Two Distinct Patterns of MYCN Protein Expression in Stage 4S Neuroblastomas: A Report from the COG Neuroblastoma Study.

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Background: Stage 4S neuroblastoma (4SNB, <1yr at diagnosis; INSS stage 1 or 2 primary; metastasis to liver, skin and/or bone marrow) is often associated with spontaneous regression and a good clinical outcome. Augmented expression of MYC-family (MYCN/MYC) protein indicates aggressive clinical behavior in NBs.

Methods: There were 185 4SNBs filed at the COG NB Pathology Reference Laboratory: Their MYCN oncogene status [Non-amplified(NA) vs. Amplified(A)], MYC-family protein expression [no-overexpression(-)/(+/-) vs. overexpression(+)] by immunohistochemistry(IHC), and INPC histopathology with a special attention to the nucleolar hypertrophy [NH, supporting increased protein expression, (-) vs. (+)] were correlated with patient survival.

Results: As shown in the Table, MYCN oncogene status (165N-A;20A) distinguished clinical outcomes of the patients. The vast majority of tumors showed MYCN NA, MYC-family protein(-)/(+/-), FH, NH(-), and excellent survivals (conventional NA tumors). Among MYCN-NA tumors, however, 11 demonstrated MYCN protein(+) with uniform and moderate intensity: they were FH(10/11), NH(-) and one showed MYC protein(+). Also found were 5 MYC protein(+) and MYCN(-)/(+/-) tumors: they were FH without NH(4/5). Among the MYCN-A tumors, 18 had MYCN protein(+) with heterogeneous and strong intensity regardless of INPC [UH(9, conventional A tumors)/FH(9)] and 15 of them had NH(+). Two tumors had MYCN protein(-)/(+/-) despite MYCN-A; both were FH and NH(-).

<table>
<thead>
<tr>
<th>MYCN oncogene</th>
<th>MYCN protein</th>
<th>MYC protein</th>
<th>INPC</th>
<th>NH</th>
<th>3-yearEFS (SE)%</th>
<th>3-yearOS (SE)%</th>
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<tbody>
<tr>
<td>Non-Amplified (165)</td>
<td>(-)/(+/-)</td>
<td>(-)/(+/-)</td>
<td>FH (-)</td>
<td>88.5 (3.1)</td>
<td>94.1 (2.3)</td>
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<tr>
<td>Conventional (147)</td>
<td>(+)</td>
<td>(+)</td>
<td>FH(9);UH(1)</td>
<td>(-)</td>
<td>100 (0)</td>
<td>100 (0)</td>
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<tr>
<td>Others (10)</td>
<td>(+)</td>
<td>(+)</td>
<td>FH (-)</td>
<td>100 (0)</td>
<td>100 (0)</td>
<td></td>
</tr>
<tr>
<td>(1)</td>
<td>(+)</td>
<td>(+)</td>
<td>FH (-)</td>
<td>100 (0)</td>
<td>100 (0)</td>
<td></td>
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<tr>
<td>(5)</td>
<td>(-)/(+/-)</td>
<td>(+)</td>
<td>FH</td>
<td>4(-);1(+)</td>
<td>100 (0)</td>
<td>100 (0)</td>
</tr>
<tr>
<td>(2)</td>
<td>(-)/(+/-)</td>
<td>(-)/(+/-)</td>
<td>UH (-)</td>
<td>100 (0)</td>
<td>100 (0)</td>
<td></td>
</tr>
<tr>
<td>Amplified (20)</td>
<td>(+)</td>
<td>(+)</td>
<td>FH(1)</td>
<td>68.6 (19.2)</td>
<td>68.6 (19.2)</td>
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MYCN expression by IHC: *Moderately and uniformly positive; **Strongly and heterogeneously positive.

Conclusion: In 4SNBs, 2 patterns of MYCN protein expression were distinguished. In MYCN-A tumors, the expression was associated predominantly with NH, showed a heterogenous and strong (+) pattern, and indicated a poor prognosis. Protein expression in MYCN-NA tumors was mostly not associated with NH, showed a uniform and moderate (+) pattern, and indicated an excellent prognosis.
Pediatric Granular Cell Tumors: Clinicopathologic and Molecular Analysis of Six Cases Including Multicentric, Atypical, Malignant, and Non-Neural Tumors

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Background: Granular cell tumors (GCTs) are uncommon in childhood. While most are solitary and benign, very rare cases of multicentric, atypical and malignant pediatric GCTs have been documented. Multicentric GCTs are a rare component of RASopathies (Noonan, neurofibromatosis I) and are sometimes familial, but most occur without a defined etiology. Despite histologic features of atypical and malignant GCTs having been described, they lack sensitivity and specificity in predicting malignancy, and are based on predominantly adult series. Molecular alterations may better define multicentric and/or malignant behavior, but they have not been characterized in GCTs. This series describes unique clinicopathologic and molecular findings in a series of pediatric GCTs.

Methods: Cases of GCTs with uncommon clinical or histologic features diagnosed at a single institution over 5 years were reviewed. Lesional areas were identified for FFPE DNA/RNA testing using the Oncomine Childhood Cancer Research Assay (Thermo Fisher).

Results: We identified 6 pediatric GCT cases including 3 multicentric GCTs (one malignant), 1 non-neural GCT, and 2 additional GCTs with atypical features. There were 12 primary lesions overall; 4 residual/recurrent lesions; and 1 metastasis to an axillary lymph node 2 years after initial diagnosis. Age of tumor onset was 4-16 yr and 5/6 patients were female. One patient with multicentric disease had a history of neuroblastoma (RASopathy negative); the others were without comorbidities. The extremities and chest wall were common sites. Most involved the dermis and subcutaneous tissue, and two demonstrated an unusual plexiform or fascicular growth pattern with perineurial involvement. An S100-negative non-neural GCT was also identified. Histologic features ranged from classical to one or more atypical features, including pleomorphism, nucleoli, spindling and mitoses. Multicentric cases were associated with both classical and atypical histologic patterns at different sites. Molecular testing identified a somatic NF1 (p.W696*) loss-of-function variant in one multicentric tumor.

Conclusion: We present a series of rare nonclassical pediatric GCTs, documenting that atypical histologic features used in adult classifications are not reliable predictors of multicentric, recurrent or malignant behavior. Pediatric GCTs also have unique microscopic features, including a fascicular pattern we describe. Ultimately, molecular markers may be the best predictors of outcome, and our finding of a probable NF1 mutation is intriguing given the association of GCTs with NF1, although the lack of somatic variants in other cases suggests that the pathogenesis may involve less-common alterations.
Utility of p53, ATRX, and H3K27Me3 Immunohistochemical Staining in Distinguishing Osteosarcoma from Histologic Mimics

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Background: Osteosarcomas (OS) are the most common primary bone malignancy in children. They may be difficult to distinguish from other bone lesions histologically and due to similar expression of lineage-specific markers such as SatB2. There is no single genetic alteration that appears responsible for OS tumorigenesis. Some cases of OS have shown p53 or ATRX mutations by immunohistochemistry, and loss of H3K27 trimethylation (H3K27Me3) has been described in rare cases. These markers have not been well-studied in potentially distinguishing OS and its mimics. We sought to compare immunohistochemical expression of these markers in OS compared to various histologic mimics.

Methods: Ten OS of various histologic subtypes and 25 histologic mimics (5 aneurysmal bone cysts, 3 chondroblastomas, 3 fibrous dysplasias, 3 osteofibrous dysplasias, 3 giant cell tumors of bone, 3 non-ossifying fibromas, 3 osteoblastomas, and 2 osteoid osteomas) were selected. Immunohistochemical staining for p53, ATRX, and H3K27Me3 was performed on all cases. p53 staining was graded on a 0-3+ scale (0=no nuclear staining, 3+ = strong nuclear staining in >50% of tumor nuclei), and ATRX and H3K27Me3 were graded based on degree of nuclear staining loss (retained=nuclear staining in >80% of tumor nuclei, lost=nuclear staining in <20% of tumor nuclei). Three pathologists interpreted each marker, and consensus was obtained.

Results: A trend toward lost ATRX staining was seen in OS compared to mimics (3/10 OS and 1/25 mimics; p=0.061). p53 staining of at least 2+ was seen in 2/10 OS and 9/25 mimics (p=0.45), while strong diffuse (3+) p53 staining was seen in 1/10 OS and 0/25 mimics (p=0.29). H3K27Me3 staining was lost in 1/10 OS and 1/25 mimics (p=0.50). Selecting for cases showing any of 3+ p53 staining, or complete loss of ATRX or H2K27Me3 staining was useful in separating OS (5/10) from mimics (1/25) (p=0.004). The single mimic was a fibrous dysplasia that showed loss of both ATRX and H3K27Me3 staining (including in the internal control), suggesting loss of antigenicity due to decalcification. Otherwise, no cases had more than one of 3+ p53 staining or H3K27Me3 or ATRX loss.

Conclusion: In this pilot cohort, individual evaluation of p53, ATRX, and H3K27Me3 staining trended toward but was not statistically significant in distinguishing OS from mimics. However, the combination of strong diffuse p53 staining, complete ATRX loss, or H3K27Me3 loss was significant. A panel consisting of these three stains may be helpful in the workup of bone tumors where OS is suspected. Expansion to a larger cohort and correlation with available molecular findings may be beneficial. However, even in a small cohort where molecular sub-groupings are unknown, this panel shows promise at distinguishing OS from non-OS bone lesions.
USP6 RNA Expression by Targeted RNA NGS to Rescue Missing Fusions
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Background: Oncogenic USP6 fusions are oncogenic drivers in aneurysmal bone cyst, nodular fasciitis and cranial fasciitis. Fusions typically result from promoter-swapping events, which drive upregulation of the USP6 protein. Since the promoter region of USP6 is relatively large, we hypothesize that a targeted RNA sequencing assay could miss fusions with alternate breakpoints. We investigated the utility of the RNA expression data within the assay to correctly identify false negative USP6-fusion cases.

Methods: Twenty-seven unique cases were evaluated with a custom RNA sequencing assay, designed for pediatric solid tumors and brain tumors, which targets 64 genes. USP6 RNA expression was determined using RNA reads with unique molecular barcodes, and was compared to expression of the GPI housekeeping gene.

Results: Five cases with USP6 fusions were identified by sequencing and 1 case had evidence of USP6 fusion by FISH but was negative by sequencing (false negative sequencing result). USP6 fusion-positive cases include 4 nodular fasciitis and 2 aneurysmal bone cysts. Twenty-two cases from the same sequencing runs were used as USP6 fusion-negative controls, of which 8 had other gene fusions and 14 were fusion-negative. The average USP6 expression in the USP6 fusion-positive cases was 781.4 reads, compared to 7.03 reads for the control cases. There was a significant difference in absolute USP6 expression between fusion-positive cases and controls (p<0.0001) and also a significant difference in the ratio of USP6 expression to GPI expression between cases and controls (p<0.0001). The false negative case had significantly higher USP6 expression than controls (177-fold), consistent with the other USP6 fusion-positive cases.

Conclusion: Analysis of RNA expression successfully identified a case with USP6 fusion which was missed by traditional sequencing fusion analysis. This analysis technique could be translated to other gene fusion targets, and would be particularly useful for those genes which undergo promoter-swapping or have variable breakpoints. Implementation of this algorithm may help to improve the sensitivity of the assay, and to improved rates of diagnosis.
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DNA Methylation and Copy Number Profiling in Pediatric BCOR-ITD

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**Background:** BCOR-ITD are recurrent genetic alterations common to different pediatric malignancies, including Primitive Myxoid Mesenchymal Tumors/undifferentiated sarcomas (PMMTI/UND), clear cell sarcomas of kidney (CCSK), and a subgroup of central nervous system high-grade neuroepithelial tumors (HG-NET). Recently, the same genetic alteration has been reported in high-grade endometrial stromal (HG-ES) sarcomas. In spite of a common gene expression profile, recent studies reveal a different immunophenotype in HG-NET and PMMTI/UND, with absent expression of OLIG2 in PMMTI/UND. The aim of the study is to explore DNA methylation and copy number variation profiling in pediatric BCOR-ITD to better define their potential contribution to the diagnostic work-up.

**Methods:** DNA methylation and copy number variation profiling were studied on 13 primary tumors (9 PMMTI/UND, 4 CCSK, 3 brain HG-NET) and relapses (3 PMMTI/UND) from 16 pts (age range 0-18 month-old). BCOR-ITD had been previously identified in PMMTI and HG-NET by FISH and/or RT-PCR. RT-PCR was newly performed in CCSK. Results were compared to the methylation sarcoma classifier version 12.2 ([https://www.molecularneuropathology.org/mnp/classifier/9](https://www.molecularneuropathology.org/mnp/classifier/9)) developed by Heidelberg University and DKFZ.

**Results:** Unsupervised hierarchical clustering analysis of DNA methylation data revealed a rather homogeneous methylation cluster for BCOR-ITD malignancies, including 3 HG-NET, 8 PMMTI/UND and 3 CCSK. One UND/PMMTI clustered with HG-ES sarcoma. Intriguingly, the only case with negative RT-PCR for BCOR-ITD, originally diagnosed as CCSK, clustered with rhabdoid tumors. Loss of INI-1 immunostaining, not performed at the time of initial diagnostic workup, confirmed the diagnosis of rhabdoid tumor. Copy number variation (CNV) profiles showed an overall flat profile in 10 of out 14 cases. Four PMMTI/UND (1 primary, 3 relapses) showed recurrent gains or losses at 1q and 10q respectively (2 cases). Other CNV included 7q and 3p gain, and 9p and 2p loss.

**Conclusion:** BCOR-ITD pediatric malignancies share a distinctive methylation profile representing a potentially useful diagnostic tool. The evidence of CNV with losses at loci of oncosuppressor genes (like PTEN and CKN2A/B), mostly in relapses, suggest these alterations may play a role in the aggressive clinical behavior of BCOR-ITD malignancies.
The Prognostic Significance of Anaplasia in Childhood Rhabdomyosarcoma: A Report From the Children’s Oncology Group

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Background: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood, and several clinicopathologic prognostic factors are well established. Among pathologic features, anaplasia has been suggested as a poor prognostic indicator, but the clinical significance of anaplasia remains unclear. Prior studies investigating anaplasia in RMS have indicated the need for a larger study with systematic analysis.

Methods: Patients enrolled on COG clinical RMS studies D9602 (n= 357), D9802 (n=80), D9803 (n=462), ARST0331 (n=335), and ARST0531 (n = 414) with prospective central pathology review were included in this study. Clinicopathologic variables including demographic information, risk group, histologic subtype (embryonal, botryoid, spindle cell, alveolar, not otherwise specified), and anaplasia were recorded along with follow-up on relapse, progression, secondary malignancy, and death. Tumor TP53 mutational status was known for 146 patients. The log-rank test and Cox regression model were used to evaluate the impact of anaplasia.

Results: A total of 1648 cases of RMS were included in the study Anaplasia was more commonly documented in embryonal RMS (27%) in comparison to other subtypes (Spindle cell-20%, Alveolar – 10%, Botryoid – 7%). On multivariate analysis, anaplasia was not an independent prognostic factor in RMS overall (OS: p=0.43; FFS = 0.56), and across all subtypes, including embryonal RMS (OS: p=0.078; FFS: p =0.16). Pattern of anaplasia (absence, focal or diffuse) was also not statistically significant (OS: p=0.15; FFS: p= 0.22). Anaplasia was observed in a majority of tumors with TP53 mutations (69%), while only a minority of tumors with anaplasia harbored a TP53 mutation (24%).

Conclusion: Anaplasia is not an independent indicator of adverse outcomes in RMS. Emerging information on the prognostic significance of TP53 mutations suggests that anaplasia may be a surrogate marker of TP53 mutations in some cases. TP53 mutation status may be investigated as a prognostic indicator in future studies.
Prognostic Significance of Inflammatory Microenvironment in Post-transplant Lymphoproliferative Disorder as a Potential Method to Identify High Risk Patients.

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Background: Post-transplant Lymphoproliferative Disorder (PTLD) includes a spectrum of conditions in the setting of immune deficiency. The vast majority of diagnoses are associated with Epstein-Barr virus (EBV) infection resulting from impaired immune response. EBV-positive lymphomas are a significant cause of morbidity and mortality in pediatric solid organ and bone marrow transplantation. The purpose of this study was to analyze the expression of PTLD microenvironment components and assess their utility as prognostic tissue biomarkers to help identify high risk patients.

Methods: Computerized search for cases of pediatric solid-organ and bone marrow transplant recipients diagnosed with PTLD after the year of 2000 was performed in the pathology database. Cases were stratified by the diagnosis, clinical outcome, and the EBV status. Expression of EBER, CD3, CD4, CD8, CD20, CD57, CD163, PD1 and PDL-1 was assessed by immunohistochemistry. The density of positive cells was expressed as a percentage of positive cells in the lesion. Student unpaired t-test was used to quantify the data.

Results: Retrospective analysis was performed for 60 pediatric patients with the pathologically confirmed diagnosis of PTLD (36 males, mean age=12.5 years). Histological classification included monomorphic PTLD (n=20, 33%), early (n=15, 25%), unclassified (n=13, 22%), polymorphic (n=11, 18%), and a classical Hodgkin lymphoma (n=1, 2%). Disease-free status or stable disease was considered a positive outcome (n=43, 72%) and mortality from the disease was considered a negative outcome (n=17, 28%). Lesions of patients with a negative outcome had a 30% lower density of CD3 infiltrate (p=0.02), a two-fold lower density of the PD1 infiltrate (p=0.007) and a two-fold increase of the PD-L1 expression (p=0.02). There was no significant difference in the density of CD3, CD163, PD1 and PD-L1 infiltrates in cases stratified by the EBV status.

Conclusion: The density of CD3, PD-1 and PD-L1 infiltrates have a prognostic impact in patients with solid organ or bone marrow transplant complicated by the PTLD. Disturbed immune surveillance and decreased T-cell function contribute to the PTLD development, progression, and the outcome, and patients with more profound immune regulation deficiencies are more likely to have a negative outcome. Tissue biomarkers may help identify high risk patients in order to strategize chemotherapy and clinical surveillance. Whether checkpoint inhibitors could be a targeted therapy option remains to be determined.
Does the Use of a Next Generation Sequencing Assay Improve Diagnostic Accuracy in Pediatric Fibroblastic/Myofibroblastic Tumors?

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**Background:** Pediatric fibroblastic/myofibroblastic tumors (PFMTs) are a heterogeneous group that range from reactive entities to aggressive sarcomas. They include benign ‘pseudo-sarcomas’, some rapidly-growing with malignant-appearing histology, as well as sarcomas, some slowly-growing with benign-appearing histology. Due to overlapping clinical presentations, histologic features, and lack of specific immunohistochemical and molecular markers, many of these lesions are signed-out descriptively or using ‘catch-all’ diagnoses, hindering optimal clinical management. Distinct molecular alterations have recently been identified within this group that could be used to improve diagnostic accuracy – or in some cases identify a targeted therapy, although most of these are considered too rare for routine testing in combined adult/pediatric labs. We sought to evaluate the use of a pediatric-specific next-generation sequencing assay to aid in the diagnosis of these challenging PFMTs.

**Methods:** Soft tissue PFMTs diagnosed at a single center over 12 years were included. Age, gender, site and size were abstracted from pathology reports. Archived slides were reviewed and lesions categorized using the most recent WHO classification. Cases that did not fit current diagnostic categories were recorded as unclassified, with the closest category and salient features noted. Lesional areas were identified for FFPE DNA/RNA testing using the Oncomine Childhood Cancer Research Assay (Thermo Fischer).

**Results:** Seventy-five cases were reviewed, and 50 chosen for molecular analysis. Of these 50, a definitive diagnosis was possible in 37 cases (74%), with 13 (26%) lacking definitive classification. Many of the defined cases were associated with known molecular alterations. We identified mutations (notably in \textit{CTNNB1}, \textit{TSC2}, \textit{TET2}), fusions (\textit{NTRK3}, \textit{ALK1}, \textit{PDGFB}, \textit{USP6}), or deletions (\textit{EGFR}) in 24% (12) of cases tested (10% pending repeat). Expected alterations were identified for most known diagnoses, notably except for infantile myofibroma, and a variant identified for an entity lacking recurrent alterations. We discovered alterations in 4 of 13 cases that were initially unclassified, enabling a diagnosis/suggested treatment.

**Conclusion:** Our review confirmed that a significant subset of PFMTs are challenging to classify using current criteria. Molecular alterations are now identified for at least half of the classified PFMTs, and we confirmed that a combined DNA/RNA assay is able to identify many of these, improving diagnostic certainty. We also identified genetic alterations in 31% of unclassified cases, suggesting a clinical utility for challenging cases. Since the assay was developed from known genetic alterations, the discovery of novel alterations in other PFMTs may require a more comprehensive sequencing approach.
Implementing Telepathology to Improve a Pediatric Fine Needle Aspiration Service: an Institutional Experience.


Background: Fine needle aspiration (FNA) of thyroid nodules and associated abnormal cervical lymph nodes was historically performed at our children’s hospital without presence of an onsite cytopathologist. This led to increased FNA passes per lesion site and inability to perform selective triage of cytologic material for ancillary studies in indeterminate nodules. We evaluated the implementation of telepathology to enable remote rapid onsite evaluation (ROSE) of FNA specimens.

Methods: We validated (100% agreement between RTIS and standard light microscopy) and implemented a real-time telepathology imaging system (RTIS) for onsite FNA. The system allows for remote real-time viewing of onsite FNA cytology preparations by a board-certified cytopathologist located at our academically affiliated adult institution. The case cohorts included 112 lesion sites pre-RTIS (01/01/18-01/31/19) and 42 sites post-RTIS (02/01/19-04/30/19). Number of passes per lesion site, sample adequacy per Bethesda system, and collection of specimens for ancillary testing for nodules with indeterminate cytology (13 pre-RTIS; 9 post-RTIS) were evaluated. Testing for differences between cohorts was performed using Mann-Whitney testing or Fischer’s exact test as appropriate.

Results: Post-RTIS, the number of FNA passes per site on both thyroid nodules and lymph nodes was significantly reduced (p=0.005, Mann-Whitney). Specifically, the percentage of patients requiring 3 or more FNA passes decreased from 47% to 21% after RTIS implementation (p=0.008, Fisher’s Exact). The relative risk of requiring 3 or more passes without RTIS was 1.5 (95% CI 1.1-1.9). Sample inadequacy was over-diagnosed by onsite versus final assessment pre-RTIS (12%) but not post-RTIS (0%). Collection of FNA specimens for indeterminate diagnosis increased from 0% to 100% post-RTIS.

Conclusion: Telepathology can improve patient care in a pediatric FNA service. This has enabled previously unavailable remote ROSE at our institution, resulting in reduced FNA passes per site, improved adequacy accuracy, and increased upfront collection of samples for ancillary testing on thyroid nodules classified as indeterminate.
Grading Fetal Vascular Malperfusion in the Placenta and Short Term Perinatal Outcome

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Background: Fetal vascular malperfusion (FVM) is a major placental determinant of remote neonatal outcome. However, its impact on more immediate perinatal complications is less well documented. The aim of this analysis is to compare the relation of grade of segmental and global FVM to other abnormal placental findings and early perinatal outcome.

Methods: 439 consecutive cases of placental FVM including 374 cases with segmental FVM (Group 1: 308 low grade, Group 2: 66 high grade), and 65 cases of high grade global FVM without segmental villous changes (Group 3), were compared. Histological FVM was diagnosed and graded according to the quantitative Amsterdam criteria, with grade subcategories determined on H&E stains only (Subgroups A) and on the recently described segmental endothelial fragmentation and villous mineralization (Subgroups B). Frequencies of 25 clinical and 40 placental phenotypes were statistically compared among the groups and subgroups.

Results: 22 abnormal clinical (88%) and 35 placental (71%) phenotypes were more common in this material than in the author’s published placental database of 3382 high-risk pregnancies. There were no differences in gestational age, intrapartum fetal distress, abnormal dopplers, fetal growth restriction and perinatal mortality among the groups. Groups 1B and 2B showed similar perinatal outcomes as Groups 1A and 2A. Groups 1 and 2 frequently showed mixed global and segmental patterns. Statistically significantly (p<0.05), Group 3 distinguished itself by higher rate of multiple pregnancy and lower rate of villous infarction and lesions of shallow placental implantation than Groups 1 and 2, and higher rate of abnormal fetal heart rate tracing than Group 1. Group 1 featured higher frequency of EXIT procedures than Group 2 and Group 3, but lower rate of umbilical cord compromise than Group 2. Group 1A showed less fetal malformations and EXIT procedures but more erythroblastosis of fetal blood than Group 1B.

Conclusion: Although lesions of global FVM of incomplete umbilical cord compromise and mass-forming fetal anomalies may be associated with scattered foci of segmental FVM, high-grade global FVM without a segmental component has similar short-term prognosis as segmental FVM, low-grade or high-grade. Various grades of segmental FVM are not associated with striking differences in short term clinical outcomes and/or associated other abnormal placental phenotypes. CD34 immunohistochemistry and iron/phosphate histochemistry-diagnosed/upgraded lesions increase the sensitivity of placental examination for FVM but carry similar short-term prognosis as older traditionally recognized lesions, low grade or high grade, diagnosable on H&E slides only, which confirms the usefulness of those stains in diagnosis of FVM.
Magnetic Resonance Imaging: Retrospective Analysis of in Vivo MRI and Placental Pathology

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Background: Magnetic resonance imaging (MRI) is a promising technique that can be used to assess placental microstructure and perfusion. Our previous work has shown changes in T2 relaxation time are associated with placental pathology in vitro. Few studies have examined the placenta in vivo and correlated the findings with placental pathologic findings. The aim of our study was to determine if there are correlations between in vivo MRI of the placenta and placental pathologic findings at delivery in patients undergoing MRI for fetal conditions. We hypothesize that important placental pathologic conditions will have altered T2 relaxation times.

Methods: The radiology database was searched for patients who underwent fetal MRI between 1/2017 and 1/2019. Singleton pregnancies with both adequate MRI images of the placenta and adequate existing placental pathology slides were selected. MRI images were reviewed and T2 relaxation time measurements were taken from placental regions of interest (ROI) near the umbilical cord insertion and away from the umbilical cord insertion. An overall “normalized T2 ratio” was calculated = mean T2 relaxation time of the two placental ROIs (msec) /T2 relaxation time of the psoas (msec) in the same image. Placental pathology findings were categorized according to standardized criteria for acute inflammation (AI), chronic inflammation (CI), fetal vascular pathology (FVM) and maternal vascular pathology (MVM). Statistical analysis was performed using IBM SSPS Statistics 22.

Results: 53 fetal MRIs were identified in the study period. For two patients, T2 images could not be retrieved and 2 sets of twins were excluded. Placental pathology reports were available from 21/49 remaining fetal MRI. Mean maternal age was 30.7 years and mean gestational age at delivery was 33.4 weeks. The normalized T2 ratio was significantly lower in cases where MVM was present (3.46 vs 5.57; p=0.031). The ratio was also significantly lower when AI was present (3.80 vs 6.88; p=0.023). No statistically significant differences in the normalized T2 ratio were seen in cases where FVM and CI were present.

Conclusion: Our data show that alterations in T2 relaxation time can be detected by MRI in placental tissue when pathologic conditions are present. The current data suggests that T2 relaxation time is shorter in AI and MVM, which contrasts somewhat with our previous work on ex vivo placentas. It is likely that both microstructural changes in the placenta tissue as well as blood flow (perfusion) in vivo contribute to alterations in T2 relaxation times in diseased placentas. Further investigation and correlation between in vivo MRI findings and placental pathology is warranted.
Placental Chronic chorioamnionitis Is Associated with Offspring Asthma in Pregnancies with Maternal History of Asthma

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Background: Asthma is the most common chronic disease of childhood, and parental asthma, prenatal environmental tobacco smoke, and prematurity are risk factors for childhood asthma. Vitamin D insufficiency is another risk factor for asthma. The Vitamin D Antenatal Asthma Reduction Trial (VDAART) was funded by NIH grant #HL091528 as a two-arm, randomized, double-blind, placebo-controlled trial to determine whether vitamin D supplementation daily at 400 IU vs. 4,400 IU to pregnant women prevented asthma and allergy in offspring at age three. Placental tissue collected during the VDAART trial allowed us to test the hypothesis that whether placental pathology is more common in offspring that develop asthma at three years of age, compared to those who did not develop asthma.

Methods: The offspring from the pregnancies studied in the VDAART trial had a maternal history of asthma, eczema, or allergic rhinitis. Pregnant volunteers in the St. Louis cohort participated in a nested case-control study, were between 18-39 yo, were enrolled between 10-18 weeks’ gestation, and consented for the study of placentas after delivery. Histopathological studies were done on 27 formalin-fixed, paraffin-embedded placental specimens, of which, 11 placental specimens were RNA sequenced for changes in gene expression was performed. Gene transcripts were quantified per sample using the DESeq2 package (version 1.26) in Bioconductor (version 3.1) with the analysis of raw RNA-seq read count data. Analyses used R statistical software (version 3.6, R Foundation).

Results: None of the demographic parameters were different between mothers of offspring with vs. without asthma at age 3 years (n= 6 and 21, respectively). There was a significant difference (P =0.047) in the proportion of chronic chorioamnionitis in the placentas of the offspring with asthma at age three (2/6), compared to the placentas of the offspring without asthma (0/21), and comparison of placentae from pregnancies with and without asthma demonstrated differential expression of HLA-DRB1 and HLA-DRB5.

Conclusion: The association of placental chronic chorioamnionitis and offspring asthma suggests placental pathology may offer risk stratification for offspring asthma and the association is worthy of further study.
Kaiser Neonatal Early-Onset Sepsis Risk Score and Histologic Evidence of Infection in Antibiotic-Treated Group B Streptococcus-Positive Mothers

M Gondim, J Hata; Norton Children's Hospital/University of Louisville, Louisville, Kentucky

Background: Group B streptococcus (GBS) is the leading cause of neonatal infection. Pregnant women are to be screened for GBS colonization, and if positive, treated with intrapartum antibiotics to prevent vertical transmission during delivery.

At our hospital, GBS colonization, with appropriate antibiotics and with or without clinical signs of chorioamnionitis, is currently a positive indicator for placenta examination. We aim to determine if the Kaiser neonatal early-onset sepsis risk score (KS), which considers gestational age, maternal temperature, duration of membrane rupture, and intrapartum antibiotics in order to estimate the rate of sepsis, can predict which GBS+ placentas will show a placental inflammatory response.

Methods: 1301 placentas were submitted from 1/1/2019 to 3/11/2019, 140 (10.7%) of which had a history of GBS+. Placenta diagnoses and clinical charts from GBS+ mothers were evaluated for the following parameters: intrapartum antibiotics, clinical indicators of chorioamnionitis (maternal fever >100.4°F, uterine fundal tenderness, maternal tachycardia (>100/min), fetal tachycardia (>160/min) and purulent or foul amniotic fluid), duration of membrane rupture, gestational age, APGAR scores, maternal age, and presence of a fetal and/or maternal inflammatory response. In 105 of 140 cases (75%) all necessary information was available. 99 of 105 (94.2%) mothers received antibiotics. A KS was calculated for each patient.

Results: 66 of 99 mothers with antibiotic prophylaxis had no histologic signs of amniotic fluid infection sequence. Patients with a low risk KS (<0.05) showed a lower percentage of fetal and maternal inflammatory responses than patients with a high risk KS (≥ 0.05).

Placentas from mothers <30 years old were more likely to show a maternal inflammatory response (21/61, 34%) than ≥ 30 years (10/38, 26%) and babies with a 1 minute APGAR score of ≤ 7 were more likely to have an inflammatory response (8/15, 53%) than APGAR > 7 (25/84, 30%).

<table>
<thead>
<tr>
<th>Kaiser Risk Score</th>
<th>Fetal Inflammatory Response</th>
<th>Maternal Inflammatory Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Risk (&lt;0.05)</td>
<td>11/71, 15%</td>
<td>20/71, 28%</td>
</tr>
<tr>
<td>High Risk (≥ 0.05)</td>
<td>7/28, 25%</td>
<td>11/28, 39%</td>
</tr>
</tbody>
</table>

Conclusion: In 67% of appropriately treated GBS+ mothers there was no histologic evidence of infection. The KS showed the expected trend, i.e. high risk KS associated with the presence of inflammation, however the numbers were not statistically significant. Likewise, the data show a possible association of inflammatory response with younger maternal age and low APGAR scores.
Tumor Neoantigens in Synovial Sarcoma for Immunotherapy

M He1, M Kaushal1, C Liu1, B Abro1, L Dehner1, J Pfeifer1; 1Washington University School of Medicine in St. Louis, St. Louis

Background: Immunotherapies that boost the ability of endogenous T cells to destroy cancer cells have demonstrated therapeutic efficacy in a variety of human malignancies. The endogenous T cell compartment is able to recognize peptide epitopes that are displayed on major histocompatibility complexes (MHCs) on the surface of the malignant cells. A class of potential cancer rejection antigens is formed by peptides that are entirely absent from the normal human genome, so-called neoantigens. These neo-epitopes are usually generated by tumor-specific DNA alterations. Prior studies have investigated immunotherapy markers such as PD-L1 IHC in synovial sarcoma (SS). The current study aimed to explore the neoantigens in SS via whole exome sequencing (WES).

Methods: With IRB approval, 7 cases of SS were subjected to normal tissue-primary tumor-metastatic tumor triad WES. Sequencing targeted 25-30M reads for normal tissue and 45-50M reads for neoplastic tissue. Characterization of HLA molecules and neoantigens was performed as follows. Briefly, for each patient, HLA alleles were detected from the WES data using ATHLATES. ATHLATES filters the sequences by comparison against all alleles of HLA genes obtained from IMGT/HLA database and then performs HLA Typing. Identified non-silent mutations from WES were further used to generate a comprehensive list of peptides 8–12 amino acids in length with the mutated amino acid represented in each possible position. The binding affinity of every mutant peptide and its corresponding wild-type peptide to the patient’s germline HLA alleles were predicted using PVACSeq. PVACSeq uses multiple MHC Class I and class II algorithms (e.g., NetMHCpan, NetMHC and SMM) to predict epitope binding. The neoantigens with highest score that reflects the lowest ic50 binding affinity of all prediction algorithms were subsequently selected for analysis.

Results: Among the seven cases, HLA typing failed in one case. Mutation-specific neoantigens were identified in 9 tumor samples from the rest of the 6 patients, ranging in number from 28 to 2,490 (median 54). Potential neoantigens were identified in both primary and metastatic tumors in 3 cases, with from 10 to 16 shared neoantigens. Among these 9 tumor samples, while each case had its own spectrum of neoantigens, recurrent neoantigens were also found with 15 genes, including ABCA12, MUC12, MUC 17, and SLC11A1, some of which have been reported in other tumors in the TSNAdb database.

Conclusion: The current study, the first to investigate potential neoantigens in synovial sarcoma, provides preliminary data and suggests that some neoantigens may be recurrent in synovial sarcoma. The findings suggest that evaluation of neoantigens may have potential use in tumor vaccine development and immunotherapy in this type of tumor.
Comparative Histopathologic Features of FGFR Fusion Pediatric and Adult Central Nervous System Tumors

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Background: The wide availability of next-generation sequencing (NGS) technologies has opened new avenues for diagnosis, prognosis, and treatment of central nervous system (CNS) tumors. At the same time, these developments have raised the question of whether these genetic alterations can be predicted by, or have a correlation with, microscopic tumor histologic features; clearly, some fusions are strongly associated with particular histologies such as BRAF-KIAA1527 with pilocytic astrocytomas and MYB-QK1 with angiocentric gliomas, while others are not. Fusions involving the fibroblast growth factor receptor (FGFR) genes have recently been identified in a subset of both pediatric and adult CNS tumors, with the latter reportedly high-grade IDH-wildtype glioblastomas with monomorphous nuclei, delicate arcuate capillaries, and microcalcifications. Little information is available for pediatric examples.

Methods: We compared histological features of FGFR fusion-positive cases: 11 pediatric/young adult (less than 21 years of age) versus 5 adult CNS gliomas.

Results: All adult examples were confirmed to be glioblastoma, IDH-wildtype. In contrast, 10/11 pediatric cases were low-grade (WHO grade I/II), 6 cases (all pediatric) involved FGFR2 fusions, 1 patient showed FGFR2 fusion and 9 cases (4 pediatric/5 adult) involved FGFR3 gene alterations. Fusion partners of FGFR genes included TACC1, TACC3, KIAA1598, THAP10, and INA gene. Histopathologic analysis according to WHO 2016 criteria for the low grade pediatric cohort revealed a large variety of tumor histological types even with the same fusion event. Histologic features showed at-least focal small round cells, calcifications and arcuate vessels in a vast majority of cases, although diagnoses had ranged from extraventricular neurocytoma (WHO grade II) to pilocytic astrocytoma, pilomyxoid astrocytoma, diffuse astrocytoma, anaplastic astrocytoma, rosette-forming glioneuronal tumor, ganglioglioma and pleomorphic low-grade glioneuronal tumor of the young. The contrast in grading differences between pediatric versus adult examples was striking even with some shared histological features; in addition, the possible diagnostic categories for pediatric examples were far broader than for adults.

Conclusion: While we conclude that all low (pediatric) and high grade IDH-wildtype (adult) tumors with at-least focal small round cells, calcifications and arcuate vessel should be tested for FGFR alterations, this study demonstrates once again (Lake et al Pediatr Blood Cancer. 2019: e28028) that the need to test all pediatric brain tumors for molecular alterations whatever the histology cannot be overemphasized.
Background: Angiomatoid fibrous histiocytoma (AFH) is a rare “fibrohistiocytic” tumor of intermediate malignancy. It has a small risk of metastasis and death. But most AFHs behave indolently. Wide complete excision of tumor is the main treatment with local recurrence in up to 15% of cases. However, treatment options for unresectable tumor and/or metastasis are not well defined. It is also known that some patients may present with a paraneoplastic syndrome including fever, malaise, and anaemia. Additionally, most AFHs have tumor-associated lymphocytic infiltrates and/or a peripheral lymphocytic cuff; these features suggest that this tumor interacts with the immune system, and immune checkpoint pathways including programmed death signaling (PD-L1/PD-L1) may play a role in the biology of this entity. PD-L1 expression in AFH has not yet been reported.

Methods: Expression of PD-L1 (clone 22C3) was assessed using immunohistochemical staining in 35 tumors from 35 patients. The percentage of tumor and intratumoral immune cells demonstrating membranous (cell surface) PD-L1 staining were scored separately. Positivity was defined as membranous expression in ≥ 1% of cells. PD-L1 status was correlated with clinicopathologic features.

Results: There were 20 females and 15 males with an age range of 2–15.5 years (median: 8 years). Four patients had a paraneoplastic syndrome and two patients had metastasis. The most common sites of primary tumor were the upper extremities (12, 34%), followed by head and neck (9, 26%), lower extremities (7, 20%), trunk (6, 17%) and adrenal gland (1, 3%). The tumor size ranged from 0.4 cm to 10.5 cm in greatest dimension. All patients underwent surgical resection and were alive with a median follow-up of 2.5 years (range: 1 month to 13 years). Two were alive with disease at the latest follow-up. Twenty tumors (57.1%) had PD-L1(+) tumor cells and 18 (51.4%) showed PD-L1(+) immune cells. Concurrent tumor and immune PD-L1 expression was observed in 17 (48.6%) tumors. There was a positive correlation between the proportion of tumor and immune cells expressing PD-L1 (r = 0.43, p = 0.009), supporting a component of adaptive PD-L1 expression. Increased tumor PD-L1 expression was significantly associated with tumor size (r = 0.362, p = 0.032). The remaining clinicopathologic features assessed did not significantly correlate with tumor cell or immune cell PD-L1 expression.

Conclusion: PD-L1 is expressed in approximately 50% of pediatric AFH, suggesting that immunotherapy with PD-L1 may be a potential option for patients with unresectable tumor and/or metastasis. Further studies in larger cohorts are needed for confirmation and validation of clinical use of immunotherapy with PD-L1 in pediatric AFH.
Melanotic Neuroectodermal Tumor Of Infancy (MNTI) Harbors A Medulloblastoma Signature By DNA Methylation Profiling

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Background: MNTI is a tumor of apparent neural crest origin morphologically overlapping neuroblastoma and a subset of medulloblastoma (MBT). Despite its favorable prognosis, it can be locally aggressive and rarely metastasize. The molecular features of MNTI are largely unknown, although epigenetic deregulation was recently suggested. We aim to explore DNA methylation and copy number profiling of MNTI and a morphologically similar pineal anlage tumor (PAT) to better delineate their histogenesis.

Methods: We reviewed 8 MNTIs and 1 PAT. Immunohistochemistry for PHOX2B (H-20, Santa Cruz Biotechnology) and DNA methylation with copy number profiling was performed in all cases. Results were compared to the methylation brain tumor classifier v11b4 (BT-C), sarcoma classifier v12.2 (S-C) and the MBT classifier group 3/4 v1.0 (MBT3/4-C), developed by Heidelberg University and DKFZ (https://www.molecularneuropathology.org/mnp/classifier/all). The methylation data was also analyzed by multidimensional scaling (MDS) analysis on the 1000 most variable islands and unsupervised hierarchical clustering, together with those derived from a cohort of 36 medulloblastoma patients from OPBG.

Results: The mean age was 12 mo (4-48 mo) with a M:F ratio of 3. All MNTIs contained nodules of small round cells alternating with larger epithelioid cells and variable melanotic pigment. Anatomic locations included maxilla (4), head/neck (2) and testicle (1). A PAT (primary and relapse) displayed similar morphology to MNTIs. Immunohistochemistry for PHOX2B was negative in all cases. The BT-C classified 66% of tumors (5 MNTI, 1 PAT relapse) in the class family MBT G3 and G4; subclass group 3 with an optimal score >0.9. The remaining 33% (2 MNTI, primary PAT) were classified in the class family plexus tumor, subclass pediatric, with suboptimal scores (>0.45). No significative methylation scores were reached in the S-C. The MBT3/4-C, classified all MNTIs as high-risk MBT G3 tumors, Subtype II (n=8, 100%) with a score >0.45. The primary PAT was classified as subtype III (score 0.99) and its relapse as subtype II/III.

MDS and hierarchical clustering analysis on the reduced 48 CpG islands signature clustered MNTI and PAT close to those of Group 3 MBT from our cohort of 36 MBT patients. Copy number variation (CNV) profiles showed multiple alterations in 33% of tumors (1 PAT and 2 MNTI). Outcome data was available in 4 MNTI, all of them in clinical remission and with a median follow-up of 60 mo.

Conclusion: MNTI share a homogenous methylation profile with group 3 high-risk MBT, a profile also seen in a PAT, suggesting that MNTI and PAT likely have a common histogenesis. Most MNTIs lacked CNV alterations while their presence in PAT may be related to a greater tendency for relapse, however further studies are needed.
Prevalence of Hippocampal Abnormalities and a History of Seizures in Sudden Unexpected Death in Childhood and its Comparison with Sudden Unexpected Death in Epilepsy and SIDS: A 16-years Single Centre Review

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Background: Sudden Unexpected Death in Childhood (SUDC) is the medically unexplained death of children aged between 1 – 18 years old. The pathophysiology behind SUDC is unknown but hippocampal abnormalities are currently thought to play a role. These abnormalities are strongly associated with epilepsy and have been previously described in Sudden Unexpected Death in Epilepsy (SUDEP). These are defined as the sudden unwitnessed death of patients known to have epilepsy after which no cause of death is ascertained during the post-mortem. With the aim to better characterize SUDC and SUDEP, we assessed our cases in order to: 1) identify similarities and differences in epidemiological features characterizing SUDC and SUDEP vs SIDS and 2) explore an association between a history of seizures (febrile or afebrile), epilepsy and hippocampal abnormalities.

Methods: A review of post-mortem (PM) reports from 2003-2018 was used to identify cases of SUDC and SUDEP. A control group of Sudden Infant Death Syndrome (SIDS) cases was also identified. Evidence of hippocampal abnormalities, patient demographics (age, gender), season of death, sleeping position, co-sleeping, parental smoking and past medical history (history of seizures, illness 72 hours prior to death and prematurity) were recorded. Statistical analysis was performed to compare the three groups.

Results: Out of a total of 1129 PMs performed in infants and children dying suddenly an unexpectedly there were: 48 SUDC cases (prevalence: 4.3%; median age: 3 y); 18 SUDEP cases (prevalence: 1.6%; median age: 8 y) and 358 SIDS cases (prevalence: 31.7%; median age: 2.5 m). Hippocampal abnormalities were found 32% of SUDC; 14/18 SUDC cases with clinical information about seizures have had them (febrile or afebrile). Hippocampal abnormalities associated with temporal lobe epilepsy were found in 4/14 SUDC cases with a history of seizures. More than half of SUDEP had hippocampal abnormalities (69%) and all had history of epilepsy or epileptic disorders (100%). SUDC and SUDEP cases were more likely to be found prone and to demonstrate hippocampal abnormalities than SIDS cases (OR = 5.553, CI: 1.606–19.204 and OR = 26.550, CI: 5.983 – 117.824 respectively). Prevalent hippocampal abnormalities were granule cell dispersion with bi-lamination in SUDC and hippocampal gliosis in SUDEP cases.

Conclusion: Our study compares SUDC, SUDEP and SIDS cases to one another and reveals similarities and differences between all 3 groups. We have identified a potential link between hippocampal abnormalities and epileptic seizures in SUDC. There should be a concerted effort towards consistent sampling and standardised description of the hippocampus and clinical correlation with history of seizures/epilepsy in PM reports.
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PD-L1/PD-1 Expression in Wilms Tumor: Analysis of 52 cases

J Chen1, A Mattis1, I Gonzalez1, R Rais1, L Dehner1, J Pfeifer1, M He1; 1Washington University School of Medicine, St Louis

Background: Wilms tumor (WT) is the most common renal tumor in children. Patients with high-risk histology or recurrent/metastatic disease have poor prognosis. At least 25% of survivors sustain long-term morbidities secondary to treatment. Immune checkpoint inhibitors (ICIs) have reformed oncology practice. Expression of PD-L1 and other immune-related markers correlate with response to ICIs in many solid tumors. It remains unclear whether WTs benefit from ICIs, and whether the expression of immune checkpoint molecules such as PD-L1 correlates with histology and clinical outcome. Our previous pilot study on 15 cases showed very limited PD-L1 expression within the tumor. There were significant CD8+ tumor-infiltrating lymphocytes, few of which express PD-1. Here we expanded the cohort to include 52 WTs and examine the expression of PD-L1, PD-1, and CD8 with a systemic approach, and correlate with histologic findings and clinical outcome.

Methods: Cases of WT were identified from departmental archives from 2000 to 2017. IHC stains were performed on whole-tissue slides with Ventana PD-L1 (SP263), PD-1 (NAT105), and CD8 (SP57) antibodies. Three pathology residents were trained by a senior pathologist and scored the slides independently. Positive PD-L1 expression was defined as membranous staining in ≥1% of cells per high-power field (HPF) by Combined Positive Score (CPS). Tumor cells and intratumoral immune cells positive for PD-1 and CD8 were assessed within the tumor and at tumor-normal interface (TNI). Expression of the markers was correlated with histology and clinical outcome.

Results: A total of 52 WTs from 41 patients were analyzed (including 6 with both primary and recurrent/metastatic tumors, 1 with pre- and post-treatment primary tumors, 2 with multiple primary tumors, and 1 with multiple metastatic lesions). Eight WTs showed anaplasia. Six WTs (6/52, 11.5%) were positive for PD-L1 (4 primary, 2 metastasis; CPS: 1-3), none of which showed anaplasia. The CPS score for WTs with anaplasia is lower than that of WTs with favorable histology (p<0.05). CD8+ lymphocytes are present in all cases, with higher counts within the tumor compared to TNI (3.0-226.3 vs 2.0-126.0 per HPF, p<0.01). The numbers of PD-1+ cells or PD-1/CD8+ lymphocytes are similar within the tumor compared to TNI (0-135.3 vs 0-69.0 and 0-60.0 vs 0-40.7 per HPF, respectively). There is a trend toward more PD-1+ cells and CD8+ lymphocytes within the tumor or at the TNI in WTs with a higher PD-L1 CPS score.

Conclusion: Our study on an expanded cohort identified a substantial fraction of WT cases positive for PD-L1 defined by CPS score, and verified our prior observation that WTs are associated with significant CD8+ lymphocytes. WTs with higher PD-L1 CPS score are likely to show higher numbers of PD-1+ cells and CD8+ lymphocytes.
Distinct Mucinous Cell Clusters in Type I Congenital Pulmonary Airway Malformations Carry Clonal KRAS Mutations

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Background: Up to 75% of type 1 (large cyst) infantile congenital pulmonary airway malformations (CPAMs) contain mucinous cell clusters (MCCs). These mucinous cells have multiple atypical histologic features, and there are rare case reports of metastatic mucinous adenocarcinoma arising in children and young adults with incompletely resected CPAMs. KRAS codon 12 mutations have been described in metastatic mucinous adenocarcinoma arising in type I CPAMs, and these mutations have also been reported within seemingly benign MCCs in type I CPAMs. Activating mutations at codon 12 of the oncogene KRAS are seen in 18% of all adult lung adenocarcinomas and 30% of lung mucinous adenocarcinomas, with p.G12D mutation being most common (39%), followed by p.G12V (29%) and p.G12C (23%). We aimed to characterize the heterogeneity of KRAS mutations in distinct MCCs located within the same infantile CPAM as a measure of their possible clonal relationship.

Methods: Ten 16 micron thick FFPE tissue sections were cut for each block, and mucinous cells were enriched via macrodissection of individual clusters. DNA was isolated from the MCCs and amplified using primers flanking exon 2 of KRAS. Primers were designed to detect mosaic mutations with allele frequency of ≥5%. PCR products were Sanger sequenced and sequences were analyzed by comparing to the reference sequence using Mutation Surveyor (SoftGenetics, PA). Statistical analysis of mutation distribution was carried out using binomial probabilities (vassarstats.net).

Results: We sequenced a total of 43 MCCs from 13 infants, with a median of 3 MCCs per patient (range 1-8 MCCs per patient). KRAS codon 12 mutations were detected in all 43 MCCs. Similar to adult lung cancers, the most common mutation was KRAS c.35G>A p.12G>D which was found in 67% of MCCs. The remaining 33% of MCCs had KRAS c.35G>T p.12G>V mutations. Interestingly, within any single patient the same KRAS mutation was detected in all MCCs. This includes one case in which eight distinct MCCs all had KRAS p.12G>D mutations, and two cases in which all five MCCs had KRAS c.35G>T p.12G>V mutations. In four cases, this distribution significantly varies from that expected by chance (p<0.05, binomial probability). Sample sizes for four additional patients are not yet large enough to determine statistical significance, and the remaining five patients had only one MCC tested each.

Conclusion: KRAS mutations were identified in all 43 MCCs tested. For the eight patients that had more than one MCC tested, all tested MCCs in the lesion had the same KRAS mutation. These findings suggest a clonal process underlying the development of these MCCs. The absence of malignant behavior in infancy argues against aerogenous metastasis of these MCCs, and instead suggests a potential field effect during development.
Centrilobular Injury Pattern is Not Indicative of Antibody Mediated Rejection in Pediatric Liver Transplant

I Gonzalez1, H Lu1, L Dehner1, M He1; 1Washington University School of Medicine, St. Louis, Missouri

Background: Acute cellular rejection (ACR) is a common complication in the liver allograft associated with significant morbidity. In recent years, centrilobular injury (CLI) has been recognized as a manifestation of rejection seen in isolation or with classic portal tract injury. CLI is thought to impart a poor prognosis and associated with a later presentation, and it has been postulated to suggest antibody mediated rejection (AMR). In this study, we sought to assess the effect of CLI in the pediatric population.

Methods: A retrospective search was done for liver allograft biopsies from 2000 to 2018. We only included patients with transplantation during childhood and allograft liver biopsies showing rejection. ACR was defined according to the Banff schema with at least two of the following features: mixed portal tract infiltrate, bile duct damage, and venular endothelial inflammation. The cases were divided into group 1 (showing both portal tract injury and CLI) and group 2 (showing portal tract injury without CLI). Complement component 4d (C4d) immunohistochemistry was performed and was considered positive in the presence of linear to granular endothelial cell staining when identified in >50% of the circumference of microvascular endothelia. Graft loss was defined as liver failure necessitating a new transplant.

Results: A total of 102 allograft liver biopsies were included, corresponding to 63 patients. 55.4% and 58.7% of the patients were male with a mean age of 7.8 ± 7.3 and 9.7 ± 8.3 years for group 1 and 2, respectively (Table 1). Although not significant, the mean time interval from transplant to biopsy was longer in the group 2 compared to group 1 (4.7 ± 6.1 and 2.8 ± 4.7 years, respectively). Both group 1 and 2 presented with abnormal liver chemistries; cases from group 1 had higher levels of bilirubin (p=0.0363) with trend for higher levels of GGT (p=0.0844) and alkaline phosphatase (p=0.0554). There was no significant difference in aspartate and alanine aminotransferase levels. Group 1 and group 2 were not significantly different in positive C4d results, chronic rejection, or graft survival (Figure 1). The mean follow-up was 9.2 ± 4.8 and 10.6 ± 6.2 years in group 1 and 2, respectively. There were no significant differences in clinical characteristics and survival between C4d positive and negative cases (Table 2, Figure 1).
<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n: 56)</th>
<th>Group 2 (n: 46)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td><strong>Gender, n, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25, 44.6%</td>
<td>19, 41.3%</td>
<td>0.7348</td>
</tr>
<tr>
<td>Female</td>
<td>31, 55.4%</td>
<td>27, 58.7%</td>
<td></td>
</tr>
<tr>
<td><strong>Age, mean ± SD (years)</strong></td>
<td>7.8 ± 7.3</td>
<td>9.7 ± 8.3</td>
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<tr>
<td><strong>Time interval from OLT, mean ± SD (years)</strong></td>
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<td>4.7 ± 6.1</td>
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<td><strong>AST, mean ± SD (Units/L)</strong></td>
<td>252.0 ± 274.9</td>
<td>214.6 ± 564.1</td>
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<tr>
<td><strong>ALT, mean ± SD (Units/L)</strong></td>
<td>290.1 ± 268.8</td>
<td>258.6 ± 383.6</td>
<td>0.6323</td>
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<tr>
<td><strong>Alkaline phosphatase, mean ± SD (Units/L)</strong></td>
<td>457.7 ± 349.3 *</td>
<td>335.8 ± 261.5</td>
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<td><strong>Bilirubin, mean ± SD (mg/dL)</strong></td>
<td>3.1 ± 5.2</td>
<td>1.3 ± 1.8</td>
<td>0.0363</td>
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<tr>
<td><strong>GGT, mean ± SD (Units/L)</strong></td>
<td>466.2 ± 597.7 *</td>
<td>288.6 ± 364.8</td>
<td>0.0844</td>
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<tr>
<td><strong>Portal tract inflammation, n, %</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>50, 89.3%</td>
<td>46, 100%</td>
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<tr>
<td>No</td>
<td>6, 10.7%</td>
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<td><strong>Lobular inflammation, n, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>46, 82.1%</td>
<td>29, 63.0%</td>
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<tr>
<td>No</td>
<td>10, 17.9%</td>
<td>17, 37.0%</td>
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<td><strong>Centrilobular inflammation, n, %</strong></td>
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<td>&lt;0.0001</td>
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<td>56, 100%</td>
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<tr>
<td>No</td>
<td>0</td>
<td>46, 100%</td>
<td></td>
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<tr>
<td><strong>C4d immunostain, n, %</strong></td>
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<td></td>
</tr>
<tr>
<td>Positive</td>
<td>8, 14.3%</td>
<td>4, 8.9%*</td>
<td>0.4048</td>
</tr>
<tr>
<td>Negative</td>
<td>48, 85.7%</td>
<td>41, 91.1%*</td>
<td></td>
</tr>
<tr>
<td><strong>Chronic rejection, n, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>20, 35.7%</td>
<td>11, 23.9%</td>
<td>0.1973</td>
</tr>
<tr>
<td>No</td>
<td>36, 64.3%</td>
<td>35, 76.1%</td>
<td></td>
</tr>
<tr>
<td><strong>Follow-up, mean ± SD (years)</strong></td>
<td>10.4 ± 5.8</td>
<td>10.7 ± 6.6</td>
<td>0.8008</td>
</tr>
<tr>
<td><strong>Graft, n, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lost</td>
<td>10, 17.9%</td>
<td>8, 17.4%</td>
<td>0.9510</td>
</tr>
<tr>
<td>Survive</td>
<td>46, 82.1%</td>
<td>38, 82.6%</td>
<td></td>
</tr>
<tr>
<td><strong>Time of graft survival, mean, SD (years)</strong></td>
<td>9.8 ± 5.6</td>
<td>10.5 ± 6.5</td>
<td>0.5376</td>
</tr>
</tbody>
</table>

1Cellular rejection with portal, lobular and centrilobular injury (Zones 1-3); 2Cellular rejection with no centrilobular injury (Zones 1 and 2); 354 patients available; 451 patients available; 545 patients available.

Abbreviations: SD - standard deviation; OLT – orthotopic liver transplant; AST – aspartate aminotransferase; ALT – alanine aminotransferase; GGT- gamma glutamyltransferase.
<table>
<thead>
<tr>
<th></th>
<th>C4d positive (n: 12)</th>
<th>C4d negative (n: 89)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7, 58.3%</td>
<td>37, 41.6%</td>
<td>0.2717</td>
</tr>
<tr>
<td>Female</td>
<td>5, 41.6%</td>
<td>52, 58.4%</td>
<td></td>
</tr>
<tr>
<td>Age, mean ±SD (years)</td>
<td>9.7 ± 9.0</td>
<td>5.0 ± 5.9</td>
<td>0.6664</td>
</tr>
<tr>
<td>Time interval from OLT, mean ± SD (years)</td>
<td>4.5 ± 4.7</td>
<td>3.6 ± 5.6</td>
<td>0.6101</td>
</tr>
<tr>
<td>AST, mean ± SD (Units/L)</td>
<td>298.3 ± 364.7</td>
<td>226.5 ± 442.5</td>
<td>0.5930</td>
</tr>
<tr>
<td>ALT, mean ± SD (Units/L)</td>
<td>324.3 ± 309.0</td>
<td>270.2 ± 330.3</td>
<td>0.5930</td>
</tr>
<tr>
<td>Alkaline phosphatase, mean ± SD (Units/L)</td>
<td>398.4 ± 149.3</td>
<td>403.9 ± 334.6</td>
<td>0.9560</td>
</tr>
<tr>
<td>Bilirubin, mean ± SD (mg/dL)</td>
<td>1.2 ± 1.2</td>
<td>2.5 ± 4.3</td>
<td>0.3103</td>
</tr>
<tr>
<td>GGT, mean ± SD (Units/L)</td>
<td>394.3 ± 606.4</td>
<td>374.3 ± 497.2</td>
<td>0.9056</td>
</tr>
<tr>
<td>Portal tract inflammation, n, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11, 91.7%</td>
<td>84, 94.4%</td>
<td>0.7087</td>
</tr>
<tr>
<td>No</td>
<td>1, 8.3%</td>
<td>5, 5.6%</td>
<td></td>
</tr>
<tr>
<td>Lobular inflammation, n, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10, 83.3%</td>
<td>65, 73%</td>
<td>0.4437</td>
</tr>
<tr>
<td>No</td>
<td>2, 16.7%</td>
<td>24, 27%</td>
<td></td>
</tr>
<tr>
<td>Centrilobular inflammation, n, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8, 66.7%</td>
<td>48, 53.9%</td>
<td>0.4048</td>
</tr>
<tr>
<td>No</td>
<td>4, 33.3%</td>
<td>41, 46.1%</td>
<td></td>
</tr>
<tr>
<td>Chronic rejection, n, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3, 25.0%</td>
<td>27, 30.3%</td>
<td>0.7041</td>
</tr>
<tr>
<td>No</td>
<td>9, 75.0%</td>
<td>62, 69.7%</td>
<td></td>
</tr>
<tr>
<td>Follow-up, mean ± SD (years)</td>
<td>11 ± 3.7</td>
<td>10.4 ± 6.4</td>
<td>0.7517</td>
</tr>
<tr>
<td>Graft, n, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lost</td>
<td>0</td>
<td>18, 20.2%</td>
<td>0.0857</td>
</tr>
<tr>
<td>Survive</td>
<td>12, 100%</td>
<td>71, 79.8%</td>
<td></td>
</tr>
<tr>
<td>Time of graft survival, mean ± SD (years)</td>
<td>11.0 ± 3.7</td>
<td>9.9 ± 6.3</td>
<td>0.5437</td>
</tr>
</tbody>
</table>

**Abbreviations:** SD - standard deviation; OLT - orthotopic liver transplant; AST - aspartate aminotransferase; ALT - alanine aminotransferase; GGT - gamma glutamyltransferase.
Conclusion: In our single center experience in pediatric allograft liver biopsies, there was no significant difference in terms of time of presentation or graft survival in those cases with or without CLI. Furthermore, the lack of significant association with C4d argues against associating CLI pattern of rejection with AMR.
Utility of Phox2B Immunohistochemical Staining in Hirschsprung Disease

M Alturkustani, S Zhou, L Wang, N Shillingford, M Warren; 1Children's Hospital Los Angeles, Los Angeles, California

Background: Suction rectal biopsy is the gold standard diagnostic test for Hirschsprung disease (HD). The diagnostic criteria for HD used in our institution include: 1. absence of ganglion cells, 2. presence of hypertrophic extrinsic nerves and 3. lack of calretinin-positive innervation in the lamina propria by immunohistochemical staining (IHC). Therefore, recognizing ganglion cells is critical. While pediatric pathologists are well-trained in recognizing the morphology of ganglion cells on H&E slides, it can be challenging for pathologists without much experience with HD to confirm the presence or absence of ganglion cells solely using H&E staining. It is especially difficult to identify sparse, single, small-sized immature ganglion cells from premature neonates.

Ganglion cells are derived from neural crest progenitors of the autonomic nervous system (ANS) lineage. A transcription factor protein, Phox2B regulates this development and is a highly sensitive and specific marker for cells of ANS-lineage neural crest origin, such as autonomic ganglia, normal adrenal medulla, paraganglioma/pheochromocytoma and peripheral neuroplastic tumors. Therefore, Phox2B IHC is a strong candidate for detecting ganglion cells in the intestine.

Methods: Phox2B IHC was performed on a total of 59 sections including: 47 sections of aganglionic segments, ganglionic segments and the transition zones from 20 initial pull-through specimens (20 HD patients, age: 6d-3y), as well as on 12 cohort rectal suction biopsies from 12 patients (2 HD and 10 non-HD patients, age: 1d-18y) including one from an ex-preterm, non-HD neonate (gestational age: 34w; age: 1d). The Phox2b IHC and the corresponding H&E and calretinin IHC were reviewed by at least 2 pathologists.

Results: Phox2B IHC highlighted ganglion cells with strong, solely nuclear positivity without any background staining in 38/38 of the pull-through and biopsy sections that were positive for ganglion cells confirmed by the routine procedure. In addition, Phox2B IHC detected immature ganglion cells that were almost impossible to identify in the H&E sections. Phox2B was completely negative in 2/2 biopsies with HD and 27/27 aganglionic sections from the pull-through specimens.

Conclusion: Phox2B IHC is a highly sensitive (100%) and specific (100%) marker for detecting ganglion cells. The strong nuclear staining with no background staining is easily recognized even at low magnification. The stain highlights sparse, single ganglion cells in different developmental phases, including immature ones that are difficult to identify by H&E staining. Therefore, Phox2B IHC adds diagnostic sensitivity, especially for premature neonates, and increases the accuracy in determining the exact transition between the aganglionic segment and the transition zone in a pull-through specimen.
Robarts and Nancy Histopathologic Indices Highlight the Significance of Pancolonic Biopsies in Pediatric Ulcerative Colitis

Background: Endoscopic biopsies are the mainstay to assess and predict disease activity, both in treatment naïve and treated ulcerative colitis (UC). In adult clinical practice, it is common to biopsy worst affected region, which in most cases is the rectosigmoid. Therefore, a limited biopsy protocol is routinely performed. There is variation in endoscopic practice among the pediatric gastroenterologists. In pediatric UC, the disease is often extensive. To date, histologic indices applied to pancolonic biopsies in pediatric UC have not been formally assessed. We aim to evaluate the significance and pattern of histologic indices in pancolonic biopsies of pediatric UC.

Methods: Sixty pediatric UC patients (<18 years old), which included 35% diagnostic biopsies and remainder on treatment, were recruited consecutively during diagnostic or follow-up procedures. UC Endoscopic Index of Severity (UCEIS; score 0-8) was scored by 4 blinded pediatric gastroenterologists. Histologic slides of all colonic segments were re-reviewed by 2 pediatric gastrointestinal pathologists concurrently using the Robarts Histopathology index (RHI; score 0-33) and Nancy Index (NI; grade 0-4). Endoscopic and histologic activities were analyzed by individual segments, rectosigmoid and pan-colonic variations (the average score of all five colonic segments). Spearman correlation test was performed to evaluate endoscopic and histologic indices.

Results: Sixty patients included in the analysis were 53% males, with a median age of 13.4 (9.8-16.1) years at the time of procedure. UCEIS medians (interquartile range (IQR)) for rectosigmoid and pancolonic variations were 4.0 (IQR 2.0-5.0) and 2.6 (IQR 0.7-4.2), respectively. Both histologic indices demonstrated a very strong correlation with each other (R>0.90). The pancolonic RHI (median 6.3, IQR ) and NI (median 1.7, IQR) correlated better with endoscopic activity compared to rectosigmoid RHI (median 7.0, IQR) and NI (median 2.0, IQR). There was progressive increase in pancolonic RHI and pancolonic NI scores with increasing endoscopic severity. The correlation between each histologic index with endoscopic activity is summarized in Table 1.

Table 1. Spearman correlations between RHI and NI and endoscopic activity

<table>
<thead>
<tr>
<th>By individual segment</th>
<th>Pancolonic</th>
<th>Rectosigmoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCEIS</td>
<td>UCEIS</td>
<td>UCEIS</td>
</tr>
<tr>
<td>RHI</td>
<td>0.72</td>
<td>0.84</td>
</tr>
<tr>
<td>NI</td>
<td>0.69</td>
<td>0.82</td>
</tr>
</tbody>
</table>

All p<0.001

Conclusion: Pancolonic variations of both indices show a better correlation with endoscopic findings, reinforcing the need of pancolonic assessment in children.
Two Unexpected Cases of Pediatric “Kaposiform” Vascular Anomalies Involving the Pancreas

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Background: Kaposiform hemangioendothelioma (KHE) and kaposiform lymphangiomatosis (KLA) are rare vascular anomalies which clinically and histopathologically overlap. Both consist of bland spindled endothelial cells (kaposiform) and malformed/abnormal vascular channels positive for PROX-1. KHE and KLA may be locally aggressively infiltrating, and associated with coagulopathy, with mortality of 10-50%. They are extremely rare in pancreas with 6 cases of KHE reported in literature and no reports of KLA. We present two clinically unsuspected cases of kaposiform vascular anomalies involving the pancreas.

Methods:

Case 1
A 16-year-old female with Hemoglobin SC disease was incidentally found to have bilateral renal cysts on an abdominal ultrasound. Further imaging also showed a locally infiltrating lymphatic malformation involving the pancreatic body and tail. Her hemoglobin was 10.8 gm/dl with a fibrinogen of 197 mg/dl. She underwent a subtotal pancreatectomy, splenectomy, and a left partial nephrectomy.

Case 2
An 11-year-old male had a 2 year history of presumed primary sclerosing cholangitis (PSC) complicated by common bile duct stricture, portal hypertension and superior mesenteric vein thrombosis, splenomegaly, chronic thrombocytopenia, and debilitating chronic pancreatitis. Imaging showed diffuse edema and fat stranding surrounding the pancreas with some atrophy. He underwent total pancreatectomy with islet autotransplantation due to his chronic pain and debilitation.

Results:

Case 1
Histology showed unremarkable pancreatic parenchyma with dilated malformed lymphatic channels. There were PROX-1 immunoreactive foci of intra and perivascular spindled endothelial cells, occasionally associated with hemosiderin, platelet microthrombi, and extravasated red blood cells. This case is unusual in that hematological/clinical findings were not typical for KLA. The renal lesion was renal cell carcinoma, NOS, which on Next Generation Sequencing (NGS) had a TSC2 mutation.

Case 2
Histology of the pancreatic head and tail demonstrated mild to moderate atrophy, mild fibrosis, and no inflammation. There were pancreatic and peripancreatic lobules of back-to-back spindled endothelial capillary sized vessels which were immunoreactive to PROX-1. There were extensive platelet microthrombi, extravasated red blood cells, and hemosiderin laden endothelial cells, consistent with KHE.

Conclusion: TSC2 mutation in the renal lesion raises a question of whether a germline mutation is present (status pending), as the latter is associated with vascular malformations, but thus far not KLA. Case 2 clinically mimicked PSC and did not present as a mass lesion. This report highlights these two rare vascular lesions occurring in a rare location and creating clinical challenge where histological recognition is critical.
Gender-Discordant Monochorionic-Diamniotic Twins both with 45, X/46, X, idic (Y) Mosaicism and a Novel Gene Mutation

**M Diamond** (co-first author), **A Inamdar** (co-first author), **W Shertz**; **1**Saint Barnabas Medical Center, Livingston, New Jersey; **2**Monmouth Medical Center, Long Branch, New Jersey

**Background:** Gender discordance among monochorionic diamniotic “identical” twins is a very rare occurrence in 45,X/46,XY patients. This karyotype is associated with poor growth and developmental delay, Turner’s syndrome, infertility in males, and germ cell tumors more often in females. We present a case of spontaneously conceived sex-discordant monochorionic diamniotic twins, both demonstrating 45,X/46,X,idic(Y)(q11.223) and a novel chromosome 3 deletion.

**Methods:** The twins were delivered preterm due to 28% growth discordance on ultrasound. Routine gross and microscopic examination of the placenta revealed a monochorionic diamniotic placenta in twins discordant for gender. Therefore, twin genotypes were evaluated via FISH and microarray studies.

**Results:** Microscopic placental examination confirmed monochorionic diamniotic twin gestation. Ultrasound demonstrated horseshoe kidney in the female twin. FISH and microarray studies revealed 45,X[2]/46,X,idic(Y)(q11.223pter)[18] karyotype in the female twin and 45,X[4]/46,X,idic(Y)(q11.223)[16] in the male twin. Microarray studies confirmed mosaicism including a single X in all cells plus a variable amount of Yq11.223pter. The results are consistent with an isodicentric Y chromosome lacking the azosperma (DAZ) complex of genes. Identical twin genotypes were confirmed via SNP markers. A novel 99 kb deletion of 3p24.3 was observed, involving exons 15-16 of transcript NM_001134381.1 of the TBC1D5 gene.

**Conclusion:** Only two other cases of an isodicentric Y chromosome have been reported in twins with 45,X/46,XY mosaicism. Gonadectomy is recommended for 45,X/46,XY females since neoplasia can develop within the first decade of life. Horseshoe kidney will require nephrology follow-up. 45,X/46,XY males with nearly normal external genitalia may be assessed periodically by ultrasound or physical examination. Loss of the azosperma complex of genes (DAZ) can result in male infertility, thus necessitating follow-up in the male twin. TBC1D5 is involved in regulating retromer, which, when defective, has been associated with Alzheimer’s and Parkinson’s disease, bacterial and viral infection and intracellular growth of *L. pneumophila*. Follow-up of the twins will be required in investigating a potential association between this novel TBC1D6 gene deletion and development of such disease processes.

In conclusion, we document a rare case of 45,X/46,X,idic(Y) gender-discordant monochorionic diamniotic twins. We report a novel 3p24.3 deletion of unknown significance. Our findings allow further insight into the exceedingly rare phenomenon of gender-discordant monochorionic-diamniotic twins.
PIK3R1 Gene: A Possible Culprit for Trans-Lineage Differentiation from Myeloid to Lymphoid Series

M Aldulescu¹, K Yap¹, S Gong¹; ¹Ann and Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois

Background: "Lineage switching" is a term used to describe the phenomenon of acute leukemias that meet standard criteria for a specific lineage (either lymphoid or myeloid) at the time of initial diagnosis, but later switch to another lineage upon relapse. Most reports of lineage switching have occurred in children and demonstrated acute lymphoblastic leukemia (ALL) to acute myeloid leukemia (AML). Myeloid to lymphoid switch has also been reported, albeit much more rarely. However, there have been no proposed molecular causes of lineage switching from myeloid to lymphoid lineage. Herein, we propose a possible molecular cause of myeloid to lymphoid-lineage switch occurring in a 2-year-old female.

Methods: The patient was a 2-year-old female presenting with a past medical history significant for t(9;11)(p22;q23), KMT2A (MLL) rearrangement-positive acute myeloid leukemia (AML-M5) originally diagnosed at 8 months of age. At that time, she underwent chemotherapy and achieved complete remission. 18 months after remission, she presented with a 2.5 cm right fronto-parietal scalp mass with concurrent relapse of AML in her bone marrow.

Results: Histologic sections of the scalp mass revealed a dense dermal infiltrate of medium-sized atypical cells positive for CD19, CD79a, weakly positive for CD20, and negative for CD10, kappa, lambda, myeloperoxidase, and lysozyme, consistent with B-lymphoblastic lymphoma (B-LBL). Sections of her relapsed bone marrow showed 3% myeloblasts with no evidence of involvement by B-ALL by morphology and flow cytometry. Genetic studies of the scalp mass and bone marrow both revealed KMT2A (MLL) rearrangement with a molecularly confirmed MLL-AF9 gene fusion, also found in B-LBL, consistent with the genetic abnormality noted 18 months prior at initial diagnosis of AML. Additionally, Sanger sequencing detected PIK3R1 mutation in the lymphoma but not in the original bone marrow specimen.

Conclusion: The development of cutaneous B-LBL from a pre-existing AML has not been described, nor has a possible molecular explanation for trans-lineage differentiation from myeloid to lymphoid lineages. Although we considered a therapy-related effect, the identical KMT2A (MLL) rearrangement found in the scalp mass and bone marrow at diagnosis strongly suggests trans-lineage differentiation. Furthermore, the presence of PIK3R1 mutation identified in the lymphoma specimen, but not in the original bone marrows, suggests that this gene may be responsible for the trans-lineage differentiation from myeloid to lymphoid series. Notably, PIK3R1 mutation has been reported in Burkitt lymphoma but not in AML.
Infantile Fibrosarcoma Presenting With Kasabach-Merritt Phenomenon, Involving The Thigh And Placenta: A Proposal Of Metastasis By Mesenchymal Migration

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Background: Kasabach-Merritt phenomenon (KMP) classically occurs with kaposiform hemangioendothelioma (KHE) or tufted angioma (TA). To distinguish KMP from less aggressive consumptive coagulopathies seen in other vascular anomalies, a strict definition of KMP requires severe thrombocytopenia, hypofibrinogenemia and anemia with a rapidly enlarging, biopsy proven KHE or TA. Very rarely, KMP has been reported in patients with infantile fibrosarcoma (IF), a diagnosis that may be delayed if the patient is too coagulopathic to biopsy. We report a case of a neonate, with KMP secondary to IF, that presented with a soft tissue mass in her leg and metastatic disease in the placenta.

Methods: Patient information was gathered and herein reported in accordance with institutional guidelines. Routine and immunohistochemical stains were evaluated in this patient’s leg mass and placenta. Next-generation sequencing was done on the leg mass, and FISH with a probe to the ETV6 locus was done on the placenta.

Results: This neonate presented with a large thigh mass extending into the inguinal region. KMP made KHE a primary consideration. Imaging studies of the thigh demonstrated an enhancing, soft tissue mass in the left groin with marked arteriovenous shunting and peripheral cysts, suggestive of either necrosis or a lymphatic component. By imaging the differential diagnosis included a vascular mass, like KHE, and sarcoma. Progression to respiratory failure led to a clinical consideration of Kaposiform Lymphangiomatosis; however, metastatic disease in the placenta was inconsistent with that diagnosis. The placenta showed microscopic tumor nodules beneath the endothelium and within the media of fetal stem vessels. Molecular studies showed that both the thigh lesion and tumor in the placenta were characterized by an NRTK3-ETV6 translocation, consistent with IF. The tumor was highly responsive to larotrectinib.

Conclusion: 1. This is the first description of infantile fibrosarcoma metastatic to the placenta. 2. The pattern of metastasis was unusual, being organized into the vascular wall of fetal stem vessels. 3. Possible pathways for this pattern of metastasis include:
   a. Re-endothelialization and organization of remote fetal vascular metastasis.
   b. Intraembryonic metastasis via mesenchymal migration. We hypothesize mesenchymal metastasis because:
      1. Tumor was detected only in the thigh-inguinal area and placenta.
      2. The physical proximity of the lower limb bud and body stalk in the 4th to 6th weeks of embryonic life makes the body stalk a plausible entry point for tumor exclusively entering the perivascular compartment of blood vessels in placental stem villi.
      3. The discrete focus of the tumor location makes a germline or field effect less likely.
MALTE Lymphoma in Gastrointestinal Tract: An Uncommon Form of Monomorphic Post-Transplant Lymphoproliferative Disorder with Both EBV-Positive and Negative Forms

L Barnea Slonim1, C Mehrhoff2, A Richardson2, S Gong2; 1Northwestern Memorial Hospital, Chicago, Illinois; 2Ann & Robert H. Lurie Children’s Hospital of Chicago, Chicago, Illinois

Background: Monomorphic post-transplantation lymphoproliferative disorders (M-PTLD) are lymphoid neoplasms arising in the setting of immunosuppression following solid organ or hematopoietic stem cell transplantation. EBV-positive extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is the only small B-cell lymphoma considered as PTLD. The latter is a relatively new concept, found in the latest WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues. EBV-negative PTLD have been reported, yet their incidence and clinical impact remain largely undefined. Here we describe two cases of GI involvement by M-PTLD, MALT lymphoma, one EBV-positive and one EBV-negative.

Methods: We searched our pathology archives from 2009 to 2019. 2 M-PTLDs were diagnosed as MALT lymphoma. Clinical history was collected from the electronic record and slides were reviewed.

Results: Case 1: A 16-year-old female status-post heart transplant, presented with melena. GI endoscopy revealed several ulcers. Stomach biopsies showed a dense lymphoid infiltrate, composed of small lymphocytes with moderate cytoplasm, slightly irregular nuclei and inconspicuous nucleoli. By immunohistochemistry, the atypical lymphocytes were positive for CD20 and BCL-2, and negative for CD5, CD10, Cyclin D1, MUM1 and BCL-6. Ki-67 proliferation index was 40%. EBER was positive. The overall findings were consistent M-PTLD, MALT lymphoma, EBV-positive. Case 2: An 18-year-old female status-post two heart transplantations, and high EBV-titers, presented with abdominal pain. GI endoscopy revealed multiple ulcers. A duodenal biopsy revealed a dense lymphoplasmacytic infiltrate consisting of small lymphocytes with moderate cytoplasm, slightly irregular nuclei and inconspicuous nucleoli. By immunohistochemistry, the atypical lymphocytes were positive for CD20, Lambda restricted, and negative for BCL-6 and CD10. Ki-67 showed a moderate proliferation rate. EBER was negative. The overall findings were consistent with MALT lymphoma, EBV-negative. Additional biopsies revealed extensive GI tract involvement. Testing for Helicobacter pylori and Campylobacter jejuni were negative. This case shared similar clinicopathologic features with the EBV-positive case and likely results from immunosuppression, suggesting that it should be classified as M-PTLD.

Conclusion: We describe two cases of M-PTLD presenting as GI MALT lymphoma. Although one case was EBER-negative, it has been described that EBV cannot be demonstrated in up to 40% of PTLDs. These cases may represent EBV-related proliferations that have lost the virus after transformation. M-PTLD, GI MALT lymphomas may be under-recognized and underreported. Future studies are warranted to identify the incidence and clinical significance of these cases.
Follicular Thyroid Carcinoma: A Rare Second Malignancy in Children with Retinoblastoma

J Kurtz¹, F Hazard²; ¹Stanford University, Stanford, California

Background: Thyroid carcinoma as a second malignancy is associated with many childhood cancers and, while potentially fatal, outcomes are improved by early detection prior to the development of metastatic disease. Children with retinoblastoma and germline RB1 gene mutations have a reported increased risk of developing medullary and anaplastic thyroid carcinoma. To our knowledge, follicular carcinoma has not been reported in association with germline RB1 mutations. Given our recent experience, we hypothesize that, although rare, children with retinoblastoma should be surveilled for follicular thyroid carcinoma to ensure early detection and good outcomes.

Methods: The Pathology archives at our institution were searched from 1999 – 2019 to identify patients age 0-18 years with retinoblastoma and subsequent thyroid carcinoma. The clinical presentation, germline RB1 mutation status, duration from the diagnosis of retinoblastoma to thyroid carcinoma, and the development of other malignancies were explored.

Results: An institutional review identified 13 second malignancies in retinoblastoma patients. Only 1 patient had follicular carcinoma of the thyroid; a 12 year old male with bilateral retinoblastoma diagnosed at age 18 months. Germline mutation testing revealed a pathologic variant in the RB1 (c. 1458del) gene. He received bilateral laser photocoagulation, chemotherapy and radiotherapy. At 12 years of age, he presented with bone pain and respiratory distress and was found to have a neck mass and nodules within his lungs, liver and right femur. Biopsies confirmed metastatic follicular thyroid carcinoma. Targeted sequencing performed on the tumor confirmed the germline RB1 mutation (c. 1458del).

Conclusion: Follicular carcinoma of the thyroid gland is a rare second malignancy in retinoblastoma patients. A 20 year review of our Pathology files yielded 13 retinoblastoma patients with second malignancies, which included rhabdomyosarcoma, osteosarcoma and undifferentiated sarcoma. Only the patient described had follicular thyroid carcinoma. A review of the literature identified 14 cases of thyroid carcinoma out of 1,290 second primary malignancies in patients with retinoblastoma. This analysis confirms routine screening for a second malignancy in retinoblastoma patients should include thyroid cancer, and follicular carcinoma should be considered, especially when evaluating small biopsies (e.g. core biopsies, aspirates) and metastatic sites.
Myogenin expression in benign skeletal muscle tumors: a potential pitfall

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Background: Myogenin is a transcription factor driving myogenesis in developing skeletal muscle and regenerating muscle. In diagnostic pathology, it is a highly specific marker for rhabdomyosarcoma. Although a few case reports have documented myogenin expression in rhabdomyoma, this marker has not been systematically evaluated in benign skeletal muscle lesions. We investigated the manner and quantity of myogenin expression in benign mesenchymal lesions with skeletal muscle differentiation in comparison to highly differentiated rhabdomyosarcomas.

Methods: Five cases of benign tumors with skeletal muscle differentiation were collected from consult files of two tertiary children hospitals. Retrospective review of the H&E and myogenin stains was conducted and myogenin staining was done in cases where not performed.

Results:

<table>
<thead>
<tr>
<th>Histotype</th>
<th>Age</th>
<th>Site</th>
<th>Histologic features</th>
<th>Desmin</th>
<th>Myogenin</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMH</td>
<td>2 yr</td>
<td>Tongue</td>
<td>Few mature SM cells, nerves</td>
<td>&gt;50%</td>
<td>Rare</td>
</tr>
<tr>
<td>RMH</td>
<td>8 yr</td>
<td>Chin</td>
<td>Few mature SM cells, nerves</td>
<td>&gt;50%</td>
<td>Rare</td>
</tr>
<tr>
<td>RMH</td>
<td>9 m</td>
<td>Anus</td>
<td>SM cells with subepithelial aggregates, nerves</td>
<td>&gt;50%</td>
<td>5-10%</td>
</tr>
<tr>
<td>Fetal Rhabdomyoma</td>
<td>1 yr</td>
<td>Ear</td>
<td>Circumscribed, densely cellular, rhabdomyoblasts</td>
<td>&gt;50%</td>
<td>&gt;10%</td>
</tr>
<tr>
<td>Benign Triton Tumor</td>
<td>3 m</td>
<td>Brachial plexus</td>
<td>Thin SM cells in fascicles and nerve fibers within perimysial sheath</td>
<td>&gt;50%</td>
<td>&gt;15%</td>
</tr>
</tbody>
</table>

RMH - rhabdomyomatous mesenchymal hamartoma, SM – skeletal muscle

Conclusion: Rare myogenin positive cells may be present in fetal rhabdomyomas and RMH. More numerous myogenin positive cells, as seen in one RMH and one benign triton tumor, might raise the possibility of a highly differentiated rhabdomyosarcoma. Although the differential diagnosis remains difficult, awareness that myogenin may be expressed even in benign lesions should prompt careful morphologic scrutiny for lack of features of malignancy in RMH and use of an appropriate immunohistochemical panel to highlight S100 positive nerve fibers and nuclear beta-catenin staining in benign triton tumor.
Aggressive Pediatric Adrenal Cortical Carcinoma With a Novel Translocation t(20;22) Fused MN1 and ZNF341

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Background: Adrenal cortical carcinoma (ACC) is a rare aggressive malignancy with a dismal prognosis. It is common to show distant metastasis at the time of diagnosis which is an independent poor prognostic factor. Genetic sequencing has played an increasingly important role in the diagnosis of cancer, especially in the pediatric population. Unfortunately, the capabilities of our technology reach beyond our knowledge. While we have accumulated an impressive list of genetic mutations and associated malignancies, there are still many others that have not been reported or described. In ACC the typical mutations seen in association with sporadic cases are somatic mutation of TP53, IGF2 or the beta-catenin/CTNNB1 in the Wnt signaling pathway with no known recurrent translocation.

Methods: In order to contribute to this growing body of knowledge, we present a case of a 3-month-old male who presented with growing lumps on head, chest, and back. Imaging studies showed multiple masses of the skull, thorax, extremities as well as a suprarenal mass. Metaiodobenzylguanidine (MIBG) scan was positive in aforementioned lesions. With the initial impression of neuroblastoma, skull lesion biopsy and subsequent adrenalectomy was performed.

Results: Microscopic sections showed a poorly differentiated tumor containing round tumor cells in a nesting growth pattern. Extensive panel of immunostains demonstrated the tumor cells were diffusely positive for SF-1, Melan-A, synaptophysin and Cam5.2 and negative for PHOX2B, desmin and NKX2.2 most consistent with widespread metastatic ACC. Cytogenetics, sarcoma FISH panel and next generation sequencing performed to show a novel translocation of t(20;22) fused MN1 and ZNF341. Despite multiple treatment attempts, the tumor continued to grow, and the patient passed away roughly four months after the initial diagnosis.

Conclusion: To our knowledge, this is the first pediatric metastatic adrenal cortical carcinoma with the novel translocation of t(20;22) fused MN1 and ZNF341.
Subcutaneous Inguinal Myxoid Liposarcoma: Rare Entity In A Rare Location

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Background: Liposarcomas are rare malignancies in pediatric population (2%). Among them myxoid liposarcomas are the most common (72%). They mostly occur in the lower extremities, retroperitoneal abdomen and pelvis. They are characterized by the recurrent translocation (12;16) (q13;p11) resulting in Fused in Sarcoma and DNA Damage Inducible Transcript 3 (FUS-DDIT3) gene fusion. Here, we report a case of a 10-year-old female with myxoid liposarcoma in a very rare location (subcutaneous inguinal).

Methods: The patient is a 10-year-old female with no past medical history, who presented with a 2 months history of left inguinal, subcutaneous mass. Ultrasound showed a well circumscribed 5.0 x 0.8 cm mass of uncertain etiology, the differential diagnosis included atypical lymph node and atypical lipomatous tumor. Subsequently the mass was surgically excised.

Results: Gross examination revealed an intact mass with homogeneous, gelatinous cut surfaces. Histologic sections showed a multinodular mass of low cellularity with enhanced cellularity at the periphery, the cells are fusiform and lie suspended individually in a myxoid matrix, without a discernible nuclear pattern or identifiable mitotic activity. A delicate plexiform capillary vascular network is present throughout the tumor with many multivacuolar lipoblasts. Fluorescence in situ hybridization (FISH) testing for DDIT3 rearrangement is positive which confirms the diagnosis of myxoid liposarcoma. The tumor was extending to the peripheral resection margins. She is scheduled for further resection.

Conclusion: The overall prognosis for myxoid liposarcomas in children is excellent, generally with surgical treatment alone. In a case with unusual superficial location, one should think about myxoid form of dermatofibrosarcoma protuberance as a differential diagnosis, in addition to lipoblastoma, and low-grade fibromyxoid sarcoma.
The Morphological Connection Between Ewing Sarcoma and Neuroblastoma Pediatric Tumors

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Background: Ewing sarcoma (ES) and neuroblastoma (NB) are two pediatric malignancies that are usually distinct based on patient age, anatomic site, and genetic profile (i.e., the presence of EWSR1 translocation in ES vs. MYCN amplification in NB). We have, however, encountered cases of translocation-positive ES which, following treatment, showed striking morphologic features of NB including ganglion-like cells and neuropil. Furthermore, ES and NB generally display overlapping immunophenotypes, creating diagnostic challenges; however, the depth and significance of this histologic and immunohistochemical (IHC) resemblance is unclear. Thus, we sought to further define the pathobiologic similarities and differences between ES and NB.

Methods: The pathology database was queried for ES cases and poorly differentiated/undifferentiated subtypes of NB. Clinical information (gender, age at diagnosis/biopsy date, diagnosis, anatomic site, stage, therapy, and outcome) was obtained from the electronic medical record. H&E slides were reviewed for overlapping histologic features: rosettes, undifferentiated areas resembling ES, ganglionic differentiation, and neuropil. A tissue microarray with triplicate cores from areas of shared morphology was subjected to IHC analysis (tyrosine hydroxylase [TH], PHOX2B, PGP9.5, TRKA, TRKB, and TRKC) and fluorescence in situ hybridization for EWSR1 rearrangement and MYCN amplification. IHC staining was semiquantitatively scored (0-3+) and averaged across all TMA cores for each case to determine mean intensity (MI); comparisons were performed via Student t-tests.

Results: We examined 275 ES cases and 215 NB cases, of which 33 ES (12%) and 28 NB cases (13%) had overlapping morphologic features. Of the ES cases, 11 had ganglion-like cells, 15 had rosettes, and 14 had neuropil; 8 cases showed multiple features. Of the NB cases, 18 cases had undifferentiated areas and 10 had rosettes. Of cases with histologic overlap, 42.4% of ES and 32.1% of NB had prior treatment. IHC showed significant differences for PGP9.5 (MI = 0.17 for ES vs. 2.30 for NB, p = 2E-10; 26% of ES cases staining vs. 96% of NB) and TRKB (MI = 1.67 for ES vs. 2.07 for NB, p = 0.02; all ES and NB cases staining). TH, PHOX2B, TRKA, and TRKC revealed no significant differences between the two tumor types. FISH demonstrated only one ES case with MYCN amplification and no NB cases with EWSR1 translocations.

Conclusion: ES and NB may show morphologic overlap in 10-15% of cases, which may present a diagnostic dilemma, especially post-treatment. However, there are specific assays – IHC for PGP9.5 and FISH for EWSR1 and MYCN – that can help differentiate these tumors, even in cases with histologic uncertainty. Thus, despite the pathologic similarities, ES and NB appear to be distinct biologic and genetic entities.
Cystic Fibrosis (CF) Mimics Biliary Atresia (BA): Histology of Liver and Extrahepatic Biliary Tree (EHBT) in Three Cases with Kasai Portoenterostomy (KHPE)

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Background: Neonatal jaundice in infants with CF, associates with meconium ileus (MI) and obstructive jaundice that may be prolonged, and often requires TPN support before clearing. Rarely, CF-related structural abnormalities of the EHBT simulate BA and KHPE may be necessary. The histopathology of the EHBT in such cases has not been examined in detail.

Methods: We report operative findings with liver and biliary remnant histology in 3 male infants with CF and prolonged neonatal jaundice who had Kasai surgery for BA 2-3 months after birth. We compared these specimens to liver histology and step-sectioned remnants in idiopathic BA. Case1. Male infant with cystic fibrosis and prolonged neonatal jaundice; KHPE at 83d. Died of liver disease in early childhood. Case2. Male infant with cystic fibrosis in-utero closed loop obstruction, meconium ileus resected, small GB; initial, cholangiogram via GB failed, but common bile duct patent; repeat cholangiogram at 37d was totally obstructed; KHPE at age of 72 days with successful drainage to date. Case3. Male infant with cystic fibrosis (homozygous F508del), meconium ileus resected, TPN-cholestasis, acholic stools and KHPE at age 65 days. Liver transplant for biliary cirrhosis at age 6 years.

Results: Sections of all three remnants differed from BA, showing hypoplastic poorly developed microcystic GB with inspissated luminal mucin, normal to immature cuboidal epithelium and incomplete muscularis. Similar malformations in the cystic duct and hepatic duct were minimally inflammed. The earlier of two sequential liver biopsy pairs were inconclusive for obstruction.

Conclusion: Rare infants with CF have microcystic maldevelopment with mucus accumulation in extrahepatic biliary tree; histology resembles obstructed vas deferens/epididymis in male CF patients who are usually sterile (Landing et al, 1969). Features of complete large duct obstruction may be difficult to detect in early liver biopsies; the pattern of injury in the liver partially simulates BA.
Secretory Carcinoma in Children and Adolescents: A Case Series

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Background: Secretory carcinoma (SC), previously known as mammary analog secretory carcinoma, is a rare salivary gland neoplasm, with only 14 reported cases occurring in children. SC presents as a slow-growing painless lesion in the head and neck. The mean age at diagnosis is 46 years, with the youngest patient being diagnosed at 7 years. The reported female to male gender distribution ratio is 1:1.2. Surgical resection with or without lymph node dissection is the standard treatment, clinical outcomes are generally favorable. SC has a specific genetic translocation (ETV6-NTRK3 fusion; t(12;15)(p13;q25)). Here we present a case series of pediatric patients with SC (ages 9-21). To the best of our knowledge, this is the only SC case series comprised of pediatric patients.

Methods: A retrospective laboratory information system data search was conducted for secretory carcinoma in pediatric population (ages≤21 years) between May 2010 and November 2019. Available clinical information and pathological data was recorded.

Results: 6 cases of SC in children and adolescents (age 9-21) were identified. None of the patients had past medical history. Female to male ratio was 1:5. The sites included parotid gland (3 cases) and minor salivary glands (1 in the hard palate and 2 in upper lip). In the 3 cases where clinical history could be obtained, the lesion was present from two weeks to five years prior to evaluation. In 3 of the cases, there was pre-resection diagnostic material (2 biopsies and 1 fine needle aspiration) which showed a low-grade salivary gland epithelial lesion, SC, and low-grade salivary gland carcinoma, respectively. All tumors were resected with negative margins. 1 case had intermediate-grade nuclei, while the other 5 had low-grade nuclei. Tumor size ranged from 0.9 cm- 2.7 cm. Cytogenetic evaluation was performed on 1 case, which demonstrated t(12;15)(p13;q25). Fluorescence in situ hybridization was performed on one case and showed an ETV6 rearrangement. The 3 children with available follow-up information had no post-surgical complications and had no recurrences in three years.

Conclusion: SC is a relatively newly-described variant of salivary gland carcinoma. While rare, this entity can be seen in the pediatric and adolescent population. These lesions are typically slow-growing, painless masses that are incidentally discovered. To date, the preferred treatment is complete surgical resection with negative margins. Patients typically have a good prognosis with no reports of recurrence in children at the time of this abstract. There may be a potential therapeutic role for recently developed NTRK inhibitors. Recognition of this neoplastic entity in pediatric patients is essential, especially for small biopsies and fine needle aspiration; while other treatment modalities are being explored.
The Role of a Safety Skills Curriculum for the Anatomic Pathology Trainee

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Background: Accidental exposure of broken skin and/or mucous membrane to tissue, blood and/or body fluid is an occupational hazard in the Pathology laboratory. Our institution is a major academic center handling a high volume of perinatal and adult specimens, with comprehensive institutional safety protocols in full compliance with OSHA, CAP and CLIA requirements, and a robust system for addressing safety incidents. Despite this, pathology trainees still experience exposures. These accidents are self-reported, with variability in reporting compliance. A rich literature addresses patient safety, but is limited for personal safety of the anatomic pathology trainee. This project seeks to define the landscape of accidental exposures in pathology trainees, including reported and unreported events, to address underlying causes, and propose interventions.

Methods: Via online anonymous survey, we asked residents and fellows their exposure experiences and reporting behavior on anatomic pathology services. Questions were multiple choice, asking number, timing, location, type of reporting, and reasons for reporting variability. Two questions solicited open-ended responses.

Results: A 73% response rate (27/37) yielded 33% (9) with an exposure in the past year and 41% (11) in the past 5 years; 37% (10) reported multiple exposures. Most (11) occurred in the gross room, 3 in autopsy, 2 in the frozen room, and 1 on cytopathology rotation. Only 18% followed proper procedures for reporting (2/11). Reasons cited included guilt/shame, unclear thinking at the time of the accident, minor injury, pressure to continue working, and perception of reporting as an unnecessary formality. Perceived contributors for accidents were high work volume and fatigue.

Conclusion: These data reveal surprisingly high rates of accidental exposure among pathology trainees, and a high rate of non-reported incidents. This highlights an urgent need for change in the way we train our residents and fellows. Safety in the laboratory relies on experiential skills, often taught one-on-one at the bench using an apprenticeship model. This approach is vulnerable to variability and gaps in training. Moreover, protocols and guidelines are often presented generally and in written format, and are not suited to skills-based learning. A lack of specific resources for safety training exacerbates this problem, but also provides an opportunity for improvement. We propose a pathology-focused and practical trainee curriculum, concentrating on gross room and autopsy suites, to include in-person group instruction using a video-based training tool, monthly review and mini-workshops, and a focus on culture shift to encourage reporting. We share our institutional experience in the spirit of promoting transparency and a broader discussion of this important issue.
Gaucheroma - An Emerging Complication in Patients With Gaucher Disease with Long-Term Enzyme Replacement Therapy

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Background: Gaucher disease (GD) is the most common lysosomal storage disease caused by GBA mutations that decreases b-glucocerebrosidase activity and causes an accumulation of glucosylceramide mainly in macrophages (Gaucher cells). Intravenous enzyme replacement therapy (ERT) using a recombinant enzyme has been the first-line treatment for GD since the early 1990s, so we are beginning to identify complications correlated with long-term use. Gaucheroma, mass-like accumulation of Gaucher cells, is one of these rare complications. The majority of Gaucheromas were located in the liver and spleen but a few were reported in the abdominal or thoracic cavity.

Methods: We present 3 cases of Gaucheromas that arose in unusual sites, including detailed histologic and ultrastructural findings.

Results: Case 1 is a 16-year-old (yo) female with GD type 3 (GBA homo, L444P) with decreased vision due to vitreous opacities, abnormal extraocular movement, bilateral worsening deafness, and intellectual disability (ID). She has been on ERT with imiglucerase for 16 years (y). Right cochlear implant surgery was attempted at 15 yo but discontinued as a mass lesion were found in the posterior mastoid region.

Case 2, an elder sister of Case 1, is a currently 24-yo female with GD type 3 (GBA homo, L444P) with femoral head aseptic necrosis, decreased vision, bilateral deafness, ID, psychiatric symptoms and growing intra-abdominal masses diagnosed at 8 yo resulting in protein losing enteropathy. She has been on ERT for 23 y. Oral substrate reduction therapy (SRT) with eliglustat was added at 21 yo. After 2 y of the combination therapy, the masses became significantly smaller by imaging.

Case 3 is a 33-yo female with GD type 3 (GBA homo, L444P) with ID, who has been on ERT for 27 y. Large soft-tissue masses on her back were noted at 26 yo. The mass progressively enlarged and reduced her quality of life. SRT was added at 31 yo. The masses promptly decreased in size. The histology of the mass lesions demonstrated a large aggregate of foamy macrophages containing a “wrinkled-tissue-paper” material (Gaucher cells). EM revealed enlarged lysosomes filled with fine tubular structures. The features confirmed the diagnoses.

Conclusion: We report 3 Gaucheromas found in unusual sites (back, abdominal cavity and mastoid), including 2 novel sites, that caused significant mass-related disability and organ dysfunction. In Case 2 and 3, the unique combination therapy was effective to reduce the size of the masses. Therefore, close image monitoring for early detection of Gaucheroma(s) is essential to avoid the mass-related disfunction before it becomes permanent damage. It is also important for pathologists to recognize this newly emerged, rare complication in GD patients with long-term ERT in order to give an accurate diagnosis.
A Malignant Mimicker: Features of Kikuchi-Fujimoto Disease in the Pediatric Population

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Background: Kikuchi-Fujimoto disease (KFD), also known as histiocytic necrotizing lymphadenitis, is a rare, benign, and usually self-limited disease, which often presents with cervical lymphadenopathy (LAD) and systemic symptoms. Though mostly reported in Asian women in their 20s, the disease can also present in children. Histologic evaluation is usually necessary to differentiate this diagnosis from malignant entities such as lymphoma.

Methods: Our pathology database was queried for cases of KFD or histiocytic necrotizing lymphadenitis from Jan 2013 to Nov 2019. Slides were reviewed to confirm diagnosis, and DNA was extracted for next generation sequencing to detect structural variants, single nucleotide variant (SNVs), insertion/deletions, and copy number variants in a panel of 340 genes. Demographics and clinical data including age, sex, race, disease location, symptoms, and laboratory values were recorded.

Results: Twelve confirmed KFD cases had a M:F ratio of 5:7, a median age of 16.3 years (range 9.6-18.6), and predominantly Asian ancestry (6/8, 4 without stated race). The most common presenting symptoms (median duration 1.5 months) included fatigue (n=10), fever (n=9), weight loss (n=6), loss of appetite (n=5), and night sweats (n=5). Laboratory values demonstrated leukopenia (n=9/11), neutropenia (n=8/11), anemia (n=6/11), thrombocytopenia (n=4/11), increased ESR and/or CRP (n=9/11), elevated AST and/or ALT (n=8/9), elevated ferritin (n=6/7), and elevated LDH (n=7/10). Eleven had cervical LAD, often bilateral, and one had bilateral axillary LAD. Morphologic examination revealed enlarged lymph nodes with necrotic foci without neutrophils but with CD68/CD163, MPO-positive histiocytes containing crescentic nuclei. Increased immunoblasts often surrounded the necrosis but contained a mixture of CD4+ T cells and CD8+ T cells, the latter more prominent. CD123+ plasmacytoid dendritic cells were also increased. Focal intact architecture with germinal centers was usually identified. None had significant increases in plasma cells or other features of lupus. While molecular analysis of 4 cases demonstrated rare population SNVs in genes including NOTCH1 (4 cases, 2 with an identical synonymous SNV), NOTCH3 (3 cases), and/or NOTCH2 (2 cases), it did not reveal any likely/pathogenic somatic or predisposition variants. Most patients had resolution of symptoms within 2-3 months, though some required steroids, anakinra, and/or plaquenil therapy.

Conclusion: This is the largest KFD series from a single US pediatric hospital, and the first study to report molecular findings. While additional molecular studies may one day help to define KFD, its etiology remains elusive. However, despite the worrisome presenting clinical picture, KFD can be differentiated from neoplasms by the above histologic features.
Monochorionic Triplet Pregnancies with Twin Reversed Arterial Perfusion (TRAP) and Acardiac Triplet: Case Series and Literature Review

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Background: In twin reversed arterial perfusion (TRAP), the acardiac twin depends on the normal heart of the “pump twin” to pump blood for both fetuses as there is no functioning circulatory system. The acardiac twin experiences reversed arterial perfusion as blood enters through the umbilical artery and exits through the umbilical vein. The acardiac twin usually does not develop or survive and there is a poor prognosis for the compensating pump twin as well. TRAP pregnancies involving monochorionic triplets are extremely rare, calculated to be 1 in 4.5 million; there are only 10 cases reported in the literature. We report a series of two extremely rare cases of monochorionic triplet pregnancies with an acardiac triplet.

Methods: Fetal and placental examination was performed, and Epic database was utilized to obtain clinical history, maternal physical examination, imaging studies, and pertinent laboratory findings. Literature review is performed using PubMed.

Results: We report two cases of monochorionic diamniotic TRAP and acardiac triplet. In one case, the mother was a 33 year old G2P1 who underwent dilation and evacuation at 20 weeks due to poor prognosis. The other case involved a 21 year old G1P0 mother presenting in advanced preterm labor at 21 weeks who elected expectant management. Ten cases of monochorionic triplets with TRAP were identified in the literature. The mothers had an average age 30.1 years (range 22 to 38 years). In only one case did the mother deliver vaginally; the remaining 9 cases involved cesarean sections. Radiofrequency ablation was performed in 3 of these cases with interstitial laser coagulation performed in 1 case; all 4 of these cases resulted in the survival of the nonacardiac twins. Only 2 of the 6 cases without intervention resulted in the survival of the nonacardiac twins. Our two cases had common radiographic features which included polyhydramnios of all fetuses. Placental features shared between both cases included being large for gestational age and velamentous insertion of the acardiac twin. The acardiac twins in both of our cases were hydropic and demonstrated developed pelvis and lower extremities. One had an omphalocele. Most of the case reports in the literature review also reported hydrops, lack of upper body development, and a well developed lower half of the body in the acardiac twin.

Conclusion: Two cases of extremely rare monochorionic triplets with TRAP and acardiac twin are herein described. Successful treatment by fetoscopic laser intervention or cord occlusion has been described in this condition, highlighting the importance of early detection.
Clinical Significance of CD31/CD68 Interpretation in Complex Heart Transplant Biopsies

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Background: Grading of cardiac antibody-mediated rejection (pAMR) requires assessment of subjective features including the problematic interpretation of the location of CD68 positive macrophages as intra or extra-vascular. A dual-stain method for CD68 and endothelial cell (CD31) markers has shown moderate interobserver agreement when routinely applied. However, most biopsies are negative for all features of rejection. Areas of diagnostic difficulty with this cocktail are not established in more complex cases. We retrospectively stained a subset of more complex biopsies to determine interpretation agreement and clinical utility of this protocol.

Methods: Transplant biopsy reports from 2013 to 2017 were reviewed, and cases were selected based on positive history of pathologic findings, clinical concern, or past rejection, as well as sufficient tissue. CD31/CD68 stain was performed, and materials were independently scored by two pathologists blind to clinical information and original diagnosis.

Results: 145 (21%) of 681 total biopsies were selected from 48 patients, including specimens with mild (44) or moderate (13) acute cellular rejection (ACR) or ischemia-reperfusion injury (10).

Interobserver agreement was best for C4d (kappa=0.82) but substantial for all categories: ACR (0.64), histologic AMR (0.64), and CD68/31 (0.75).

In 14 cases (12 patients), disagreements interpreting either histology or CD31/68 were due to concomitant cellular rejection (8), guilty (1) and/or abundant extravascular macrophages (5). 4 patients were clinically asymptomatic and not treated, 2 had treated AMR, 3 had treated ACR, and 1 had C4d positive pAMR. 2 cases with abundant macrophages were treated as biopsy negative AMR based on positive donor specific antibodies (DSA) and clinical symptoms.

12 specimens from 7 patients reached a diagnosis of pAMR2 based on macrophage staining despite negative or sub—threshold (50%) C4d staining. 4 of 7 patients (9 specimens) had been previously treated for C4d-positive AMR. 2 patients were clinically stable, with one later requiring treatment with IVlg for persistent pAMR1h. The final patient had increasing DSAs and patchy C4d and required escalation of therapy for persistent biopsy findings.

Conclusion: Interobserver agreement for CD31/CD68 staining is substantial and better than for histologic pAMR alone. Positive staining may persist as C4d becomes patchy/negative following treatment for AMR, or it may support a new diagnosis of pAMR in cases where C4d staining is only patchy. Staining may be difficult to interpret when abundant macrophages are present following either AMR or ACR; however, the presence of intra or abundant extra-vascular macrophages in the absence of ACR may rarely correlate with clinically significant AMR in patients with clinical symptoms and rising DSA.
Sloughing Esophagitis in the Pediatric Age Group: Clinicopathologic Characteristics of Twelve Cases

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Background: Esophagitis dissecans superficialis, also known as sloughing esophagitis (SE) is a benign, often self-limiting condition of uncertain etiology. SE is well reported in adults and rarely reported in children. Herein, we present an analysis of clinicopathologic characteristics in a pediatric case series of SE.

Methods: Our electronic database (01/2010 – 12/2019) was queried for esophageal biopsies containing words “sloughing” and “dissecans” in patients aged 0 – 21 years. Cases of eosinophilic esophagitis (EoE) were excluded. Cases were re-reviewed by one practicing and one trainee pathologist. Histologic features required for inclusion were “two-tone” appearance of mucosa and sloughing or detachment of superficial squamous epithelium with parakeratosis. Sites of biopsy, sites of involvement, fungal stains as well as the presence and degree of inflammation were recorded. Endoscopic images were re-evaluated by a pediatric gastroenterologist. A chart review was performed for medical history, medication history, endoscopic findings, treatment, and follow up.

Results: A total of fourteen patients were identified. Slides from one patient were unavailable for review and another patient’s biopsy failed histologic inclusion criteria. The remaining twelve patients were included in our study whose ages ranged from 1-19 years (mean: 16 years), including three males and nine females. Though clinical symptoms varied, 8/12 (67%) had abdominal pain, and 6/12 (50%) had nausea and vomiting. Only 2/12 (17%) experienced dysphagia. Six cases showed classic findings with a neutrophilic inflammatory infiltrate layered between the sloughing layer of superficial epithelium and underlying mucosa, five cases had minimal or no inflammation, and one case displayed severe acute inflammation. With one exception, most cases (11/12, 91%) showed no evidence of fungal organisms (Grocott Methanamine Silver stain was performed on 11 cases). Sloughing was visible endoscopically on 3 cases, 7 had other mucosal abnormalities, 2 were normal. 50% of patients were on three or more medications at the time of endoscopy. Eight patients (75%) patients were on medications for depression/anxiety with at least five patients (42%) reporting marijuana/cannabis oil exposure. Symptomatic resolution occurred within one month for 5/12 patients, while 5/12 had continued symptoms, and 2 patients were lost to follow up. One patient presented with recurrent SE and another with EoE 6 years after diagnosis of SE. No patients had a history of cutaneous bullous disorders.

Conclusion: SE appears to be multifactorial in children. Histologically, inflammation can be variable, ranging from none to severe acute inflammation. An association with medications for depression/anxiety and marijuana exposure is possible and needs to be explored further.
Colon Metabolomic Changes in SIDS

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**Background:** The etiology of sudden infant death syndrome (SIDS) is thought to be multifactorial with abnormal serotonin metabolism as a possible pathogenetic factor. Colon is the primary site of serotonin synthesis and metabolic activity of the gut microbiota influences this process. The infant gut microbiome has been linked to SIDS susceptibility, which raises the possibility that infant gut microbiota, which is in a state of developmental flux, may cause an abnormal gut metabolome and abnormal serotonin synthesis in SIDS. This pilot study compares the metabolome in colonic tissue from SIDS cases to non-SIDS controls.

**Methods:** Research ethics board approval was obtained for this study. The study included fresh frozen colonic autopsy tissue from 23 cases of SIDS without a history of co-sleeping (average age 90 d, range 29-224 d; 17 M:6 F) and 20 cases of non-SIDS controls (average age 100 d, range 16-253 d; 14 M:6 F ). All non-SIDS control cases had a defined cause of death not involving the gastrointestinal system or an infectious etiology. Untargeted metabolomic analysis was performed using an Agilent 6530 quadrupole-time of flight mass spectrometer (QTOF), Agilent 1290 binary ultra performance liquid chromatography, and MassHunter data acquisition software. Reverse phase (RP) positive ion and hydrophilic interaction (HILIC) chromatography with negative ion mode were run to identify hydrophilic and hydrophobic compounds. The QTOF was tuned for low masses, operated in high resolution mode, and used Agilent's standard reference mass solution providing corrected mass accuracy of 2-3 ppm. Data processing used Agilent software packages. T-tests with Benjamini-Hotchberg correction were performed on filtered data to determine statistical significance.

**Results:** Approximately 3500 entities in RP and 1600 entities in HILIC were identified. No statistically significant difference was observed in hydrophilic or hydrophobic metabolite levels between the SIDS and non-SIDS control cohorts. Levels of serotonin in the SIDS cohort (mean area 112403) were almost twice that in the non-SIDS controls (mean area 67712), but this did not reach statistical significance (p=0.17). Tryptophan levels were similar in both groups (p= 0.49). 3-indolepropionic acid, a tryptophan metabolite produced by gut flora, was not detected in either cohort.

**Conclusion:** In this pilot study no statistically significant difference was found between hydrophilic and hydrophobic metabolites detected in colonic autopsy tissue from infants diagnosed with SIDS compared to age-matched non-SIDS controls. Serotonin levels were increased in the SIDS cohort but the difference was not statistically significant; whether this is a true result or false negative related to insufficient study power is unclear.
Hydrops Fetalis in Hawaii: An Epidemiological Study

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Background: Hydrops fetalis is associated with causal conditions (fetal, placental, or maternal), body system abnormalities, developmental or genetic disorders, intrauterine infections, metabolic disorders, and idiopathic forms. In the United States (US), White Americans make up the largest racial group and structural cardiac defects are the leading cause hydrops fetalis. This is in contrast to the state of Hawaii where Asians make up the largest racial group in the state. Our specific aim of the study is to report the causes of hydrops fetalis in Hawaii and compare our results to the causes of hydrops fetalis elsewhere in the United States. Such an epidemiological study has not been previously reported.

Methods: CoPath database was accessed for hydropic fetuses including spontaneous abortions, intrauterine fetal demises, pregnancy terminations, and autopsy reports. Epic was accessed for clinical and epidemiological features (maternal age, gestational age, ethnicity of the fetus) and genetic testing results. The causes of hydrops were categorized as follows: fetal conditions, maternal conditions, placental conditions, organ specific congenital anomalies, developmental or genetic disorders, intrauterine infections, and idiopathic/unknown.

Results: 20 fetal hydrops cases were found in our database from 2006 until 2018. The ethnic distribution was 55% Asians (11), 20% Native Hawaiian and Other Pacific Islander (4), 5% Black or African American (1), and 20% Two or more ethnicities (4). 50% of the fetuses were females and 50% males (10 fetuses each). The most common etiologic category was idiopathic/unknown (35%, 7 cases). The remaining etiologies included 25% developmental or genetic (5 cases: 2 Turner syndrome, 2 multiple congenital abnormalities, and 1 chromosomal abnormality), 25% fetal conditions (5 cases: 3 homozygous thalassemia, 1 hemorrhage, 1 tumor), 10% pulmonary system (2 cases: diaphragmatic hernia and pulmonary hypoplasia), and 5% placental (1 case: chorangioma). In our study, alpha thalassemia (Bart hemoglobinopathy) was the most common known etiology. In contrast to the demographics of the US in general, in our study, Asians were the largest ethnic group and there were no fetuses of White or American Indian, or Alaska Native ethnicity. There were also no cases with cardiovascular or infectious etiologies for hydrops.

Conclusion: Although the most common etiologic category for fetal hydrops in Hawaii based on the results of our study was unknown/idiopathic, the most common known etiology was alpha-thalassemia. This is likely related to the predominantly Asian ethnicity of the population of Hawaii, and contrasts with the leading cause of fetal hydrops from other US studies.
Curation of Clinically Actionable Variants in Pediatric Cancers within the Clinical Genome Resource (ClinGen)

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Background: The Clinical Genome Resource (ClinGen) Somatic Cancer Working Group is a multi-institution team engaged in developing processes, resources, and standards to support accurate classification of somatic variants in cancer. There remains a dearth of resources for interpretation of childhood cancer variants, given the focus on adult cancers in current guidelines and knowledgebases. The genomic landscape of pediatric cancers is fundamentally different than adult tumors, with a lower mutational burden and a higher incidence of oncogenic gene fusions; this difference demands a different approach to variant interpretation. Herein we describe the goals, progress and impact of the Pediatric Cancer Taskforce (PCT), created within the ClinGen Somatic Cancer Working Group to drive curation efforts of clinically actionable alterations in childhood cancers.

Methods: The PCT consists of 39 geneticists, pathologists, oncologists, and bioinformaticians with expertise in pediatric tumors. Under guidance of the expert members, 12 volunteer-curators contribute to curation activities. In collaboration with the Clinical Interpretation of Variants in Cancer (CIViC) team at Washington University in Saint Louis, variants are curated for clinical utility using the curation and data-sharing platforms, CIViC knowledgebase and the ClinVar database. PCT subgroups focus on specific genes, variants and tumor types for review, curation, adding relevant evidence items to the database. Final assertions regarding significance of curated variants are created in CIViC during monthly group conference calls.

Results: Based on their clinical impact and the limited representation within clinical knowledgebases, the PCT has prioritized nearly 40 variants and fusions associated with pediatric cancer as the current focus. The PCT has active curation efforts for common variants in pediatric sarcoma and brain tumors, targetable kinase fusions in Ph-like B-lymphoblastic leukemia, and NTRK-fusions agnostic of tissue histology. The work of the PCT includes 157 evidence items, of which 65 have already been accepted, and 6 assertions in CIViC. In addition, the PCT works to implement appropriate tagging of evidence using ontology terms, to enhance search efforts for pediatric-specific data. The PCT has collaborated with the ClinGen Somatic Cancer Working Group to establish an NTRK fusion expert panel.

Conclusion: As molecular alterations are increasingly relevant to the care of children with cancer, the ClinGen PCT will work to develop standards, processes and resources for efficient and accurate determination of clinical relevance of pediatric cancer variants. We welcome new curators and team members.
High-Grade Sarcoma With PTCH1 Mutation: Report of a Unique Case With Histologic Features of Sclerosing Epithelioid Fibrosarcoma

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Background: PTCH1 is a membrane-bound receptor for Hedgehog (Hh) ligands; heterozygous germline PTCH1 mutation causes the tumor predisposition Gorlin syndrome (nevoid basal cell carcinoma syndrome). Somatic PTCH1 mutations have been identified in medulloblastoma, a variety of carcinomas including basal cell carcinoma, and rhabdomyosarcoma, but otherwise PTCH1 mutations are not described in pediatric soft tissue sarcomas which are typically driven by chromosomal translocation/gene fusions. Sclerosing epithelioid fibrosarcoma (SEF) is a distinctive malignant tumor of variable cellularity, composed of uniform, round to oval cells set in a hyalinized collagenous matrix. SEF tumor cells typically express MUC4 and may demonstrate EWSR1 or FUS gene rearrangements; however these features are not uniform and SEF is considered a genetically heterogenous tumor. We report a unique high-grade sarcoma with histologic features of SEF characterized by homozygous PTCH1 inactivation.

Methods: Clinical records, radiographic images, pathology reports, and all H&E and immunohistochemical slides were reviewed.

Results: A 14-year old girl with sickle cell disease (Hgb SS) presented with a one year history of a left thigh "knot" and slowly growing mass. Radiologic studies showed an 8.5 cm ovoid mass within the left rectus femoris, with no evidence of metastatic disease. Biopsy of the tumor showed a variably cellular malignant neoplasm composed of uniform round cells with bland oval nuclei, finely granular chromatin, a moderate amount of clear cytoplasm, rare mitoses, and a small focus of possible necrosis. Hypercellular areas contained sheets of tumor cells with minimal matrix, while hypocellular areas contained cords and nests of tumor cells with abundant hyalinized collagenous stroma. Extensive immunophenotyping was inconclusive; tumor cells expressed SATB2, patchy Cyclin D1 and Nkx2.2, but did not express MUC4 or myogenic, epithelial, nerve sheath, melanocytic, vascular, myoepithelial, glial, or lymphoid markers. Molecular testing revealed frameshift mutation of PTCH1, multiple copy number variations including partial 9q loss (including PTCH1) and partial 12q amplification (including CDK4 and MDM2), and no evidence of known or novel gene fusion. Subsequent resection showed a circumscribed and lobulated tumor with peripheral gross venous invasion and negative resection margins. Overall histology was similar to biopsy material, with the additional findings of focally high mitotic activity (26 per 10 hpf), definitive necrosis, and a single focus of spindled tumor morphology.

Conclusion: This is the first report of PTCH1 mutation in a pediatric non-rhabdomyomatous soft tissue sarcoma. Routine molecular profiling of such tumors may further define the role of Hedgehog signaling in pediatric neoplasia.
Gauging the Effectiveness of Pediatric Pathology Fellowship Training for Pediatric Dermatopathology Specimens: Interpreting the Preliminary Results for A National Survey

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Background: The field of medicine is constantly evolving towards higher levels of subspecialization. Even within the already highly subspecialized field of pediatric pathology, pediatric dermatopathology is a niche that many fellowship trained pediatric pathologists find challenging. To gauge the effectiveness of current fellowship training strategies for pediatric dermatopathology, the University of Texas Southwestern Dermatology and Pediatric Pathology departments developed a joint study specifically comparing levels of confidence between pediatric pathologists and dermatopathologists for interpretation of pediatric dermatopathology cases.

Methods: We prepared a survey which consists of twenty-one questions and respondents self-select as pathologists or dermatopathologists. Additional questions quantify the dermatopathology cases viewed by the physician and their comfort level with these specimens. This study was introduced at the Fall 2019 Society for Pediatric Pathology meeting and disseminated online through pediatric pathology forums. Preliminary data for the pediatric pathology arm of this survey was compiled and analyzed.

Results: Thirty-three people took the survey but only twenty-six self-identified as fellowship trained in pediatric pathology, and results are restricted to those physicians. Most people (~70%) identified as practicing for over 6 years, and the vast majority practice in an academic setting. Pathologists’ weekly workload varied between >100 total cases (~42%) and 100-300 cases (~54%). Of these cases, 81% of people estimated only 1-10 cases were dermatopathology specimens. Although most respondents described their training experience as adequate, a significant number (~27%) described their fellowship exposure as inadequate, and 81% of respondents reported no formal dermatopathology training during fellowship. Many pathologists described themselves as mostly comfortable interpreting these cases, but the majority of pathologists varied between feeling neutral to somewhat uncomfortable. ~69% of pathologists were less comfortable handling pediatric dermatopathology cases than other specimens and 70% of people refer cases to colleagues for consultation. Comfort levels also varied for different types of specimens, with most people professing comfort with neoplastic and melanocytic lesions, and the majority of people uncomfortable with inflammatory dermatoses.

Conclusion: Analysis of initial data indicates pediatric dermatopathology training is an area of improvement for fellowship programs. Dedicated time during training including formal lectures and rotations with dermatopathologists would be useful tools in raising trainee confidence. With more data, further insight into these trends will emerge, including comparisons with the ongoing dermatopathology arm of the study.
Tissue Transglutaminase and Deamidated Gliadin Peptide in Tandem: A Comparison Of Sensitivity and Specificity in a Pediatric Population

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Background: Diagnosis of celiac disease is evolving and serologic diagnosis without biopsy is gaining traction. In symptomatic patients with tissue transglutaminase IgA (TTG) greater than 10x upper limit of normal (ULN), some forego biopsy. Our goal was to understand the performance of the Bioplex 2200 TTG assay against biopsy. Deamidated gliadin peptide IgG (DGP) has been used as an additional test by our clinicians and understanding its performance on the same platform was a secondary goal.

Methods: Patients who had TTG drawn from June 2017 through November 2018 had serum stored frozen. If the patient underwent biopsy within 6 months after TTG testing, frozen serum was used to measure DGP. All biopsies were reviewed by the study pathologist and Marsh grade was assigned. Sensitivity and specificity for TTG and DGP were calculated using biopsy as the gold standard. Calculations were made using TTG and DGP greater than or equal to 15 U/ml and 150-2501 U/ml (normal for both is less than 15 U/ml) and Marsh greater than or equal to 1 and greater than or equal to 2.

Results: For TTG 445 samples were included, and for DGP 432. Using Marsh greater than or equal to 1, for greater than or equal to 15 U/ml, the sensitivity of TTG was 76.4% and specificity was 92.4%; DGP was 57.7% and 86.0%. For 150-2501 U/ml, TTG sensitivity was 30.3% and specificity was 100%; DGP was 22.7% and 97.9%. Using Marsh greater than or equal to 2, for greater than or equal to 15 U/ml, the sensitivity of TTG was 96.9% and specificity was 91.6%; DGP was 71.2% and 85.8%. For 150-2501 U/ml, TTG sensitivity was 40% and specificity was 99.7%; DGP was 28.8% and 97.8%.

Conclusion: TTG outperforms DGP in pediatric patients. For greater than or equal to 15 U/ml, sensitivity is better with TTG for Marsh greater than or equal to 1 and Marsh greater than or equal to 2. In all cases, TTG had greater specificity than DGP. Bioplex TTG performs as well as biopsy in patients with 10x ULN.
TLE1 and BCOR Expression in Pediatric Angiomatoid Fibrous Histiocytoma: Diagnostic Utility and Pitfalls

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³Washington University in Saint Louis, Saint Louis, Missouri; ⁴University of Oklahoma Health Sciences Center, Oklahoma, Oklahoma; ⁵Cincinnati Children’s Hospital, Cincinnati, Ohio; ⁶Rhode Island Hospital, Providence, Rhode Island

Background: Angiomatoid fibrous histiocytoma (AFH) is a rare soft tissue neoplasm, most often arising in the deep dermis and subcutis of the extremities of children and young adults. Diagnosis can be challenging due to its significant morphological spectrum and a lack of reliable immunohistochemical (IHC) markers. Additional IHC markers may be helpful in the evaluation of difficult or atypical cases. Recently, we encountered a challenging case of primary adrenal AFH with diffuse metastases. In the evaluation of this case, to our surprise, both TLE and BCOR demonstrated strong and diffuse nuclear positivity. Prompted by this experience, we evaluated TLE and BCOR expression in AFH.

Methods: Thirty-five pediatric AFHs diagnosed between 2000 and 2017 were retrospectively collected from 6 institutions. The pathologic diagnosis was confirmed by at least two pathologists. Representative sections from each tumor were stained with both anti-TLE1 and anti-BCOR antibodies. An additional 9 cases of other fibrohistiocytic lesions were also analyzed for TLE1 expression. An IHC composite score (score range: 0-9; weak: <4; moderate: 4-6; strong: >6) combining both extent (0, <25%; 1, 25-49%; 2, 50-75%, 3, >75%) and intensity (0-absent; 1-weak; 2-moderate; 3-strong) of nuclear staining was used. IHC staining was performed in a majority of cases in the initial evaluation of lesions. Molecular studies were also performed in a number of cases.

Results: We found that TLE1 was moderately to strongly expressed in 100% of AFHs (63% of cases with a composite score of 9, and 37% of cases with a composite score of 6). In comparison, TLE1 was negative in 5 dermatofibromas; 1 atypical fibrous histiocytoma; and 1 juvenile xanthogranuloma, while demonstrating weak positivity in 1 inflammatory myofibroblastic tumor and 1 atypical fibrous histiocytoma. BCOR immunoreactivity was present in 94.3% of AFHs with 65.7% of cases showing moderate to strong staining and 28.6% of cases showing weak staining. EWSR1 rearrangement was documented in 15 of 16 cases analyzed, including 4 cases with an EWSR1-CREB1 fusion and 1 case with an EWSR1-ATF1 fusion. Ten cases were analyzed by FISH only, thus fusion partners were unknown. AFHs were positive for CD99 (16/16) and variably positive for desmin (16/20), CD68 (18/22), and EMA (6/14).

Conclusion: TLE1 and BCOR are highly sensitive IHC markers for AFH, suggesting potential diagnostic utility in combination with other IHC markers such as CD99 and desmin. This study also illustrates a diagnostic pitfall in differentiating AFH from other tumors such as synovial sarcoma and BCOR sarcoma by using these markers.
Birth Weight: Placental Weight Ratio in Autopsy of Term Stillbirth

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Background: Stillbirth is the fifth leading cause of death worldwide. In this disease category, the term stillbirth is a standard, but unsettling and uncertain designation to the grieving loved ones. Per United States National Vital Statistics report in 2016, complications related to placenta, umbilical cord and fetal membranes and fetal death of unspecified cause are the first and second leading cause of term stillbirths, respectively. We undertook this retrospective study to evaluate the potential parameters such as birth weight: placental weight or fetoplacental ratio (f/p) to gain some insight into the potential mechanism of term stillbirths without specified causes of death in our institution.

Methods: With the approval of Institutional Review Board, 37 term stillbirths were retrieved from the Pathology database from January 2000 – August 2018. Stillbirths from singleton and multiple pregnancies were included. The final autopsy reports were reviewed and the following were tabulated: Final anatomic gestational age, fetal weight, placental weight, cause of death, contributory factors, gross and microscopic findings in the placenta, umbilical cord and fetal membranes. Fetoplacental ratios were derived and standard references were utilized to categorize the fetal weight, placental weight and fetoplacental ratio into small (<10th percentile), appropriate (10-90th percentile) and large (>90th percentile) for gestational age.

Results: The identifiable causes of death in 22 of 37 term stillbirths were categorized into fetal, placental, maternal and fetomaternal hemorrhage. 15 of 37 (40.1%) did not have a specified cause of death (unknown). The f/p ratios for all term stillbirths are summarized in Table 1. Abnormal f/p ratios were identified in 7 and small for gestational age (SGA) placentas were identified in 6 of these 15 cases.

Table 1. Fetoplacental ratio in 37 term stillbirths.

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Fetoplacental ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of term</td>
</tr>
<tr>
<td></td>
<td>stillbirths(%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>15 (40.5)</td>
</tr>
<tr>
<td>Fetal</td>
<td>7 (18.9)</td>
</tr>
<tr>
<td>Placental</td>
<td>7 (18.9)</td>
</tr>
<tr>
<td>Maternal</td>
<td>6 (16.2)</td>
</tr>
<tr>
<td>Fetomaternal hemorrhage</td>
<td>2 (5.4)</td>
</tr>
</tbody>
</table>

Conclusion: While f/p ratio has its limitations (i.e. when both fetus and placenta are large, normal or small), an abnormal f/p ratio (<10th and >90th percentile) can provide insight into potential cause of death or contributory factor in term stillbirths with otherwise unspecified cause. SGA placenta by itself could attribute to adverse pregnancy outcomes. We recommend the evaluation of f/p ratio in term stillbirths.

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Background: Our institution has seen increased sequencing requests for lymphatic and vascular malformations (L-VM), especially using interventional radiology-guided needle biopsies (IR-NB). We assessed the adequacy of limited fresh frozen (FF) and formalin-fixed paraffin-embedded (FFPE) tissue from IR-NB for sequencing.

Methods: We retrospectively analyzed L-VM samples tested on a next-generation sequencing panel from 10/2018-10/2019. We evaluated size, number of cores, lesional content (endothelial component ≥10%), and tissue type (FF or FFPE) tested. We evaluated sequence result as variant detected (positive), no variant detected, or insufficient quantity/quality.

Results:  

<table>
<thead>
<tr>
<th>n, specimens sequenced</th>
<th>IR-NB</th>
<th>Excision/Punch</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>average # cores (range)</td>
<td>6 (1-13)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Maximum dimension of tissue, cm (range)</td>
<td>1.0 (0.5-2.0)</td>
<td>1.6 (0.3-5.6)</td>
<td>1.5 (0.3-5.6)</td>
</tr>
<tr>
<td>FF:FFPE</td>
<td>8:4</td>
<td>4:7</td>
<td>12:11</td>
</tr>
<tr>
<td>≥10% lesion</td>
<td>6/12</td>
<td>7/11</td>
<td>13/23</td>
</tr>
<tr>
<td># without pathologic evaluation</td>
<td>4</td>
<td>2</td>
<td>6/23</td>
</tr>
<tr>
<td># positive sequence result</td>
<td>6/12</td>
<td>3/10</td>
<td>9/22</td>
</tr>
<tr>
<td># positive result AND ≥10% lesion</td>
<td>3/6</td>
<td>3/6</td>
<td>6/12</td>
</tr>
<tr>
<td>average Variant Allele Fraction (range)</td>
<td>0.08 (0.02-0.16)</td>
<td>0.36 (0.5-0.23)</td>
<td>0.10 (0.02-0.23)</td>
</tr>
</tbody>
</table>

Positive variants were detected in HRAS, MAP2K1, PIK3CA (n=3), IDH1 (n=2), and BRAF. One case contained a copy number alteration (partial 5p gain of SDHA and TERT).

Conclusion: The majority of cases (22/23), irrespective of biopsy type, produced DNA quantity/quality sufficient for a molecular result. One FFPE had degraded DNA insufficient for testing. Lesion ≥10% on pathologic assessment was associated with a higher positive rate; however, two samples <10% lesion still had positive results. We conclude that pathologic assessment of L-VM alone is not predictive of positive sequence results and testing should proceed if lesion <10%.
Pediatric Renal Cell Carcinomas: A Single Institutional Study of 20 Cases

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**Background:** According to recent literature, renal cell carcinomas (RCC) in children constitute more than 4-5\% of all renal tumors, surpassing clear cell sarcoma and malignant rhabdoid tumors of the kidney. The aim of this study is to review the characteristics of RCCs in young patients and determine their genetic background.

**Methods:** Cases were identified by screening for RCC in patients < 25 years in our pathology database from 2001 to 2018. H&E and IHC slides were reviewed and additional ancillary studies were performed or reviewed.

**Results:** In total, 20 tumors were identified. The ages ranged from 3 to 23 years (median, 12 years; mean age 12.4 years; M/F 1:1). The mean diameter was 4.8 cm (range, 1.1-11 cm). Patients were known to have renal cystic disease (4), tuberous sclerosis (2), neuroblastoma (1), retinoblastoma (1), glial neoplasm (2), sickle cell trait (1), family history of hereditary leiomyomatosis-RCC (HLRCC, 1) and RCCs (1). Using the WHO classification as a reference, tumors were classified as MiT translocation RCC (30\%), Papillary RCC (15\%), Chromophobe RCC (10\%), Tuberous sclerosis-associated RCC (10\%), Medullary RCC (5\%), Fumarate hydratase RCC (5\%), and Unclassified (20\%). One tumor was diagnosed as a renal papillary tumor.

**Conclusion:** In summary, renal cell carcinomas in young patients are significantly associated with a previous history of renal cystic disease and cancer predisposition conditions. MiT translocation RCCs are the most common. Further genetic testing has been ongoing to delineate fusion partners of TFE3 and characterize the unclassified tumors.
Rhabdomyoblastic Differentiation in Wilms Tumor Portends Worse Prognosis

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Background: Classic triphasic Wilms tumor (WT) can have a heterologous rhabdomyoblastic (RMB) stromal component in approximately 8-18% cases. While favorable WT has excellent overall prognosis, the outcome with RMB differentiation is largely uncertain. With worse prognosis, rhabdomyosarcoma may be synchronous or metachronous with WT, including in Beckwith-Wiedemann and DICER1 syndromes. After index cases of triphasic WT with metastasis of solely the stromal RMB component, we wanted to study our experience of RMB differentiation in WT.

Methods: Cases coded as WT from 2005 - 2019 were pulled and reviewed to select triphasic WT with stromal RMB differentiation. Rhabdoid tumor and primary renal rhabdomyosarcoma were excluded. Slide morphology, IHC, clinicoradiologic data, and follow-up were obtained and analyzed. Anaplasia required nuclear hyperchromasia and enlargement (x3) and atypical mitoses.

Results: Seventeen cases of “Rhabdomyoblastic Wilms” were identified from 69 WT reviewed. There were 7 males, 10 females, age range 4 days to 13 years, and median age was 4 years. All cases had varying proportions of blastema, epithelium, and stroma. RMB component varied from immature round and plump epithelioid cells with eccentric cytoplasm, to maturing spindled cells with cross striations, to fully mature skeletal muscle in one post-treated metastasis. Only two cases (12%, one diffuse, one focal) had anaplasia. IHC on RMB component revealed that all cases were positive for desmin and myogenin (myf4), WT1 (cytoplasmic), focal PAX8, INI1-retained, and negative for keratin and synaptophysin. Nine (53%) cases had metastatic disease to lung>lymph node>inferior vena cava, one adrenal, pancreas, and spleen and one drop metastasis to bladder. Two pure RMB metastases to lung (mature) and bladder (immature/maturing) were in patients without known syndrome. NGS of both WT primary (non-anaplastic, stage II) and RMB metastasis of the latter case revealed striking overlap in chromosome copy number gains (1q, 7, 8, 11, 12, 20) and gene amplifications. Two other patients with lung metastases died of disease (12%), one with 60% stromal component.

Conclusion: Wilms tumor with heterologous rhabdomyoblastic differentiation comprises one-fourth of our WT and portends worse prognosis, approximately half with metastases and rare death from disease. It can be challenging to decipher syndromic-associated rhabdomyosarcoma from metastasis of pure RMB differentiation due to overlapping morphology and phenotype; common chromosome copy number gains suggest a non-germline clonal relationship between primary and metastasis. Even without anaplasia, increased metastases, death, and presence of RMB in the metastasis propose that this component may be better designated as “rhabdomyosarcomatous component” and treated accordingly.
Assessment of Histopathologic and Ultrastructural Features of Wilson Disease in Pediatric and Adult Patients

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Background: Wilson disease (WD) is an autosomal recessive condition caused by a defect in copper-transporting ATPase, which leads to copper accumulation in hepatic parenchyma and subsequent liver and neurodegenerative disease. Histomorphologically, WD in the liver manifests as varied patterns including fatty liver disease, acute and chronic hepatitis and cirrhosis. Initial clinical presentation in most cases is vague and may not be indicative of WD and therefore an accurate and early diagnostic workup may get delayed. Routine light microscopic findings most often generate a differential for potential etiologies. In our experience, electron microscopy (EM) is a valuable diagnostic aid to consider Wilson disease early in the diagnostic workup. This study examines the histologic and ultrastructural features of pediatric and adult WD.

Methods: 24 pediatric and 14 adult WD patients who underwent a diagnostic liver biopsy were analyzed. All cases had a clinically confirmed diagnosis of WD. Review of routine light microscopic slides and archival EM images (where available) was performed by three expert pathologists. Liver disease patterns for steatosis, steatohepatitis, hepatitis and cirrhosis were evaluated.

Results: 24 pediatric cases (≤18 years old), included in the analysis were 16 males, with mean age of 11 (3-18). The 14 adult patients (>18 years old) included 10 males, with mean age of 32 (18-52). Steatotic pattern of liver injury with or without steatohepatitis was observed in most adult and pediatric cases, 71.4% and 62.5%, respectively. Only 3 adult and 2 pediatric patients had microvesicular steatosis. The pattern of fibrosis in all cases was non-biliary and mostly presented at an advanced stage in adults (78.6%). Contrary to adults, 20.8% pediatric cases had no fibrosis. Hepatitic pattern of disease was more prevalent in adults (28.5%). Copper stain was positive in 50% adult cases and only in quarter of the pediatric biopsies. This may be related to the advanced degree of fibrosis. Only 5 out of 14 adult cases and 23 pediatric biopsies had routine EM performed. All cases of pediatric WD showed mitochondrial changes including pleomorphism and dilated cristae but no paracrystalline inclusions (PCI). The most consistent EM finding in adult cases included mitochondrial pleomorphism and fat droplets with 2 of the 5 adult cases showing PCI.

Conclusion: This study evaluated histopathologic features of pediatric and adult WD for the first time. Importantly, it highlights the significance of EM findings, which can play a critical role in guiding a timely workup and management of these patients before they develop advanced hepatic fibrosis or neurological disease.
Chondrosarcoma and Mesenchymal Chondrosarcoma: A Study of 12 Pediatric Cases and Review of the Literature

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Background: Chondrosarcoma and mesenchymal chondrosarcomas are rarely encountered tumors in pediatrics. The majority of chondrosarcomas in this age group are of the conventional or intramedullary type and are characterized as malignant neoplasms composed of cartilage producing tumor cells. Extraskeletal examples of conventional chondrosarcomas have been described. On the other hand, mesenchymal chondrosarcoma represent a unique variant characterized by a biphasic histological pattern composed of small blue cells (primitive chondrocyte stem cells) with islands of well-differentiated cartilage. They are predominantly intraosseous, but are often seen extraskeletal within soft tissue with meninges being the most common location. Here we are presenting a series of various pediatric conventional chondrosarcomas and mesenchymal chondrosarcoma cases.

Methods: With IRB approval we collected de-identified cases form 2000 to 2017, from various institutions, of conventional chondrosarcomas and mesenchymal chondrosarcomas. Age at presentation, gender, location, histological type, grade, stage, genetic mutations, and outcome were obtained.

Results: 8 patients with conventional (Grade 1) chondrosarcoma, 5 males and 3 females ranging in age from 12-17 years old, were identified. 3 of 8 cases had multiple resections of benign osteochondroma before developing chondrosarcoma. 6 patients had lesions involving the extremities (5 lower and one upper) and 2 had lesions involving the pelvic bones. 3 patients showed underlying genetic abnormalities including 13q14, 9p21, and 17p13 loci aberrations. One out of the eight patients died (5 years survival of 87.5%). On the other hand, 4 patients with mesenchymal chondrosarcomas, 3 males and 1 female ranging in age from 4-11 years old, were identified. 3 of the 4 mesenchymal chondrosarcomas involved the maxilla and frontal bone, the forth one occurred in the fibula. All mesenchymal chondrosarcomas were solitary osseous lesion. Two of the four died (5 year survival of 50%). All the patients of the cohort received chemotherapy and radiation therapy.

Conclusion: Chondrosarcoma can develop from osteochondroma and having multiple osteochondromas is a risk factor for developing osteosarcoma. Therefore, patients with multiple osteochondromas should be followed closely with a high index of suspicion for subsequent malignant transformation. Also, small blue cell tumors, especially of the head and neck region, should include mesenchymal chondrosarcoma as a differential. Our cohort of patients with mesenchymal chondrosarcomas, being a younger age group, showed a worse prognosis than expected and confirms the aggressive nature of mesenchymal chondrosarcoma.
TERT Promoter Mutations in Hepatocellular Neoplasms-Not Otherwise Specified (HCN-NOS)

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Background: HCN-NOS (also known as transitional liver cell tumor (TLCT)) is a provisional diagnostic category included in the International Consensus Classification to describe a subset of malignant pediatric liver tumors with equivocal/overlapping histological features of hepatoblastoma (HB) and hepatocellular carcinoma (HCC), and is now being treated as high-risk HB in the on-going Pediatric Hepatic International Tumor Trial (PHITT). It is frequently diagnostically challenging to classify tumors in this category as classical HB or pediatric HCC based on histopathology alone. Telomerase reverse transcriptase (TERT) promoter mutations have been reported to be associated with aggressive clinicopathological characteristics in several cancers. A previous study detected TERT promoter mutations in 2/4 HCN-NOSs but not in any classical HBs (0/43), suggesting that TERT promoter mutation may be a useful molecular marker for HCN-NOS. However, the finding has yet to be confirmed.

Methods: We studied 12 patients classified as HCN-NOS (age range: 4-12.5 years) and 10 patients classified as classical HB (age range: 0.3 to 3.5 years). TERT promoter mutations were assessed by direct Sanger sequencing of genomic DNA from formalin-fixed and paraffin-embedded tumor tissue. TERT protein expression was assessed by immunohistochemistry in 9 HCN-NOSs. Correlations between TERT promoter mutation status and a number of clinicopathological features were analyzed for any significant associations by Pearson chi-square.

Results: TERT promoter mutations were detected in 5/12 (41%) HCN-NOSs including two at the c.1-124C>T (C228T) mutation hotspot, two at the c.1-146C>T (C250T) mutation hotspot and one with a novel c.1-138C>T mutation, a variant of unknown clinical significance. A c.1-146C>T (C250T) mutation was also identified in 1/10 (10%) classical HBs, an HB with a crowded fetal histology from a 3.5 year old male. Eight of 9 HCN-NOSs showed weak (n=3) to strong (n=5) TERT immunostaining. TERT protein expression did not significantly correlate with mutation status. We found no statistically significant associations between the status of TERT promoter mutation and the clinicopathological features in our limited HCN-NOS patient cohort.

Conclusion: Our study demonstrates that TERT promoter mutations are frequent in HCN-NOSs and rare in classical HBs. Further study of the prognostic value of TERT promoter mutations in both HB and HCN-NOS is warranted.
Fine Needle Aspiration (FNA) for Superficial Lymphadenopathy: A Retrospective Analysis of Accuracy and Pathologist Utilization of Ancillary Testing at a Large U.S. Pediatric Hospital

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Background: In patients with superficial lymphadenopathy, studies have shown that FNA can often obviate the need for tissue biopsy. However, such studies have typically focused on general/adult and non-U.S. populations with different disease epidemiology and do not always describe how often patients need a second diagnostic procedure. Additionally, few studies have addressed pathologist utilization patterns for FNA ancillary testing in this setting.

Methods: Lymph node FNA cases (232) were queried by keyword from pathology records (2005-2018) at a tertiary U.S. pediatric hospital and reports screened. Cases were excluded if FNA was performed for an existing neoplasm (65), had a concurrent tissue biopsy (29), or had <60 days of documented clinical follow-up (48), leaving 90 cases for analysis. FNA accuracy was determined by concordance with a subsequent tissue biopsy or clinical follow-up diagnosis.

Results: Lymph node FNAs with on-site evaluation were performed by pathologists (67) or interventional radiologists (23) on 44 males and 46 females of average age 8 years (1-17 years) from various sites (neck 68, submandibular 9, supraclavicular 4, parotid 4, face 2, other 3). Patients were symptomatic for 9 months on average. FNA diagnoses included 55 reactive (polymorphous lymphoid), 10 atypical lymphoid, 10 granulomatous, 7 acute inflammation, 6 lymphoma (or suspicious), and 2 suboptimal. A subsequent similar-site tissue biopsy was performed in 21 patients (23%) on average 8 months after the FNA. Excluding suboptimal cases, FNA overall had a 95% (84/88) concordance rate with the final clinical/tissue biopsy diagnosis. The 4 discordant cases were diagnosed with Hodgkin lymphoma on subsequent tissue biopsy after an initial FNA diagnosis of reactive (2), granulomatous (1), and acute inflammation (1). Most atypical FNAs (8/10) had a final non-neoplastic diagnosis. Although pathologists frequently ordered FNA flow cytometry (on 45/90 overall and 31/55 reactive FNAs), it identified a neoplasm in only 2 cases, both with atypical monotypic smears. Cultures (28/90) were applied mostly in FNAs with granulomatous (8) or acute (6) inflammation but grew organisms in only 4 cases. Culture types employed (aerobic vs. anaerobic bacterial, AFB, fungal) were highly variable; all anaerobic and fungal cultures were negative. A cell block was made in 32 cases, including 19 reactive FNAs.

Conclusion: FNA is very accurate in characterizing superficial lymphadenopathy in children. However, clinicians and patients/families should be advised of the need for a second diagnostic procedure in approximately one-fourth of cases. The potential overutilization of FNA ancillary testing in reactive-appearing smears deserves additional study as it may reduce the cost-effectiveness of FNA relative to tissue biopsy.
Pathologic Characterization of Pediatric Umbilical Lesions and Their Association with Postoperative Complications

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Background: Umbilical lesions in the pediatric population are common and often require surgical excision. In some cases, umbilical lesions may represent an underlying umbilical cord anomaly with a higher rate of postoperative complications. The terminology utilized for the pathologic diagnosis of umbilical lesions is not uniform, and may vary based on institution. The purpose of this study is to review the incidence and pathology of umbilical lesions that have undergone excision and their association with postoperative complications.

Methods: A retrospective review was conducted of patients undergoing excision of an umbilical lesion between 2011 and 2018. All pathology specimens were independently reviewed and classified in the following five categories: fibrous lesion, epidermal inclusion cyst (EIC), omphalomesenteric duct remnant (OMDR), urachal remnant and other. Patient demographics, perioperative data and outcomes were collected and analyzed.

Results: One-hundred and three patients were identified with a mean age of 3.6 years. Excised umbilical lesions were more common in male patients, accounting for 66% of all patients. Fifty-two lesions were classified as fibrous lesions, 32 as EIC’s, 14 OMDR’s, 1 urachal remnant and 4 classified as other. The other category consisted of melanocytic nevi, an infantile hemangioma and an epidermal nevus. The fibrous lesion category was further subdivided into those containing granulation tissue (11), keloid morphology (5), fibrous umbilical polyp (3) and conventional cicatrix morphology (33). OMDR and urachal remnants were associated with a higher rate of postoperative complications as compared to all other umbilical lesions (13% vs 1%, p=0.009).

Conclusion: Fibrous lesions and more specifically a conventional cicatrix was the most commonly excised umbilical lesion, followed by an EIC. OMDR and urachal remnants were associated with a higher rate of postoperative complications. To ensure appropriate post-operative management, pathologists should recognize the presence of gastrointestinal or urachal tissue in these lesions. Standardized pathology terminology of umbilical lesions may additionally allow for optimal communication of the characterization of these lesions.
Diagnosis of Hair Disorders with Scanning Electron Microscopy: An Update

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Background: Hair shaft and bulb disorders may be acquired or congenital. These disorders are often secondary to structural alterations in hair fibers and cuticle. Although, light microscopy may identify certain features in some conditions and syndromes, scanning electron microscopy (SEM) rapidly identifies hair shaft and bulb disorders, which may be the first sign of an underlying syndrome.

Methods: Archives were searched for hair shaft and bulb disorders examined by SEM over a 12 year period. A total of 133 cases were identified.

Results: Pili canaliculi et trianguli (54 cases) is characterized by longitudinal hair shaft grooving, and triangular or heart-shaped cross-section appearance. Autosomal dominant familial inheritance may occur. Associated abnormalities include cataracts, abnormal bone development, alopecia areata and lichen sclerosus. Loose anagen hair (34 cases) is characterized by bending of the hair bulb (hockey stick appearance), and may be associated with coloboma, Noonan syndrome, hypohidrotic ectodermal dysplasia, Ectrodactyly–Ectodermal Dysplasia–Clefting syndrome, trichorhinophalangeal syndrome, Nail–Patella syndrome, neurofibromatosis, trichotillomania, woolly hair, AIDS, and alopecia areata. Pili torti (20 cases) is characterized by hair shaft twisting and flattening with fracturing, leading to unruly thin fragile eyebrows, eyelashes and scalp hair. Associations include non-progressive mental deficiency, sensorineural hearing loss, hypogonadism, ectodermal dysplasia, Menkes and GRACILE syndromes, citrullinemia, and other hair shaft abnormalities. Trichorrhexis nodosa (16 cases) is characterized by hair shaft beaded nodal areas with fraying and fracturing. This disorder may be genetic (arginosuccinicaciduria, citrullinemia) or acquired. Trichoschisis (9 cases) is characterized by impending "clean" hair shaft breaks, and may be associated with trichothiodystrophy (TTD, brittle hair, low sulfur content), an autosomal recessive neuroectodermal disorder (>100 variable features, 8 subgroups, impaired DNA repair).

Conclusion: Scanning electron microscopic examination rapidly identifies features of many hair shaft and bulb disorders and allows for appropriate categorization. Diagnosing these disorders is of importance in guiding clinical evaluation and possible genetic testing, as well as providing early diagnosis of an associated syndrome, anticipating associated medical conditions, and predicting prognosis.
Multinodular Goiter Leading to Diagnosis of Minimally Invasive Follicular Thyroid Carcinoma and DICER1 Syndrome in an 18-year-old Female

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Background: DICER1 syndrome is an autosomal dominant tumor syndrome caused by germline DICER1 mutations. The benign and malignant tumors are typically of pediatric and adolescent onset, and include pleuropulmonary blastoma, cystic nephroma, nasal chondromesenchymal hamartoma, embryonal rhabdomyosarcoma of the cervix, and multinodular goiter, among others. The patient in this case is an 18 year-old female with a five year history of bilateral multinodular goiter and a 2.2 cm nodule in her right thyroid lobe.

Methods: The patient underwent fine needle aspiration of multiple thyroid nodules, followed by total thyroidectomy with routine histology and immunohistochemical stains which were reviewed by three pathologists, including two expert consultations. Subsequently, peripheral blood was analyzed for DICER1 mutations.

Results: The cytology was read as “suspicious for follicular neoplasm.” The total thyroidectomy specimen revealed an encapsulated minimally invasive carcinoma with a predominantly solid pattern, confined to the thyroid, without vascular invasion. The background thyroid tissue showed unusual encapsulated, cystic nodules, some showing papillary hyperplasia and nuclear atypia. These unusual features prompted constitutional genetic analysis for DICER1 mutation and analysis of the patient’s peripheral blood revealed a heterozygous pathogenic mutation in the DICER1 gene.

Conclusion: This previously undiagnosed young adult presented with multinodular goiter and her diagnostic work-up led to diagnosis of minimally invasive carcinoma in the presence of DICER1 syndrome. Due to her diagnostic work-up, this patient will be able to receive appropriate screening for DICER1 associated tumors along with genetic counseling for herself and for her family. Not only does this case represent a rare presentation of a tumor syndrome, it highlights the importance of recognizing the unusual features in the patient’s multinodular goiter that lead to her constitutional testing for DICER1 syndrome.
The Spectrum of Sub-Saharan African Pediatric Pathology: A novel, systematic epidemiological assessment at a national referral hospital in Kampala, Uganda

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Background: There is a dearth of reliable pathologic epidemiologic data from the world’s most underserved countries, particularly in sub-Saharan Africa. No trained pediatric pathologist currently works on the continent south of Egypt and north of South Africa, and cancer surveillance and treatment plans are severely hampered by the lack of accurate diagnostic information. Makerere University’s hospitals serve as a national referral system for Uganda. Previously, pathology records at Makerere were not filed and existed on paper only, rendering epidemiology and research difficult.

Methods: Paper anatomic surgical pathology reports at Makerere University were digitized and entered into a database for the years 2016, 2017 and 2018. Cases for patients aged 18 and under were selected and the diagnosis as rendered by the Ugandan pathologist was recorded. Each case was assigned a category based on its organ system and the diagnostic category; cases were not independently reviewed. Epidemiologic data were calculated.

Results: A total of 2810 cases from 2016-2018 were identified; 423 in patients under 18 (15.1%), with an average age of 10.8 years. Amongst infants, the most common diagnostic category was congenital (15/28 cases, 53.6%), with inflammatory (4, 14.3%), traumatic (3, 10.7%), and malignant neoplasms (2, 7.1%) following. The most common diagnosis was Hirschsprung disease (5, 17.9%). Amongst young children, the most common category was malignant neoplasms (26, 33.3%), with congenital (15, 19.2%), infectious (8, 10.3%) and inflammatory (8, 10.3%), and benign neoplasms (7, 9.0%) following. The most common diagnosis was Wilms Tumor (11, 14.1%). Amongst school-aged children, the most common category were both malignant neoplasms and inflammatory (26, 24.8%), followed by benign neoplasms (21, 20.0%) and infectious (14, 13.3%). The most common single diagnosis was lymphoma (8, 7.6%). For teenagers, the most common category was malignancy (67, 31.6%), followed by benign neoplasms (56, 26.4%), inflammatory (32, 15.1%), and infectious (22, 10.4%). The most common diagnosis was fibroadenoma (23, 10.8%). The most common malignant diagnoses for all ages were lymphomas (32, 7.6%), rhabdomyosarcomas (29, 6.9%), Wilms tumor (12, 2.8%), osteosarcoma (12, 2.8%), nasopharyngeal carcinoma (7, 1.7%), and Kaposi’s sarcoma (5, 1.2%). Conditions less commonly encountered include tuberculosis (24, 5.7%), rheumatic heart disease (7, 1.7%), and ruptured bowel secondary to typhoid (2, 0.5%).

Conclusion: This is the first systemic epidemiologic pathology-based study to be conducted in a sub-Saharan university hospital. Diagnoses as rendered differ greatly from published WHO surveillance data for the region. Implications for treatment are profound.
Evaluating the Anticipated Molecular Alterations for Pediatric CNS Neoplasms Using a Targeted DNA and RNA Sequencing Gene Panel

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Background: Molecular annotation of pediatric brain tumors has become an essential component of pediatric pathology. Providing this information up-front as an integrated diagnosis in our pathology reports gives our clinical counterparts multiple options, as most tumors they treat eventually become chronic diseases. While the genomic landscape of pediatric low-grade glioma is well known, the genomic landscape of pediatric high-grade glioma is still evolving. Molecular testing of brain tumors also occasionally results in alterations that may allude to an underlying cancer predisposition syndrome.

Methods: All pediatric brain tumors from June 25, 2019 to December 12, 2019 were analyzed by a targeted DNA and RNA sequencing gene panel. The results were integrated with the histologic diagnoses as addenda. Additional comments were annotated if (1) the molecular test result was unique or unexpected and (2) if molecular information suggested an underlying cancer predisposition syndrome.

Results: 54 cases in total were submitted that contained adequate DNA and RNA for analysis and significant findings are shown below:

Histologic diagnoses for cases submitted
7 embryonal tumors (6 Medulloblastoma, 1 AT/RT)
10 ependymomas (3 MPE, 2 grade II Ependymoma, and 5 grade III Ependymoma)
2 epithelial tumors (1 Choroid plexus carcinoma, 1 Craniopharyngioma)
29 glial/glioneuronal tumors (1 DNT, 8 Pilocytic astrocytoma, 6 Ganglioglioma, 4 Anaplastic astrocytoma, 1 Pilomyxoid astrocytoma, 1 Anaplastic PXA, 1 Congenital glioblastoma, 1 High grade glioma NOS, 3 Low grade glioma NOS, 3 Low grade glioneuronal tumor NOS
3 Meningioma
1 Schwannoma
1 High grade neuroepithelial tumor
1 Encephalocele with giant cell fibroblastoma-like areas

Of the 29 glial/glioneuronal tumors, 20 contained either a BRAF-KIAA1549 fusion or BRAF V600E mutation. No rare or unusual mutations were seen however rare fusions were observed:

1 GOPC-ROS1 fusion
1 PPP1CB-ALK fusion
1 FAM131B-BRAF fusion

Molecular alterations also suggested an underlying predisposition syndrome in 12 cases
NF2 mutation in 4 cases (3 meningioma, Schwannoma)
NF1 in 1 case (Ganglioglioma)
TP53 mutation in 2 cases (2 Anaplastic astrocytoma)
RB1 mutation 2 cases (Congenital glioblastoma, Low grade glioma)
DICER1 mutation in 1 case (Encephalocele with giant cell fibroblastoma like areas)
TSC1 in 1 case (Pilocytic astrocytoma)
SMARCB1 in 1 case (AT/RT)

Conclusion: Molecular testing of pediatric brain tumors provides essential information for treating oncologists; moreover testing using both a DNA and RNA gene panel often discloses unusual or rarely occurring fusions. Occasional ancillary testing and genetic counseling may be also be indicated as a high frequency of tumors may have abnormalities that suggest an underlying predisposition syndrome.