependymomas, and one glioma was changed from low to high grade.

Conclusion: Thus, standard assessment of histologic and immunohistochemical features is highly accurate for the broad classification and grading of pediatric CNS tumors. However, molecular testing and expert consultation are useful in the refinement of diagnosis and grade within the revised 2016 WHO classification, as well as the assessment of more difficult tumors such as anaplastic ependymomas. Additionally, molecular testing may identify therapeutically targetable molecular alterations in an important subset of cases.

7. Towards A More Accurate Characterization Of Histiocytoid Cardiomyopathy (HC): Implicating New Genes In The NDUF Family And Renaming The Condition

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Background: Many hypotheses have been proposed in the etiopathogenesis of HC, including cytochrome b missense mutations in complex III of the electron transport chain, MERRF gene mutations in complex IV, and X-linked Xp22 mutations. However, the first breakthrough in the molecular characterization of HC was the identification of a de novo nonsense mutation in exon 2 of the *NDUFB11* gene, which encodes a structural component of complex I of the electron transport chain, in two cases. This finding was confirmed 2 years later by another HC case report from Rahei et al. We have since analyzed new families from the HC registry. Additionally, over the past several years, we have demonstrated that the condition is neither histiocytic nor a cardiomyopathy. We therefore aim, in addition to reporting the newly identified genes to Society members, to propose an overdue renaming of the condition.

Methods: Utilizing our registry of over 150 individuals, we performed whole exome sequencing of 10 members of one familial case and 3 family members in one sporadic case. Supporting the proposed new name, we stained 50 sections from the registry samples for CD68 to exclude the histiocytic nature of the cells of origin.

Results: The familial case was composed of 2 parents, 6 living siblings (4 female and 2 male) and 2 deceased (6-month-old male and 9-month-old female). The other family had a sporadic case and consisted of 2 parents and one deceased child. Our analysis showed no mutations in the parents and living siblings. Therefore, our identification of a de novo missense mutation in *NDUFB9* in male and female siblings, who died 7 years apart, suggests a familial tendency of this condition. In the sporadic case, we identified a nonsense mutation in *NDUFA1*, located on Xq24. In our staining for histiocytic markers, all 50 cases stained negative for CD68.

Conclusion: Our data implicates 2 additional members of the *NDUF* family (*NDUFB9* and *NDUFA1)* in the etiopathogenesis of this condition for its role in complex I of the electron transport chain. Our discovery of the same *NDUFB9* mutation in 2 deceased siblings also suggests a familial tendency in the condition. These findings support and expand upon our previous work on the role of the *NDUF* family. Our findings show that the cells of origin in these cases are not histiocytic, nor has the disease ever been classified by consensus data as a cardiomyopathy. Therefore, we propose a more accurate name for the condition: Arrhythmogenic Conduction System Hamartoma (ACSH). We will adopt this name in further publications.

8. Dissecting The Cardiac Conduction System: Is It Worthwhile?

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Background: Dissection of the conduction system (CS) is not performed routinely at autopsy or during evaluation of cardiac explants, being perceived as a last resort in identifying the cause of death, and possibly considered unrewarding. Histologic changes are mostly reported in forensic practice, in adult patients with sudden death. We studied the value of consistently performing such dissections in the appropriate clinical context in our pediatric patients.

Methods: Patients with unexplained sudden death (autopsy cases) or severe, inexplicable arrhythmias (both autopsy and surgical cardiac explants) were included. Dissection of the sinoatrial (SA), atrioventricular (AV) nodes and bundle of His (BH) was performed following standard techniques, in addition to routine examination of ventricular walls, valves and vessels. Masson trichrome stain was used to better identify the CS structures. The pathologic changes observed within the CS components were recorded and correlated with findings in other sections from the heart.

Results: 20 patients (12 male, 8 female; 8 days to 19 years of age) were included: 12 autopsies and 8 surgical explants. 13 patients had a prior history of cardiac disease (cardiomyopathy, valvular abnormalities, ASD/VSD, etc). Indications were sudden death (11 cases) or severe arrhythmias (9 cases). CS components were retrieved in all instances: SA node was identified in 8 cases, AV node in 15 and BH in 13. Pathologic changes within any of the CS structures were present in 17/20 patients (85%). Of these, 13 (76%) had findings that mirrored those found in other cardiac structures, including inflammatory changes related to active myocarditis (3), fibrosis (2), post-transplant rejection/coronary allograft vasculopathy (2), ischemia (1), BH/bundle branches endocardial fibroelastosis (1), histiocytoid cardiomyopathy (1), rhabdomyoma (1), prominent cardiomyocyte hypertrophy (1) and vascular fibromuscular dysplasia (1). Four cases (24%) had abnormalities restricted to CS: BH with suture material and giant cell reaction following VSD repair (1); BH with fibrosis and calcifications (1 patient with congenital complete AV block), intimal fibroplasia of SA node artery (1 case with sudden death), and inflammatory changes of CS (1 case with AV block).

Conclusion: Pathologic changes within the CS are present in a high percentage (85%) of pediatric cases with known cardiac diseases presenting with unexplained sudden death or severe arrhythmias. Most frequently the findings mirror those observed in other cardiac structures and are variable. However, in a significant number of cases (4/20, 20%) the changes are restricted to CS and likely explain the patients' symptoms or the cause of death. Systematic dissection of the CS adds valuable information in a selected cohort of patients.

9. Calretinin-Immunopositive Mucosal Neurites in the Squamocolumnar Junction: A Supplemental Diagnostic Technique for Hirschsprung Disease

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Background: Diagnosis of Hirschsprung disease (HD) relies upon confirmation of aganglionosis in suction rectal biopsies taken 2 cm above the pectinate line/squamocolumnar junction (SCJ), proximal to the physiologic hypoganglionic zone (HZ). One limitation is the risk of false negative results with overly proximal biopsies in short- or very short-segment HD. On the other hand, overly distal biopsies from the HZ and SCJ are considered inadequate. Targeting the SCJ for mucosal biopsies has three advantages: 1. The SCJ provides a histopathologic landmark for accurate localization of biopsies. 2. These distal most biopsies reduce the risk of false negative results. 3. Low mucosa-only biopsies are less invasive than proximal suction rectal biopsies reducing the risk of perforation. Calretinin immunohistochemistry (CIHC) is an established ancillary technique for the diagnosis of HD. One caveat is that CIHC can give a false negative result in a biopsy taken too proximal in the transition zone (TZ) or proximal aganglionic segment. This retrospective pilot study evaluated whether CIHC distinguishes between HD and non-HD biopsies from the SCJ.

Methods: The present study followed from a preliminary analysis of infant anorectal canal autopsy specimens, which demonstrated calretinin staining of mucosal neurites in non-HD cases in the HZ extending to the SCJ, and complete absence of calretinin staining in a HD case. We retrospectively identified 11 rule-out HD rectal biopsies that included the SCJ and performed CIHC on each biopsy. Mucosal mast cells were used as an internal positive control. Three experienced pediatric pathologists independently examined the H&E and calretinin-stained slides, blinded to the clinical information. The presence or absence of HD in each case was subsequently ascertained from the electronic medical record.

Results: All observers correctly identified each of the biopsies from HD patients (N = 3) based on the complete absence of calretinin-positive mucosal neurites. All observers correctly identified biopsies from non-HD patients (N = 8) by the presence of calretinin-positive mucosal neurites extending to the SCJ. Calretinin-immunoreactive mast cells were present in each biopsy, providing an internal positive control.

Conclusion: CIHC can be used in the HZ to diagnose HD. A larger, prospective study of mucosa-only biopsies from the SCJ is warranted. Targeting the SCJ could be more sensitive, more specific, and less invasive. The limitation of false-negative results with CIHC staining in the TZ or proximal aganglionic segment may be negligible in this location. Lastly, endoscopic mucosal mapping of calretinin staining starting from the SCJ and moving proximally could provide a basis for surgical planning and medical management in HD.

10. Clinical And Histologic Features Of Barret's Esophagus In A Pediatric Population

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Background: There are only few reports of Barrett's esophagus (BE) in children. We aimed to describe the clinico-pathologic features of BE in a cohort of pediatric patients to identify associations with other disorders, clinical manifestations, endoscopic findings and outcome.

Methods: Patients diagnosed with BE (intestinal metaplasia-IM- in esophageal mucosa) between 1992 and 2016 were identified by a search in our pathology information system. Retrospective review of medical records and archived glass slides was performed.

Results: We identified 24 patients (7 girls, 17 boys, 5-20 years of age, mean age = 13. 9 years at presentation) with a histologic diagnosis of BE (presence of IM). "Esophagitis" was the most frequent clinical presentation (15). A neurologic disorder was the most frequent underlying disease (7 with cerebral palsy). Body mass index (BMI) was available in 14 patients and was significantly increased in 2. The most frequent endoscopic finding was esophageal mucosa suspicious for BE (7). The patients underwent a combined total of 219 esophageal biopsies (distal, mid and/or proximal segments) through the years and 67 of theml (30.6%) showed IM. In cases with questionable IM on routine HE sections (41.8%), Alcian blue stain was performed and confirmed presence of goblet cells. The most common location of the IM was the distal esophagus (35 biopsies), followed by the mid-esophagus (n=9) ; the proximal esophagus was not involved in any of the cases. . Fourteen patients had no esophageal biopsies before the one diagnosed as BE. The remaining 10 patients had previous biopsies showing only gastric metaplasia (3), features of reflux plus ulceration (2), gastric metaplasia plus ulceration (1), ulceration only (1), reflux plus acute esophagitis (1), acute esophagitis with ulceration (1) and eosinophilic esophagitis (EoE, 1). The most common additional histologic gastrointestinal finding associated with BE was inactive chronic gastritis (6). Interestingly, EoE was observed in 3 patients (2 with no previous biopsies, 1 with EoE in a previous biopsy) . Follow-up (1 month-17 years) was available in 15 patients. BE continued to be observed in 10 biopsies from those patients. No definitive dysplasia was observed, but one biopsy showed features considered indeterminate for dysplasia.

Conclusion: The most frequent clinical association was a neurologic disorder, particularly cerebral palsy. Obesity was not a frequent association. Endoscopy was suspicious in only a minority of cases, therefore warranting histologic evaluation. EoE can be associated with BE. All of our patients had a good outcome.

11. Sources of Error In Pediatric Surgical Pathology: A Review Of Amended Reports

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Background: Amended reports are used to alter information in a pathology report, as opposed to addended reports, which are used to only add (but not alter) information in a report. Several studies of amended reports have been performed in adult surgical pathology, but no such studies have been performed in pediatric surgical pathology. We hypothesized that the types of error and organs affected would differ in a pediatric only practice compared to typical adult pathology settings.

Methods: Retrospective review of all amended reports over a 10-year period (2007-2016) at a tertiary care children's hospital was performed. Using the taxonomy published by Meier et al, amended reports were classified into one of four defect types by the apparent cause of the amendment: report defect, specimen defect, misidentification, or misinterpretation. Amended reports were stratified by organ site, defect discoverer, mode of discovery, and clinical significance. Statistical comparison to published adult data was performed using the Chi-squared test considering a p-value of less than 0.05 as significant. Descriptive statistics were used to characterize the specific nature of the errors.

Results: Our average amendment rate was 2.43 per 1000 reports, within the published range reported for adult practices. There was a significantly different distribution of error types compared to published adult data ($p < 0.0001$) mostly driven by many fewer report defects in pediatric practice. The distribution of organs affected by amended reports was also significantly different in pediatric practice compared to adults ($p < 0.0001$) with endoscopic gastrointestinal biopsies as the most common organ in pediatrics compared to skin in adults. In most instances (74%), both original and amended reports involved non-neoplastic diagnoses; however, in a subset of cases (11%), there was a diagnostic change between a non-neoplastic and neoplastic entity.

Conclusion: The types of error prompting amendment and the specimen sites affected by amendments differ significantly between pediatric and adult practices. This implies that best practices directed at reducing amendment rates designed to reduce error in adult practice may not be appropriate for the pediatric setting. Future work will be needed to define pediatric-specific best practices for report error reduction.

12. Diagnostic yield by indication for submission of singleton placentas from term births

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Background: Significant placental pathology has been shown to correlate with adverse outcomes. Submission to pathology depends on clinical judgment, but additional guidance is necessary. Previously published College of American Pathologists (CAP) and Royal College of Pathologists (RCP) guidelines are based on expert opinion alone and objective data are needed to determine which indications best predict placental lesions.

Methods: A local placental database encompassing a 10 year period (N=8002) with clinical history and outcomes was used to explore indications for submission that 1) predict high grade lesions (>2X overall prevalence), 2) are not associated with significant pathology, and 3) potentially alter clinical management. Lesions were defined by Amsterdam criteria and we used/developed high grade subgroups in seven categories: histologic chorioamnionitis (HCA), maternal vascular malperfusion (MVM), fetal vascular malperfusion (FVM), VUE, fetal stromal-vascular abnormalities, meconium myonecrosis, and increased NRBC.

Results: The best predictors of high grade lesions were SGA (<-1 SD from population mean), LGA (>+2 SD), IUFD, Apgar 5 <6, severe preeclampsia (PE), pregestational diabetes, maternal fever, prolonged rupture of membranes (ROM), in vitro fertilization (IVF), substance abuse, >42 weeks gestation, oligohydramnios, polyhydramnios, prolonged/late decelerations, clinical IUGR, abnormal placental weight, high fetoplacental weight ratio (FPR), UC >70 cm, and hypercoiled UC. Indications not associated with significant pathology were mildly elevated birthweight (+1-2 SD), chronic or gestational hypertension without PE, obesity, vaginal bleeding, meconium, nonreactive fetal heart rate, and peripheral UC. Clinical indications yielding potentially actionable diagnoses were a) clinical amnionitis, maternal fever, prolonged ROM, Apgar 5 <6 (HCA/extended neonatal antibiotics), b) low placental weight, SGA (<-1SD), high FPR, clinical IUGR, IUFD, severe PE, oligohydramnios (MVM/aspirin in next pregnancy), c) Apgar 5<6, low FPR, long UC, hypercoiled UC, UC entanglements (FVM/neonatal head cooling), and d) IVF, substance abuse, low placental weight, marginal/membranous UC (VUE/early monitoring for recurrence).

Conclusion: Guidelines for placental submission have been criticized as too generous (CAP) or parsimonious (RCP). Optimization is needed to ensure that important specimens are examined while scarce resources are not squandered on placentas with a low probability of significant findings. This study begins this process by ranking indications according to their yield of high grade and/or actionable placental lesions.

13. Do Increased Aspirated Amniotic Fluid Squames Correlate with Acute Fetal Asphyxia? An Autopsy Study.

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Background: It is a generally held concept that increased numbers of aspirated amniotic fluid squames is one of the autopsy findings supporting a diagnosis of acute fetal asphyxia, the massive aspiration of squames into distal airways being an indicator of terminal gasping. Despite the importance of distinguishing an acute mode of fetal death from subacute or chronic modes, few studies specifically evaluate fetal autopsy findings and their relationship to the time course of fetal death. In this study, we evaluate the quantity of aspirated squames in 3rd trimester stillborns with death caused by placental abruption (acute asphyxia) and compare them to unexplained stillbriths with evidence of subacute or chronic modes of death.

Methods: We identified 15 autopsies on 3rd trimester singleton stillborns with clinical placental abruption and with placental findings consistent with that diagnosis (abruption group). Thirteen of these 15 stillborns also had thymic petechiae, and all had absence of severe acute thymic involution, autopsy findings also supporting acute asphyxia. Thirty 3rd trimester stillborns with unexplained stillbirth comprised the control group; none of these had thymic petechiae and all had severe acute thymic involution, findings supporting a subacute or chronic mode of death. The deliveries occurred between 2005 and 2013, and all the stillborns had complete autopsies. The slides of the lungs were reviewed by 2 perinatal pathologists, who at the time of review were blinded to the clinical history and all other autopsy findings. Intra-alveolar squames were semi-quantitatively scored as follows: 0, no squames seen; 1+, squames seen singly or in small groups in scattered alveoli; and 2+, squames seen in many alveoli and at least focally in compact clusters. Clinical information was obtained from the maternal medical record for both groups and compared.

Results: In all cases the squames were patchily distributed. At least a few-intra-alveolar squames were identified in all cases, and therefore none received a score of 0. In the abruption group, the intra-alveolar squames were scored as 1+ in 12 (80%) and as 2+ in 3 (20%), while in the control group the squames were scored as 1+ in 20 (67%) and as 2+ in 10 (33%) cases (p=NS). Comparison of maternal demographic data and gestational age between groups revealed no significant differences.

Conclusion: The abruption group (acute asphyxia) did not have increased intra-alveolar squames when compared to the control group. Quantification of aspirated squames at autopsy did not aid in separating stillborns with acute asphyxia from those with a subacute or chronic mode of death.

14. Utilization Of A Synoptic Report To Improve Placental Pathology Examination

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Background: Placental examination after delivery plays an important role in the continuum of maternal and newborn care. As seen in other areas of pathology, synoptic reporting may improve quality and completeness of pathology reports over conventional narrative reporting. Here, we evaluate if a synoptic reporting format developed from evidence-based guidelines and the 2015 Amsterdam consensus improves the completeness of placental examination compared with conventional narrative reports.

Methods: Placentas from singleton pregnancies sent for Pathology between October 1, 2013 to December 31, 2014 were randomly selected for inclusion (n=100). Histology slides for each case were examined independently by two experienced placental pathologists, who were blinded to previous histological findings. Kappa scores were calculated to assess inter-observer agreement between the pathologists. To assess completeness of the reports, the percent of lesions noted using the synoptic report but missing in the narrative report, and the percent of lesions noted in narrative report but missing in the synoptic report, were calculated.

Results: The inter-observer agreement for lesion severity between the pathologists ranged from 0.124 to 1.0. Higher agreement was observed for well characterized lesions (e.g., chorioamnionitis fetal stage: 0.894 [0.710-0.961]) and for rare lesions (e.g., chorionic hemosiderosis: 1.0 [1.0-1.0]). Lower agreement was observed for less characterized lesions (e.g., distal villous immaturity: 0.294 [0.050-0.488]). As noted in the Amsterdam criteria, villous maturation, chorioamnionitis, and villitis are of significance clinically and should be examined during a routine placental pathology examination. While all synoptic reports contained the presence/absence and severity of each of these high prevalence lesions, they were only commented on in 85%, 43% and 24% of narrative reports, respectively. The narrative reports missed an average of 47% of the lesions that were noted with the synoptic report, while 28% of the lesions that were noted in the narrative reports were not indicated as being present in the synoptic reports.

Conclusion: We have demonstrated that synoptic reporting significantly increased the completeness and detail of placental examinations compared with traditional narrative reports. The findings of low inter-observer agreement for some lesions may highlight the inherent challenge associated with assigning discrete severity grades for a continuous morphology or may reflect the need for heightened specialized training focused on the updated diagnostic criteria in the 2015 Amsterdam consensus. The next steps in this audit will include a discussion to refine severity definitions for lesions with high discrepancies and adjust the synoptic template into a user-friendly format.

15. Gastroschisis is Associated with Placental Distal Villous Immaturity

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Background: Gastroschisis is a congenital abnormality characterized by visceral herniation through an abdominal wall defect. The cause of gastroschisis is unknown, though it has been linked to risk factors including young maternal age, smoking, and alcohol use during pregnancy. To date, the only established placental correlate is amniocyte vesicular change. Our goal is to characterize placental villous morphology associated with gastroschisis, specifically distal villous immaturity (DVI), which we hypothesize to be associated with gastroschisis based on our clinical experience.

Methods: With IRB approval, we conducted a retrospective slide review of placentas of cases with gastroschisis at our institution between October 2013 and June 2018. Additionally, we selected gestational age and gender matched control groups of placentas: one with a previous diagnosis of DVI and one with normal villous morphology. All placentas were randomized and reviewed by a perinatal pathologist, who was blinded to the groups, and DVI and amniocyte vesiculation were assessed.

Results: 23 gastroschisis cases, with average gestational age of 35 weeks, were studied. Average gestational ages of the previously diagnosed DVI (n=20) and normal villous morphology (n=23) groups were 38 and 35 weeks respectively. Gastroschisis was associated with increased placental DVI (p=0.007) and increased amniocyte vesiculation (p=0.003) compared to the control group. Based on the normal and DVI groups, kappa agreement between current slide review and initial pathology diagnosis was 0.292, indicating fair agreement. For DVI, the percentage of gastroschisis cases positive was 69.6 and of normal controls 26.1. For vesicular changes, the percentage of gastroschisis cases positive was 52.2 and of normal controls 8.7.

Conclusion: Our study shows that gastroschisis is associated with placental DVI. This association may be due to: 1. a common upstream factor contributing to both gastroschisis and DVI or; 2. DVI may be a consequence of the altered placental and amniotic environment in the context of gastroschisis. The kappa score between current slide review and initial diagnosis of DVI morphology emphasizes the difficulty of making this diagnosis, especially in the absence of clinical context.

16. A Method for Identification and Localization of Bacteria in Formalin Fixed Paraffin Embedded Crohn's Disease Biopsies

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Background: The cause of Crohn's disease is unclear but may involve an aberrant gastrointestinal (GI) mucosa inflammatory response in genetically susceptible individuals to a luminal trigger. The trigger is unknown but the granulomatous inflammation characteristic of Crohn's disease suggests that intramucosal bacteria resistant to clearance may be involved. This pilot study describes a 2 step method using microbiome analysis and chromogenic in situ hybridization (CISH) to identify and localize bacteria in pediatric Crohn's disease FFPE GI biopsies.

Methods: Ethics board approval was obtained. For microbiome analysis, FFPE biopsies from 19 Crohn's and 15 normal patients were pooled by site to yield 6 samples: Crohn's upper GI, Crohn's terminal ileum (TI), Crohn's colon, normal upper GI, normal TI, and normal colon. DNA was extracted and amplified using tagged V6 region primers with the resulting products sequenced by Ion Torrent next-generation sequencing. The number of reads per operational taxonomic unit (OTU) was used as a measure of relative abundance. OTUs without species information, <10 reads in 1 or more sites, likely to represent contaminants, or with total reads in Crohn's samples of <105% of normal samples in 2 or more sites were excluded.

Tissue for *A. muciniphila* and *B. ovatus* CISH was selected from a separate patient population (up to 67 sections from up to 29 Crohn's patients, 60 sections from 12 normal patients). Positive bacterial controls were generated with type strains; precipitated bovine serum albumin was used as a negative bacterial control. CISH probes were complimentary to the target OTU sequence and 5' or 3' digoxin labeled. Competitive and scrambled probes were used to demonstrate probe specificity and an X centromeric probe used as a tissue DNA positive control. CISH was performed using a polymer detection kit. Staining in tissue samples was scored positive or negative based on control staining

Results: Microbiome analysis identified *A. muciniphila, B. ovatus, B. uniformis, P. andersonii, A. otitis, B. longum, P. mendelii, A. octavius, R. cervicalis,* and *P. acnes* as relatively more abundant in Crohn's samples. The positive and negative CISH controls showed appropriate staining. Occasional positive *A. muciniphila* signals were seen in luminal fecal material but intramucosal positive signals for *A. muciniphila* and *B. ovatus* were not seen in 67 and 35 separate Crohn's biopsies from 29 and 18 individuals respectively. All normal biopsies were negative for both A*. muciniphila* and *B. ovatus* CISH.

Conclusion: CISH based on OTUs identified by microbiome analysis is a valid method for investigation of intramucosal bacteria in FFPE biopsies. *A. muciniphila* and *B. ovatus* are not detectable by this CISH assay in GI mucosa from pediatric patients with Crohn's disease.

17. Diagnostic Value Of BRAF VE1 Immunohistochemistry As A Reliable Marker Of Neoplasms Harboring BRAF V600E Mutation In Pediatric And Histiocytic Neoplasms.

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Background: The BRAF gene is responsible for regulation of cell growth and proliferation. Numerous neoplasms are now recognized to harbor the BRAF-V600E mutation highlighting its importance as a diagnostic, therapeutic and prognostic marker. Although mutational assessment is usually performed by DNA-based techniques, several studies have highlighted the significance of BRAF VE1 immunohistochemistry (BRAF-IHC) among different cancers. Nonetheless, the value of BRAF-IHC as a surrogate of molecular testing is still uncertain and limited data has been published in pediatric patients. The aim of this study is to assess BRAF-IHC diagnostic utility as compared to confirmatory molecular techniques in a series of cases with emphasis on pediatric and histiocytic neoplasms.

Methods: 100 cases were retrieved from our pathology files under IRB approval. The BRAF-IHC was performed on formalin-fixed paraffin-embedded (FFPE) tissue using the anti-BRAF V600E (VE1) mouse monoclonal antibody and OptiView DAB detection system and OptiView Amplification Kit (Ventana Medical Systems, Tucson, AZ). The immunoreactivity was graded per the percentage of stained tumor cells (positive: ≥10% of cells with diffuse dark and granular 2-3+ cytoplasmic staining; negative: 0-1+, including focal and punctate staining. BRAF mutational analysis (BRAF-PCR) was performed by PCR amplification of the BRAF exon 15 (c599-601) on FFPE for initial validation, while consult material was performed with a variety of methods. Frequency of mutant allele detection ranged from <1-20%, dependent on molecular method.

Results: Neoplasms included central nervous system (CNS) (n=28), thyroid (n=15), bone and bone marrow (n=14), skin (n=32), soft tissue (n=5) and visceral/lymph node (n=6), including 54 histiocytic neoplasms. The median age was 9.5 years (range: 0.1 – 69 y). Concordant correlation was achieved in 92% (53/63), with a sensitivity of 88% and specificity of 96%, positive predictive value of 96% and negative predictive value of 89%. The remaining 8% of the cases showed discrepancies among the molecular and BRAF-IHC methods.

Conclusion: BRAF-IHC is a sensitive and reliable marker for BRAF-V600E mutational analysis in a wide-range of pediatric and histiocytic neoplasms including decalcified specimens. False negative staining can occur in a subset of decalcified specimens and caution is needed. False positive staining is rare but may reflect another mutation in BRAF or low level mutant allele burden if sensitive, quantitative molecular analysis (i.e. allele specific PCR) are not used. We recommend that if focal strong staining (2-3+, in ≥10% of cells) is present with a negative molecular result, investigation with more sensitive molecular assays should be considered, especially for histiocytic lesions.

18. H3K27me3 in the Pathologic Investigation of Disorders of Sexual Development

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Background: H3K27me3 is a marker of histone methylation that can be utilized, via immunohistochemical staining (IHC), to identify inactivated X chromosome material (i.e., Barr bodies). Use of this marker in the practical evaluation of gonadal biopsies in the setting of disorders of sexual development (DSD) is unexplored.

Methods: Over a 2-year period, a single attending pathologist prospectively applied H3K27me3 IHC to gonadal biopsies in the setting of DSD.

Results: Detailed findings were as follows:

Age (yr)	Phenotype	Genotype	Key Pathologic Features	H3K27me3 IHC (dots/nucleus)
1	Ambiguous genitalia; uterus and undescended gonads present	45,X0/46,XY	1) Left streak gonad with ovarian-type stroma and primitive cord-like structures; germ cells 2) Right dysgenetic testis with germ cell hypoplasia	1) 0 dots 2) 0 dots
1	Ambiguous/virilized genitalia; clitoromegaly	46,XX	Ovary with no specific pathologic findings	1 dot
2	Ambiguous genitalia; undescended gonads with complex hypospadias	46,XY	Dysgenetic testis with germ cell hypoplasia	0 dots
3	Ambiguous genitalia, hypospadias, chordee	49,XXXXY	Dysgenetic testis with germ cell hypoplasia	≤3 dots
12	Severe hypospadias; undescended testes	46,XX *SRY*-neg. by FISH	Dysgenetic testis with germ cell hypoplasia	1 dot

Conclusion: Our study shows that H3K27me3 IHC corroborates the clinical karyotype in DSD gonads, and thus can serve as an important component of an integrated pathologic diagnosis in this setting. We suggest its use for 1) routine pathologic investigation of DSD, 2) evaluation for potential sex chromosome mosaicism, and 3) as a complement to or surrogate for germline and/or somatic karyotyping.

19. Significance Of Glutamine Synthetase Expression In Hepatoblastomas

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Background: Hepatoblastomas (HB) account for 47% of pediatric liver malignancies and are histologically classified into epithelial and mixed HB. Epithelial HB consists of well-differentiated fetal (WDF), crowded fetal (CF), embryonal (E), small cell undifferentiated (SCU), and blastemal cell components. Beta catenin mutations have been described in almost 90% of HB and the type of mutation, viz. exon 3 deletion versus point mutations are believed to determine the histologic phenotype of the tumor (deletion in fetal and point mutation in embryonal HB), reflected by nuclear B-cat expression. Glutamine synthetase (GS) is a known activation marker of B-cat as well as a possible downstream target, and a possible differentiation marker in HB. The correlation of GS with B-cat nuclear expression has not been assessed in detail in large series of HB. The aim of our study was to assess this correlation.

Methods: 68 HB patients and 80 specimens from a tertiary care Children's Hospital were analyzed after IRB approval. H&E slides from biopsy and resection specimens were analyzed to determine cellular composition, evidence of necrosis, and post-chemotherapy effects. Immunohistochemistry (IHC) for B-cat and GS were then performed using the Ventana automatic stainer and their staining pattern was scored as 1-3(average for intensity and distribution) besides their localization in the cell (nuclear, cytoplasmic, or membranous). Appropriate positive and negative controls were run. In normal liver B-cat expression is usually membranous only, while GS is restricted to the cytoplasm of a single perivenous layer of hepatocytes.

Results: The average score for nuclear B-cat staining was 1.42 for CF cells, 1.00 for WDF cells, 2.24 for E cells, and 1.90 for the blastemal/SCU components. The average score for GS staining was 2.39 for the CF cells, 2.16 for WDF cells, 0.33 for E cells, and 0.04 for the blastemal/SCU cells. Nuclear beta catenin had a greater degree of staining in the E and blastemal/SCU cells compared to the WDF and CF cells. CF and WDF cells had a greater degree of GS staining on average compared to the E and blastemal/SCU cells.

Conclusion: The correlation of B-cat and GS is paradoxical. GS is overexpressed in fetal components despite low nuclear B-cat expression, suggestive of a role in differentiation, but also indication B-cat mutation. In contrast, E and Blastema/SCU areas show strong nuclear B-cat, but there is almost no GS, suggestive of a role in differentiation only. The expression of GS appeared to be diffuse in all areas of differentiated tumors in treated HB irrespective of B-cat nuclear staining, also supporting this role in differentiation. Embryonal HB tumors are known to have a worse prognosis than fetal HB, suggesting a role for assessment of GS expression in prognosticating HB.

20. Analysis Of MOC31 (Epcam) Expression In Hepatoblastomas.

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Background: Hepatoblastoma (HB) is a pediatric neoplasm composed of immature liver precursor cells (hepatoblasts). The epithelial cell adhesion molecule (EpCAM/MOC31) is a cell-surface glycoprotein expressed in the fetal liver, whereas characteristically lost in adult hepatocytes. Prior molecular-based studies have proposed those HBs recapitulating earlier stages of liver development (embryonal, fetal crowded and macrotrabecular histologic subtypes) consistently express EpCAM/MOC31 in contrast to HBs recapitulating later stages of development (fetal and pure fetal histologic subtypes) however, this issue has not been fully addressed from an immunohistochemical perspective. The aim of this study is to better characterize the differential expression of EpCAM/MOC31 among different histologic subtypes of HBs.

Methods: 43 cases were retrieved from our pathology files under IRB approval. The EpCAM/MOC31 immunohistochemistry (MOC31-IHC) was performed on formalin-fixed paraffin-embedded (FFPE) tissue using the anti- EpCAM/MOC31 mouse monoclonal antibody (1:50 dilution; Cell Marque Corporation, Rocklin, CA) and the Ventana Benchmark XT platform. The immunoreactivity was graded based upon the percentage of positive membranous staining (Negative: 0; <10%: 1+; 10-50%: 2+; >50%: 3+) as well as per histologic component. Appropriate positive and negative controls were used.

Results: The median age was 2 years (range: 0.6 – 9y) with a male to female ratio of 1.7. HBs included crowded fetal (38/43; 88%), embryonal (29/43; 67%), well-differentiated fetal (12/43; 28%), blastemal/small cell (18/43; 42%) and other (8/43; 19%) histologic components. The EpCAM/MOC31 IHC demonstrated a membranous staining pattern and was consistently strongly positive (2+ to 3+) in those HBs with embryonal (83%) and crowded fetal (79%) components in contrast to the well-differentiated fetal (33%), blastemal/small cell (6%) and other (25%) groups (mesenchymal, glandular and pleomorphic fetal features).

Conclusion: HBs featuring more primitive histologic features (embryonal and crowded fetal components) show strong EpCAM/MOC31 expression whereas HBs demonstrating well-differentiated fetal component, blastemal/small cell or mesenchymal elements are characteristically negative. These findings correspond with prior molecular studies suggesting that EpCAM/MOC31 expression is progressively lost in the transition from crowded to well-differentiated fetal morphology and its presence in HBs recapitulating immature stages of development might have implications in treatment response as well as survival although these observations deserve further study. The significance of negative staining in blastema/SCU is interesting despite these components being considered immature and needs further studies.

21. Pseudoglycogen Inclusions Of Hepatocytes: Diverse Contexts, Contents, And Types.

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Background: Ground glass cytoplasmic inclusions (GGCI) in hepatocytes, first described in chronic Hepatitis B infection, occur in many clinical scenarios (Lafora disease, type IV glycogenosis, adult polyglucosan body disease, and fibrinogen storage disease). GGCI have been identified in the setting of both bone marrow and solid organ transplant, immunosuppression, kidney dialysis, medications including cyanamide and iron chelation therapy, hepatocellular carcinoma, and HIV infection. Except in hepatitis B, hepatocellular carcinoma and type IV glycogenosis, these inclusions are composed mainly of glycogen or another related complex polysaccharide. Beyond this, little is known about the biology or functional significance of glycogen containing GCCI.

Methods: Twelve patients with GGCI in needle biopsies of liver allografts and one native liver post dialysis were identified in our archives. Clinical information, including a detailed medication history, was extracted from the medical record. The PAS stain, and Immunohistochemical staining for beta tubulin, LAMP2, and cytochrome oxidase were performed to characterize the components of the inclusions.

Results: Comparison of glycogen-rich GGCI in liver allograft and the post-dialysis native liver showed differences in immunohistochemical staining, particularly striking with anti- beta tubulin, that suggest that there may be two types of glycogen inclusions. The PAS stain with and without diastase showed more complete digestion with diastase in the Type I inclusions, indicating a more dominant glycogen component in liver allograft cases. Type 1 inclusions had highly variable excess beta tubulin staining compared to those designated type 2 in the native liver with chronic renal insufficiency, which were universally strongly reactive for beta tubulin. Lamp2 reactivity was more intense in type 2 inclusions but normal apical localization was absent in both types. Mitochondrial content was minimal in both types.

Conclusion: Glycogen-predominant GGCI have been reported mainly in pediatric liver allografts and occasionally in other contexts. They usually are seen in immunosuppressed patients on multiple medications. Our observations suggest that these inclusions may differ in cytoarchitectural characteristics enough to suggest fundamentally different subsets that may be related to different clinical contexts. The significance is yet unknown. Further observation, particularly in native livers, may shed important insight. Collaboration between institutions will be crucial due to the rarity of this finding outside of liver allografts.

22. Fantastic Yeasts and Where to Find Them - The Pathology of Neonatal Non-Albicans Candidiasis

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Background: Non-*albicans Candida* species such as *C. parapsilosis* and *C. glabrata* have emerged as prevalent pathogens in premature infants. The aim of this study was to systematically delineate the histopathologic findings in neonatal non-*albicans* candidiasis.

Methods: We performed a retrospective clinicopathologic analysis of extremely premature (23-28 weeks gestation) infants diagnosed with invasive candidiasis. Archival autopsy tissues were subjected to periodic acid-Schiff, methenamine-silver and anti-*Candida* (immuno)histochemical stains, as well as dual anti-*Candida* and anti-cytokeratin or anti-CD31 immunofluorescence assays. In addition, we studied the prevalence of intestinal *Candida* colonization in a consecutive autopsy series of extremely premature infants.

Results: Based on positive postmortem blood and/or lung cultures, invasive candidiasis (3 non-*albicans*, 11 *C. albicans*) was diagnosed in 14/187 extremely premature infants examined between 1995 and 2017. In contrast to the well-known inflammatory and tissue-destructive phenotype of congenital *C. albicans* infection, invasive non-*albicans* candidiasis/candidemia caused by *C. parapsilosis* and *C. glabrata* was inconspicuous by routine hematoxylin-eosin-based histopathologic analysis despite a heavy fungal presence detected in intestines, lungs and blood by targeted (immuno)histochemical assays. Intestinal colonization by *Candida* species was identified in 16/26 (61%) extremely premature neonates who had lived for at least one week, as assessed by anti-*Candida* immunostaining.

Conclusion: Invasive neonatal non-*albicans* candidiasis/candidemia appears to have no distinct histopathologic signature. Based on the notoriously low sensitivity of fungal blood cultures and the observed high frequency of *Candida* intestinal colonization (>50%), it is likely that non-*albicans* candidiasis/candidemia may be underdiagnosed in (deceased) preterm infants. Routine inclusion of targeted (immuno)histochemical fungal detection strategies in the perinatal autopsy may lead to deeper insight into the prevalence and clinical relevance of neonatal non-*albicans* candidiasis.

23. Aspiration Cytology of Cystic Bone Lesions in Pediatric Patients

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Background: Bone cysts are uncommon and primarily affect the pediatric population. Their treatment often involves aspiration, and fluid is typically submitted for cytologic evaluation. Existing series studying bone cytopathology primarily include adult patients, with high rates of malignant disease. We aimed to characterize the clinicopathologic features of pediatric bone cysts aspirated at a single pediatric institution.

Methods: Patients under age 21 who underwent cytologic evaluation of a bone cyst from 1994 to 2018 were identified via departmental archival review. During the study period, cytologic evaluation of aspirated bone fluid routinely included preparation of 1 H&E- and 1 Wright-Giemsa-stained cytospin. Clinical data were reviewed. Cytologic findings were categorized as positive, no malignancy, atypical, or suspicious. Additional reported comments were also evaluated.

Results: 526 samples from 367 patients (68% male) were identified. Age at cytologic sampling ranged from 1 month to 21 years (mean, 9 years; median, 10 years). Anatomic sites included: humerus (n=297), femur (n=143), tibia (n=32), foot (n=17), fibula (n=8), pelvis (n=8), radius (n=5), other (n=16). All specimens except 1 (525/526) were negative for malignancy. In only 2 of these negative cases were there findings indicating a specific diagnosis beyond benign cyst: 1. An 8-year-old boy with a suspected humeral unicameral bone cyst that showed Langerhans cell histiocytosis. 2. A 2-year-old girl with a suspected femoral Brodie's abscess that showed osteomyelitis (corroborated by positive Gram stain and cultured Kingella kingae). One case with atypical cells (1/526) was from a 17-year-old boy with suspected recurrent osteosarcoma.

Conclusion: Our findings indicate a low malignancy rate (0% positive, 0% suspicious, <0.2% atypical) in cystic pediatric bone lesions undergoing aspiration. In particular, all lesions preoperatively viewed as benign were negative for malignancy. This study provides benchmark data for pediatric centers that could be used to guide clinical practice.

24. High Concordance Between FUS-CREBCL2 And 1 Fusion Proteins And MUC4 Expression Of Histologically Characteristic Low-Grade Fibromyxoid Sarcoma (LGFMS): Analysis Of 13 Cases.

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Background: Low-grade fibromyxoid sarcoma (LGFMS) is a mesenchymal neoplasm first described by Evans in 1987. The histology of LGFMS is relatively bland, however, the tumor is known to metastasize late after presentation. Males are more often affected than females with a median age at presentation of 29 years, but can affect a wide range of ages with cases reported from 3-78 years of age**.** Typically, the disease is characterized by a balanced translocation, t(7;16)(q32-q34;p11), fusing the 5' end of *FUS* to the 3' end of *CREB3L2* or in a minority of tumors *CREB3L1* on chromosome 11p11 creating a FUS-CREB3L2 fusion protein. MUC4 overexpression has also recently been found to be specific for *FUS* positive LGFMS cases. Recently, DOG1 has also been implicated in approximately 40% of cases of LGFMS.

Methods: With Institutional Review Board approval we searched for cases of LGFMS. Selected cases were searched for the typical genetic translocations via FISH and RT-PCR and also searched for MUC4 expression by immunohistochemical staining.

Results: 13 patients were identified and included in the analysis. FISH studies looking to detect the characteristic translocation producing the FUS-CREB3L2 and 1 fusion proteins were performed. 13/13 FISH samples yielded the FUS gene rearrangements. Out of 13 cases that were tested with RT-PCR, 12 were positive for FUS-CREB3L2, 1 was positive for FUS-CREB3L1. 13 cases were used for MUC4 immunohistochemical analysis, and 12/13 were positive for MUC4. After compiling the data, there was a 95% concordance rate between FISH/PCR results and MUC4 expression.

Conclusion: Genetic testing of low-grade fibromyxoid sarcoma tissue specimens using a combination of FISH and RT-PCR, demonstrated the presence of *FUS* gene rearrangements in all of our cases. MUC4 immunohistochemistry was 95% concordant with molecular studies. MUC4 is thus a useful marker for diagnosis and in predicting the presence or absence of *FUS* gene rearrangement. Larger numbers of molecularly characterized cases are needed to better delineate the presence, specificity, and clinical importance of these molecular abnormalities and their correlation with MUC4 expression.

25. Retinoblastoma: A Review of 84 Cases from a Single Institution from 1983-2017 with Emphasis in the Genetic Prognostic Factors and Outcome

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Background: Retinoblastoma (RB) is a rare disease yet the most common pediatric primary ocular malignancy, occurring in 250-350 children annually in the USA. Knudson proposed the two-hit hypothesis implicating mutations in RB1 and silencing of its gene product, pRB. In recent years, it has been shown that two RB1 mutations are neither necessary nor sufficient to develop RB. Hypermethylation of numerous genes, including MSH6, CD44, PAX5, GATA5, TP53, VHL, GSTP1, MGMT, and CDN2, are associated with development of RB. Over the past 10 years, management of RB has dramatically changed as a result of the success of ophthalmic artery and intravitreous chemotherapy.

Methods: We performed a retrospective review of charts of patients treated for RB from 1983-2017. Age and clinical characteristics at diagnosis, treatment, outcome, genetic analysis and follow-up are described.

Results: We identified 97 cases of RB. Of the 97 cases, 13 were omitted from analysis due to insufficient charting. There were 51 males and 33 females with age at diagnosis ranging from 8 weeks gestation to 5 years 4 mos. The majority of patients had no family history of RB. Patients with bilateral RB presented significantly earlier (5.82±6.32 mos, n=35) than patients with unilateral RB (21.33±15.16 mos, n=47, $p<0.001$). One presented at 17 mos with metastatic RB. One patient presented at 5 mos with trilateral RB. Since 2003, 59% of patients required unilateral enucleation. Prior to 2003, 70% of patients required enucleation and 16% of enucleations were bilateral. The mortality rate was 2.38% from 1983-2018.

Since 2003, patients with RB were referred for evaluation of germline RB1 mutations. Other mutations are not evaluated. The follow up rate has been poor, with 64% of patients completing genetic testing. Germline RB1 mutation is found in 55% of cases. Patients with germline mutations have a lower rate of enucleation (60% vs 70% without germline mutation) and are more likely to be sent to outside hospitals for localized therapy.

Conclusion: Treatment for RB has changed significantly over the past 30 years. With localized therapies, there has been an increase in eye preservation. Genetics counseling is an integral component in evaluation of all cases of RB. Results of testing to identify the aforementioned new genes is not yet available, however, correlation of genetic analysis with prognostic outcome will be reported in the future manuscript. As survivors mature, genetic analysis provides clinical guidance for monitoring and counseling in family planning.

26. Clinical, Pathological and Loss of Heterozygosity Differences in Wilms Tumors between Asian and non-Asian Children

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Background: Wilms tumor demonstrates significant inter-ethnic epidemiological, histological and outcome differences, and is rare and poorly studied among Asians. We compared the clinicopathological, and loss of heterozygosity (LOH) profile and survival outcomes of Asian and non-Asian patients with Wilms tumor.

Methods: Clinical charts and histological slides from patients with malignant renal tumors over a period of 20 years were retrospectively reviewed. We adapted a genotyping assay to determine 1p36 and 16q21-22 LOH in formalin-fixed paraffin-embedded (FFPE) specimens, and compared these characteristics between Asian and non-Asian patients.

Results: Fifty-three (79.1%) Asian and 14 (20.9%) non-Asian patients had Wilms tumors. Compared to non-Asians, Asians were younger (mean 4.6 and 4.0 years, respectively), had more equal gender distribution (female:male=1.8 and 1.0, respectively), fewer tumors with unfavorable histology (25.0% and 4.2%, respectively, P=0.02), and less advanced disease at presentation, yet similar nodal metastases rates (19.1% and 20.0%, respectively). No Asian patients had bilateral tumors. Our adapted genotyping assay accurately determined LOH in FFPE specimens <10 years post-fixation. Among 30 Asian patients, 1p and 16q LOH was each detected in 5 (16.7% each) patients, respectively – similar to rates reported in other ethnicities. Yet following similar treatment with National Wilms Tumor Study regimens, 15-year event-free and overall survival for Asian patients were 93% and 97% respectively.

Conclusion: Despite similar nodal metastasis and LOH rates, Asian patients had fewer unfavorable histology tumors, lower-stage disease, and better survival outcomes. The bases for these differences and implications on treatment strategy for these patients warrant further study.

27. Is FNAC Beneficial In Evaluation Of Mass Lesions In Pediatric Population?

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Background: Fine-needle aspiration cytology (FNAC) is a technique well accepted as a diagnostic procedure in the adult population. FNAC also has been recently recommended for the accurate evaluation and diagnosis of childhood tumors. reported benefits including safety, minimal trauma, and cost-effectiveness. However, FNA is not universally accepted as a diagnostic tool in pediatric patients. Previous publications have focused on FNA of specific anatomic sites, mainly thyroid. In this 3-year retrospective study, we present our experience with FNA in pediatric patients 18 years of age and younger excluding thyroid cases.

Methods: Computer-generated searches were performed in our database from 2015 to 2017. FNA material from tumors and masses in patients 18 years of age and younger were analyzed. FNA diagnoses were correlated with the final histopathological diagnoses and clinical follow-up.

Results: A total of 39 FNAs were performed in children between 2015 and 2017. 16 pediatric FNA cases were identified and included in this series after excluding all thyroid cases. The age of study sample ranged from 5 months to 18 years of age (mean 12.3 years). Of the 16 patients, 5 were females and 11 were males. Regardless of the gender, the most frequently aspirated anatomic sites were liver (3) and bone (3), followed by kidney (2), lung (2), anterior mediastinum (1), salivary gland (1), lymph node (1), soft tissue (1), ovary (1) and testis (1). The cytologic diagnoses were stratified into four categories: negative for malignant cells (NFMC) (11), positive for malignant cells (3), atypical (1) and non-diagnostic (1). Final histopathological diagnoses were available for 11 cases. There was concordance between the FNA diagnosis and final histopathologic diagnosis in 6 cases with FNA diagnosis NFMC, and 3 with FNA diagnosis positive for malignant cells (Wilms tumor, Ewing sarcoma, and Desmoplastic small round cell tumor). The case with FNA diagnosis of atypical had a final histopathological diagnosis of lymphoma, and the case with FNA diagnosis of non-diagnosis had a final histopathological diagnosis of the benign fibro-osseous lesion. Of the 11 cases with a diagnosis of NFMC, 7 showed cytopathologic features suggestive of fungal infection, and blood cultures were positive for fungal infection in three cases while four cases were negative. Regardless, all patients were subsequently treated with antifungal and antibiotic. Follow-up was available for all 16 cases with a mean of 10 months and showed that three patients died from the disease.

Conclusion: Our experience showed FNA is a beneficial technique in providing guidance for rapid clinical therapeutic decisions, especially in malignant and infectious cases in pediatric patients.

28. Proximal Collecting Tubule Deficiency in Angiotensin-Converting Enzyme Inhibitor and Angiotensin II Receptor Blocker Fetopathy

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Background: Angiotensin-Converting Enzyme Inhibitors (ACEi) and Angiotensin II Receptor Blockers (ARB) are widely used for several renal and cardiac indications. Multiple reports since the 1980's have documented ACEi/ARB-related fetal anomalies. Initially postulated to be a teratogen, it is now felt these medications act more as toxins, with discontinuation resulting in a reduction in the phenotype. The two most prominent anomalies include hypocalvaria and the phenotype of renal tubular dysgenesis with oligohydramnios, Potter-like facies, and absent proximal convoluted tubules (PCTs), the latter being identified but not quantified to date in ACEi/ARB fetopathy. Our study aimed to quantify the reduction of PCTs in infants with prenatal exposure to ACEi/ARBs and correlate this reduction with extent of exposure.

Methods: We identified five cases of fetal exposure to ACEi/ARBs who underwent autopsy at our institution between 2011 and 2018. We compared autopsy pathology parameters with five gestational age (GA)-matched controls who did not have renal or cardiovascular findings and minimal maceration changes. After reviewing routine kidney sections, immunohistochemistry (IHC) using antibodies to CD10 and epithelial membrane antigen (EMA) were used to quantify the PCT percentage of all tubules per high power field by two pathologists. Each case had three separate HPFs evaluated which were then averaged.

Results: The median GA was 28 weeks (range 27-32 weeks) with three liveborn and two stillbirths. One mother discontinued therapy at 23 weeks, 7 weeks prior to delivery. Four mothers had pan-gestation exposure for a median of 27.5 weeks exposure (range 27-32 weeks). The average PCT percentage was 19.0% + 12.3% in ACEi/ARB fetopathy patients (range 3.5%-34.3%). This was significantly less ($p<0.0001$) than normal controls which showed a PCT percentage of 52.8% + 4.4% (range 49.9%-58.1%). The fetus with in utero cessation of medication showed an average PCT percentage of 34.2% while those with continued exposure throughout gestation showed a median PCT percentage of 15.2% (range 3.6%-29.0%). One case demonstrated renal vein thrombosis with extensive calcifications. Four of the five cases showed hypocalvaria.

Conclusion: ACEi/ARB exposure mimics renal tubular dysgenesis and shows a reduction in the PCT number. *In utero* cessation of medication may reverse loss of PCT. Due to the small sample size, we were unable to draw any conclusions between ACEi/ARB dosage, duration, cessation, or other maternal factors and reduction in PCT percentage. Future larger studies may benefit from calculating the PCT percentage to correlate with post-natal renal function and maternal factors including medication type, dosage, duration, and time from medication cessation.

29. Correlating Magnetic Resonance Imaging Findings with Placental Pathology

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Background: Noninvasive methods to identify placental pathologic conditions are being sought. Magnetic resonance imaging (MRI) is a non-invasive technique that provides anatomic information and potentially defines pathologic structural alterations. Diffusion weighted MRI is a means of studying the placenta in vivo as restricted motion of water molecules in the placenta highlights structural and perfusion alterations. The study objective was to examine fixed tissue slices of placenta by MRI and correlate the images with placental pathologic findings defined by routine gross and histologic examination.

Methods: After institutional review board approval, a search of the surgical pathology database identified six formalin-fixed placentas for an initial pilot study. Four cases had pathology and two were normal. The H&E-stained slides were reviewed by an expert placental pathologist and diagnosis given for maternal vascular malperfusion (MVM), high grade chronic villitis of unknown etiology (VUE) and massive perivillous fibrin deposition (MPVFD). For MRI evaluation, representative placental slices were selected (2 cm long, 10 mm thick) and rehydrated in PBS. Imaging was performed on a Bruker Avance14.1T microimager. Diffusion weighted images were acquired from 16 slices using TR/TE 8000ms/27ms, b=1200s/mm2, slice thickness 0.5mm, and in-plane resolution 100µm x 100µm. T2 maps were obtained from the same slices.

Results: In T2-weighted images, the normal placental structure (N=2) appeared as multiple compactly arranged hexagonal structures with relatively bright outlines surrounding a low signal intensity region (ie darker center). This multiplex hexagonal structure showed varying degrees of aberrations with placental pathology. In MVM, some hexagonal structures were retained, but there was partial collapse of the normal organization. The rim and the center of the hexagons had increased signal intensity, compared to normal controls. Multiple dilated vessels were seen also. In VUE, the multiplex hexagonal tissue organization was not well visualized with a generalized and somewhat homogenously increased signal intensity and multifocal dilated vessels noted. Lastly, in MPVFD, there was loss of hexagonal tissue structure with heterogeneous T2 signal intensity. Overall, the background had a higher intensity than normal and some areas showed very bright T2 hyperintensity.

Conclusion: This retrospective study was undertaken to correlate histologic pathologic findings with MRI findings. Our study results suggest that placental microstructure can predominantly be defined as a multiplex hexagonal structure with a predictable MRI signal and that recognizable, unique changes are present in placental microstructure in varying pathologic conditions.

30. Bone Growth in Intrauterine Growth Restriction: A Correlative Histological and Micro-CT Study

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Background: The pathological basis for the short bones that accompany fetal asymmetrical growth restriction is poorly explored.

Methods: We explored the architecture and mineralisation of femoral bone at midshaft and at the metaphyses in a series of seven fetuses (gestational age range 17-35 weeks) with severe growth restriction due to uteroplacental insufficiency, compared to six age-matched normal controls using histology and quantitative micro-computed tomography. 5 cases of growth restriction were due to maternal malperfusion, one was due to twin-twin transfusion, and one to massive perivillous fibrin deposition.

Results: At femoral midshaft, IUGR bones were smaller (lower Total Volume, TV) , and had lower bone volume (BV) and bone surface area than controls, but bone mineral density and bone volume as a proportion of total volume (BV/TV) were relatively preserved. In the metaphyseal cross sections, TV and BV were both decreased over age matched controls, and again BV/TV was relatively preserved, as was trabecular thickness . However, trabecular number and bone mineral density were reduced. In a marked exception to the general trend, BV/TV was remarkably reduced in the case of massive perivillous fibrinoid deposition. Placental insufficiency results in smaller amounts of bone, with impaired mineralisation, and fewer bony trabeculae in metaphyseal bone, although cortical bone mineral density is relatively preserved.

Conclusion: These results imply that placental insufficiency most compromises deposition and remodeling of new trabecular bone, with relative sparing of cortical bone, and that there is considerable heterogeneity of effect, even within growth retardation of placental origin.

WITHDRAWN

31. Environmental Influences on Chronic Villitis of Unknown Etiology

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Background: Chronic villitis (CV) is an infiltration of chorionic villi by maternal immune cells, most frequently CD8+ T-lymphocytes. Infectious etiologies are identified in <10% of cases, with the remainder dubbed villitis of unknown etiology. The histologic similarity of CV to transplant rejection and the increased prevalence of alloantibodies suggest a mechanism similar to autoimmune and alloimmune diseases {Lee 2011}.

There is evidence of environmental triggers for several autoimmune diseases, including: seasonality in onset and relapse of multiple sclerosis {Spelman 2014}; variation by birth month in risk of autoimmune thyroid disease {Krassas 2007}; childhood trauma prior to manifestation of type 1 diabetes {Huffhines 2016}; and post-infectious systemic illnesses, such as rheumatic fever.

Methods: Time series data were retrieved from the pathology database of our institution from July 1, 2011 through June 30, 2018 and grouped by week, then smoothed over an 8 week window. Total case volume by year was retrieved for comparison. Weather, ultraviolet flux, pollution, and influenza prevalence data were retrieved from public data sets. Patients with placental pathology reports were retrieved from the de-identified patient data set of a second institution. CV cases and presence of selected diagnoses (by international classification of disease [ICD]9/10) and patient demographics were assessed. We performed multivariate logistic regression.

Results: CV cases ranged from 8 to 56% of cases in a week. Chronic villitis shows seasonal variation with peaks in the fall (range: September 18 to December 28). Peaks in recent years were higher, with the exception of a dramatic spike in 2012-13. The seasonal variation followed the expected peak in temperature, ultraviolet light exposure, and pollution during the summer. Variation in peak summer pollution levels, as measured by air quality index (AQI) roughly correlated with variation in peak CV.

In multivariate regression, African American ethnicity (OR 0.60, 95% CI 0.46-0.78, p<0.001), cervical dysplasia (OR 0.53, 95% CI 0.33-0.84, p = 0.008), and tobacco use (OR 0.72, 95% CI 0.53-0.97, p = 0.031) were protective against CV.

Conclusion: This is the first study to show seasonality in CV. There is a peak in the fall but considerable year-to-year variation, which shows some correlation with air pollution. The small number of years, prior negative studies (e.g. {Becroft 2005}), and presence of other time-sensitive factors, such as changing diagnostic criteria argue for caution. African American ancestry, smoking, and cervical dysplasia were all protective. HPV infections show seasonality related to UV flux {Hrushesky 2005} and smoking is a risk factor for cervical dysplasia {Mzariko 2015}. Conversely, air pollution is considered proinflammatory.

32. Immnohistochemical Profile of Chronic Inflammatory Lesions of the Placenta

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Background: Chronic inflammatory lesions of the placenta, such as chronic villitis (CV) and chronic deciduitis (CD), have been associated with signs of stress in pregnancy and socioeconomic disadvantage. However, the biologic mechanisms underlying these associations are not well understood. The aim of this study is to examine the chronic inflammatory cell populations in the basal plate of the placenta with CV and CD and compare them with cases lacking these chronic inflammatory lesions. We investigated specific subsets of inflammatory cells at the maternal-fetal interface including CD3+ T cells, CD56+ NK cells, and CD68+ macrophages/monocytes.

Methods: Placental tissue was collected as part of a prospective study. 53 placental tissue samples were included, none of which were submitted for clinical placental pathology. Representative H&E samples of each placenta included membranes, umbilical cord, fetal surface and basal surface with intervening chorionic villi. Histologic characterization based on H&E slides of the chronic inflammatory infiltrates was performed. Within the villous parenchyma, CV, was characterized as lymphohistiocytic infiltrates of the villi. On the basal plate and/or in the parietal decidua of the membranes, the presence of lymphoplasmocytic infiltrate was characterized as CD. Other chronic inflammatory lesions sought included chronic chorionitis/chorioamnionitis and basal chronic villitis. In addition, one representative formalin-fixed paraffin-embedded block containing basal surface and central chorionic villi was submitted for CD3, CD56, and CD68 immunohistochemical staining. Five random high-power fields (HPF) of basal plate were examined and the total number of positive cells was counted. Statistical analyses were performed using the sum and mean number of positive cells.

Results: 21/53 (40%) placentas had at least one chronic inflammatory lesion (6 cases with CV and 15 with CD). The most prevalent type of chronic inflammatory cells in the basal plate were CD3 positive T-cells (mean CD3 range = 2-36 cells/HPF; sum CD3 range = 9-182 cells; mean CD56 range 0-9 cells/HPF; sum CD56 range = 1-46 cells; mean CD68 range 1-17 cells/HPF; sum CD68 range = 9-87 cells). While no statistically significant differences were seen in the mean and sum of CD3, CD56 or CD68 positive cells in the basal plate when placentas were stratified by presence or absence of histologic chronic inflammatory lesions,there was a trend toward more prominent numbers of CD3+ T-cells when other chronic inflammatory lesions were present, especially CD.

Conclusion: These data show that the most prominent chronic inflammatory cells seen in the basal plate are CD3+ T cells and suggest there may be an association between an increase in these cells and the histologic lesions of chronic placental inflammation.

33. Maternal Vascular Malperfusion Dilemma

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Background: Inadequate spiral artery remodeling leads to maternal vascular malperfusion (MVM) and has been linked to fetal growth restriction. The MVM diagnosis requires compilation of multiple placental findings. In this study, we aimed to determine the associations with specific placental findings and small for gestational (SGA) infants and develop an MVM score.

Methods: We used a case-control design and a standardized protocol to examine prospectively collected placentas of appropriate for gestational age (AGA, n=60) and SGA (n=78) neonates. Placentas were evaluated for findings associated with MVM (See Table 2). The odds ratios (OR) of the associations between placental pathologies among SGA neonates were compared to AGA group. A cumulative MVM score was calculated: 1 for each significant finding in Table 2, and 2 when increased syncytial knots involved >30% and infacts >10% of the parenchyma (maximum 9). Univariate testing for continuous and categorical variables was conducted.

Results: AGA and SGA infants had the following characteristics (Table 1):

Table 1	**AGA (N=60)**	**SGA (N=78)**	***P* value**
GA (wks)*	39.1±0.04	38.9±0.1	*NS*
Placental Weight (g)*	500±10.5	349.6±7.6	<0.0001
Birth Weight (g)*	3592±47	2576±36	<0.0001

* x±sem

Distribution of placental findings associated with MVM is listed in Table 2:

Table 2	**AGA(N=60)**	**SGA (N=78)**	**Fisher's exact test**	**OR (95% CI)**
SGA placenta	15 (25%)	69 (88.5%)	<0.0001	23 (9.3-57)
Decidual vasculopathy	4 (6.7%)	17 (21.8%)	0.0166	3.9 (1.2-12.3)
syncytial knots	8 (13.3%)	34 (43.6%)	0.0002	4.88 (2-11.6)
Distal villous hypoplasia	0	1 (1.3%)	NS	2.34 (0.1-58.6)
perivillous fibrin deposition	3 (5%)	22 (28.2%)	0.0003	7.46 (2.1-26.4)
Villous fibrinoid necrosis	1 (1.7%)	4 (5.1%)	NS	3.19 (0.4-29.3)
EVT islands	5 (8.3%)	21 (26.9%)	0.0077	4.05 (1.4-11.5)
Implantation site trophoblastic giant cells	11 (18.3%)	27 (34.6%)	0.0366	2.36 (1.1-5.3)
Infarcts	4 (6.7%)	17 (17.9%)	0.017	3.9 (1.2-12.3)

The median (25^{th}-75^{th} %) cumulative MVM score among variables associated with an SGA infant was higher in SGA group (3.0 [1.8 – 4.0]) compared to AGA (0.5 [0 – 1.0]) (P<0.0001). The odds of an SGA neonate was 12.9 folds higher (95% CI: 5 – 33.7) when MVM score was ³3.

Conclusion: The results demonstrate associations among SGA infants and multiple placental findings which are components of MVM. The most prominent association was a small placenta. The cumulative scoring system might be used as an objective tool when studying maternal vascular malperfusion.

34. An Immunohistochemical Re-evaluation of Placental "Adrenocortical" Heterotopias

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Background: Rare nodules of adrenocortical tissue are described in the human placenta, and are reported as distinct from hepatic nodules. Fewer than 10 cases of adrenocortical heterotopia of the placenta have been reported. Jaques and Qureshi reported the largest series to date with 5 cases in 2003, including 2 from a previous report. A mechanism for adrenocortical tissue heterotopia has been elusive. Based on our experience with three cases, we sought to re-assess the cell lineage of purported adrenocortical nodules in the placenta.

Methods: Three placentas containing small nodules of tissue identical to those described as adrenocortical nodules were identified. Two cases were identified prospectively in a routine perinatal practice, the third had been previously diagnosed as an adrenocortical nodule. Paraffin embedded sections were immunostained utilizing antibodies for SF-1 (Steroidogenic factor-1, R&D systems, 1:100 dil), HepPar1 (Dako, 1:200) and Arginase-1 (Sigma, 1;200).

Results: The cases were 41, 33 and 32 weeks gestation singleton placentas. Each case showed a single subchorionic focus of cuboidal cells with clear cytoplasm, small round central nuclei and a rich associated vasculature. The foci measured between 0.05 and 0.4cm in greatest diameter. No nested architecture was present. Occasional cells were binucleate. No associated foci of extramedullary hematopoiesis were seen. In one case, continuation of the nodule with a stem villous was present. Immunostaining showed no nuclear immunoreactivity for SF-1 in any of the 3 nodules. Each was strongly positive for Arginase-1, and showed diffuse cytoplasmic and membranous HepPar1 reactivity.

Conclusion: Our immunohistochemical findings suggest that these nodules of cleared cells termed “adrenocortical”, based upon the histologic resemblance to adult adrenal cortex, are actually nodules of hepatic parenchymal tissue with clear cytoplasm, likely from residua of yolk sac elements. Inhibin and Melan-A staining is often used to support adrenocortical differentiation. However, these antibodies can be difficult to interpret, and may feature non-specific cytoplasmic reactivity. SF-1 is a nuclear stain that is easier to interpret. SF-1 production is noted in the embryofetal adrenal from 10 weeks gestation, suggesting that the absence of SF-1 in these lesions is not due to immaturity. In addition, the histology of these nodules does not resemble fetal adrenal tissue. HepPar1 and Arginasae-1 are easy to interpret and specific for hepatic differentiation in this context. We suspect the diagnosis of placental "adrenocortical" ectopia/heterotopia will likely disappear in the era of modern immunohistochemical evaluation, as more and more these lesions are recognized as hepatocytic.

35. Mismatch Repair Gene Immunohistochemistry in Products of Conception Can Diagnose Congenital Constitutional Mismatch Repair Deficiency

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Background: The genomic stability is maintained by mismatch repair (MMR) genes. Heterozygous germline mutations in MMR genes cause Lynch syndrome. Homozygous (bi-allelic) mutations in MMR genes lead to constitutional mismatch repair deficiency (CMMRD), characterized by predisposition to hematological, brain and gastrointestinal malignancies in early childhood. Immunohistochemistry (IHC) is a sensitive and specific test in detecting MMR deficiency in CMMRD-associated tumors.

Methods: We present the MMR-IHC staining pattern on various tissue samples from a single family (mother and product of conceptions respectively). The mother (a Lynch-syndrome case) had several pregnancies, one of which lived till age of 3 and found to have Glioblastoma and consequently was diagnosis as CMMRD for MSH2. As the father was also a Lynch-syndrome case, the family opted for terminations-of-pregnancy (TOPs) with the following pregnancies, due to the high probability of having descendants with homozygous deletions.

Results: Endometrial, colonic mucosa and maternal side of the products of conception tissue showed intact IHC expression for all four MMR proteins. The fetal side (chorionic villi) showed loss of expression of MSH-2 and MSH-6.

Conclusion: MMR-IHC staining of the product of conception can identify the CMMRD status, and offer a confident result to parents opting for TOPs, without undertaking genetics/molecular studies. IHC can also potentially be applied as an affordable test to amniocentesis/villus sampling for prenatal diagnosis of such cases.

36. Perinatal Mortality Audit In The Emilia-Romagna Region Within The Period 2014-2016: The First Italian Experience

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Background: Perinatal Mortality audit are strongly recommended to analyze the cause of death, associated risk factors, adequate maternal medical assistance, and to improve counseling for future pregnancies. In the Countries where this kind of surveillance has been applied it has been found effective to reduce the stillbirth rate.

According to recent Italian laws on stillbirth, in the Emilia-Romagna Region, a local group was instituted in 2014 composed of gynecologists, neonatologists, medical geneticists, microbiologists and dedicated perinatal pathologists. A specific protocol in case of stillbirth was accurately elaborated and widely introduced in every hospital. The same regional group regularly meets every 4 months to discuss difficult cases and to evaluate the adequate maternal prenatal care.

Methods: To evaluate the regional stillbirth rate, Intrauterine Fetal Death (IFD) was defined according to WHO as fetal death after the 22ndweek of gestational age or the delivery of a stillborn baby of at least 500 g if the gestational age is not known.

According to these criteria, for every IUD a "stillborn clinical chart" was prepared including maternal clinical history, risk factors, medical investigations on both parents, placental and fetal autopsy findings. Cause of death was classified with the ReCoDe system (relevant condition at death), which takes into account placental and fetal causes such as intrauterine fetal growth restriction.

Results: Within the period 2014-2016 the perinatal audit registered 330 fetal deaths out of 107.528 deliveries. Early IUDs, happened between the 22ndand the 27thweeks of gestational age were 80 (0,74‰). Late IUDs were 250 (2,33‰).

Main causes of death were attributed to placental pathologies (38,5%) including placental insufficiency, chorioamnionitis, and abruption. Fetal pathologies (intrauterine growth restriction, congenital anomalies) were found relevant in 17,6%. Cord accidents (true knots, cord entanglement) were observed in 17,6%. Maternal pathologies, mainly diabetes and hypertension, were observed in 7,6%. However, in 14 cases (4,2%) the cause of death remained unexplained.

Statistically significant risk factors for IUD were the following: 1) maternal age more than 30 years; 2) early gestational age; 3) maternal BMI > 25; 4) fetal growth restriction.

Conclusion: The stillbirth rate registered in the Emilia-Romagna region is low and similar to the European Developed Countries. Prenatal care was adequate in most of the cases.

A major contribution to define the cause of death was given by placental examination and fetal autopsy performed by dedicated perinatal pathologists.

37. Increasing The Value Of Autopsies In Patients With Brain Tumors In The Molecular Era

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Background: Many pediatric brain tumors are associated with high morbidity and mortality, which is due to insufficient understanding of tumor biology. Research allocation from surgical specimens is challenging due to small amount of tissue, but brain tumor autopsies are a valuable resource. This study reviews the brain tumor autopsy practice at our institution, and describes their use beyond the autopsy report.

Methods: We reviewed the autopsies in the interval 2007-2017, and the gross and histologic examinations for the ones with brain tumors. For research consented autopsies, we analyzed the method of tissue triaging (bioregistry, frozen and fresh tissue, and cerebrospinal fluid) and documented their use.

Results: Of 1602 deaths (636 with autopsies), 96 had a diagnosis of brain tumor (56 consented for autopsy). DIPG and other high-grade gliomas (HGGs) accounted for the greatest proportion of diagnoses (63% of brain tumor autopsies). In particular, 88% (22/25 cases) of all DIPG deaths resulted in autopsy. Fourteen DIPGs (56%) were contributed to DIPG registry. Additionally, 18 separate brain tumor autopsy cases were consented for biobanking and research; these cases were used successfully for DNA-based studies, and 4 were used successfully for RNA-based studies. Cell lines were successfully generated from 3 tumors (12 attempted). Success rates for these studies were postmortem interval-dependent.

Conclusion: We describe our institutional pediatric brain tumor autopsy experience and demonstrate the utility of autopsy-derived tissue for research, particularly for HGGs. Our experience demonstrates a wide utilization of brain tumor autopsy material in translational research, and might encourage a wider purpose of brain tumor autopsy material in the molecular era.

38. A Novel Tetratricopeptide Repeat Domain 7A (TTC7A) Mutation in a Newborn with Multiple Intestinal Atresia and Combined Immunodeficiency

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Background: Hereditary multiple intestinal atresia and combined immunodeficiency (MIA-CID; OMIM 243150) is an extremely rare and invariably lethal disorder. This condition is characterized by multiple intestinal obstructions with atretic sites occurring at various locations throughout the small and large intestines. These intestinal features are associated with a spectrum of immunodeficiency, with very few children surviving beyond the first year or two of life. The genetic basis of this condition has recently been linked to autosomal recessive mutations in TTC7A gene. In our patient, we demonstrate two novel deletions of the TTC7A gene involving a multi-exonic heterozygous deletion (exon 12-15) and a homozygous exon 15 deletion producing a severely affected neonate with MIA-CID and very early onset inflammatory bowel disease.

Methods: Targeted single-gene duplication/deletion testing using comparative genomic hybridization was carried out at Cincinnati Children's Cytogenetics and Molecular Genetics Laboratories. Protein expression status was evaluated by TTC7A immunofluorescence staining pattern, which was carried out at SickKids IBD Centre at Toronto's Hospital for Sick Children

Results: Two large, previously undescribed, exonic deletions were identified in the TTC7A gene. The first deletion identified is between 15.4 kb and 16.6 kb in length and corresponds to a heterozygous deletion of intron 11 to intron 15 (exon 12-15). The second deletion identified is between 3.1 and 3.3 kb in length and corresponds to a homozygous deletion of intron 14 to intron 15 (exon 15). Both of these mutations are strongly predicted to be a null protein expression based on the negative immunofluorescence staining pattern.

Conclusion: In the past several years, multiple centers have reported autosomal recessive mutations in TTC7A gene in patients with multiple intestinal atresia and immunodeficiency. Here, we present a neonate who was investigated for MIA-CID after pathologic examination of multiple atretic bowel segments queried this diagnosis. Molecular studies revealed two deletions within the TTC7A gene by single gene deletion and duplication analysis. The patient described here expresses a severe phenotype of MIA-CID with multiple large and small bowel atresias, combined immunodeficiency, and very early onset inflammatory bowel disease with additional, previously undescribed renal and cardiac abnormalities. The null expression of the TTC7A gene within gastrointestinal enterocytes, as confirmed by Immunofluorescence studies, suggests the total loss of TTC7A protein function, consistent with the severity of the phenotype.

39. A Primary Epithelioid Vascular Tumor of Bone with Atypical Features and FOS Gene Rearrangement: A Case Report

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Background: Primary vascular tumors of the bone encompass a variety of neoplasms ranging from benign to malignant. Differentiating these lesions can be challenging due to tumor multifocality, aggressive radiologic appearances, and overlapping histologic features. In addition, benign lesions are frequently associated with atypical histologic appearances mimicking malignancy, further complicating the classification of these tumors. We present a primary epithelioid vascular tumor of bone with atypical histologic features and FOS gene rearrangement.

Methods: A 15 year old boy presented with a two month history of wrist fullness and pain. MRI revealed a 4 cm distal radius, metaphyseal, enhancing, marrow replacing mass, with paraosseous extension. A core needle biopsy was non-diagnostic and an open biopsy was subsequently performed. The lesion was composed of atypical epithelioid cells with vesicular chromatin, prominent nucleoli and significant mitotic activity, up to 5 in 10 high power fields. The cells were arranged in vasoformative configurations as well as cellular areas of solid growth. The lesional cells expressed ERG and CD31 with weak and patchy CK AE1/3. FOS gene rearrangement was identified by FISH.

Results: This epithelioid vascular tumor of the bone had atypical histologic features and an aggressive radiologic appearance that were suggestive of malignancy. Our differential diagnosis included epithelioid hemangioma (EH) and epithelioid angiosarcoma (AS). FOS gene rearrangement has been associated with EH, a benign, locally aggressive tumor. The patient received a complete curettage and is without recurrence or disease progression at four months follow up.

Conclusion: The classification of primary vascular tumors of bone is challenging due to overlapping morphology, the presence of aggressive radiologic appearances and histologic atypia suggestive of malignancy in benign tumors, as illustrated in this case. EH and AS have drastically different prognoses and treatment options. FOS gene rearrangements occur in 59% of skeletal EH and have not been identified in AS. The integration of morphology and molecular changes has allowed for better characterization of these entities.