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PLATFORM I:

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Placental Cell-Specific Extracellular Vesicle (EV) Changes Throughout Gestation Quantitated by Nanoscale Flow Cytometry

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Background: Extracellular vesicles (EVs) are virus-sized lipid-bound vesicles released by cells as either small EVs (eg. exosomes), medium-sized EVs (eg. microvesicles), or larger cell fragments. Others have suggested that placental alkaline phosphatase (PLAP) positive EVs increase with gestational age and may be relatively increased early in pregnancy in women who develop preeclampsia. However, PLAP is not specific for placental EVs and multiplex antibody labeling is required to measure cell-specific events in plasma. We employ nanoscale high resolution flow cytometry to reliably image, count, and isolate cell- and size-specific EVs in banked plasma. The objective of this study was to quantitate placental cell-specific EVs from floating villous syncytiotrophoblast (STB), invasive extravillous trophoblasts (EVTs), and spiral artery plug-cells throughout gestation compared with MRI-based uteroplacental blood flow quantitation and pregnancy outcomes.

Methods: Retrospective study of 351 banked plasma samples collected from 6-32 weeks' gestation and tested using published *International Society of Extracellular Vesicle* approved methods, controls, and validation assays. Cell-specific EV populations were targeted as STB (PLAP/CD66f/CD63), EVT (PLAP/HLA-C/CD63), Plug Cell (PLAP/CD56/CD63), platelet (CD41/CD61/CD9) and endothelial cell (CD31/CD41neg/CD9) internal controls. For scientific rigor, samples were tested in triplicate using a *BD FACSymphony*. Results were reported as counts/ul of plasma and compared between gestational ages and outcomes by ANOVA.

Results: PLAP positive EVs reproducibly changed with gestational age with EVTs comprising the source of most of these events. STB-related EV concentrations decreased with gestation (note: >1um events were excluded by 2500g centrifugation). Spiral artery trophoblast plug-cell EVs fell below EVs increased markedly by 32 weeks'. Platelet EVs peak in the second trimester.

Conclusion: This data suggest that most of the PLAP+ EV events in pregnant plasma arise from EVT, not floating villi. Relationships between cell-specific EV counts and obstetric outcomes is preliminary based on only 28 cases of early onset PET/IUGR and require validation.

Eosinophilic/T-cell chorionic vasculitis: a rare but increasingly common placental lesion that does not appear to recur

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Background: Eosinophilic/T-cell chorionic vasculitis (ETCV) is an incidental finding in third trimester placentas mostly near or at term. It is characterized by the presence of eosinophils and CD3+ T lymphocytes infiltrating single or multiple chorionic vessels and/or stem villous vessels. The etiology and clinical significance is unclear, however it is often associated with villitis of unknown etiology, chronic chorioamnionitis and intravascular thrombi. The incidence and recurrence risk of ETCV is unclear, as is whether incidence and degree of multifocality have been stable over time. The objectives of our study were to (1) characterize the incidence and recurrence risk of ETCV in a large sample of placentas examined by a team of pediatric-perinatal pathologists, and (2) test for temporal and seasonal differences in incidence.

Methods: Twelve years (2010-2022) of placenta pathology reports verified by 8 pediatric-perinatal pathologists in Calgary, Alberta, Canada were retrieved from the Cerner Millennium lab information system, and candidate reports were identified using a Perl script searching for 'eosinophil'. Diagnoses of ETCV were validated in candidate reports by pathologist review. Incidence of ETCV was determined per placenta and per patient, and recurrence risk was calculated among patients who had an initial diagnosis of ETCV and a subsequent placenta examined. Generalized estimating equations were used to examine temporal and seasonal associations with ETCV incidence while accounting for clustering by pathologists.

Results: Over the follow-up period there were 38 127 placentas examined from 34 702 patients. Among these, there were 328 cases of ETCV, for an overall incidence of 86 per 10 000 placentas examined and 94 per 10 000 patients. We received more than one placenta from 46 mothers who had a diagnosis of ETCV. Among these, 15 had a diagnosis of ETCV and at least one subsequent placenta examined, however there were no recurrences. The incidence of ETCV significantly increased 23% per year (Odds Ratio = 1.23, 95% confidence interval: 1.16 to 1.31), from 11 per 10 000 in 2010 to 292 per 10 000 in 2022. This temporal change was observed among all pathologists, and we will provide observations suggesting that multifocality is also increasing over time. No variation was attributable to season.

Conclusion: In a large sample of placentas examined by pediatric- perinatal pathologists, ETCV was relatively rare and did not recur however its incidence steadily increased over a 12-year period.

Immunohistochemical Interferon Gamma Expression in Chronic Intervillositis of Unknown Etiology

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Background: Chronic intervillositis of unknown etiology (CIUE) is a rare cause of recurrent early pregnancy loss. Upregulated interferon gamma (IFNg) expression in syncytiotrophoblast has been shown in a small cohort of high grade CIUE cases suggesting this may play a role in pathogenesis; however, the extent of IFNg expression in this disease remains unclear. This study compares IFNg expression in CIUE to placenta from non-CIUE spontaneous early pregnancy losses and normal early gestations by immunohistochemistry.

Methods: Ethics approval is obtained for a tissue-based study comparing late first to mid second trimester archival placental tissue from high grade CIUE (CHG; 18 cases), low grade CIUE (CLG, 12 cases), elective termination (NP, 13 cases), euploid spontaneous pregnancy losses (ESPL, 18 cases), and aneuploid spontaneous pregnancy losses (ASPL, 17 cases). 2 cases of third trimester pregnancy loss associated with placental COVID19 infection are included as examples of chronic histiocytic intervillositis of a known etiology. Atypical mycobacterial granuloma is employed as a positive control. IFNg expression is assessed on whole tissue sections by automated immunostaining and graded semiquantitatively by multiplying staining intensity (3+ same as control staining; 2+ between 3+ and 1+; 1+ just visible at 200x; 0+ no staining visible at 200x) by distribution (1 <5%; 2 5-50%; 3 >50%) for a score from 0-9. Data are compared by 2-tailed independent t-test.

Results: Focal positive syncytiotrophoblast IFNg staining is seen in 35% of CHG, 42% of LGH, and 4% of non-CIUE cases (0% NP, 0% ESPL, 12% ASPL). IFNg expression in CIUE placenta is characterized by luminal orientation in syncytiotrophoblast while the ASPL cases show non-luminally oriented cytoplasmic staining. IFNg is not expressed in other placental cell types. The average IFNg grade is 1.3 (range 1-9) for CHG, 0.7 (range 1-2) for CLG, 0 for NP and ESPL, and 12% (range 1-2) for ASPL. There is a statistically significant difference between CIUE and non-CIUE placenta ($p = .0015$) but not between CHG and CLG (overall $p = .42$; positive cases only $p = .17$). Occasional lymphocytes in the maternal intervillous space strongly express IFNg and these appear more abundant in areas with more prominent histiocytic inflammation. COVID19 placental tissue does not express IFNg but IFNg-positive lymphocytes are present in the intervillous space.

Conclusion: A subset of high grade and low grade CIUE express IFNg suggesting this represents a distinct subgroup of CIUE. IFNg expression appears to correlate with grade implying IFNg expression is related to inflammation intensity although the difference is not statistically significant. Future studies of CIUE, particularly those assessing therapies, may benefit from separate assessment of IFNg positive cases.

Pathologic Lesions Attributed to Shallow Implantation and Decidual Hypoxia Correlate With Maternal Vascular Malperfusion and Related Obstetric Conditions

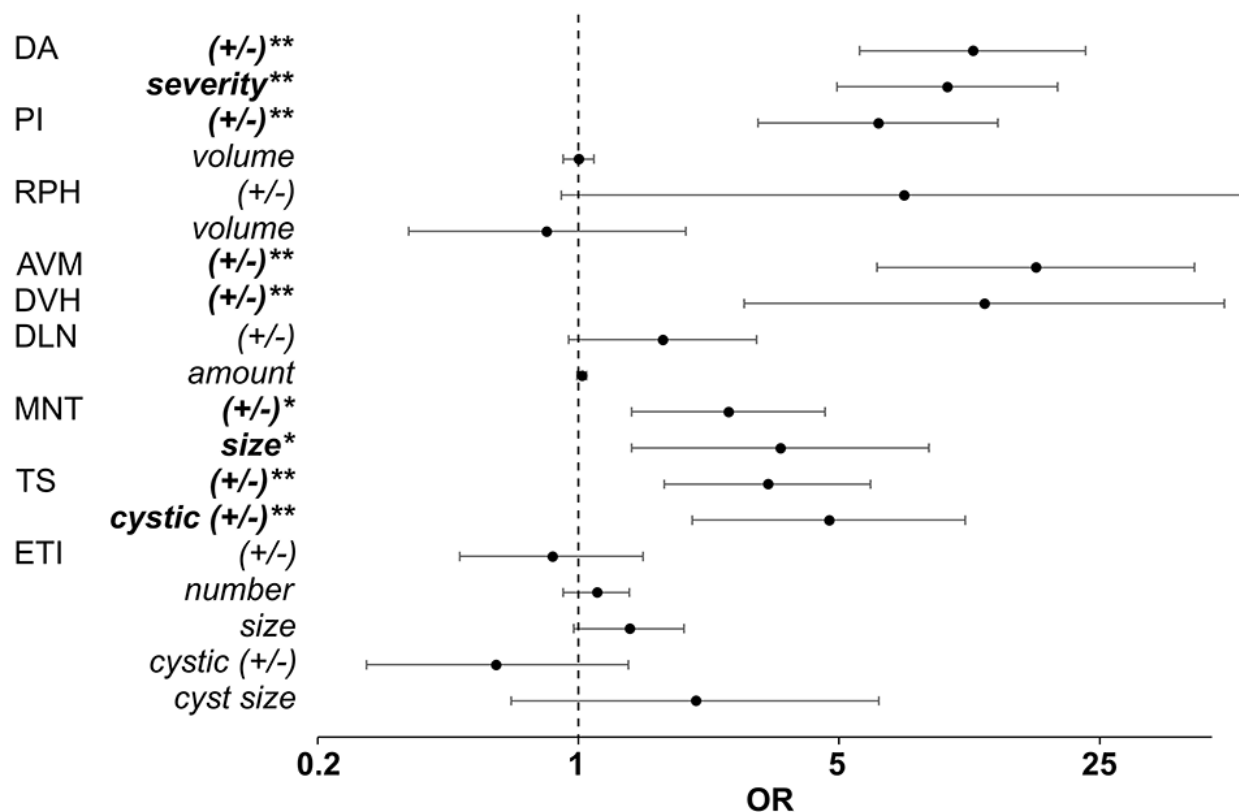
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Background: Maternal Vascular Malperfusion (MVM) encompasses a constellation of findings related to impaired maternal-placental circulation. Findings associated with placental hypoxia and/or shallow implantation, not fully endorsed by the Amsterdam guidelines as part of the MVM spectrum, include: decidual laminar necrosis (DLN), basal plate multinucleated implantation trophoblast (MNT), and excess trophoblast islands of the placental disc (ETI), including the so-called “trophoblast septa” (TS). We explored the association between these lesions and MVM as well as related obstetric morbidities.

Methods: Placentas reviewed at our institution with a diagnosis of MVM within a 5-year period were reviewed to document DLN, MNT, ETI and TS. Established features of MVM were also recorded including placental weight, accelerated villous maturation (AVM), decidual arteriopathy (DA), retroplacental hematoma (RPH), placental infarct (PI) and distal villous hypoplasia (DVH). An equal number of maternal age- & GPA-status-matched controls without MVM diagnosis or documented MVM-related disorders were chosen. Obstetric morbidities including hypertension (HTN), pre-eclampsia (PET) including with severe features (sPET), gestational diabetes (GD) and fetal growth restriction (FGR) were recorded, and their relationship with individual placental features was analyzed.

Results: 200 cases were included (100 in each MVM and control groups). DLN was more frequent in MVM vs control placentas (43 vs 31%), although the difference was not statistically significant. DLN extent (estimated as % of the membrane roll involved) showed statistical correlation with PI volume and AVM (when present), as well as with GD. Prevalence and linear extent of MNT (in mm along basal plate) were significantly higher in MVM vs control placentas (47 vs 26%). Moreover, MNT size was positively correlated with HTN, PET and sPET. Interestingly, MNTs were inversely associated with GD. TS were significantly more common in MVM placentas than controls (43 vs 19%) and correlated with HTN. There was no statistical correlation between ETI and MVM or related morbidities. FGR was not significantly more frequent in placentas with DLN, MNT or TS. In turn, placental hypoplasia, DA, AVM and DVH correlated with several obstetric morbidities including FGR.

Odds Ratios for MVM with various placental lesions



** $p < 0.001$ * $p \leq 0.05$

Conclusion: MNTs and TS often occur in placentas with pathologic characteristics of MVM. They also correlate with obstetric morbidity in the current gestation, underscoring the importance of reporting these lesions and, potentially, considering them part of MVM. DLN is related to placental infarction, further supporting the theory of acute hypoxia as an initiating event for this lesion, possibly related to MVM. No association between ETIs and MVM or associated obstetric conditions was found.

Comparison of Placental Pathology Reports Finalized by Generalist Pathologists Versus Perinatal Pathology Expert: A Call to Action

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Background: Preterm birth (PTB) is a leading cause of neonatal morbidity and mortality, and examination of the placenta plays an important role in explaining individual patient outcomes and guiding our understanding of the underlying mechanisms leading to preterm. Due to a limited number of perinatal pathologists, many preterm placentas are reviewed by general pathologists. The aim of this study was to compare diagnoses between generalist pathologists (GP) and a perinatal pathologist (PP).

Methods: This is a secondary analysis of selected placentas from a large study of placental pathology in recurrent PTB, which included placentas from spontaneous PTB examined between 2009-2018. We included all placentas that were originally signed out by a GP and then subsequently re-examined by a specialist PP as part of the parent study. All pathology diagnoses were coded into 4 categories (acute Inflammation (AI), chronic Inflammation (CI), fetal vascular malperfusion (FVM), maternal vascular malperfusion (MVM)) based on histologic lesions identified in the original reports for the GP and second review by the single PP. Kappa statistics were used to assess inter-observer agreement.

Results: 331 placentas were included, representing cases finalized by 17 GPs. Median gestational was 34.4 weeks and ranged from 20.1 weeks to 36.9 weeks. The prevalence of all 4 placental diagnostic categories was higher for the PP. Kappa was highest for AI at 0.50 (weak agreement). However, there was no agreement for MVM (kappa 0.063), CI (kappa 0.0026), and FVM (kappa -0.018). Amongst common lesions such as infarction and fetal acute inflammation there was minimal to weak agreement (kappa 0.39-0.49). Chronic basal deciduitis with plasma cells was also common, present in PP review in 32% of cases but only 0.3% of cases finalized by GP (kappa 0.006). The more uncommon lesions had no to minimal agreement: chronic villitis (kappa 0.3), retroplacental hematoma (kappa 0.14), fetal vascular thrombi (kappa -0.017) and diffuse chorioamnionic hemosiderosis (kappa 0.18).

Conclusion: There is no agreement between GP and PP when assessing placental pathology other than AI, and weak agreement even for AI. These findings are a call to action to implement educational efforts and structural/organizational changes to improve consistency of placental pathology reporting.

A Revised Placental Diagnostic Template Responds to Clinicians' Need for Outcome and Recurrence Data

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Background: A recurring theme in placental pathology is the difficulty clinicians have in reading placenta pathology reports. A second theme is the irrelevance that many diagnoses have in clinical practice. One recent editorial in a major OB/GYN journal argues that far too many placentas are submitted for pathology largely due to tradition. In order make placental pathology reports more clinically useful, we composed a template for diagnoses with comments that add clinical relevance.

Methods: An academic obstetrician composed and sent a survey to community women's health care clinicians to screen their impressions of current placental pathology reports. Subsequently, two perinatal pathologists constructed a new placental diagnostic template in which major placental diagnoses were categorized according to the Amsterdam Placental Workshop Group Consensus Statement. Additional diagnoses were included that were not within the Working Group Statement. Concise footnotes were written to place each diagnostic category into clinical context with respect to recurrence and poor outcome.

Results: The survey included 69 clinicians. A majority of clinicians (63%) found placental reports not useful; however, a majority (65%) would modify care of the patient's next pregnancy if they were told that a certain placental finding predicted future pregnancy risk. Major placental diagnostic categories in the revised placental diagnostic template included maternal and fetal acute inflammatory responses; and chronic inflammatory, maternal vascular malperfusion, fetal vascular malperfusion, and thrombotic lesions; and six additional diagnostic categories including placenta accreta spectrum, hypervascular lesions, perivillous fibrinoid lesions, delayed villous maturation, abnormal membranous insertion, cord pathology, and meconium deposition.

Conclusion: There is a need for pathologists to provide categorized placental diagnoses and explanatory comments that provide the value-added information related to poor outcomes and recurrence. This new placental diagnostic template will be used for several months and a follow-up clinician survey will gauge clinicians' opinion of its utility.

PLATFORM II:

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Increase in pediatric thrombotic amputations during the SARS-CoV-2 pandemic: manifestation of macrophage activation syndrome

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Background: This study was inspired by the sudden unexplained increase in pediatric amputations during the SARS-CoV-2 pandemic.

Methods: With appropriate IRB approval, pathology files were searched for all amputations from Jan 2017 to May 2022. All available slides on thrombotic amputations of 2020 and 2021 were reviewed. Additional immunohistochemical stains for CD3, CD20 and CD163 were performed. Medical records were reviewed.

Results: Total yearly amputations from 2017 to 2020 ranged from 17 to 19; they increased to 26 in 2021. They remained stable in etiologies such as oncologic, diabetic, traumatic, congenital anomalies, and infectious, but rose for thrombotic/ischemic etiology. Between Jan 2020 and Oct 2021, 10 children (M:F 1:1), ranging from 36 days to 19 years in age underwent lower extremity amputations secondary to large vessel thrombosis (compared to 2 in 2017 and 0 for 2018-2019). All except 3 were previously healthy. Five were African American, 3 Caucasian, and 2 Hispanic. At admission, 4 were SARS-CoV-2 positive (RT-PCR), 2 showed elevated SARS-CoV-2 IgM antibody suggestive of recent exposure/infection, and 4 were negative or non-tested. One was vaccinated 6 months prior (2 doses) with reported recent COVID-19 exposure. Four had co-existing viral positivity including Influenza B, parainfluenza virus type 3, Parvovirus B19, and HSV-1. Six had secondary bacterial sepsis during the course of illness. At presentation, 8/10 had cardiac, renal and/or respiratory failure; 6/10 showed all three. Seven were started on ECMO at or immediately after presentation. Elevation in BNP was seen in 7, CRP in 9, and ferritin in 7. All were diagnosed with compartment syndrome and underwent multiple fasciotomies before amputations. Tissue was available as thrombectomy, amputation specimens, and autopsy. Admission to amputation interval ranged from 2 days to 3.5 months. Three patients died of multiorgan failure. Histopathology review showed microthrombi (10/10), medium/large vessel thrombi (10/10), intravascular macrophages (9/10), extravascular macrophages (9/10), vasculitis (6/10), and myositis (5/10). Histologic lympho- and hemophagocytosis was seen in 7/10 cases. Immunostains showed scant T and B cells with abundance of CD163 positive foamy macrophages. No such cases have been seen since Oct 2021 to May 2022.

Conclusion: Sudden unexplained rise in pediatric amputations was noted during the SARS-CoV-2 pandemic. Histopathology showed large, medium and small vessel thrombosis. Clinical elevation of inflammatory markers in conjunction with histologic abundance of macrophages and occurrence of lympho- and hemophagocytosis suggests macrophage activation syndrome as a likely thrombotic etiology.

Effectiveness of Sigmoidoscopy in Disease Surveillance of Pediatric Patients with Ulcerative Colitis

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Background: Ulcerative colitis (UC) is a remitting and relapsing disease with a desired outcome of endoscopic and histologic remission after initiation of disease course altering therapy. Patients who achieve endoscopic and histologic remission have significantly lower risk of relapse and other disease complications like dysplasia or carcinoma compared to those with persistent endoscopic or histologic activity. Disease surveillance usually involves repeat colonoscopy at regular intervals. Recent data in adults suggests findings in the left colon on flexible sigmoidoscopy can be highly predictive of concordant findings in the right colon and overall disease activity. To date, similar research and data are lacking in children. The efficacy of flexible sigmoidoscopy compared to colonoscopy warrants investigation in disease surveillance of children.

Methods: A retrospective chart review of children with UC seen at UHCMC from 2010-2021 with two colonoscopies approximately one year apart was performed. Patient demographics, clinical disease activity, laboratory values and endoscopic findings were collected. The Mayo Endoscopy Score was used to assess the findings in the right and left colon. Histologic findings were assessed using the Geboes Score. Histologic remission was defined as complete mucosal normalization or chronic architectural changes in the absence of neutrophilic infiltrate (Grades 0 and 1, respectively). Weighted kappa correlation was used to assess concordance between left and right colon endoscopic and histologic findings. Receiver operator curve (ROC) analysis was used to assess the accuracy of left-sided findings in predicting right-sided findings.

Results: Of the 57 patients, 31 (54.4%) were male, mean age at diagnosis was 13.5 ± 4.1 years, and 53 (93%) were Caucasian. Mean time between index and repeat colonoscopy was 11.3 months. On repeat colonoscopy, endoscopic findings on the left colon moderately correlated with findings on the right colon [$\kappa = 0.53$ (0.30-0.75)], similarly histologic findings for the left and right colon had a moderate correlation [$\kappa = 0.41$ (0.17-0.64)]. Area under the ROC curve (AUC) was 0.89 for left sided findings to predict endoscopic remission anywhere in the colon and 0.98. Longer intervals between the index and follow-up colonoscopy did not affect odds of concordance.

Conclusion: Endoscopic and histologic disease activity in the left colon correlated with right sided findings on repeat assessment. Left sided disease activity was highly accurate for identifying endoscopic and histologic disease remission. This data suggests that flexible sigmoidoscopy may be considered in place of colonoscopy in surveillance of children with UC. The feasibility of this less invasive procedure can positively impact the patients and ultimately the healthcare system.

Immunological and Pathological Features of Multiple Intestinal Atresia Accompanied By Severe Combined Immunodeficiency Patients with TTC7A Mutations

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Background: Mutations in the *tetratricopeptide repeat domain 7A (TTC7A)* gene have recently been shown to cause early-onset inflammatory bowel disease or multiple intestinal atresia accompanied by severe combined immunodeficiency (MIA-SCID), a disease with a median survival of less than 12 months of age without curative treatment. Allogeneic hematopoietic stem cell or bone marrow transplantation has been a treatment option.

Methods: Here we report the immunological and pathological findings of two patients with TTC7A-deficiency, who are alive up to 2 years post bone marrow transplant. Both patients presented a severe gastrointestinal disease (severe diarrhea, gut failure, and multiple intestinal atresia) and immunodeficiency soon after birth. The molecular sequencing studies confirmed loss of function mutations of *TTC7A* gene. With close monitoring and bone marrow transplants, both patients are currently alive 2 years post- bone marrow transplant.

Results: The flow cytometry of peripheral blood showed the decrease in absolute concentration of total natural killer, T, B, naïve CD4 and CD8 T cells, consistent with severe combined immunodeficiency. In addition, there was a relative increase in cells with a well-differentiated effector memory phenotype as defined by patterns of CD45RO, CCR7, and CD57 expression. Majority of the B cells showed atypical transitional or innate-like B1 phenotype: CD10+(lo), CD38hi, CD20+, CD5dim, CD21lo, IgM+(hi), and IgD+. In one of the two patients, there was a modest increase in CD4+ CD8+ double positive T cell population.

Post-bone marrow transplant, none of the patients could be weaned off parenteral nutrition. A gastrointestinal biopsy was performed on one patient, which showed persistent intestinal inflammation and abnormal epithelial features including loss of apicobasal polarity of the epithelial cells, and occasional epithelial apoptosis. Although a possible graft-versus-host-disease (GVHD) was proposed, the morphological findings and no response to GvHD treatment are most consistent with an ongoing primary disease as opposed to GVHD phenomenon.

Conclusion: Our findings suggest the variable spectrum of immunology presentation associated with TTC7A deficiency. Although BMT is a feasible treatment for TTC7 deficiency, that may improve the immune dysfunction, it did not correct the epithelial phenotype or enteral tolerance.

Pathologic Findings in Post-Transplant Biopsies from Pediatric Lung Transplant Patients Requiring Retransplantation for Bronchiolitis Obliterans

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Background: Pediatric lung transplantation is an option for end-stage lung disease in children. The main challenge for graft longevity is bronchiolitis obliterans (BO). The aim of this study is to systematically study pathologic changes in lung post-transplant biopsies among pediatric patients who developed BO with allograft failure requiring retransplantation.

Methods: Retrospective chart review of pediatric patients requiring 2nd lung transplant for BO between 2003-2018 was performed. Slides were reviewed and scored by consensus between two pathologists (NCS, DLR) with acute cellular rejection (ACR), small airway inflammation and BO documented. Presence of eosinophils was scored as 0=none, 1=rare and 2=easily identified. Presence of neutrophils (PMN) was scored as 0=none-rare, 1=<10/hpf, 2=10-30/hpf and 3=>30/hpf. C4d immunohistochemistry was scored as 0=negative, 1=focal (<50%) and weak, 2a=diffuse (50%) and weak, 2b=focal and strong and 3=diffuse and strong.

Results: Ten patients required a 2nd transplant and 71 specimens were adequate for review, including 67 transbronchial (TBB) and 4 wedge biopsies (WB). M:F ratio was 1:4. The most common indication for 1st transplant was cystic fibrosis (n=6). Median age at 1st transplant was 11.8 yearsrs (0.4-19.1) and median time to 2nd transplant was 5.3 yearsrs (2-14). BO was diagnosed in 8 patients (11 TBBs and 3 WB). The remaining 2 patients overall had 10 TBBs; 3 had no small airways and 2 (one each patient) had intraalveolar foamy macrophages. BO was confirmed on explant. ACR was seen in 17 biopsies from 6 patients; of these, 10 biopsies from 4 patients were ≥A2. Concomitant small airway inflammation was noted in 9 biopsies from 5 patients, most commonly in association with higher grades of ACR. Isolated small airway inflammation was rarely seen (4 biopsies). Overall, 16 biopsies had no small airways present, all TBBs. Eight patients (80%) had tissue eosinophilia (score=2) in 14 biopsies. Rare eosinophils were noted within 3 months post-transplant in 6 patients (75%). Eight patients (80%) had tissue neutrophilia (score ≥2) in 19 biopsies, seen in 6 patients (75%) within 3 months of transplant. Of the biopsies with neutrophilia, 7 had C4d IHC stains available; 4 had score 0, 2 had score 1, and 1 had score 3. Four patients required early retransplantation, within 3 years. Of these, 1 had repeated episodes of ≥A2 ACR stemming from the first post-transplant biopsy, and 2 had early-onset and persistent tissue eosinophilia and/or neutrophilia.

Conclusion: Female patients are disproportionately represented among lung transplant pediatric patients who develop BO with allograft failure. Allograft eosinophilia and neutrophilia, even when mild, may predict worse outcomes and should be actively sought.

Immunohistochemical Assessment of Methylation in Primary Bone Tumours

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Background: As the two most common primary bone tumours, osteosarcoma (OS) and Ewing sarcoma (EWS) possess starkly different genetic profiles, from non-recurrent and heterogenous changes in OS to classic translocations often involving EWSR1 in ES. Chemotherapy after diagnosis is often followed by resection of the primary mass with assessment of treatment response, which may intensify therapy. Although 5-year overall survival rates have been static for those with localized disease, development of metastases predicts markedly worse outcomes for both of these bone tumours. Identifying potential biomarkers that may impact event-free and overall survival could support future clinical trials attempting to improve the outcomes in OS and EWS. Relative to conventional genetics, little is known about epigenetics in OS and EWS. We aim to characterize the methylation and phosphorylation status in OS and EWS using common histone markers found in primary diagnostic biopsies.

Methods: We created tissue microarrays (TMA) from 58 OS and 45 EWS cases that were identified with a primary diagnostic biopsy tissue block available from 2002-2020. Clinical charts were reviewed to evaluate each patient's demographics, post-therapy response, development of metastatic disease, and overall survival. Control tissues including bone marrow and other tumours were included in the TMAs. We evaluated 5 histone H3 residues using immunohistochemistry, including H3K27me3, H3K9me3, H3K4me3, H3S10T11phospho, and H3S28phospho. We utilized a dichotomous scoring system, with either low (<50%) or high (>50%) nuclear staining of tumour cells.

Results: For OS, diagnostic biopsies that showed low H3K27me3 nuclear staining were associated with poor treatment response (<90% necrosis) at the time of definitive excision ($P < 0.05$). Although interesting trends were seen, there were no other significant associations between pre-treatment biopsy H3K27me3 immunoexpression and overall or event-free survival. Immunoexpression of all other antibodies for OS and EWS did not yield any significant results.

Conclusion: In this pilot study, we identified H3K27me3 as a potential biomarker in OS diagnostic biopsies which may predict a poor neoadjuvant response, while other histone residues for OS and EWS provided no further diagnostic or prognostic value at this time. Although further studies with a larger patient cohort are needed to explore the role of H3K27me3 in OS, these encouraging results support the expanded evaluation for risk stratification of other histone modification markers.

Undifferentiated Embryonal Sarcoma of the Liver: A Molecular Genetic Study of Six Cases Highlighting Chromosomal Instability and Recurrent Biallelic Inactivation of TP53

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Background: Undifferentiated embryonal sarcoma of the liver (UESL) is a rare and aggressive liver malignancy. The genomic alterations of UESL are largely unexplored, though TP53 mutations and translocations involving 19q13.4 containing the chromosome 19 microRNA cluster (C19MC) have been described. The aim of this study is to explore and better define the genomic landscape of this neoplasm.

Methods: Parallel chromosomal microarray analysis and DNA- and RNA-based targeted deep sequencing were performed on UESLs diagnosed and treated at our institution from January 2018 to June 2022.

Results: Six patients (median [range] age, 10.5 [0.8–18.3] years; 3 females) were assessed. Most patients presented with abdominal pain. The median tumor size was 19.7 cm (range: 7.5–25 cm). Three tumors had ruptured capsules. All tumors revealed sheets of spindle or stellate shaped cells with inconspicuous nucleoli and ill-defined cell borders in a myxoid background, along with scattered bizarre multinucleated giant cells and eosinophilic hyaline globules. Five of 6 patients underwent gross surgical resection of the tumor followed by chemotherapy; the three patients with tumor rupture also received whole abdominal radiation. One patient had UESL diagnosed on biopsy and was receiving neoadjuvant therapy prior to definitive resection. Clinical follow-up data were available for 5 patients (median duration: 14 months; range: 2–42 months) and all patients were alive.

Molecularly, 4 of 6 tumors revealed *TP53* mutations (two missense, one frameshift, one truncation) along with extremely complex chromosomal gains and losses affecting every autosome, including loss of heterozygosity (LOH) ($n = 3$) or deletion ($n = 1$) of 17p, which contains *TP53*. In addition, all four tumors showed segmental chromosomal gain in 19q13.42-q13.43, which includes C19MC. A fifth tumor harbored *TP53* missense mutation in a subset of neoplastic cells (variant allele frequency 9%) without LOH/deletion of 17p. Instead, this tumor demonstrated LOH in 3q27.1q29, gain and homozygosity in 11p11.2. The remaining (youngest) patient had both UESL and mesenchymal hamartoma of the liver identified in the hepatectomy specimen. Interestingly, the UESL showed no genetic alterations at all. Other variants of unknown clinical significance that were each detected once included *ABL1*, *CDKN2A*, *DICER1*, *FGFR2*, *MET*, and *PAX5*. High-level gene amplification of *MYCN* and *CDK4* was seen once, in the same tumor. No clinically significant RNA fusions on our panel were identified in any tumor.

Conclusion: Biallelic inactivation of *TP53* and widespread chromosomal instability including segmental chromosomal gain in 19q13.42-q13.43 are frequent molecular alterations in our cohort of UESL.

PLATFORM III:

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Improvements to a pediatric and perinatal autopsy service with use of an integrated lab informatics system and mobile workstations

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Background: Performance of an accurate pediatric or perinatal autopsy requires documentation of many body weights/measures and comparison to reference ranges. Historically at our institution, this was a multi-step laborious process whereby autopsy values were initially hand-written, checked against printed reference ranges, and entered into the lab informatics system (LIS) at a later date. We sought to digitally integrate autopsy reference ranges into our LIS and use mobile Workstations-On-Wheels (WOWs) for real-time data entry to increase efficiency, reduce errors, and improve staff satisfaction.

Methods: Iterative improvements were made as follows: paper reference ranges were standardized by group consensus, then entered into excel spreadsheets; reference ranges, selectable by age/gender and linked to discrete components, were built into the LIS with real-time flags for out-of-range values; WOWs with LIS access were stationed at each autopsy table. The number of autopsy values and reference ranges requiring documentation per case and the number and complexity (number of genders/ages) of reference ranges requiring review were recorded pre- versus post- implementation. Staff satisfaction was assessed by interview.

Results: Prior to digital improvements, an autopsy performed at our facility required the documentation of 37 pediatric or 48 perinatal patient weights/measures, followed by equal numbers of reference ranges, written then entered in the LIS, totaling 148 pediatric or 192 perinatal values per case. Each autopsy required review of 44 pediatric or 41 perinatal reference ranges, stratified by 2 genders and 30 postnatal or 33 prenatal age ranges. Due to the time-consuming nature of this work, it was often completed after release of the body, precluding correction of potential errors. Following implementation of digital improvements, all patient weights/measures are entered directly into the LIS at the time of autopsy. For each case, patient age and gender are selected within the LIS, generating the appropriate reference range for each value and alerting the staff if there is an out-of-range value. No hand-written documentation of patient values, review of reference range charts, or manual recording of reference ranges by staff are required. On average, this represents a 4-fold reduction in the number of data points requiring staff documentation. Staff report a marked reduction in time spent on these tasks (several hours vs. <30 minutes), increased satisfaction, and reduced errors.

Conclusion: The implementation of integrated reference ranges within the LIS and introduction of WOWs to our pediatric and perinatal autopsy service has resulted in significantly increased efficiency, accuracy, and staff satisfaction.

FOXF1 Mutations in Three Fetuses with Patchy Distribution of Intrapulmonary Shunt Vessels: Insight into Progression of Alveolar Capillary Dysplasia with Misalignment of the Pulmonary Veins (ACDMPV)

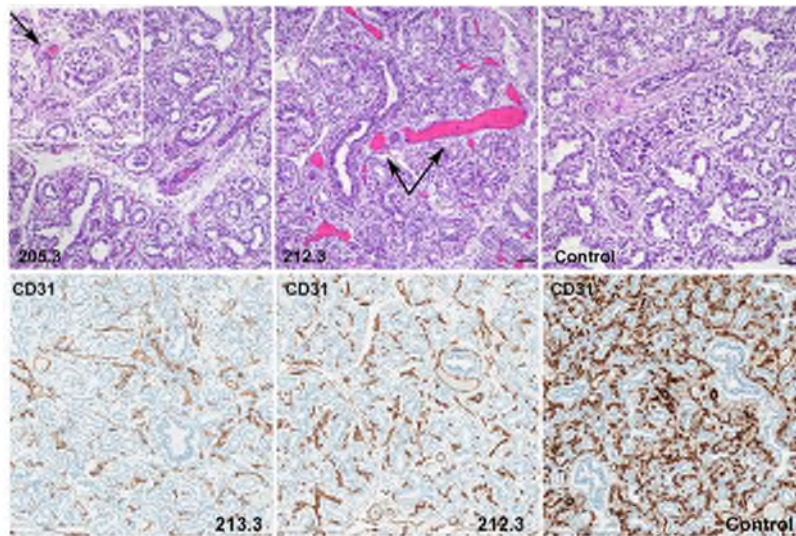
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Background: ACDMPV is a rare lung developmental disorder named based on distinctive abnormalities of the vasculature and acinar units. The presence of intrapulmonary bronchial shunt vessels (“misaligned veins”) adjacent to small pulmonary arteries is required for pathologic confirmation of ACDMPV and invariably accompanied by a hypertensive arteriopathy, reduced/centrally placed septal capillaries and frequent arrest in lung development. Most infants are born term, present with persistent pulmonary hypertension of the newborn, and die within the first month of life despite aggressive support. Few cases of delayed presentation and longer survival have been described, some demonstrating patchy versus uniform intrapulmonary vascular shunts on histology. Heterozygous SNVs or CNV deletions involving FOXF1 and/or its lung-specific enhancer on chromosome 16q24.1 have been found in 80-90% of histologically-verified ACDMPV patients who commonly (50-80%) have extrapulmonary malformations. Forkhead box F1 (FOXF1), is a mesenchymal transcriptional factor essential for lung development, mediating lung angiogenesis and alveolarization.

Methods: In this report, we detail the pulmonary pathology in three unrelated fetuses terminated at 21 weeks gestation for multiple anomalies, later found to have heterozygous CNV deletions encompassing *FOXF*, *FENDRR*, and their enhancer. We use immunohistochemistry to highlight the vasculature, which is compared to age-matched cases with similar anomalies. Our findings add to the rare descriptions of ACDMPV in fetal life.

Results: All fetuses had multiple anomalies involving the cardiac, gastrointestinal, and genitourinary systems. ACDMPV was not suspected on initial histologic evaluation by experienced pediatric pathologists. Lung weights were normal for gestational age and an arrest in acinar development was not apparent. Upon receiving the molecular results, in two cases (213.3, 205.3) the presence of intrapulmonary shunt vessels was retrospectively seen in few bronchovascular bundles (insert image). Shunt vessels (arrows) were more readily detected in case 212.3, a historical case from over 20 years ago. While none of the cases showed significant arterial hypertrophy, all exhibited a notable reduction in capillaries compared to controls with similar cardiac anomalies, seen on CD31 immunostaining.



Conclusion: These cases show that a diminished capillary bed is a more consistent feature of ACDMPV in fetal life than the presence of shunt vessels or arterial changes. Detailed characterization of cases of ACDMPV in the second trimester suggests that a primary defect in FOXF1 mediated angiogenesis results in progressive intrapulmonary vascular shunting and hypertensive pulmonary arteriopathy, more prominent in the term neonatal lung.

Assessing time of death according to microscopic stillbirth features: a cluster analysis approach

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Background: Previous studies identified microscopic changes of isolated fetal tissues and placental pathologies associated with different time of death to delivery of stillborn fetuses. These studies used identifiable time of death for validation, which is reliant upon last known fetal movement or heartbeat. The objective of this study was to utilize unsupervised machine learning to identify which features were associated with clinically-relevant interval from time of death to delivery. This method will allow for grouping of relevant variables without bias of subjective last fetal movement (i.e. grouping based on pathologic features alone).

Methods: Data were derived from the Stillbirth Collaborative Research Network (SCRN), which collected comprehensive pathologic data from singleton stillbirths delivered at 5 geographic regions in the US from 2006-2009. Lesions were characterized according to standard protocols published previously. Stillbirths included in these analyses had complete internal postmortem examination. Cause of death (COD) was identified according to Initial Causes of Fetal Death Evaluation (INCODE). Features were chosen a priori based on previous literature for entry into k-modes cluster analysis (clusters according to most common features within groups), including loss of nuclear basophilia, villous karyorrhexis, chorionic plate vasculopathy, calcifications of basal surface, and umbilical cord degeneration.

Results: A four-cluster solution was derived (Table 1). Parturients in Cluster 1 were likely to report reduced fetal movement and have placental anomaly-related stillbirth COD. Stillbirths in Cluster 2 were more likely to display severe maceration (Grade IV-V) and placental COD; parturients within this group were also likely to endorse reduced fetal movement. Cluster 3 was associated with obstetric-related COD; stillbirths in this cluster displayed numerous basophilic changes in contrast to Cluster 4. Cluster 4 was associated with intrapartum death and displayed no basophilic changes.

Table 1: Comparisons across clusters

		Cluster 1 (n = 100)	Cluster 2 (n = 104)	Cluster 3 (n = 25)	Cluster 4 (n = 150)	p-value
Maternal history	Age (years)	27.2 ± 6.7	28.4 ± 6.7	29.3 ± 7.0	27.2 ± 6.7	0.3
	Race (minority)	33 (33.0)	41 (39.4)	12 (48.0)	66 (44.0)	0.3
	Ethnicity (Hispanic)	36 (36.0)	38 (36.9)	9 (36.0)	47 (31.3)	0.8
	Education (years)	13.5 ± 3.2	12.9 ± 2.9	13.5 ± 2.3	12.5 ± 3.0	0.046
	Pre-pregnancy diabetes	7 (7.6)	4 (4.0)	1 (4.0)	9 (6.5)	0.8
	Pre-pregnancy hypertension	13 (14.1)	13 (13.0)	4 (16.0)	19 (13.7)	0.9
Prenatal history	Had prenatal care	89 (97.8)	93 (93.0)	25 (100.0)	124 (89.2)	0.04
	Reduced fetal movement	64 (69.6)	69 (70.4)	11 (44.0)	52 (38.0)	<0.001
	Alcohol in pregnancy	34 (37.0)	38 (38.0)	8 (32.0)	58 (41.7)	0.8
	Smoking in pregnancy	14 (15.2)	15 (15.0)	2 (8.0)	34 (24.5)	0.1
Stillbirth findings	Weight (g)	1493 ± 1181	1338 ± 1212	1216 ± 1021	1292 ± 1203	0.4
	Sex (female)	47 (47.0)	48 (46.2)	10 (40.0)	63 (42.0)	0.8
	Maceration grade IV-V	8 (8.0)	37 (35.6)	2 (8.0)	6 (4.0)	<0.001
Cause of death*	Obstetric	28 (28.0)	26 (25.0)	17 (68.0)	80 (53.7)	<0.001
	Intrapartum	7 (7.0)	2 (1.9)	6 (24.0)	45 (30.2)	<0.001
	Cord abnormality	25 (25.0)	26 (25.0)	5 (20.0)	17 (11.4)	0.012
	Placental abnormality	69 (69.0)	60 (57.7)	11 (44.0)	69 (46.3)	0.003
Loss of nuclear basophilia	Renal cells - isolated	<u>57 (57.0)</u>	0 (0.0)	12 (48.0)	12 (8.0)	<0.001
	Renal cells - majority	17 (17.0)	<u>101 (97.1)</u>	6 (24.0)	9 (6.0)	<0.001
	Hepatocytes - isolated	11 (11.0)	2 (1.9)	<u>24 (96.0)</u>	5 (3.3)	<0.001
	Hepatocytes - majority	11 (11.0)	2 (1.9)	<u>24 (96.0)</u>	5 (3.3)	<0.001
	Myocytes - inner	<u>85 (85.0)</u>	<u>100 (96.2)</u>	7 (28.0)	5 (3.3)	<0.001
	Myocytes - outer	<u>83 (83.0)</u>	<u>89 (85.6)</u>	1 (4.0)	1 (0.7)	<0.001
	Bronchial epithelium	46 (46.0)	<u>86 (82.7)</u>	2 (8.0)	7 (4.7)	<0.001
	Gastrointestinal tract - majority	<u>96 (96.0)</u>	<u>104 (100.0)</u>	<u>18 (72.0)</u>	14 (9.3)	<0.001
	Adrenal glands - majority	94 (94.0)	103 (99.0)	11 (44.0)	16 (10.7)	<0.001
Placental and umbilical cord findings	Villous karyorrhexis	27 (27.0)	<u>68 (65.4)</u>	<u>13 (52.0)</u>	16 (10.7)	<0.001
	Chorionic vasculopathy	26 (26.0)	36 (34.6)	<u>15 (60.0)</u>	35 (23.3)	0.001
	Disc calcifications	4 (4.0)	3 (2.9)	0 (0.0)	2 (1.3)	0.5
	Umbilical cord degeneration	19 (19.0)	18 (17.3)	3 (12.0)	12 (8.0)	0.042

P-values <0.05 are considered significant (bold) according to Chi-Square analysis across clusters. Underlined values are those identified via k-modes cluster analysis as most common within clusters. All values given as mean ± standard deviation or number (percent). *According to INCODE evaluation (assigns cause of fetal death when postmortem evaluation is available).

Conclusion: These clusters represent distinct, clinically-relevant groups of stillborn fetuses separated according to microscopic postmortem changes. Cluster 4 may represent intrapartum stillbirths; cluster 3 may represent stillbirths due to obstetric causes with more time from fetal death to delivery than cluster 4; cluster 2 may represent extended time from fetal death to delivery; and cluster 1 may represent a group with fetal death to delivery interval between cluster 1 and cluster 3. Future analyses will externally validate these findings according to known time periods from fetal death to delivery. This information may be of utility for pathologists in determining time of fetal death and in testifying in medicolegal cases.

Tapering of the Ductus Arteriosus in Stillbirth: Correlations with Placental Pathology and Cause of Death

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Background: The ductus arteriosus (DA) is an important vascular shunt between the pulmonary artery and aorta in fetal life which allows blood from the right heart to enter the systemic circulation and bypass the fetal lungs. The DA diameter typically is uniform and equal to the pulmonary artery diameter. In fetal autopsies the DA sometimes appears tapered with a distal diameter less than the proximal diameter, but this phenomenon is not well-studied in the literature.

Methods: We searched our Pathology database for stillborn fetal autopsies examined between Jan 2017 and Jan 2022 where a tapered DA was described. For each autopsy case identified, the closest (in time) age-matched (± 1 -2 weeks gestation) control fetal autopsy without tapering of the DA reported was selected for a 1:1 matching of cases and controls. We abstracted demographic, clinical, and pathology data from the autopsy and placental pathology reports. Autopsy data included heart weight, DA diameter, cause of death (COD), and evidence of fetal hypoxia in the brain, liver, and bone marrow. Placental pathology included evidence of umbilical cord abnormalities, acute inflammation, chronic inflammation, fetal vascular malperfusion (FVM), and maternal vascular malperfusion (MVM).

Results: We identified 50 cases with tapered DA and 50 matched controls. Gestational age at delivery ranged from 18 weeks to 38 weeks. Maternal and fetal demographic characteristics did not differ significantly between cases and controls. COD related to placental pathology was common in cases and controls; however, COD determined to be related to an umbilical cord accident/FVM was significantly more prevalent in the cases versus controls. Signs of hypoxic stress in the fetal tissues did not differ between cases and controls. A DA ratio (distal diameter/proximal diameter) was calculated in 31 autopsies where DA measurements were provided in the autopsy report. The values ranged from 0.29 – 0.67 with mean of 0.487. A ratio <0.5 was present in 10/31 (32%). In a subanalysis, DA ratio <0.5 was associated with a higher prevalence of COD related to placental insufficiency/MVM than DA ratio ≥ 0.5 (50% vs 9.5%, $P=0.022$ Fisher's Exact test). Cord accident/FVM occurred as a COD in 40% of those with DA ratio <0.5 and 48% of those with DA ratio ≥ 0.5 .

Conclusion: Tapering of the DA is seen in stillborn fetuses with COD related to both FVM and MVM. Our findings suggest that pathologic conditions which affect either fetal or maternal blood flow in the placenta, can alter blood flow within the fetus and potentially reduce the blood flow into the systemic vasculature. This may occur through mechanisms affecting forward flow such as obstruction of umbilical blood flow or mechanisms leading to high systemic vascular resistance as expected in MVM.

Macroscopic lesions of maternal and fetal vascular malperfusion in stillborn placentas: results from the Stillbirth Collaborative Research Network

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Background: Lesions of maternal vascular malperfusion (MVM) and fetal vascular malperfusion (FVM) are common in placentas associated with both stillbirth and live birth. The objective of this study was to identify lesions present more commonly in stillborn placentas and which findings are most indicative of MVM and FVM in stillborn placentas without microscopic pathologic evaluation. Such information is valuable when microscopic evaluation is not available, especially in low-resource settings.

Methods: Data were derived from the Stillbirth Collaborative Research Network (SCRN), which collected comprehensive pathologic data from singleton stillbirths and livebirths delivered at 5 geographic regions in the US from 2006-2009. Lesions were identified according to standard protocols published previously and categorized as either MVM or FVM according to the Amsterdam Placental Workshop Group Consensus Statement and macroscopic “umbilical cord at risk” findings. Lesion frequency was compared between stillbirths and live births. Multivariate logistic regression was used to determine the odds of stillbirth with macroscopic findings of MVM or FVM.

Results: Of those in the SCRN study, 595 stillbirths and 1,305 live births were analyzed. There were more individuals of minority race in the live birth sample (71.6% in livebirths versus 37.1% in stillbirths, $p < 0.001$) and those who delivered stillbirths had a higher average body mass index (26.4 ± 7.0 in live births versus 27.5 ± 7.2 in stillbirths, $p = 0.001$); otherwise, there were no demographic or health history differences in the two groups. FVM lesions (85.2%) were marginally more common in stillbirths compared to MVM lesions (81.3%). Macroscopic findings of both MVM and FVM were more common in stillbirths versus livebirths ($p < 0.001$). These lesions were more common in stillbirths versus preterm livebirths and in stillbirths versus term livebirths as well ($p < 0.001$). Odds ratios of macroscopic MVM and FVM lesions for stillbirth, adjusted for gestational age at delivery, maternal race (minority), ethnicity (Hispanic), age, and history of hypertension or diabetes, were 1.48 (95% CI 1.30 - 1.69) and 1.34 (95% CI 1.18 - 1.53), respectively.

Table 1: Macroscopic lesions of maternal and fetal vascular malperfusion

Lesion	Live birth (n = 1305)	Stillbirth (n = 595)	p-value*
Any FVM lesion	774 (59.3)	496 (85.2)	<0.001
All macroscopic umbilical cord findings	630 (48.3)	357 (61.3)	<0.001
Marginal insertion	158 (12.1)	117 (20.1)	<0.001
Velamentous insertion	19 (1.5)	25 (4.5)	<0.001
Length of unprotected cord (cm)	0.13 +/- 1.09	0.47 +/- 2.13	<0.001
Furcate insertion	36 (2.9)	9 (1.6)	0.12
Length of unprotected cord (cm)	0.08 +/- 0.46	0.15 +/- 1.28	0.8
Knots			
True	13 (1.0)	6 (1.0)	0.9
False	184 (17.3)	68 (23.3)	0.02
Single umbilical artery	28 (2.2)	41 (7.2)	<0.001
Increased cord coiling (>3 coils/10 cm)	323 (26.2)	178 (33.2)	0.003
Nuchal cord entanglement	38 (20.0)	35 (50.7)	<0.001
Any MVM lesion	856 (65.6)	473 (81.3)	<0.001
All macroscopic findings	570 (43.7)	370 (63.6)	<0.001
Placenta small for gestational age	511 (39.8)	313 (54.8)	<0.001
Retroplacental hematoma	78 (6.1)	126 (22.7)	<0.001

FVM = fetal vascular malperfusion. MVM = maternal vascular malperfusion.

*Comparison between stillbirths and live births, Chi-Square test or Mann-Whitney test. P-values ≤0.003 considered significant according to Bonferroni correction.

Conclusion: Macroscopic features of MVM and FVM are associated with higher odds of stillbirth versus live birth even when controlled for gestational age and maternal factors. This information is useful for pathologists when microscopic examination is not available.

Placental lesions associated with stillbirth by gestational age, according to feature importance: results from the Stillbirth Collaborative Research Network

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Background: Previous studies have identified placental lesions commonly found in placentas associated with stillbirth across a range of gestational ages (GAs) at delivery. The objective of this study was to build upon this information by using measures of feature importance to identify lesions associated with stillbirths at different GAs.

Methods: Data were derived from the Stillbirth Collaborative Research Network (SCRN), which collected comprehensive pathologic data from singleton stillbirths delivered at 5 geographic regions in the US from 2006-2009. GAs at stillbirth were categorized as: <28 weeks (extreme preterm birth [PTB]), 28-33'6 weeks (early PTB), 34-36'6 weeks (late PTB), >37 weeks (term). Lesions were identified according to standard protocols published previously and compared across GA categories. We then identified and ranked the most discriminating and/or correlated placental features, as well as those that were similar across GA ranges, using an advanced mapping technique, Kernel Principal Covariates Regression (KPCovR).

Results: Of those enrolled in SCRn, 445 stillbirths had complete placental data for these analyses, which included 210 (47.2%) extreme PTB, 85 (19.1%) early PTB, 62 (13.9%) late PTB, and 88 (19.8%) term births. Lesion comparisons are shown in Table 1; the following lesions were significantly different across ranges: acute funisitis, acute umbilical arteritis; acute chorionic vasculitis; diffuse terminal villous immaturity; parenchymal infarct; retroplacental hematoma; accelerated villous maturity; nucleated fetal red blood cells; placental disc hemorrhage, syncytial knots, and trophoblast proliferation; decidual vasculopathy; and multifocal amniocytes, calcifications, and meconium. When KPCovR was applied, the first principal covariate indicates there are four lesions of those listed above (acute funisitis, nucleated fetal red blood cells, reactive amniocytes, and meconium) that distinguish GA ranges among all stillbirths.

Table 1: Lesions according to gestational age

	Extreme PTB	Early PTB	Late PTB	Term	p-value*	Correlation with 1st Kernel Principal Covariate**
Acute funisitis	28 (13.3) ^y	2 (2.4)	1 (1.6)	2 (2.3)	<0.001	
Acute umbilical arteritis	10 (4.8) ^w	0 (0.0)	1 (1.6)	0 (0.0)	0.030	
Acute chorionic vasculitis	22 (10.5)	1 (1.2)	2 (3.2)	4 (4.5)	0.012	
Diffuse terminal villous immaturity	6 (2.9)	3 (3.5)	8 (12.9) ^w	5 (5.7)	0.022	
Parenchymal infarct	44 (21.0)	37 (43.5) ^y	16 (25.8)	20 (22.7)	<0.001	
Retroplacental hematoma	52 (24.8) ^w	17 (20.0)	9 (14.5)	5 (5.7)	0.001	
Accelerated villous maturity	40 (19.0) ^y	28 (32.9)	23 (37.1) ^w	0 (0.0)	<0.001	
Nucleated fetal red blood cells (RBCs)	45 (21.4) ^y	29 (34.1)	28 (45.2)	31 (35.2)	0.001	
Placental disc Hemorrhage	83 (39.5) ^w	26 (30.6)	15 (24.2)	19 (21.6)	0.009	
Syncytial knots	22 (10.5)	29 (34.1) ^w	8 (12.9)	25 (28.4)	<0.001	
Trophoblast proliferation	45 (21.4)	16 (18.8)	4 (6.5) ^w	9 (10.2)	0.012	
Decidual vasculopathy	41 (19.5)	19 (22.4)	5 (8.1) ^w	7 (8.0) ^w	0.009	
Multifocal						
Reactive amniocytes	16 (7.6)	6 (7.1)	14 (22.6) ^w	32 (36.4) ^w	<0.001	
Calcifications (disc and/or umbilical)	1 (0.5)	3 (3.5)	0 (0.0)	7 (8.0) ^w	<0.001	
Meconium	41 (19.5)	29 (34.1)	16 (25.8)	43 (48.9) ^w	<0.001	

*Chi-Square difference test; p<0.05 significant. **Represented as correlation coefficient (standard error); significant for absolute value <0.3. ^yPost-hoc analysis indicates significantly higher/lower relative to all other ranges. ^wPost-hoc analysis indicates significantly higher/lower relative to 1-2 other ranges. Lesions not significant: thrombi of or disrupted fetal vessels, chorionic vascular degeneration, chorioamnionitis, chorangioma, chorangiomatosis, chorangiosis, chorionic or disc karyorrhexis, acute umbilical phlebitis, umbilical vascular lesion, punctate or gross umbilical hemorrhages, true knot, single umbilical artery, umbilical degeneration, irregular disc lobulations, disc inclusion bodies, deciduitis, amniotic bands, disc fibrin deposition, avascular villi, terminal villous hypoplasia, intravillous thrombus, diffuse villous fibrin deposition, intervillitis, acute or chronic villitis, abnormal membrane insertion, abundant blood clots, or multifocal edema, hemosiderin, amnion nodosum, or necrosis.

Conclusion: There are distinct placental lesions present across GA ranges in stillbirths; these lesions are identifiable using comparative analyses and sophisticated feature selection. Further validation of these data using such methods may be able to identify lesional changes across gestations that relate to fetal morbidity and mortality.

POSTERS

Resident Recruitment Award Recipient

A case of Apert syndrome with tracheal cartilaginous sleeve: Demonstration of wintergreen oil tissue clearing technique for tracheal malformation visualization.

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Clinical information. Female fetus of a 44 year old mother, G2P0A1, found to have multiple malformations (cloverleaf skull, facial dysmorphism, syndactyly, cardiac anomaly) on ultrasound. Therapeutic termination was done by injection of intracardiac lidocaine and subcutaneous fetal fentanyl at 24 5/7 weeks gestational age.

Pathological assessment. Pertinent external findings: The head is abnormally shaped with hypertelorism, frontal bossing, flat nose and nasal bridge, wide open mouth, thin lips, exophthalmos, low-set ears and cleft palate. The limbs are normally formed, but the hands and feet show bilateral total cutaneous syndactyly of four digits and opposable triphalangeal thumbs and big toes.

Pertinent internal findings: **Cardiovascular System.** The pulmonary venous return to the heart is to the right and left atrium respectively, but there is agenesis of ductus venosus with the umbilical vein connected directly to the right atrium. **Respiratory System.** The laryngobronchial tree shows absence of cartilaginous rings and replacement by a tracheobronchial cartilaginous sleeve (single cartilaginous tubular structure), highlighted by toluidine blue staining and wintergreen oil tissue clearing (Figure 1A-C) and by histomorphology of tertiary bronchi (Figure 1D-E). The lung lobation is abnormal with a single left lung lobe. The hepatobiliary system is unremarkable. **Gastrointestinal system.** There is partial malrotation of the small bowel. The genitourinary, endocrine and hematopoietic systems are unremarkable. **Central Nervous System.** The transverse suture is fused with enlarged and widening of median suture. Microscopy shows extensive malformation involving the caudate nucleus and the adjacent germinal matrix. The two structures are not clearly separated, with large islands of partially mature neurons resembling caudate nucleus present in the germinal matrix and conversely, numerous ectopic islands of germinal matrix extending into the caudate nucleus and beyond into the internal capsule. In the medulla, the pyramids are small and internally rotated.

Genetics. A QF-PCR chromosomal microarray done by amniocentesis were normal, after which a comprehensive developmental disorder NGS panel was ordered and revealed an heterozygous sequence variant in the *FGFR2* gene, designated c.755C>G, which is predicted to result in the amino acid substitution p.Ser252Trp. This variant has been documented to be pathogenic for Apert syndrome^{1,2}. This variant is classified as pathogenic and occurs both in familial and sporadic cases. Targeted testing for the *FGFR2* c.755C>G (p.Ser252Trp) variant in the biological relatives was found negative.

Interpretation/Discussion. The *FGFR2* c.755C>G (p.Ser252Trp) heterozygous variant, in association with the findings of craniosynostosis, midface hypoplasia and syndactyly of the hands is diagnostic of Apert syndrome. Other mutations in *FGFR2* are known to cause other disorders including Crouzon syndrome, Pfeiffer syndrome, Antley-Bixler, Beare-Stevenson cutis gyrata Jackson-Weiss syndrome, LADD syndrome, Saethre-Chotzen syndrome, Bent bone dysplasia syndrome and Axenfeld-Rieger anomaly³. Tracheal cartilaginous sleeve (TCS) consists in vertically fused tracheal cartilaginous rings coalescing in a solid cylinder that can extend from the subglottis down to the carina or bronchi. It can either be complete or have a posterior membranous septum and can be found as often as in 70-80% of cases of craniosynostosis syndromes such as Apert, Crouzon, Pfeiffer and Goldenhar syndromes⁴. The mortality rate is over 90% at 2 years in patient with TCS⁵. Therefore, while it can be diagnosed by endoscopy, it is often diagnosed post-mortem. Landing and Wells initially described this “dissection-staining-clearing” methods in which wintergreen oil (oil of Gaultheria or methyl salicylate) can be used to clear soft tissue after staining with

toluidine blue to visualize tracheobronchial malformations⁶. Briefly, after fixation and dissection, the trachea is placed in 0.25% toluidine blue/70% ethanol for 24-48hr, then differentiated by progressive wash from 70% ethanol to absolute ethanol. Ethanol is then exchanged for xylene, and the soft tissue of the trachea is then cleared by immersion into methyl salicylate, which is also used to preserve the specimen. This method, which stains cartilage blue and soft tissue light blue/green, allows to visualize tracheal and other cartilaginous malformation more distinctly than with histology alone and is particularly useful in the setting of congenital syndromes and malformations (Figure 1 F).

Conclusion. We here present a case of Apert syndrome associated with tracheal cartilaginous sleeve. We highlight the cartilage abnormality with the use of wintergreen oil technique, which unambiguously demonstrates a circumferential and continuous cartilaginous sleeve from the subglottis to the bronchi. This staining technique is easy to perform, and combined with histological examination, provides further details on tracheal malformations and thus can help to further classify these defects.

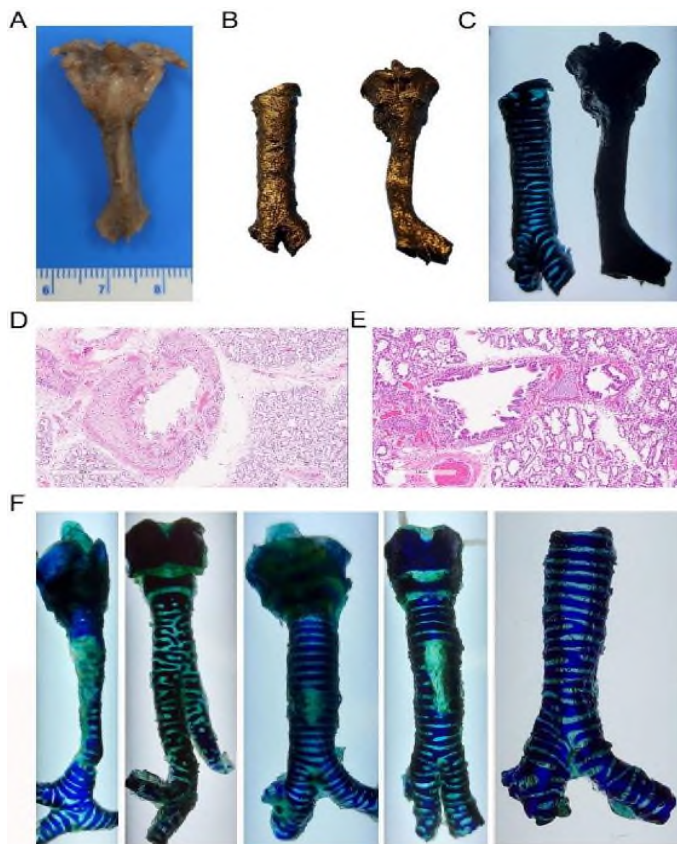


Figure 1. A. Dissected trachea prior to fixation (current case). B-C. Fixed, cleared and stained tracheas with (C) and without (B) transillumination (left: normal, right: tracheobronchial cartilaginous sleeve, current case). D. Tertiary bronchi, current case. E. Tertiary bronchi, normal case of similar gestational age. F. Demonstration of wintergreen oil technique; from left to right; trachea bronchus with tracheomalacia, incomplete tracheal duplication⁷, central anterior cartilage defect (iatrogenic) (x2), normal trachea.

Resident Recruitment Award Recipient

Histiocytic sarcoma with NF- κ B pathway alterations in a 13 year old female

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Introduction

Histiocytic sarcoma is a high-grade myeloid neoplasm that has only rarely been reported in children. In both the pediatric and adult populations, these tumors typically demonstrate alterations in the RAS-MAPK pathway, the PI3K pathway, or the tumor suppressor gene CDKN2A, and in some cases carry a molecular signature which suggests transdifferentiation from another hematolymphoid malignancy. Here, we report a case of histiocytic sarcoma in a pediatric patient with no history of leukemia or lymphoma. Our case highlights the challenge – commonly encountered in pediatric pathology – of integrating clinical history, histology, and complex molecular findings into the diagnosis of a rare disease when little research has been dedicated to its presentation, diagnosis, or treatment in children.

Case Report

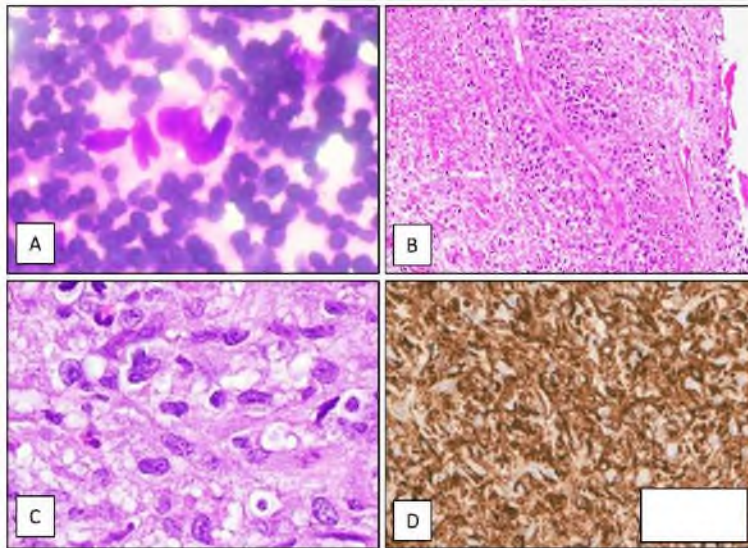
A 13 year old female presented to the emergency department with fever and bulky right-sided cervical, supraclavicular, and axillary lymphadenopathy. She also described three months of persistent abdominal pain and diarrhea; these symptoms had initially been attributed to acute gastroenteritis, but had not improved with antibiotics, and were now associated with an unintentional weight loss of almost fifty pounds. Imaging demonstrated masses suggestive of conglomerate lymphadenopathy in the right neck, right axilla, anterior mediastinum, and pericardiophrenic region; masses in both lungs and the right kidney; and destructive osseous lesions in the sternum, right humerus, sacrum, and pelvis.

An excisional biopsy of the right neck mass (presumed lymph node) was performed, and lesional tissue was sent for intraoperative touch preparation and frozen section. The touch preparations showed few large plump-to-spindled cells with irregular nuclear contours, nuclear hyperchromasia, and abundant pale cytoplasm in a background of blood elements. The frozen sections showed sheets of the same pleomorphic cells (characterized by irregular and folded nuclei with occasional grooves, conspicuous nucleoli, and abundant vacuolated cytoplasm) with a mixed inflammatory infiltrate including focally prominent eosinophils. The lesional cells were observed to infiltrate into skeletal muscle and demonstrated prominent angiocentricity with large intervening areas of necrosis. No lymph node tissue was seen.

Permanent sections recapitulated the histologic findings seen on frozen section, and a panel of immunohistochemical stains was performed. The lesional cells were positive for CD163, CD68 (focal), CD4, and CD45; and were negative for ALK, CD20, CD3, S100, CD1a, CD23, CD21, MUM1, CD30, CD15, and MPO. EBER ISH was also negative. Ki-67 demonstrated a proliferation index of approximately 30% (focally up to 50%). Flow cytometry and cytogenetic studies were noncontributory due to poor specimen viability. Next generation sequencing (DNA/RNA) revealed variants of possible significance (Tier 2) in the NF- κ B pathway genes NFKBIA (p.Gln111*), NFKBIE (p.Tyr254fs), and SOCS1 (p.Met1fs); and in the class 1 major histocompatibility complex component gene B2M (p.Ser16fs). Germline testing was also performed on the patient's saliva, and showed none of these mutations.

Given this constellation of histologic, immunohistochemical, and molecular genetic findings, a final diagnosis of histiocytic sarcoma was rendered.

There is no standard therapy for histiocytic sarcoma in children. The patient was treated initially on an anaplastic large cell lymphoma protocol and later switched to a B cell lymphoma protocol (including rituximab) after the molecular results were finalized.



Touch preparation (A, 400x) showing rare atypical histiocytyoid cells; frozen section (B, 200x) showing tumor cells with prominent angiocentricity in a background of necrosis; permanent section (C, 200x, H&E) showing sheets of atypical histiocytyoid cells with scattered mixed inflammatory cells. The tumor cells were positive for CD163 (D, 200x)

Discussion

Histiocytic sarcoma is a clinically aggressive malignancy of mature myeloid-derived tissue histiocytes. Like many hematolymphoid neoplasms, it is diagnosed more frequently in adults but demonstrates a bimodal age distribution, with peaks at 0-29 years and 50-69 years (1). Patients may present with localized disease in the skin, soft tissue, gastrointestinal tract, or central nervous system; however, disseminated disease is common and is uniformly associated with a grim prognosis (2-6). In both children and adults, histiocytic sarcomas have been reported in association with preexisting hematolymphoid neoplasms (such as B- or T-lymphoblastic leukemia, in children; or follicular lymphoma, in adults), but most cases appear to arise *de novo* and the clinical suspicion for a histiocytic or even hematolymphoid process may be low at the time of initial biopsy (7-10).

Histologically, histiocytic sarcomas display overt features of malignancy, including marked pleomorphism, necrosis, mitotic activity, and soft tissue invasion. Most show histology that is at least suggestive of histiocytic differentiation, with abundant eosinophilic cytoplasm and peripherally-located nuclei with prominent irregular folds. Still, the pathologist's differential diagnosis is broad and – depending on the clinical presentation and histologic features present – may include other histiocytic processes (such as Langerhans cell histiocytosis or follicular dendritic cell sarcoma); other soft tissue sarcomas; large cell lymphomas; poorly differentiated carcinoma; and even melanoma. Immunohistochemical stains for markers such as CD163, CD68, and/or lysozyme are required to confirm histiocytic lineage. CD1a and langerin – which are definitionally negative in histiocytic sarcoma – may be used to exclude Langerhans cell histiocytosis, while CD23 – also negative in histiocytic sarcoma – can help to exclude follicular dendritic cell sarcoma. Likewise, other hematolymphoid markers, cytokeratins, and melanoma markers may be included depending on the particular histologic features identified at biopsy (2, 11).

Molecular testing is also becoming a standard component of the workup for histiocytic sarcoma, often by next generation sequencing. While a particular genetic signature is not required to make a diagnosis of histiocytic sarcoma, molecular test results may provide substantial treatment benefit for patients; for example, identification of a canonical BRAF(V600E) mutation – which has been reported in a subset of histiocytic sarcomas – may prompt treatment with selective kinase inhibitor therapy (12). Recent studies have suggested that other MAPK pathway mutations, which are common among a

variety of histiocytic proliferations, are also frequently seen in histiocytic sarcoma and may provide even more potential therapeutic targets for this historically difficult-to-treat malignancy (13).

A 2019 molecular study of 28 histiocytic sarcomas by Shanmugam et al. showed mutations in MAPK pathway genes (such as KRAS, NRAS, and BRAF) in 57%, PI3K family mutations (such as PTEN, MTOR, or PIK3R1) in 21%, and single-gene deletion of the tumor suppressor CDKN2A in 46% (14). Cases within this series which were diagnosed in the context of a pre-existing B-cell malignancy were more likely to show aberrant somatic hypermutation and NF- κ B pathway mutations than their de novo counterparts, though neither of these findings were specific for secondary (transdifferentiated) histiocytic sarcoma. The results of this study reinforce the findings of Chen et al. in 2009, who reported a high frequency of clonal immunoglobulin gene rearrangement – classically considered a marker of lymphoid neoplasia - even in some de novo histiocytic sarcomas (15).

While these two studies represent some of the larger investigations into the molecular pathogenesis of histiocytic sarcoma to date, neither focused on the pediatric population, and each included only two children (age 12 and 16 in the former; and age 8 and 12 in the latter). Histiocytic sarcoma – already rare in adults – is even less common in children, and much of the available literature consists of small case series or autopsy case reports in which complex molecular testing was not performed. While research has shown that classically “lymphoid” molecular alterations can be seen even in de novo histiocytic sarcomas, caution must be exercised in applying data collected from medically-complex older adults to otherwise previously healthy children. The sequencing results in our case showed several somatic NF- κ B pathway alterations, and once these results were finalized, the clinical team at our institution opted to treat the patient with a B cell lymphoma protocol to cover the possibility (no matter how uncertain) of a transdifferentiated lymphoid malignancy. Ideally, more systematic sampling of this patient’s multifocal disease would be performed, though the desire for a comprehensive diagnosis must be balanced against the patient’s tenuous clinical status and poor prognosis regardless of any additional diagnostic information.

This case highlights both the excitement and the challenge of pediatric pathology. The pursuit of a perfect diagnosis – utilizing all available literature, molecular testing, histology, and complex clinical history – is an interesting puzzle to solve, but on the other side of the laboratory door is a living child for whom treatment requires timely and actionable results. In our case, time will tell whether our patient’s histiocytic sarcoma responds to the B cell chemotherapy protocol, hopefully shedding more light on the pathogenesis and presentation of this unusual malignancy in the pediatric population.

Resident Recruitment Award Recipient

Diffuse Glioneuronal Tumor with Oligodendroglioma-like Features and Nuclear Clusters (DGONC) – A Novel Pediatric Brain Tumor Entity

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Background: Epigenomic analysis has emerged as a tool that enables profiling of oncogenic lineages of different brain tumors and provides improved sub-classification of existing tumor entities¹. Diffuse Glioneuronal tumor with Oligodendroglioma-like features and Nuclear Clusters (DGONC) is a newly proposed provisional neuroepithelial tumor type described by World Health Organization (WHO)² which requires methylome profiling for clinical diagnosis. Available literature suggests DGONC is most prevalent in the pediatric population without brain regional predilection³. These rare cases show consistent monosomy of chromosome 14 and frequent histological presentation of perinuclear haloes and nuclear clusters. Here we report a DGONC case suspected on histomorphologic examination and confirmed by methylation profiling²⁻⁴.

Clinical History: This 15 year-old previously healthy male presented with two months of frequent seizures, characterized by impaired awareness with variable emesis, tinnitus, jaw locking, lip smacking, and secondary generalization. His verbal memory had also subjectively declined. Electroencephalogram (EEG) revealed spike and sharp discharges as well as subclinical seizures in the left temporal region. Brain MRI showed a 2.3 x 2.0 x 1.7 cm non-enhancing space-occupying lesion in the left mesial temporal lobe with regional mass effect upon the amygdala and hippocampus (Fig. A). The initial differential diagnosis included low-grade glioma, ganglioglioma, and focal cortical dysplasia. The lesion was resected using a transcortical approach (Fig. B). The final pathologic diagnosis was established on the resected specimen.

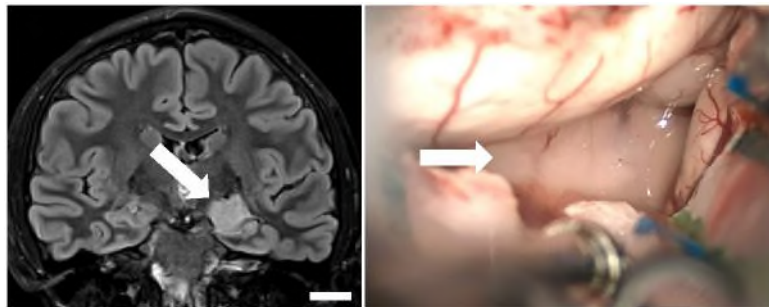


Figure A: T2 FLAIR MR image showing a space-occupying lesion (pointed by the arrow) in the left mesial temporal region. Scale bar = 2.0 cm. **Figure B:** Intra-operative image showing gross appearance of the lesion *in vivo* (pointed by the arrow). The white vascular structure that situates superior to the lesion is the hippocampus.

Case Findings: The tumor was moderately cellular and composed of sheets of monotonous cells with round nuclei and scant cytoplasm (Fig. C). A few nuclear clusters and entrapped cortical neurons were present (Fig. D). Rare perinuclear clearing, an oligodendroglioma-like feature, was seen (Fig. E). Tumor cells were diffusely positive for Olig2 (Fig. F) and negative for GFAP (which highlighted entrapped reactive astrocytes). ATRX was retained. Neither overexpression of p53 nor IDH1-R132H mutation were identified by immunostaining. Only rare mitotic figures were present, and the Ki-67 proliferation index was approximately 3-4 %. The constellation of features was suggestive of a low-grade glial/glioneuronal tumor, and a diagnosis of DGONC was favored. Next generation sequencing revealed loss of one copy of chromosome 14. No other pathogenic variants were identified. The specimen was further evaluated by methylation profiling performed at the National Institute of Health (NIH), and the classifier confirmed the diagnosis of DGONC with a high confidence score. Of note, in the most recent WHO guidelines, DGONC is characterized by common oligodendroglioma-like histological features, nuclear clusters, and occasional neuronal differentiation². While our case contained small cells with round nuclei and occasional nuclear clusters, perinuclear haloes and definitive neuronal

differentiation were not present. Additionally, the previously described micro- or macro-calcifications and vascular proliferation^{2,4} were also not found by histology or imaging.

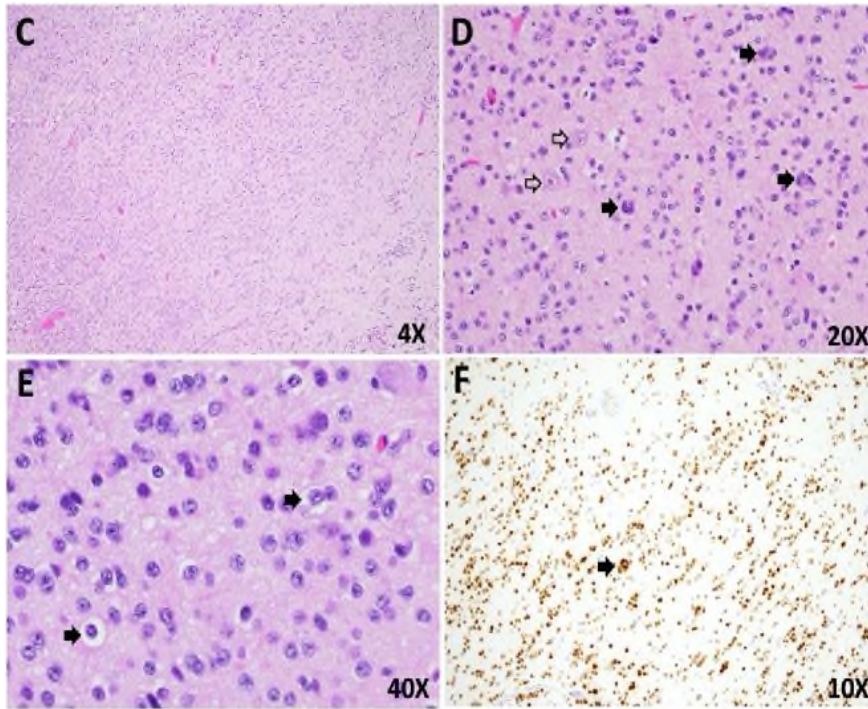


Figure C: H&E stain showing the temporal cortex overrun by sheets of monotonous small to medium-sized round cells in moderate cellularity.

Figure D: H&E stain showing tumor cells with round nuclei, scant cytoplasm, and vesicular chromatin. A few nuclear clusters (black arrows) and occasional entrapped cortical neurons (open arrows) are present in the background.

Figure E: H&E stain showing rare tumor cells with perinuclear clearing (black arrow), an oligodendroglioma-like feature.

Figure F:

Immunohistochemical stains showing tumor cells with diffuse nuclear positivity for Olig2. Nuclear clusters were occasionally seen (black arrow).

Discussion and Conclusion: DGONC is a new entity that was first differentiated and classified by its distinct DNA methylation profile¹. Immunohistochemical, morphological, and other genetic features were subsequently surveyed on limited available specimens for further characterization^{3,4}. A total of 34 cases (predominantly in the pediatric population) have been reported to date^{3,4}. While the convergent characteristics of DGONC include monosomy 14 and frequent presence of perinuclear haloes and nuclear clusters, the absence of these features does not entirely rule out the DGONC diagnosis. Histologic features of DGONC can mimic low-grade entities such as oligodendroglioma, neurocytoma, and ependymoma as well as high-grade entities including anaplastic oligodendroglioma and primitive neuroectodermal tumors³. Improved histomorphological characterization has been limited by the paucity of cases and should be pursued as more cases are identified through methylation profiling. Notably, DGONC shares morphological features and methylation profile spatial proximity with CNS neuroblastoma^{1,2}. Consistent loss of chromosome 14 heterozygosity has been previously identified in neuroblastoma⁵, a finding similar to monosomy 14 found in DGONC. It is hypothesized that loss of tumor suppressor genes on 14q contributes to the tumorigenesis of neuroblastoma⁵. Combined epigenomic and genomic analyses focusing on chromosome 14 will permit exploration of both disease entities' pathogenesis and testing the hypothesis that DGONC and CNS neuroblastoma may be two sub-categories of CNS embryonal tumor^{1,2}.

Maternal Vascular Malperfusion of the Placenta and Measures of Cardiac Structure in Stillbirths

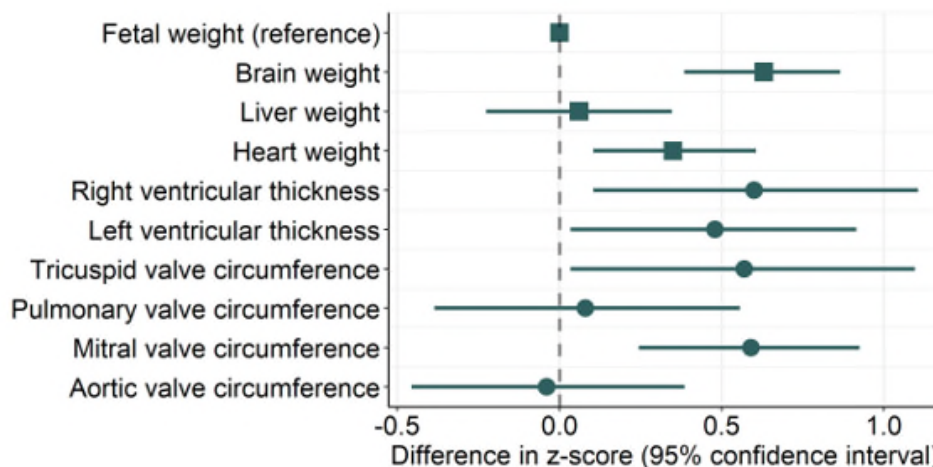
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Background: Maternal vascular malperfusion (MVM) is a constellation of findings in the placenta associated, in severe cases, with placental insufficiency, intrauterine growth restriction (IUGR) and fetal demise. Sparing of brain weight is a known feature of IUGR fetuses, but measures of cardiac structure are not well-studied. The objective of our analysis was to investigate whether MVM in the placenta is associated with changes in cardiac measures among stillbirths.

Methods: This study utilized a cohort of 32 stillbirths submitted to pathology with MVM as the main placental finding/cause of death. Fetal weight and measures of cardiac size, including heart weight, right and left ventricle thicknesses, and circumferences of the tricuspid valve, pulmonary valve, mitral valve, and aortic valve, were abstracted from autopsy reports. Measures were standardized by gestational age using published reference ranges. Differences in standardized cardiac measures as compared to standardized fetal weight were calculated, where differences <0 indicated that heart measures were smaller than expected for fetal size, differences of 0 indicated similar heart size and fetal weight, and differences >0 indicated larger heart size relative to fetal weight. One-sample t-tests were used to determine if differences in standardized heart size and fetal weight were different from 0 (heart size measures consistent with fetal weight).

Results: The median gestational age among stillbirths with MVM was 27 weeks (range: 20 to 35 weeks) and 34% ($n=11$) were small for gestational age (SGA; fetal weight <10 th percentile). The mean difference in z-score for heart weight relative to fetal weight was 0.4, which was significantly different from 0 ($p<0.01$), indicating heart sizes were larger than expected for fetal weight (Figure 1). In comparison, the mean difference in z-score for brain weight vs. z-score for fetal weight was 0.6, while the mean difference in liver weight vs. fetal weight was 0.1, indicating that heart weight may be spared in MVM, similar to brain weight. Right ventricular thickness, left ventricular thickness, tricuspid valve circumference, mitral valve circumference were also significantly larger relative to fetal weight.

Figure 1. Difference in z-score comparing organ weights and heart measures to fetal weight



Conclusion: In this cohort, stillbirth due to MVM and subsequent placental insufficiency was associated with relative sparing of heart weight and other heart measurements, albeit to a lesser extent than brain weight. The significance of these cardiac findings in liveborn infants with MVM needs further study.

Hemoglobin A1c May Underestimate Maternal Hyperglycemia And Its Consequences In Perinatal Demise Associated With Gestational Diabetes Mellitus

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Background: Gestational diabetes mellitus (GDM) is diabetes diagnosed for the first-time during pregnancy. In poorly controlled GDM, excess maternal blood glucose might cross the placenta into the fetal circulation. The affected infants are typically large-for-gestational-age (LGA), with puffy face, excess adipose tissue, and pancreatic islet hyperplasia, and are termed “infants of diabetic mothers”. Sub-optimally controlled GDM is associated with increased stillbirth risk. Thus, in the event of stillbirth, maternal HbA1c testing is recommended by the professional associations of obstetricians and gynecologists in Canada, the US and the UK to determine whether maternal glucose is truly under control. Yet, a recent study found that maternal HbA1c could underestimate maternal glycemia in mid-to-late gestation. Thus, we hypothesize that in late gestation pregnancies associated with GDM, features of “infants of diabetic mothers” could be present in stillbirths and neonatal deaths despite a “non-diabetic” maternal HbA1c at/around the time of delivery.

Methods: This is a descriptive study of fetal/neonatal autopsies at a tertiary care children’s hospital. Autopsy reports (01/01/2015-04/30/2022) were extracted from a laboratory information system. Inclusion criteria were: (1) fetus/neonate delivered in 3rd trimester; (2) birthweight (BW) and/or femoral length >90th %tile; (3) normal cytogenetics results; (4) mother with GDM; and (5) a “non-diabetic” maternal HbA1c of $\leq 6.1\%$ at/around the time of delivery. The autopsy reports, photos, x-rays, and microscopic slides were reviewed and assessed for features of “infants of diabetic mothers”.

Results: 10 autopsies (9 stillbirths, 1 early neonatal death) met inclusion criteria. 9 were complete autopsies, and 1 was limited to an external exam, x-rays, and cytogenetics testing. Maternal HbA1c ranged from 5.3-6.1% (median: 5.7%). In all 10 cases, at least 1 feature of “infants of diabetic mothers” was found: BW >90th %tile (n=6), femoral length >90th %tile (n=10), excess adipose tissue (n=6), puffy face (n=6), and cardiomegaly (n=4). 4 cases had sections of well-preserved pancreatic tissue (i.e., not severely autolyzed), of which 3 showed pancreatic islet hyperplasia, when compared to gestational-age-matched controls. In addition, a small placenta and/or an elevated BW:placental weight ratio (n=5) and hypoxic-ischemic encephalopathy (n=7) were noted.

Conclusion: In pregnancies of women with GDM, features of “infants of diabetic mothers” were present in the fetuses and a neonate at autopsy, despite a “non-diabetic” maternal HbA1c at/around time of delivery. Thus, even though maternal HbA1c testing is standard practice to assess maternal glycemic control in stillbirth investigations, it might underestimate 3rd trimester maternal hyperglycemia in GDM pregnancies.

Hypoplastic Ductus Venosus in a Second Trimester Fetus

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Background: During intrauterine life, the fetus relies on 3 physiological shunts: the ductus arteriosus, foramen ovale and ductus venosus. Developmental abnormalities of the ductus arteriosus and foramen ovale have been well documented in pathology literature. In contrast, published cases of ductus venosus anomalies with pathologic descriptions are sparse. This discrepancy is likely in part because the ductus venosus, being situated within the liver, can be difficult to examine at autopsy, particularly if the fetus were small.

Methods: We described an autopsy case of a second trimester fetus with a hypoplastic ductus venosus.

Results: This was a termination of pregnancy at 24 weeks gestational age (GA), to a 40-year-old G5P1 woman, due to severe intrauterine growth restriction. The mother had a history of preeclampsia. First trimester screening was positive for trisomy 21, but both noninvasive prenatal screening and rapid aneuploidy detection (amniocentesis) were negative for common aneuploidies. At 19+4 weeks GA, a 2-vessel cord was identified. At 23 weeks GA, the fetus measured small-for-gestational-age, and Doppler measurements were abnormal. Absence of ductus venosus was noted, with umbilical venous blood draining through the liver to the inferior vena cava. Fetal echocardiogram was negative. An autopsy was performed. Postmortem examination revealed a female fetus. Overall measurements were compatible with 22 weeks GA. No major external abnormalities were identified. Internal examination revealed a normal right umbilical artery, and a hypoplastic and atretic left umbilical artery. The umbilical vein was of normal size; it entered the liver and connected with the right and left portal veins. The ductus venosus was present, but it was focally severely hypoplastic with a very small lumen. The ductus venosus joined the left hepatic vein, which then connected with the inferior vena cava. No cardiac abnormalities were noted. The placenta was small and showed evidence of maternal vascular malperfusion, which could explain the abnormal uterine artery Doppler and intrauterine growth restriction.

Conclusion: Ductus venosus agenesis/absence is the most frequently described anomaly of the ductus venosus. Yet, in the majority of reported cases, the diagnosis of absent ductus venosus was made by prenatal ultrasound. Here, we described an autopsy case of a 24-week-GA fetus with a prenatal ultrasound diagnosis of absent ductus venosus. At autopsy, a hypoplastic ductus venosus was found. To our knowledge, hypoplastic ductus venosus has not been previously reported. This case illustrates that in fetuses with suspected ductus venosus agenesis/absence, it is important to carefully dissect and examine the ductus venosus.

Massive Subchorionic Thrombohematoma and Maternal Cardiac Dysfunction: A Case Series

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Background: Massive subchorionic thrombohematoma (MST), also known as Breus' mole, is a rare and poorly understood entity defined as a substantial collection of clotted blood in the intervillous space, immediately beneath the chorionic plate, measuring >1 cm in thickness with >50% involvement of the fetal surface of the placenta. The presumed pathophysiology is an aberrant collection of maternal blood, although this data is limited. MST has previously been associated with maternal thrombophilia and following thrombolytic therapy.

Methods: Four cases of MST diagnosed by pathological examination at our institution from 1/1/2021-7/1/2022 were identified using our laboratory information system. Maternal medical history, prenatal imaging, antenatal complications, gestation at delivery, and pregnancy outcome were extracted from the medical record.

Results: We report a novel association of MST and maternal cardiac dysfunction, illustrated by four cases at a high-risk obstetric reference center covering a large portion of the southeastern United States. Two of these mothers had surgically repaired complex congenital heart disease, one had heart failure secondary to SARS-CoV-2 myocarditis, and one had longstanding high output heart failure due to severe sickle cell disease with resulting severe anemia. All of these pregnancies were complicated by intrauterine growth restriction (IUGR). Grossly, all four placentas had extensive fetal surface involvement by thrombohematoma (90-100%), but with variable degrees of placental volume replacement (range: 10-80%). Two of the four mothers had documented enoxaparin administration during pregnancy; however, there were no common medications given to all four mothers. None had recognized thrombophilic disorders. Only one of four lesions was definitively identified on prenatal ultrasound. Of these four cases, three were live births, though only one infant survived past fifteen days of life. This surviving child had the least affected placenta grossly, was delivered at term, and was born to the only mother who did not require admission to the intensive care unit.

Conclusion: We posit abnormal maternal hemodynamics are the final common pathway in the development of MST. Previous studies have shown blood within the thrombohematoma to be of maternal origin; furthermore, there is correlation within our case series between the largest lesions and the mothers with the most hemodynamic instability. IUGR is likely secondary to decreased placental reserve capacity from these space-occupying lesions. High fetal/neonatal morbidity and mortality rates underscore the need to further characterize this pathophysiologic process. That only one of four cases was detected on prenatal ultrasound illustrates the importance of both ante- and postnatal clinical and pathologic recognition.

Placental Dysmaturity within a Large Cohort of SARS-CoV-2-Positive Pregnant Women

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Background: From 25 February 2020 to 30 June 2021, experienced perinatal pathologists examined 975 placentas, macroscopically and microscopically, of SARS-CoV-2- positive mothers enrolled in a national prospective study, adopting the Amsterdam Consensus Statement protocol. The main results included the absence of specific pathological findings for SARS- CoV-2 infections, even though a high proportion of placentas showed signs of inflammation, including chorioamnionitis, funisitis, villitis, chronic histiocytic intervillitis, and fibrin deposition. In this further analysis, we focused our attention on placental maturity in SARS-CoV-2 infection as, according to recent literature, this feature has scarcely been considered.

Methods: All the maternal and placental data were collected by an online database system. Placental maturation was evaluated in 975 placentas from SARS-CoV-2 positive pregnant women according to the onset of maternal infection. Gestational age (GA) at the time of infection included placentas less than 14 weeks up to 41, but pathological analyses were carried out at delivery, in the third trimester. Parenchymal maturation was classified as follows: consistent with GA, immature, dysmature (also known as delayed villous maturation), and hypermature (or accelerated villous maturation). Incidence of maternal diabetes was also calculated as may affect placental maturation.

Results: Among 975 placentas, 29 cases were missing (data not inserted by pathologists). On the whole, placental maturation was consistent with GA in 686 cases, immature in 77, hypermature in 48, and dysmature in 135. According to the gestational age at which SARS-CoV-2 infection was diagnosed, the results were as follows: - < 14 weeks: 27 placentas consistent with GA, 2 immature, 4 hypermature, 5 dysmature - 14-27 weeks: 95 placentas consistent with GA, 10 immature, 6 hypermature, 22 dysmature - >=28 weeks: 559 placentas consistent with GA, 65 immature, 38 hypermature, 107 dysmature. Incidence of maternal diabetes was quite low in any kind of placental maturation, as reported below: - Immature: 1 / 77 (1.3%) - Consistent with GA: 8/686 (1.2%) - Hypermature: 2/48 (4.2%) - Dysmature: 4/135 (3%)

Conclusion: In our population, 260/975 cases (26.7%) presented abnormal placental maturation, and among them, about a half (135/260), showed dysmaturity. According to maternal GA at onset of infection, anomalous development was mainly diagnosed after 28 weeks. Incidence of maternal diabetes was very low and unlikely correlated with the histological findings. If SARS-CoV-2 infection plays a role in determining placental abnormal maturation, or instead, if this anomaly may be a permissive feature to the virus, has yet to be investigated.

Time Required For Gross Examination of Second and Third Trimester Singleton Placentas by Pathologists' Assistants

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Background: In North America, workload measurement in pathology is primarily based on specimen complexity. For example, the Canadian Association of Pathologist (CAP-ACP) Workload Model categorizes surgical pathology specimens into 6 levels (level 1 to level 6), largely based on the time required to perform gross and microscopic examination of a specimen. The CAP-ACP Workload Model recognizes that in some laboratories, Pathologists' Assistants (PAs) gross all or most of the specimens. PAs are highly trained, certified allied healthcare professionals who play a critical role in the delivery of health care. While their presence and role in anatomical pathology are expanding, few studies have investigated how long it actually takes PAs to perform gross examinations. As our laboratory has a large perinatal pathology service, and placenta is one of the most commonly encountered specimens, we attempted to answer the following question: How long does it take for a PA to gross a second-to-third trimester placenta?

Methods: 7 PAs certified by the Canadian Council of Certification of Pathologists' Assistants participated in this study. Each PA grossed at least 10 second- and third- trimester singleton placentas, and electronically recorded the start and end date and time of the examination in the pathology report in Cerner Millennium Laboratory Information System. Gross examination and dissection of the placenta were performed according to a previously published, standardized protocol, and gross findings were recorded via dictation and transcribed using Dragon Speech Recognition software. Cases that required direct pathologist supervision and consultation or ancillary studies (e.g., sampling for cytogenetics analysis) were excluded. The average and standard deviation (sd) of grossing times were calculated for each PA, and an overall average and 95% confidence interval were calculated using a mixed linear regression model, with grossing times nested within PAs.

Results: Each PA grossed at least 10 second- and third- trimester singleton placentas. The mean grossing times for each PA ranged from 11.0 (sd = 2.0) to 17.8 (sd=4.5) minutes. The overall average grossing time was 14.1 minutes, with a 95% confidence interval of 10.9 to 17.3 minutes.

Conclusion: Given that there is a dearth of information on grossing times of PAs, the results of this study provide valuable information that could be helpful in evaluating workload measurement and in assessing PA staffing needs. The methodology of this study is also easily adoptable for other specimen types and by other laboratories.

Ki-67 Staining of Atypical Trophoblasts: The Jury is Still Out

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Background: Islands of atypical trophoblasts are a rare occurrence in routinely processed placentas. These can raise the possibility of persistent trophoblast disease or malignancy. A recent review of a series of these cases concluded that atypical trophoblasts had low proliferative activity and were likely a degenerative change. We review two cases, which show similar cells. A metastatic breast cancer and a primary choriocarcinoma are shown for comparison.

Methods: Four cases of placentas with atypical findings were examined for clinical history, gross exam, and histologic exam with H&E, and BRST2, Inhibin, and Ki-67 immunostaining.

Results: Case 1: A 383 gram (75-90th percentile) placenta delivered at 32 weeks in the setting of preterm premature rupture of membranes and spontaneous labor. Maternal history was significant for complete hydatidiform mole four years prior. Patchy areas of severely atypical, large cells with clumped or smudged chromatin were located in intervillous trophoblastic islands and placental septa. The cells were negative for BRST2, focally positive for Inhibin, and 20% positive for Ki-67. Case 2: A 667 gram (>90th percentile) placenta delivered at 40 weeks by cesarean section for fetal macrosomia. Maternal history was non-significant. Severe atypia was seen in a focus of cells within an intervillous trophoblastic island. The cells were 30% positive for Ki-67. The area of interest was not present on BRST2 or Inhibin stain. Case 3: A 93 gram (<3rd percentile) placenta delivered at 26 weeks for impending maternal hemodynamic collapse due to metastatic ductal breast carcinoma. Islands and sheets of cells with atypical nuclei were present in the intervillous space with patchy central necrosis. BRST2 was strongly positive. Inhibin was negative. Ki-67 was 70%. Case 4: A 465 gram (25-50th percentile) placenta delivered at 38 weeks by cesarean section for fetal intolerance of labor in the setting of poor fetal growth and low biophysical profile score. Maternal history was non-significant. Infiltrative between and surrounding the villi were highly pleomorphic trophoblast cells with large vesicular nuclei, prominent nucleoli, and occasional multinucleation. BRST2 was negative. Inhibin was strongly positive. Ki-67 was 80%.

Conclusion: Atypical trophoblast cells can be distinguished from choriocarcinoma and metastatic cancer by histology and immunohistochemistry. However, in our cases, Ki-67 was >10% and therefore did not distinguish clearly between atypical trophoblasts and malignancies. Our cases only have 1 to 5 months' follow-up; long term follow-up is necessary to determine whether these more mitotically active cases are aggressive.

An Unusual Case of Fetal Myocardial Injury Leading to Intrauterine Fetal Demise: A Possible Sequela of Maternal COVID Infection?

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Background: Intrauterine myocardial injury/infarction (MI) is exceedingly uncommon and the few reported cases occur most commonly with congenital cardiac anomalies, particularly anomalous coronary arteries. Here we present a case of MI in a fetus resulting in intrauterine fetal demise (IUFD). A 36-year-old G3 P1011 mother with a history of methylenetetrahydrofolate reductase (MTHFR) mutation (double heterozygote; may lead to a procoagulable state) on prophylactic aspirin and recent COVID-19 infection during pregnancy (5 weeks prior, recovered) presented at 28 weeks gestational age with one day of decreased fetal movement, at which time IUFD was diagnosed.

Methods: Induction of labor with misoprostol was performed, and the fetus was delivered stillborn vaginally without complications. A full autopsy including histologic examination (including placental histopathology and immunohistochemistry) was performed.

Results: Autopsy examination of the fetus revealed normal development, including a structurally normal heart. However, histologic examination of the heart demonstrated a discrete region of intramyocardial fibrosis, dystrophic calcification, and giant cell reaction involving a large portion of one ventricle, consistent with MI (Fig 1). Immunostains for infectious causes of myocarditis (including COVID, cytomegalovirus, herpes simplex virus, parvovirus, adenovirus, and toxoplasmosis) were negative. Histologic examination of all other organs was unremarkable. Gross examination of the placenta was remarkable for a hypercoiled umbilical cord, and histologic examination revealed a hypoplastic umbilical artery with partial smooth muscle necrosis as well as a single, non-occluding stem villus thrombus, without histologic evidence of downstream fetal vascular malperfusion.

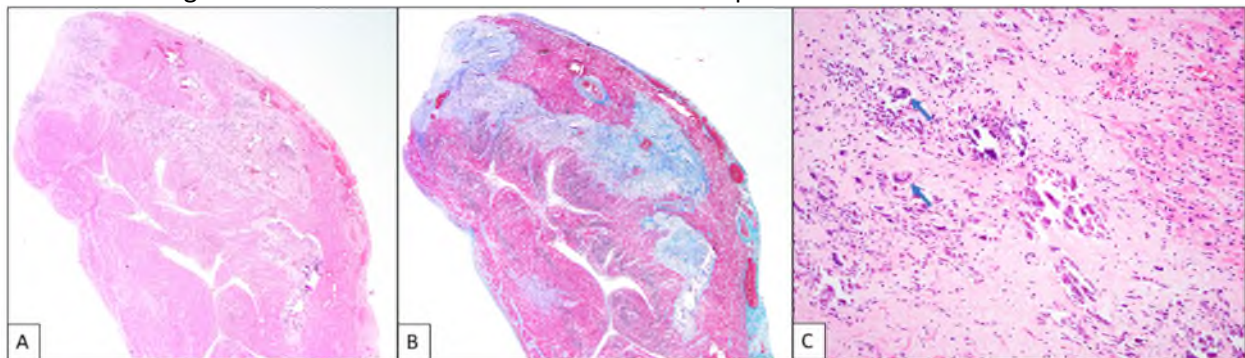


Figure 1. Fetal ventricle with discrete region of intramyocardial fibrosis and calcification, consistent with post myocardial injury repair (2x) (A). Extensive regional fibrosis is demonstrated on trichrome stain (2x) (B). 20x field from the area of myocardial scarring demonstrating fibrosis, dystrophic calcifications, and giant cell reaction (arrows) with adjacent normal myocardium (C).

Conclusion: The cause of demise was concluded to most likely be due to fetal myocardial injury, but the etiology is unclear. The differential diagnosis includes a thromboembolic phenomenon possibly leading to infarction (given maternal history of MTHFR mutation and recent COVID infection), myocarditis (possibly burned-out phase), coronary artery abnormalities that were not appreciated on fresh dissection of the heart, and ventricular dysplasia/aneurysm (least likely given histologically normal surrounding myocardium). This rare case is an example of a lethal myocardial injury in an otherwise structurally normal fetal heart.

Successful Delivery of Healthy Term Neonate Alongside a 2.8 cm Loose Fragment of Retained Calvarial Bone Tissue

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Background: Retained products of conception (RPOC) occur when fetal parts or placental tissue remains in the uterine cavity following delivery, spontaneous abortion, or artificial abortion. RPOCs may cause immediate postpartum complications including pelvic pain and menorrhagia with the potential to prevent or complicate subsequent pregnancies in a mechanism of action similarly attributed to intrauterine devices. Within RPOCs, retained fetal bone remains an exceptionally rare occurrence.

Methods: A medical record chart review was performed and the pathology reports along with gross images and operative notes were compared to literature information found from an online library system keyword search, including “retained fetal bone” and “intrauterine bone”.

Results: This is a case of a 33-year-old female who had a previously terminated pregnancy (with laminaria placement for dilation and evacuation) for agenesis of the corpus callosum and absence of the cavum septum pellucidum two years prior at approximately 21 weeks gestational age presenting two years later for spontaneous vaginal delivery of a healthy 38 week, 2-day old baby which was immediately followed by spontaneous vaginal expulsion of a calvarial bone tissue fragment (measuring 2.8 x 2.5 x 0.2 cm, 0.9 g) onto the patient bed, which was assumed by the clinical team to be the retained laminaria and was sent to pathology for examination. Previous case reports involving retained fetal bone fragments have been documented during workup for infertility, menorrhagia, and dysmenorrhea and have yielded intrauterine fragments of bone originating from a variety of fetal structures, including the spine, skull, and long bones measuring up to 1.5 cm in length in some cases. A single case report of a successful pregnancy with a previously known intrauterine fetal ossicle (dimensions not given) was found in our literature search, belonging to a 19th week gestational miscarriage which was later found embedded in the placental parenchyma on histopathological analysis. Thus, the present case represents the first noted and likely largest recorded loose fragment of retained calvarial bone tissue found after a successful pregnancy.

Conclusion: A successful pregnancy in the presence of retained fetal bone fragments is noted to be an exceptionally rare occurrence. Our case describes an unlikely situation of a successful term pregnancy occurring alongside a notably large, 2.8 cm loose fragment of retained calvarial tissue from a previously terminated pregnancy.

Discordant Cell-Free DNA and Fetal Autopsy Findings in the Setting of Monosomy X

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Background: Non-invasive prenatal testing (NIPT) via sequencing of cell-free placental DNA (cfDNA) is now widespread and shows improved sensitivity/specificity over serum screening tests for common aneuploidies. Reliable diagnosis may occur when NIPT is combined with prenatal imaging and, where indicated, invasive testing such as chorionic villous sampling or amniocentesis. However, these approaches have limitations, and multiple limitations may result in incorrect interpretation. We report a case of discordance between cfDNA results (low-risk X,Y male) and autopsy morphology of a hydropic mid-gestation fetus, in the setting of limited imaging and failed amniocentesis. Postmortem microarray confirmed a diagnosis of Turner syndrome (TS).

Methods: A 26-year-old P2012 mother presented for anatomy scan at 16 weeks, which showed large cystic hygroma, subcutaneous edema, and one-week growth lag. cfDNA testing at 17 weeks resulted as low-risk X,Y with no aneuploidy. Ultrasound at 20 weeks revealed fetal demise. Genitalia and cardiac views were limited. The fetus was clinically assigned as male. Amniocentesis sample was insufficient for genetic analysis.

Results: At autopsy, the 156 g macerated fetus had a large nuchal fluid collection and subcutaneous edema. Growth was consistent with 17 weeks of gestation, an approximate 1-2 week growth lag including estimated demise to delivery interval. The external and internal genitalia were female and grossly normal. No definitive aortic arch abnormality was identified. A single umbilical artery was present. Anatomy was otherwise normal (including cardiac and renal). The placenta was small for gestation (89 g), with dysmature chorionic villi showing abnormal villous contours and frequent trophoblastic pseudoinclusions. Based on the autopsy findings, microarray was performed on paraffin-embedded fetal ovarian tissue and resulted as 45,X with no mosaicism.

Conclusion: Sex chromosomal aneuploidies (SCA) have lower sensitivity and positive predictive values (PPV) than autosomal aneuploidies (AA). TS is one of the most common SCA (1/2000 live births) with variable genotype presentations and frequent mosaicism. Up to 12% of patients with TS have Y chromosome material, and confined placental mosaicism is reported. For cfDNA of TS, reported PPV is 26% and sensitivity is 83-91% depending on laboratory and platform. This case highlights the potential for discordance between cfDNA and autopsy morphology in cases of sex chromosome aneuploidy in fetal life, especially when other prenatal testing is limited (e.g. failed amniocentesis and limited imaging). Evaluation of the fetus and placenta is the gold standard for morphologic assessment in cases of fetal demise, and can provide crucial diagnostic information and guide further ancillary testing.

Putting It All Together: Postmortem Diagnosis of a Rare Ichthyosis Syndrome

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Background: Neu-Laxova syndrome (NLS) is a rare autosomal recessive disorder characterized by ichthyosis along with multiple congenital anomalies. We present a case in which autopsy findings raised suspicion of NLS, prior prenatal testing identified loss of heterozygosity in the gene region of interest, and definitive testing on DNA from cryopreserved fibroblasts obtained at autopsy confirmed the diagnosis.

Methods: A 26-year-old G8P2141 mother presented to obstetrics with a history of parental consanguinity and multiple stillbirths/infant deaths. At 21 weeks EGA ultrasonography (US) revealed an abnormal calvarium, flat facial profile, and clenched fingers/toes. Follow-up US at 25 weeks EGA showed growth restriction (<3rd %ile) and abnormal brain development in addition to the previously identified abnormalities. At 35 and 5/7 weeks EGA the mother presented with vaginal bleeding and decreased fetal movement, and the female infant was born vaginally. She developed respiratory failure and after multiple failed intubation attempts ultimately passed away within hours of birth. Her mother consented to an unrestricted autopsy.

Results: Postmortem examination revealed a small female infant (crown-heel length 30.9 cm, N=44 ± 4.4cm) with microcephaly (head circumference 21.3 cm, N=31.9 ± 1.6cm). There was hypertelorism, bilateral cataracts, low set abnormal ears, broad nasal bridge, cleft palate, and micrognathia. Multiple contractures of the upper and lower extremities were present, along with bilateral rocker bottom feet. The skin was taut and shiny with fissures at flexion sites. The brain displayed abnormal cortical and cerebellar development with abnormal gyration and cytoarchitecture. Fluorescence in situ hybridization showed normal patterns for chromosomes 13, 18, 21, X, and Y, and DNA deletion/duplication array revealed no clinically significant DNA copy number variants. Review of maternal history revealed that loss of heterozygosity (LOH) testing on an amniocentesis specimen from a prior pregnancy revealed a 7.92 Mb region of homozygosity involving chromosome 1p13.2-p11.2, the region of the *PHGDH* gene. Targeted sequencing of DNA from cultured fibroblasts from autopsy was then performed, revealing a homozygous sequence variant in the *PHGDH* gene which matched that previously described in another NLS patient.

Conclusion: NLS is a lethal autosomal recessive malformation syndrome caused by mutations in genes involved in L-serine synthesis. It is postulated that the lack of adequate serine supply could impair nucleotide synthesis, affecting cell proliferation and development of the central nervous system and ectoderm. The features identified at autopsy along with the family history and prior LOH testing were able to guide targeted gene analysis to establish the diagnosis of NLS in this family.

A Rare Case of Urorectal Septum Malformation Sequence (URSMS)

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Background: A rare anomaly called urorectal septum malformation sequence (URSMS) was seen in one autopsy case at our institution. This entity is a combination of anomalies arising from failure of migration of and/or fusion of the urorectal septum with the cloacal membrane. There is a spectrum of severity. Our particular case represents complete URSMS, as it is defined as a lack of perineal (urethral and vaginal in females) and anal opening with ambiguous/absent genitalia, which is an invariably fatal condition. The incidence of URSMS sequence is 50,000-250,000 neonates.

Methods: An external examination and autopsy were performed by a resident physician and attending physician on a 29 week and 5 day old neonate.

Results: Congenital defects identified include genital, lower urinary, and rectoanal agenesis and a persistent cloaca. There is no external genitalia. Upon opening, the peritoneum contains 950 mL of serous fluid and the cloaca contained 1.5 L of serous fluid. Severe dilation of the cloaca led to anhydramnios and severely hypoplastic lungs as well as club feet seen externally. The right kidney is entirely cystic and has a small ureter that ends blindly. The left kidney has dilated calyces and a dilated ureter leading to the large cloaca. The cloaca and colon end blindly. There is no urethra, rectum, or anus. There are no identifiable ovaries or fallopian tubes. Microscopically, the cloaca is composed of vaginal tissue and primitive Müllerian structures, which is consistent with tissue of cloacal origin.

Conclusion: During normal embryologic formation, the cloaca is the most distal part of the primitive hindgut, with a cloacal membrane at the end. Extra-embryonic mesoderm proliferates as urorectal septum, and embryonic rotation causes urorectal septum to approximate the cloacal membrane. The urorectal septum divides the cloacal membrane into the urogenital membrane and anal membrane. Rupture of these membranes by apoptotic cell death occurs around the 7th week, which results in patent perineal and anal opening. In complete URSMS, there are two theories of etiology of the error: 1) failure of migration to and/or fusion of the urorectal septum with the cloacal membrane, which leads to persistence of the cloaca and cloacal membrane, failure of normal differentiation of the external genitalia, and absence of the urethral and vaginal openings and an imperforate anus; 2) Mechanical interference due to persistence of the cloaca and lack of development of internal genital organs. Genetic testing prior to death was normal. Multiple environmental and genetic factors have been implicated as causes of URSMS, but nothing is definite. Alterations in genes like sonic hedgehog and homeobox genes lead to caudal mesodermal deficiency. These alterations can be spontaneous or caused by teratogens.

Disseminated Congenital Syphilis in a Stillborn Infant

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Background: Syphilis cases among reproductive age women in the United States have been increasing rapidly in the last decade. Rates of congenital syphilis in the US have more than tripled from 2013 to 2018. Congenital syphilis can have devastating outcomes including miscarriage, stillbirth, low birth-weight, fetal hydrops, and neonatal infection. Of these complications, stillbirth is common, occurring in up to 40% of untreated pregnancies. Despite the recent surge in congenital syphilis, this entity can be difficult to recognize. Syphilis in stillborn fetuses poses additional diagnostic challenges, particularly in the presence of decomposition or autolysis.

Methods: This case report describes findings in a stillborn baby with disseminated syphilis. The autopsy was performed at our home institution. Gross and microscopic examinations were performed, including immunohistochemistry (IHC).

Results: Gross examination showed desquamation, moderate to marked edema, and subtle roughened patches on the plantar surfaces. Body cavities contained adhesions, edema, hydrocele, and hepatosplenomegaly. The liver showed disproportionately marked autolysis. The placenta (per outside surgical report) was large with hemorrhage, focal fibrin deposition, and opaque fetal membranes. Microscopic sections showed autolysis with nonspecific signs of acute to chronic tissue injury and vasculitis. Extramedullary hematopoiesis was present. No plasma cell infiltration was seen. Placenta showed villous edema, chronic villitis, and marked chronic and active intervillitis. The fetal membranes showed acute chorioamnionitis. No funisitis was seen. Syphilis stain showed marked spirochete infiltration in all major organs, particularly within vasculature. The most substantial permeation was seen in the lymph nodes, liver, pancreas, spleen, and adrenal glands.

Conclusion: The cause of death is often difficult to determine in stillborn autopsies. Even though syphilis is a well-known disease, it is often under-recognized. This autopsy demonstrated a much more profound infiltration of spirochetes than was initially suspected based on initial gross and microscopic findings. Given the subtle and nonspecific findings coupled with the surge of cases in recent decades, it is likely that syphilis can be missed on stillborn autopsy. We suggest having a low threshold for suspecting syphilis and utilizing syphilis IHC, even in the absence of classic gross or histologic findings. Correctly identifying syphilis on stillborn autopsy could guide the clinical team to the correct treatment in the mother. Conversely, a missed diagnosis may delay adequate treatment, with potentially devastating consequences.

Disseminated HSV infection in a neonate: A case report and discussion.

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Background: Neonatal herpes simplex virus infection is a serious and often life-threatening condition. Newborns can be contaminated in-utero via transplacental transmission, during delivery (from genital tract of mom), or the postnatal period (HSV-1 infection). Optimal management requires prompt and accurate diagnosis, particularly in newborns, in order to prevent complications, but its implementation is often delayed because of lack of symptoms and signs and while awaiting test results. We report a case of neonatal herpes caused by type 1 herpes simplex virus contracted in the postnatal period. The mother was negative for HSV infection during pregnancy. This case illustrates the importance of addition of antiviral treatment in the neonate who develops fever and neurological symptoms

Methods: A 34-year old mother delivered a male infant at 41 weeks gestation by emergency caesarean section for failure of progress of labour. The baby had Apgar scores 4, 9, 10. The baby was well and afebrile until day 4

Results: On day five, the infant developed fever and increase in mouth breathing, was commenced on IV antibiotics (although blood culture was negative). Fever continued and on day 11, vesicular scalp lesions were identified and acyclovir was started immediately. The CSF culture came back positive for HSV-1. Subsequently, the baby developed disseminated HSV-1 infection involving CNS, skin, eye with deranged LFT and developed multiorgan failure. Then the infant developed seizures requiring multiple anticonvulsants, significant DIC requiring massive transfusion protocol, hypotension, metabolic acidosis, hyperkalaemia secondary to acute kidney injury. Finally the infant went into cardio-respiratory failure in the setting of secondary hyperkalaemia and acute kidney injury and succumbed to death on day 16.

Conclusion: Disseminated HSV infections in the neonate is a rare event and diagnosis of the disease can be difficult and delayed. As the disease can cause significant morbidity and mortality, the neonatologists should keep HSV infection high on their differential while managing a neonate with fever and neurological symptoms even if the mother has no documentation of HSV infection. Diagnosis and initiation of antiviral treatment are the key to prevent significant morbidity and mortality in the neonates. References: 1. Neonatal Herpes Infection: Case Report and Discussion; Jordan C. White and Susanna R. Magee The Journal of the American Board of Family Medicine November 2011, 24 (6) 758-762; DOI: <https://doi.org/10.3122/jabfm.2011.06.100257> 2. A Rare Case of Neonatal Disseminated Herpes Simplex Virus (HSV) With Concomitant Methicillin-Sensitive Staphylococcus aureus (MSSA) Bacteremia Sykes, Stephanie R. DNP, APRN-CNP, NNP-BC; Sharpe, Elizabeth L. DNP, APRN-CNP, NNP-BC, FAANP, FAAN

Congenital chondroid metaplasia in the heart: report of two cases

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Background: Congenital cardiac chondroid metaplasia is a rare cause of conduction defects and, if unrecognized, leads to sudden infant death. We present two cases with this unusual finding and different outcomes.

Methods: Case 1: A 4-month-old male infant diagnosed with congenital heart block soon after birth due to cardiogenic shock and acute renal failure. He also had transposition of great vessels and tricuspid valve atresia. He received a pacemaker and ECMO (extracorporeal membrane oxygenation) immediately and then a heart transplant. Grossly, the explanted heart had pacemaker wires attached to the epicardial surface along with adhesions. There was a common atrial chamber with tricuspid atresia, hypoplastic right ventricle, dilated and hypertrophic left ventricle with membranous ventricular septal defect and transposition of the great vessels. Right and left atrio-ventricular sections and sections of the atrium and ventricles were submitted. Case 2: A 28-day-old, term born female infant experienced a brief episode of gasping breaths followed by cardiac arrest. An autopsy was performed. Grossly, there was cardiomegaly with probe patent foramen ovale, widely patent ductus arteriosus and hypertrophy of the left ventricle. Standard sections and sections of the AV and SA node were taken.

Results: Histologically, both the hearts showed chondroid metaplasia in the atrial wall/ central fibrous body, around the AV node and hypertrophic cardiomyocytes in the left ventricle. Fibrosis in the SA node was present in case 2. Additional findings in the explanted heart included epicardial fibrosis with dystrophic calcification, chronic inflammation, and foreign body giant cell reaction from previous surgeries.

Conclusion: Chondroid metaplasia of central fibrous body and/or AV node is a rare finding reported in the literature especially in cases of sudden death where the conduction system has been examined, as well as some stillborn autopsies where a cause of death was not apparent. Though the significance of chondroid or osseous metaplasia in the AV node has not been established, it could potentially lead to a pathologic cardiac conduction causing fatal arrhythmia. In both of our cases, chondroid metaplasia as well as cardiac conduction defects were present. A relationship between the two findings seems quite likely. Hence, we propose that routine and careful examination of the AV node be performed, even in routine autopsies and all explanted hearts or at least those with endocardial cushion defects. This would help in expanding our knowledge regarding the true incidence of chondroid metaplasia and its role in causing conduction block or sudden cardiac death.

Dysmorphic hepatic duct-like structures in biliary atresia (BA) remnants at Kasai surgery may provide a window into the pathogenesis of BA

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Background: A well-vascularized collagen collar (CC) is a defining feature of a normal HD. A novel hepatic duct-like structure (HDLS) with a well-defined CC and reactive myogenesis (RM) around a lumen lined by degenerating cholangiocytes is occasionally identified in excised proximal remnants of sporadic biliary atresia. A detailed comparison of HDLS in remnants to control HD from infants may highlight structural features that are pertinent to the pathogenesis of BA.

Methods: HD-like structures (HDLS) were identified in the most proximal levels of 3/17 remnants by a well-defined collagen collar (CC) defined by Masson trichrome stain and reactive myogenesis (RM) using immunohistochemistry for SM-MHC-2. The CC of HDLS appeared to be deficient in capillaries. We applied Aperio® morphometry software to compare lumen size, collagen collar (CC) area, CD34+capillary pixel density/unit area and capillary distribution patterns in 3 HDLS to 7 control HD. In both groups, overall capillary density of the CC was compared to capillary density within 30 millimicrons of the cholangiocyte basement membrane (Figure).

Results: In BA remnants, the lumens of HDLS are narrow indicating stenosis compared to controls. Inflammation is absent in HDLS; the cholangiocytes that line HDLS are degenerate with sloughing. In HDLS, the area of the CC and the ratio of collar area to lumen area is greater than in normal HD. Overall capillary density/unit area of the CC in HDLS overlapped with controls. However, the normal expected high density of peribiliary capillaries in the immediate subepithelial peribiliary stroma observed in all control HD is absent in HDLS. Specifically, the capillary density in the peribiliary stromal area within 30 millimicra of the cholangiocyte basement membrane is significantly higher in controls than in HDLS in BA remnants.

Conclusion: HDLS with CC and RM are stenotic and have undervascularized subepithelial stroma, suggesting that extrahepatic peribiliary stroma in BA may be intrinsically abnormal. Failure during development to form the rich peribiliary capillary plexus typical of a normal HD may create a local perfusion deficit that contributes to cholangiocyte failure in BA.

Pediatric Angiosarcoma - An Institutional Study

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Background: Angiosarcoma (AS) is an exceptionally uncommon malignant vascular neoplasm in children. Few published reports exist on the clinical presentation, histology, genomic features, treatment, and outcomes in the pediatric population. Thus, the aim of study was to examine a defined series of this rare tumor in our pediatric institution.

Methods: During a 23-year period (1999 to 2022), clinical, pathologic, genomic, treatment, and outcome data were collected from eight patients with angiosarcomas at a quaternary care children's hospital.

Results: Eight children with angiosarcoma (M/F: 4/4; aged 8 months-17 years) were analyzed. Primary tumor sites included 4 visceral, 2 bone, 1 soft tissue, and 1 cutaneous. Morphologic AS subtype included 4 NOS, 3 epithelioid, and 1 granular cell. Immunostains performed on a subset of cases showed the following: CD31+, CD34+, and vWF+ (n=6); D2-40 focal+ (n=2). Genetic testing was performed on 4 tumors and 4 patients had associated underlying disorders, as follows: 1 patient with central cutaneous lymphatic anomaly had a tumor with hypermutated phenotype, 1 patient with *PTEN* hamartoma syndrome had a tumor with *PTEN* mutation, 1 patient with Li Fraumeni syndrome had a tumor with *TP53* mutation and *NOTCH1-ROS1* fusion, 1 patient had dyskeratosis congenita, and 1 patient had negative tumor genetics. Treatment included the following: biopsy with adjuvant therapy (n=2), complete resection (n=3), complete resection with adjuvant therapy (n=1), and no therapy (n=2). Four patients died of disease (overall survival 1-21 months), and three patients are alive and in clinical remission (follow-up 3-6 years); one patient (hypermutated tumor phenotype) is alive with unresectable disease at 2 years 1 month on dual check-point inhibition with Nivolumab and Ipilimumab.

Conclusion: AS in children has varied sites of origin, morphologic subtypes, somatic genomic abnormalities, and associated underlying conditions. Prognosis has been historically poor due to the extremely aggressive nature of these tumors. Complete surgical resection can be curative in some patients with localized disease. Genetic testing is emerging as a means to guide therapy, particularly in patients with unresectable disease, as in the patient with a hypermutated tumor phenotype for which targeted check-point inhibition has shown good response to date.

HMGA2-NCOR2 Fusions in Pediatric Giant Cell-Rich Tumors of Bone and Soft Tissue

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Background: Giant cell-rich tumors of bone and soft tissue can represent diagnostic conundrums. HMGA2-NCOR2 fusions have been recently identified in a subset of giant cell-rich tumors of bone and soft tissue. We report the two youngest patients with this entity, both in unusual sites.

Methods: Clinical information was obtained by standard chart review. Pathology reports, genomic reports, and histologic sections were reviewed.

Results: The first case was a 7-year-old male with a 5.8 cm mass of the left distal humeral metadiaphysis. Histological examination revealed oval to spindled mononuclear cells with mild nuclear atypia, abundant osteoclast-type giant cells, and small foci of necrosis. Mononuclear cells were immunopositive for CK7, CK19, and AE1/3 and negative for desmin and clusterin. Molecular testing showed no evidence of H3F3A alteration or USP6 fusion. A targeted gene fusion panel detected an in-frame HMGA2 exon 3 (NM_003483.4) to NCOR2 exon 20 (NM_001077261.3) fusion gene, which was confirmed by Sanger sequencing. The patient underwent curettage and bone grafting. At 1-year follow-up, there was no clinical evidence of recurrence. The second case was an 8-year-old female with a history of high-risk neuroblastoma diagnosed at age 3 and a germline pathogenic APC mutation, who presented with a mass centered in the left external auditory canal. Histological examination revealed round to oval mononuclear cells with scattered multinucleated giant cells, chronic inflammatory cells, and patchy hemorrhage. Mononuclear cells were immunopositive for CK7, CK19, AE1/3, CD68, CD163, F13a, MITF, and SMA and negative for desmin, CD34, S100, CD1a, and Langerin. A targeted fusion panel identified an in-frame HMGA2 exon 4 (NM_003483.4) to NCOR2 exon 16 (NM_001077261.3) fusion, which was confirmed by Sanger sequencing. At 3-year follow-up, there was no clinical or imaging evidence of recurrence.

Conclusion: To the best of our knowledge, these two cases represent the youngest patients to date with HMGA2-NCOR2 fusion positive giant cell-rich tumors of bone and soft tissue. The unusual locations of metadiaphysis of a long bone and ear canal led to diagnostic conundrums in both cases. Genetic testing ultimately revealed the correct diagnosis. Of note, no known association between high-risk neuroblastoma, APC mutation, and this lesion has been reported to date. Although rare, a high level of suspicion for this gene fusion should be maintained when faced with giant cell-rich tumors in unusual locations in the pediatric population.

A Novel Case of an Infant with a Benign Mesenchymal Scalp Lesion associated with Somatic BRAF V600E Mutation, Skull Defect, and Intracranial Vascular Anomaly

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Background: BRAF gene abnormalities have been described in several entities, including a subset of pediatric mesenchymal lesions and vascular anomalies. We describe a unique case of an infant with a scalp-based benign mesenchymal lesion with somatic BRAF V600E mutation, underlying skull defect, and temporal lobe vascular anomaly.

Methods: Clinical information was obtained by standard chart review. Histopathology and genomic results were reviewed.

Results: A term male infant was born with a right frontal scalp lesion, clinically most suggestive of congenital hemangioma. Imaging revealed a heterogeneous soft tissue density with serpiginous vessels within the scalp, an underlying skull defect, and a non-contiguous high-flow vascular anomaly along the right temporal lobe, the latter not amenable to vascular intervention. The patient presented at 4 months of age with acute symptomatic seizures attributed to right middle cranial fossa hemorrhage in the context of underlying intracranial vascular anomaly. Biopsy and partial excision of the right scalp lesion were performed at 1 month and 6 months of age, respectively. Operatively, a >5 cm subcutaneous mass was seen with numerous small veins penetrating the outermost layer of the skull and the soft tissue mass. The bone was irregular, but the bony defect seen on previous imaging was no longer present. Histologic examination of the mass showed a proliferation of bland spindled to ovoid mesenchymal cells in a loose collagenous to myxoid background with scattered aberrant vessels, including collections of capillaries and occasional small to medium vessels with prominent perivascular spindle cells. No nuclear atypia or pleomorphism were present, and mitotic activity was inconspicuous. Immunohistochemistry showed the following: spindled to oval mesenchymal cells were CD34+, S100 focal+, EMA-, GFAP-, SMA-, BRAF-, SOX10-; perivascular spindle cells were SMA+; vascular endothelium was CD31+, CD34+, ERG+, GLUT-1-. Molecular analysis using the OncoPanel Illumina HiSeq2500 assay revealed a BRAF c.1799T>A (p.V600E) mutation in 34% of reads. The patient, who is now 7 months old, is doing well without seizure recurrence, further intracranial hemorrhage, or significant focal deficits.

Conclusion: This is a novel case of an infant with a scalp-based benign mesenchymal lesion associated with a BRAF V600E mutation, underlying skull defect, and temporal lobe vascular anomaly. To the best of our knowledge, such a case has not been reported.

Non Immune-Complex Mediated Mesangial Proliferative Glomerulonephritis Progressing to Atypical Post-Infectious Glomerulonephritis in Recessive Dystrophic Epidermolysis Bullosa: Adding to the Spectrum of Renal Complications

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Background: Epidermolysis Bullosa (EB) consist in a group of rare bullous skin diseases affecting the epidermal-dermal junction. Recessive dystrophic epidermolysis bullosa, generalized severe (RDEB-GS), in which autosomal-recessive COL7A1 mutations lead to absent or markedly reduced collagen VII protein levels, is one of the most severe EB subtypes, with multisystem involvement and a high mortality rate. A minority of these deaths is attributed to chronic renal failure, secondary to varied pathologies including post-infectious glomerulonephritis, renal amyloidosis, focal segmental glomerulosclerosis, IgA nephropathy and post-renal obstruction. A recent case series has also shown glomerulonephritis with dominant C3 in 4 patients. Here, we present a case report of a patient who developed previously unreported renal pathology in RDEB-GS.

Methods: Review of clinical history and pathological findings (case report).

Results: Our patient, a five year old male, known for RDEB-GS with severe cutaneous, mucosal and gastrointestinal disease, developed gross hematuria and persistent proteinuria. A renal biopsy performed at six and a half years old, showed diffuse mesangial proliferation with focal segmental glomerular sclerosis, small mesangial deposits and tubuloreticular inclusions on electron microscopy but negative immunofluorescence. The patient was treated with steroids and ACE inhibitor at the time, with a follow-up biopsy fifteen months later showing similar findings. There was progressive increased in proteinuria in the following years, until the patient developed acute renal failure at eighteen years of age in the context of skin infection. A biopsy was performed, which in addition to the previously reported findings, showed cellular crescents, C3 segmental coarse deposits on immunofluorescence and subepithelial “humps” on electron microscopy. Given the new occurrence of low C3 serum levels and skin culture positive for staphylococcus aureus, the findings were consistent with atypical post-infectious glomerulonephritis with diffuse mesangial proliferative features. The patient received pulse corticosteroids, but developed acute pancreatitis and passed away the following month.

Conclusion: In this case, we add to the current spectrum of renal pathology associated with RDEB-GS by describing atypical post-infectious glomerulonephritis developing after longstanding non immune-complex mediated mesangial proliferative glomerulonephritis, of unclear pathogenesis. We hope that this case report bring awareness of possible renal complications in this disease, leading to optimal treatment. Collaterally, we believe that this knowledge can be instrumental in unravelling the pathogenesis of renal pathologies in other patients.

Rare Case of Malignant Transformation in Recurrent Lipoblastomatosis

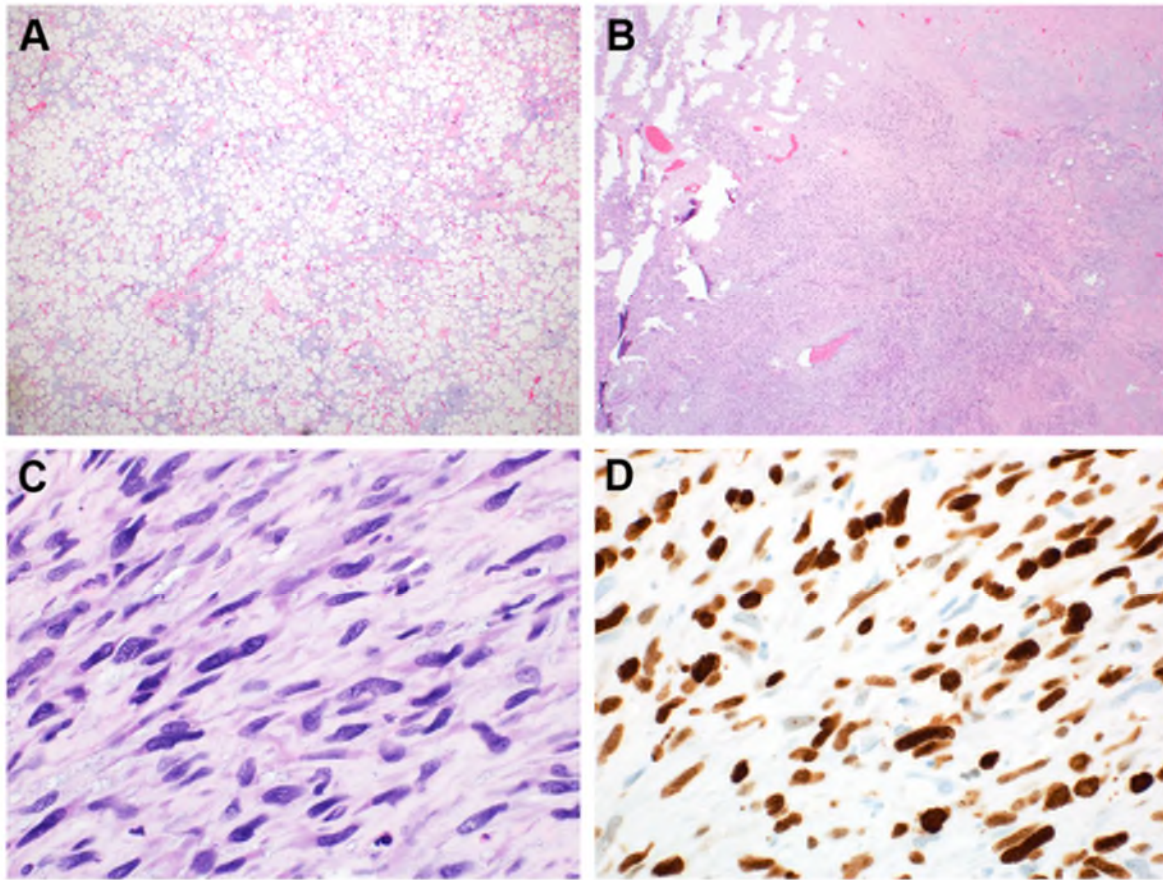
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Background: Lipoblastoma/ lipoblastomatosis is a benign neoplasm of embryonal white fat that may present as a superficial circumscribed mass or a diffuse and infiltrative tumor in deep soft tissue. Local recurrence has been reported in 15-20% of tumors, but these tend to be benign, without risk of malignant transformation. Histologically lipoblastoma shows hypocellular lobules of adipocytes in various stages of differentiation separated by fibrous septa and myxoid stroma. Most lipoblastomas exhibit a structural alteration of 8q11-q13 leading to rearrangement of PLAG1. We report an unusual case of a 23-year-old patient with a lipoblastoma, initially diagnosed at 2 years old, who has had multiple recurrences with progressive histologic changes, which in the most recent sample was morphologically similar to dedifferentiated liposarcoma.

Methods: The patient initially presented at 2 years of age with a retroperitoneal mass. This mass was resected and showed lobules of adipocytes in various stages of differentiation with myxoid areas, consistent with lipoblastoma. She remained disease free for 17 years until she presented with her first recurrence in the left pelvis. She subsequently had a total of 6 recurrences with resections. Cytogenetics were performed on 3 recurrences. Clinical, radiologic, histopathologic and molecular data were reviewed.

Results: The patient had multiple clinically aggressive recurrences despite surgical resection, cryoablation, and radiotherapy. Her most recent recurrence was a large (22.5 cm) subcutaneous pelvic mass arising approximately 22 years after initial diagnosis of lipoblastoma. Histologic examination showed that most of this tumor was similar to previous samples and composed of adipocytes in various stages of differentiation with extensive myxoid change. However, the tumor had a distinct nonlipogenic hypercellular region comprised of hyperchromatic spindle cells demonstrating an increased nuclear to cytoplasmic ratio, variably prominent nucleoli, and high proliferation index (Ki-67 >95%). The morphology in this focal area resembled dedifferentiated liposarcoma. The karyotype continued to show der(14)t(8;14) associated with PLAG1 rearrangement, but additionally showed multiple new abnormalities including del(9)(p24p21), del(13)(q32q34), der(14)t(8;14)(q12;q24), and der(20)t(17;20)(q23;p13), consistent with clonal evolution of this tumor. FISH testing for MDM2

amplification and DDIT3 rearrangement were negative.



Text Box: Figure 1. Histologic examination of recurrent lipoblastomatosis with focal malignant transformation. Low power magnification shows a well-differentiated lipomatous tumor with focal myxoid background consistent with recurrent lipoblastomatosis (A [H&E 4x]). These histologic features were similar to the patient's previous recurrences (not pictured). A focal cellular area was noted within the tumor (B [H&E 2x]). The cellular area was composed of hyperchromatic spindle cells with increased nuclear to cytoplasmic ratio (C [H&E 40x]). Ki-67 showed a high proliferation index (>95%) (D [Ki-67 40x]).

Conclusion: To our knowledge, this is the first case report of recurrent lipoblastomatosis showing histologic features resembling dedifferentiated liposarcoma. This high-grade morphologic transformation occurred in conjunction with cytogenetic evidence of clonal evolution of the tumor and increased frequency of clinical recurrence over two decades.

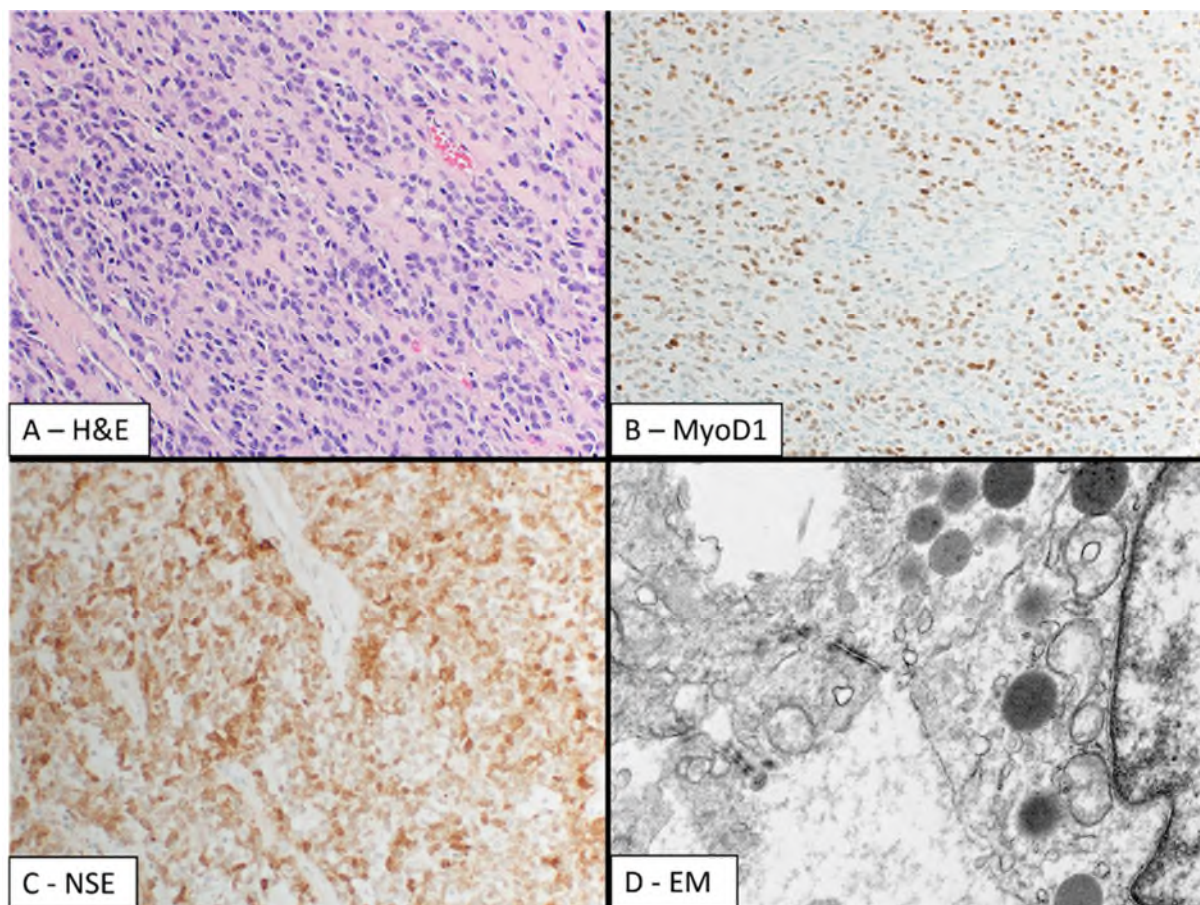
RREB1::MRTFB Fusion+ Oropharyngeal Mesenchymal Neoplasm with Neuroendocrine and Rhabdomyoblastic Phenotype

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Background: The RREB1::MRTFB fusion has been described extensively in ectomesenchymal chondromyxoid tumors (EMCMT). EMCMTs occur in the anterior dorsal tongue and consist of bland spindle cells with pale eosinophilic cytoplasm separated by thin fibrous septa. This fusion has also been reported in a few cases of biphenotypic sinonasal sarcoma (BSNS). We report on a RREB1::MRTFB+ oropharyngeal tumor with a unique phenotype.

Methods: An 18-year-old male presented with difficulty breathing. Imaging revealed a posterior midline oropharyngeal soft tissue mass. Grossly, the 5.8 x 3.3 x 2.7 cm polypoid mass had a lobulated surface. Cut surfaces were tan-gray and soft to slightly gelatinous. Histologically, the tumor was composed of clusters of monomorphic small round blue cells with ovoid nuclei displaying fine chromatin and inconspicuous nucleoli with scant amounts of pale to eosinophilic cytoplasm and separated by delicate fibrous septa. Rare mitotic figures were identified without calcification, necrosis, or hemorrhage.

Results: Immunohistochemical (IHC) staining revealed the neoplastic cells to be immunopositive for muscle markers (MyoD1, myogenin, desmin), neuroendocrine markers (NSE, synaptophysin, CD56, dot-like cytoplasmic CD99) and vimentin. They were immunonegative for epithelial markers (pancytokeratin, EMA, CAM5.2), chromogranin A, β -catenin, GFAP, SMA, MSA, CD45, CD3, CD19, CD117, and CD163. Ki67 proliferation index was low. HPV and EBER in situ hybridization were negative. Electron microscopy (EM) revealed discohesive cells with rudimentary cell junctions, prominent Golgi apparatus, and dense core neurosecretory granules of varying sizes (150 nm – 500 nm), consistent with neuroendocrine differentiation. No rhabdomyoblastic features were seen. Cytogenetics studies found t(6;16)(p24;p13.1) and FISH was negative for FOXO1 rearrangement. Next generation sequencing revealed a RREB1::MRTFB fusion and no DICER1 or other variants.



Conclusion: The histological, EM, and IHC findings are consistent with a mesenchymal tumor with neuroendocrine and rhabdomyoblastic differentiation. While the RREB1::MRTFB fusion could support the diagnosis of EMCMT, the tumor was negative for S100 and GFAP. Only five cases of RREB1::MRTFB+ extra-glossal mesenchymal neoplasms that were not reported as either EMCMTs or BSNS are reported in the literature, including the prior biopsy of this lesion (Case 5 <https://doi.org/10.1002/gcc.23082>). However, the additional IHC and EM examination revealed features subsequently found in that biopsy but not reported in the other tumors with this fusion. This combination of neuroendocrine and rhabdomyoblastic differentiation represents a unique biphenotypic RREB1::MRTFB+ oropharyngeal mesenchymal neoplasm and suggests the need for further examination of this class of tumors.

MYH::USP6 Fusion in a Proliferative Myositis-like Soft Tissue Mass

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Background: Nodular fasciitis (NF) and proliferative myositis (PM) share clinical and histopathologic features suggesting a spectrum of disease; however, to our knowledge, the USP6 rearrangement characteristic of NF has not to date been reported in PM. In fact, the recent identification of FOS rearrangement in PM suggests that at least some PM might be genetically and biologically distinct from NF.

Methods: A PET-avid right paraspinal muscle mass was detected in an 8-year-old girl, 5 months after early termination of chemoradiation therapy (due to parental discretion) for an ipsilateral primary renal sarcoma harboring two DICER1 variants in the setting of normal germline DICER1 testing. The paraspinal mass (3.9 x 2.8 x 1.5 cm) was at the level of the lumbosacral junction; it was FDG-avid and T2-hyperintense. An ultrasound-guided percutaneous biopsy was obtained.

Results: Histologic examination revealed a fasciitis-like fibroblastic proliferation involving skeletal muscle. Cells ranged from plump/ganglion-cell-like to focally elongate and feathery. Extravasated red blood cells were noted. In areas, the proliferation dissected through skeletal muscle, imparting a "checkerboard" architectural pattern histologically indistinguishable from PM (Figure 1). Lesional cells were positive for SMA and caldesmon (patchy) and negative for desmin. Molecular analysis revealed a fusion between MYH9 (NM_002473.4) exon 1 and USP6 (NM_004505.3) exon1. At last follow-up, 6 weeks following paraspinal biopsy, the mass was stable in size and the patient remained in remission from her renal sarcoma.

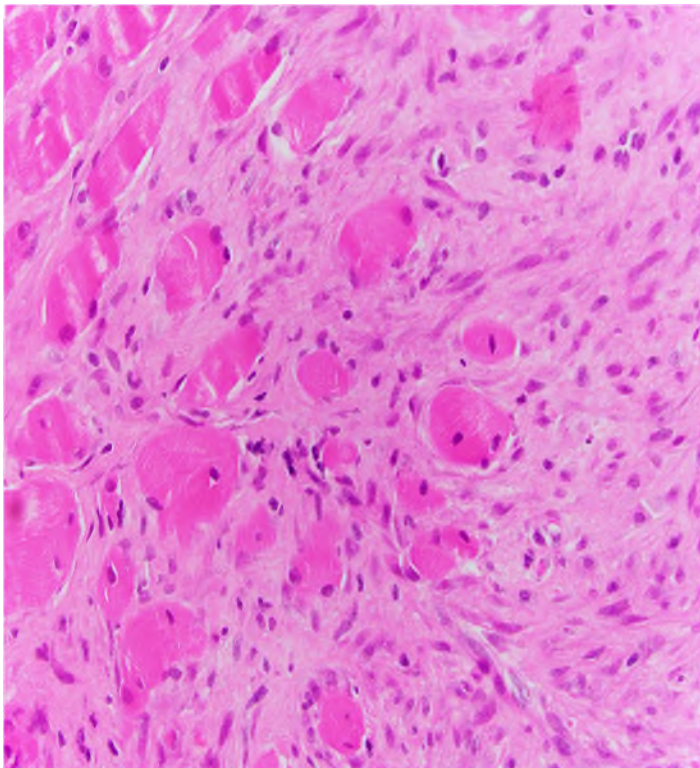


Figure 1. Plump fibroblasts proliferating between muscle fibers create a "checkerboard" pattern, classic for proliferative myositis.

Conclusion: Our finding of MYH::USP6 rearrangement in a pediatric soft tissue mass with features indistinguishable from PM is novel and suggests that at least a subset of PM may contain USP6 rearrangement and may be pathobiologically related to NF, as might be surmised based on clinical and histopathologic features. Whether FOS-rearranged PM and USP6-rearranged “PM” warrant subclassification based on their molecular findings remains to be determined. To our knowledge, neither PM nor NF have been reported to occur in patients with DICER1 tumors; however, an association with trauma has been proposed, and it is difficult to exclude whether proximity to local surgery or flank radiation played a role in this case.

Inhalational Constrictive Bronchiolitis Due to Particulate Matter Pollution Exposure

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Background: Particulates <10 micrometers (PM10) can embed within the lung, leading to significant respiratory disease, usually via a cumulative exposure that becomes more pronounced with advanced age. It is rare to see substantial pollution-related sequelae in pediatric pathology samples, particularly in the United States (US), where rigorous carbon emission standards and public health practices reduce particle pollution to a minimum compared to other areas of the world.

Methods: Shortly after moving from his native country of Nigeria to northern New England, a 9-year-old boy presented to his pediatrician with a 2-year history of wheezing and exertion-induced asthma. He was referred to a pulmonologist for further work-up. PFTs revealed a moderate obstructive ventilatory defect, with minimal bronchodilator response. Extensive mosaic attenuation with substantial air trapping on expiration and mild bronchial wall thickening was noted on CT scan. A working diagnosis of bronchiolitis obliterans was made. At age 11, he developed a spontaneous left-sided apical pneumothorax that resolved with chest tube placement. Lung from the apical left upper lobe, lingula and left lower lobe was sampled via wedge biopsy at the time of pleurodesis.

Results: Biopsy samples showed extensive anthracotic-pigment-laden macrophages, many located in collections around airways that were chronically remodeled, with variable subepithelial fibrosis, smooth muscle hyperplasia, and sparse chronic inflammation; features were consistent with inhalational constrictive bronchitis/bronchiolitis. Given the patient's years in Nigeria (a country harboring cities among the WHO's top most air-polluted list), additional history confirming chronic exposure to indoor cooking while there, and the histologic pattern, the inhalational exposure was attributed to carbon-rich air pollutants in Nigeria. Clinical follow-up included reviewing indoor cooking and burning practices with his family to minimize potential ongoing exposures.

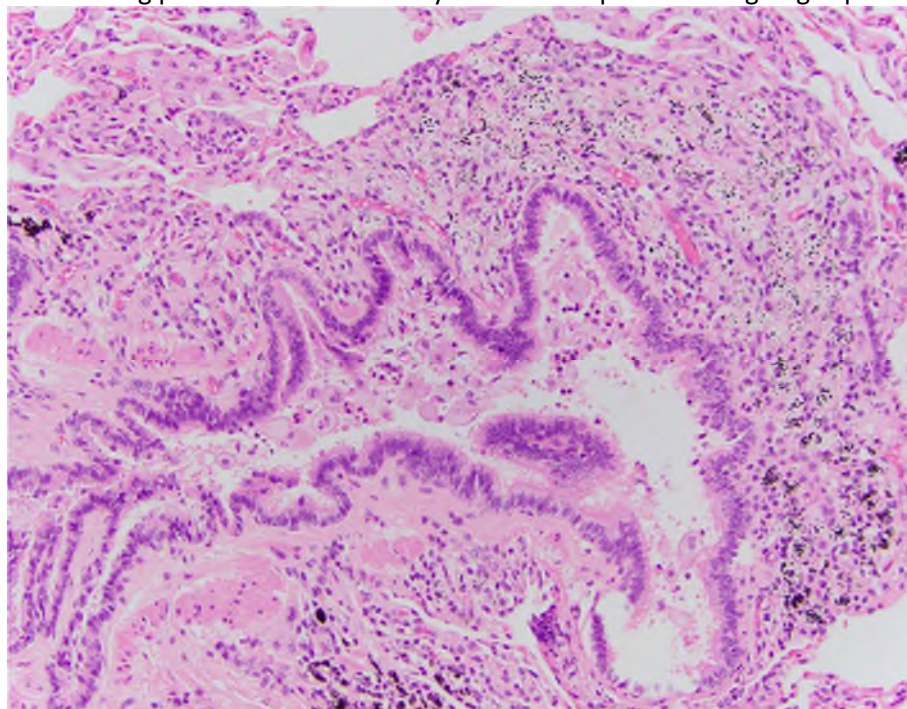


Figure 1. Constrictive bronchiolitis, characterized by chronic airway remodeling accompanied by innumerable macrophages containing fine black particulate matter

Conclusion: We highlight a case of inhalational constrictive bronchiolitis due to air pollutants, a disorder that is rarely encountered in pediatric biopsy specimens in the US. Awareness of this entity is particularly important to health management for a vulnerable immigrant population in this country. Furthermore, our case highlights the tragedy of a preventable disease that is more widespread in countries with high levels of particulate pollution.

Cellular Neurothekeoma on the Tongue of A 3 Year Old Girl, An Uncommon Lesion in an Unusual Location, A Case Report

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Background: Cellular Neurothekeoma (CN), an entity of possible fibrohistiocytic histogenesis, can be challenging to diagnose. CN is most often a well-defined, circumscribed, lobular dermal lesion, which usually occur on the head and neck, and upper body of young female patients. CN tend to have a whorled architecture and lesional cells are mostly epithelioid. There are variations with some cases having a prominent myxoid background, a subset showing dense collagen (desmoplastic CN) and others having nevoid features. Herein, we describe an example of CN occurring in the tongue of a child.

Methods: The clinical presentation, gross and histologic examination, and immunohistochemical (IHC) work-up, are reviewed.

Results: A 6 mm round pedunculated lesion was incidentally discovered by the otolaryngologist on the posterior tongue of a 3 year-old girl who was being assessed for tympanic membrane disease of the ear. The excised lesion had a slightly indurated surface. Histopathologic examination revealed a circumscribed, un-encapsulated with an overall nested pattern of arrangement and a central area of dense collagen with associated ectatic vessels and permeating lesional cells. The lesional cells were spindled and epithelioid. Focally 3 mitoses were seen in one high power field (HPF), but the overall mitotic rate was 5/10HPFs. At the base of the lesion was a large nerve. Hemosiderin and hemosiderin-laden macrophages were sprinkled through the lesion. A rare vessel containing lesional cells was noted. Collagen trapping occurred in the peripheral areas. Margins were negative. IHC staining demonstrated diffuse nuclear reactivity with MiTF and variable moderate to strong membranous staining with D2-40 of the lesional cells. CD163, S100, cytokeratin, smooth muscle actin, CD31, ERG, Melan A and c-KIT were non-reactive. The morphologic and immunophenotypic features are most consistent with CN.

Conclusion: CN may have a mixture of histologic subtypes and can occur in the tongue. It must be considered in the differential diagnosis of spindled and epithelioid lesions with a nested pattern when considering entities of a fibrohistiocytic, peripheral nerve sheath, glomus, mast cell or melanocytic derivation. Appropriate conservative clinical follow-up is indicated when atypical features are present, but a good outcome is to be expected

Orbital and peri-orbital pathology in pediatrics: an institutional perspective on its challenges and diagnostic dilemmas

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Background: The orbital and peri-orbital region offers a diverse medical and neoplastic pathology. Primary neoplasms of the orbit can arise from ocular structures and surrounding mesenchymal tissues. In addition, a systemic autoimmune process or secondary metastasis can present as orbital masses. We examined our institution's experience to define the prevalence of pediatric ocular pathology and to highlight diagnostically challenging cases.

Methods: Records of all orbital and per-orbital biopsies and resections at our institution (1975 to May 2022) were reviewed. Final diagnoses were grouped using general classifications.

Results: 835 cases were identified. 127 (15.2%) were functional/cosmetic surgeries, 58 (6.9%) were foreign body associated, 425 (50.9%) were benign neoplasms, 96 (11.5%) were malignant neoplasms, 6 (0.7%) were immune-mediated, and 53 (6.0%) were miscellaneous. 13 challenging cases were chosen for discussion (table).

Table

Diagnosis	# of procedures to reach diagnosis	Immunophenotype	Molecular/Ancillary Test
Desmoplastic small round cell tumor	1	Positive Desmin, and WT1 (C-terminus).	EWSR1-WT1 fusion
Mesenchymal chondrosarcoma	1	N/A	N/A
Sclerosing epithelioid fibrosarcoma	4	Negative AE1/AE3, S100, Desmin, SMA, MyoD1, WT1, and synaptophysin.	EWSR1-CRE3L1 fusion
EBV-associated smooth muscle tumor	1	Positive SMA, weak S100, negative CD45, and GFAP.	EBER positive
Teratoid medulloepithelioma	1	N/A	Negative for <i>RB1</i> mutation,. No DICER1 performed
Myeloid sarcoma	2	Positive MPO, lysozyme, and CD56. Subset positive CD117, CD34, and CD68.	Flow cytometry confirms immunophenotype.
Anaplastic large cell lymphoma	2	Positive CD30, CD4, CD56, and ALK.	N/A
Juvenile angiofibroma	1	Negative GFAP, Olig2, and EMA.	N/A
Cranial fasciitis	2	Positive SMA. Negative ALK and S100.	N/A
Juvenile psammomatoid ossifying fibroma	1	N/A	N/A
Eosinophilic angiocentric fibrosis	2	Scant plasma cells: Positive CD138. IgG and IgG4 inconclusive.	Serum IgG4 elevated. NGS molecular testing negative

ANCA-associated vasculitis	1	Macrophages: Positive CD68 and 163. Negative S100 and CD1a.	p-ANCA positive EM: Granular osmophilic deposits within the cytoplasm.
Ceroid-lipofuscinosis	1	N/A	

Conclusion: This review outlines the range of orbital pathology in the pediatric population. Limited sampling of this region can hinder histological interpretation and ancillary testing, leading to diagnostic delays or errors. Therefore, broad differentials, including rare primary sarcomas, secondary malignancies, and autoimmune disorders, must be considered to avoid diagnostic pitfalls.

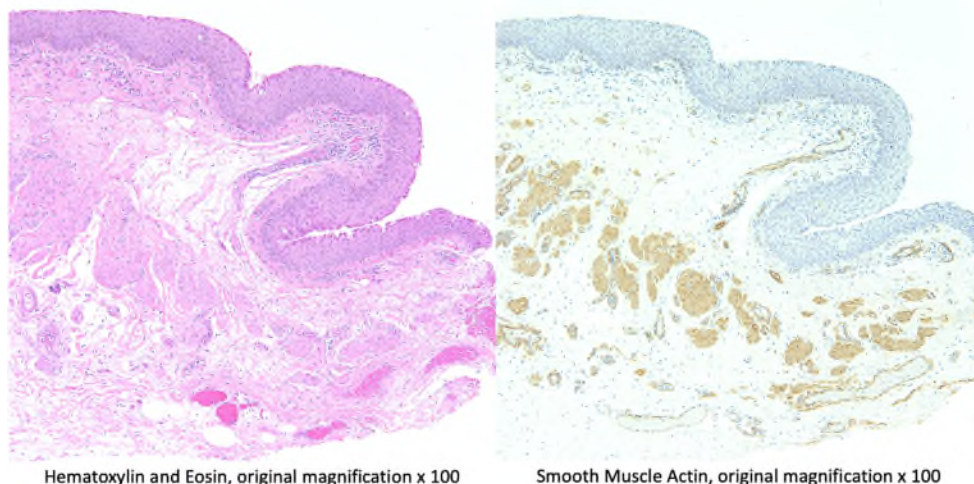
Presentation of a Smooth Muscle Hamartoma in the Bulbar Conjunctiva of an Adolescent Male

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Background: Lesions developing in the conjunctiva can represent a variety of diagnoses in children and adolescents. One uncommon lesion that can develop in the epibulbar tissue is a smooth muscle hamartoma, a disorderly expansion of smooth muscle cells, which has been discussed sparingly in the literature. We describe the first case of a conjunctival smooth muscle hamartoma in an adolescent patient.

Methods: This is a retrospective case report.

Results: A 17-year-old male presented with a large, non-tender lesion of the right bulbar conjunctiva that developed one year prior to presentation and did not improve with medical management. He had no previous medical or ocular history. The lesion was excised and histopathologic examination disclosed morphologically benign smooth muscle bundles that stained positively for smooth muscle actin, vimentin, and desmin. These findings were consistent with the diagnosis of smooth muscle hamartoma.



Conclusion: While there have been few reports of congenital smooth muscle hamartomas in the conjunctiva, this is the first reported case of an acquired smooth muscle hamartoma in the bulbar conjunctiva, a diagnosis that should be considered in the differential diagnosis for adolescents with similar lesions.

Papillary Intralymphatic Angioendothelioma in a Child with PIK3CA-related Overgrowth Spectrum: Implication of PI3K/AKT Pathway in Vascular Tumorigenesis

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Background: Papillary intralymphatic angioendothelioma (PHILA), also known as Dabska tumor, is extremely rare and its pathogenesis is unknown. Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA)-related overgrowth spectrum (PROS) is a heterogeneous group of disorders caused by genetic mosaicism for activating mutations of PIK3CA and characterized by asymmetric overgrowth with underlying vascular malformations. An association between PHILA and PROS has not been previously reported.

Methods: The case is a part of the institutional review board-approved retrospective study of histology and genetics of rare pediatric tumors. The patient is a 3 year-old girl with clinical and genetic diagnoses of PROS and a slowly growing splenic mass. Targeted Illumina sequencing using PCR primers flanking the PIK3CA gene region on chromosome 3 was performed on DNA obtained from cord tissue of the patient and blood of her both parents with the appropriate consent. The patient's splenectomy specimen was examined by routine pathology and immunohistochemistry and the diagnosis was confirmed by 2 independent pathologists.

Results: The patient clinical characteristics included hydrocephalus, global developmental and motor delay with intractable epilepsy and hemihypertrophy with multilocular hyperintense structures in subcutaneous tissues of the left upper arm and chest, consistent with lymphatic malformation. The sequencing of the patient DNA found a pathologic variant (c.1357G>A) in the *PIK3CA* gene (NM_006218.3), with the variant allele fraction of 10.6%. Sequencing of the parents' DNA was negative, confirming the *de novo* status of the variant in the proband. A small splenic mass detected by the patient's surveillance imaging was slowly growing for a period of 1 year. The splenectomy revealed a 4-cm well delineated and vaguely lobular tumor with histological and immunohistochemical features diagnostic of PHILA. The tumor morphology was characterized by morula-like three dimensional intravascular projections composed of tall cells with plump cytoplasm, occasional intracytoplasmic vacuoles, and round nuclei with dispersed chromatin, consistent with an immature endothelial phenotype. This was further supported by expression of CD31, CD34, ERG, FLI-1, caldesmon, and under expression of D2-40.

Conclusion: The data implicates *PIK3CA* in the pathogenesis of PHILA and broadens the spectrum of phenotypic expressions of PROS. The observed immature phenotype of the tumor cells can be explained by the arrest of final steps of specialized lymphatic endothelial differentiation as a result of *PIK3CA* mutation, in keeping with the known role of PI3K/AKT/mTOR pathway in the progression of vascular progenitors to mature endothelial cells.

High-Grade Osteosarcoma Without Complex Karyotype or Common Driver Mutations: A Single Institution's Recent Experience

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Background: Osteosarcoma (OS), an overall uncommon tumor, is the most common bone malignancy. It has a bimodal age distribution, the first peak of which occurs in adolescence. With more advanced understanding of its molecular underpinnings, identification of certain driver mutations (e.g. in TP53 and RB1) and complex karyotype has become a surrogate marker of high-grade malignancy. In this study, we present our institution's recent experience with high-grade OS without the characteristic molecular findings.

Methods: Chromosomal microarray analysis and DNA- and RNA- based targeted deep sequencing were performed on biopsy or resection specimens obtained at our institution or referred in consultation from outside hospitals.

Results: Only two patients at our institution received a diagnosis of high-grade OS without typical molecular genetic findings. The first patient presented to medical attention at age 10 years with right leg pain. Biopsy of a destructive tibial mass with periosteal reaction yielded sclerotic bone with a small amount of neoplastic osteoid harboring non-atypical osteocytes. Chromosomal microarray did not reveal pathogenic copy number alterations; next generation sequencing identified a NF1 point mutation of unknown clinical significance (c.5513C>G, NM_001042492.2). A diagnosis of high-grade conventional OS, sclerotic osteoblastic type, was rendered. Thirty months after neoadjuvant chemotherapy and surgical resection, the patient developed a 4.9 cm right proximal humeral mass. Biopsy revealed similar histology and molecular findings as the initial biopsy, consistent with metastasis. The second patient presented to an outside hospital at age 16 years with left knee pain. Biopsy of a distal femoral mass was performed and high-grade conventional OS, chondroblastic type, was diagnosed. Staging investigations showed metastasis to the bilateral lungs. The patient underwent neoadjuvant chemotherapy and surgical resection of the primary. Forty months later, after progression of the pulmonary metastases, representative blocks of the primary tumor were reviewed at our institution, with agreement with the original histopathologic diagnosis. A representative block of a pulmonary metastasis was subjected to molecular testing; chromosomal microarray did not reveal copy number alterations and next generation sequencing identified a TSC2 point mutation of unknown clinical significance (c.4418A>G, NM_000548.4).

Conclusion: Although complex molecular abnormality is a hallmark of OS, a complex karyotype or mutations in TP53 and RB1 may not be necessary to cinch a diagnosis of high-grade malignancy. The clinical, radiologic, histomorphologic, and molecular findings should all be considered when entertaining a diagnosis of OS.

Histopathologic “Evolution” in Pediatric Primary Intracranial High-grade Sarcoma – a Key that Unlocked the Correct Diagnosis

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Background: Soft tissue sarcomas account for ~7% of all pediatric cancers. However, primary intracranial sarcomas are rare. They can arise from smooth muscle cells of the vasculature, pluripotent mesenchymal cells of embryonic rests, or from the inner layers of arachnoid and pia. They pose a diagnostic challenge due to lack of differentiation, and significant histological variability. They may have an associated tumor defining genetic alteration such as DICER1 mutation. RNAase IIIb domain of DICER1 plays role in maturation of miRNA (micro-RNA) and gene silencing. Loss of function mutation in this domain leads to dysregulated gene silencing and carcinogenesis.

Methods: A 13-year-old African American girl presented with acute onset of headache and vomiting, and was found to have an intracranial hemorrhage. Brain MRI showed a right sided intraparenchymal lesion, with frontal cortical involvement, and leptomeningeal enhancement. She was operated twice at six weeks’ interval. The lesion was a high-grade, spindle cell neoplasm with high proliferation index (Ki67> 90%). Multiple immunohistochemical (IHC) markers were negative (AE1/AE3, desmin, caldesmon, myogenin, S100, SOX-10, pan-melanoma, CD99, beta-catenin, EMA, PR, and p53); the tumor cells only expressed vimentin, collagen IV, and STAT6. It was diagnosed as solitary fibrous tumor/hemangiopericytoma (SFT/HPC), WHO grade 3. The STAT6 IHC was subsequently found to be spurious (newly added antibody was under validation). The lesion was however, an undifferentiated high-grade sarcoma, not otherwise specified (NOS). Despite receiving chemotherapy, the tumor recurred after nine months. A significant morphologic observation in the recurrent tumor prompted further studies to identify a suspected genetic alteration.

Results: Histomorphology showed multiple foci of cells with overt rhabdomyoblastic differentiation arising in a background of a high-grade sarcoma. These cells were positive for desmin, myogenin and myoglobin IHC stains. Of note, repeat STAT6 IHC was negative. Targeted next generation sequencing (NGS) based assay revealed following: a missense variant (p.E1705V) and a nonsense variant (p.Y1417Ter) in DICER1, a missense variant in KRAS (p.G12V), and partial exon 5 deletion in TP53. These results helped categorize our case as a “spindle cell sarcoma with rhabdomyosarcoma-like features, DICER1 mutant”.

Conclusion: The emergence of overt rhabdomyoblastic foci in this previously undifferentiated high-grade sarcoma helped us suspect, and subsequently establish the diagnosis of the newly defined entity of spindle cell sarcoma with rhabdomyosarcoma-like features, DICER1 mutant. Prompt molecular studies in pediatric undifferentiated high-grade sarcomas are indicated for precise diagnosis and classification, and likely targeted treatments in the future.

Embryonal Tumor with Multilayered Rosettes and Familial BRCA2 Mutation: A New Association?

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Background: Embryonal tumor with multilayered rosettes (ETMR) is a relatively rare, aggressive, and rapidly progressive CNS tumor. In most cases, ETMR exhibits a well-described chromosome 19 miRNA cluster (C19MC) alteration; a subset of cases with an alternative molecular finding are seen in DICER1 cancer predisposition syndrome. We report a case of ETMR occurring in the setting of familial BRCA2 mutation – a novel association that has not yet been reported.

Methods: An 18-month-old female presented to the hospital after experiencing a week of twitching of the neck and arms, head tilt, vertical diplopia, eye drooping, and difficulty walking. Her family history was notable for a maternal breast cancer with known BRCA2 mutation. MRI revealed a well-defined, nonenhancing 2.8 x 2.9 x 3.0 cm expansile right pontine mass.

Results: On histology, the tumor was composed of poorly differentiated embryonal-appearing cells with very focal rosette formation (figure 1); rosettes were primarily monolayered. A neuropil-like matrix was difficult to discern. There was weak immunoreactivity for synaptophysin in the neuropil-like matrix that was present in the less cellular areas. iFISH identified amplification of C19MC miRNA cluster on 19q13, corresponding with a diagnosis of ETMR. Further molecular testing of tumor tissue revealed a BRCA2 mutation identical to the known maternal germline BRCA2 mutation. Chemotherapy was initiated and she is currently clinically stable with ongoing outpatient follow-up.

Conclusion: ETMR is a rare, aggressive, and rapidly progressive tumor. While these tumors display a broad histologic spectrum, the C19MC alteration is consistent with this diagnosis regardless of histologic appearance. On histology, ETMR typically displays frequent and characteristic multilayered rosettes with abundant neuropil. In this case the rosettes were rare and the neuropil was difficult to discern. The focal weak synaptophysin positivity provided a clue to the tumor's identity, and C19MC amplification by iFISH established the diagnosis. ETMR has been described in the context of DICER1 cancer predisposition syndrome. Our patient did not have DICER1 syndrome; however, the patient's tumor showed a BRCA2 mutation identical to the maternal germline BRCA2 mutation. Our case may represent a previously unknown association with ETMR and BRCA2 which highlights a potential syndromic association that can have a significant impact on the care of other family members of patients with this diagnosis.

Against the Grain: A Mature Cystic Teratoma Without Neuronal Differentiation in a Patient with Anti-NMDA Receptor Encephalitis

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Background: Anti-N-methyl-D-aspartate (NMDA) receptor encephalitis due to a paraneoplastic syndrome arising from autoantibodies produced in ovarian teratomas has been recently characterized. The condition is mediated by autoantibodies produced by cross reactivity with NMDA receptors in teratomas, therefore brain tissue and inflammation are the mainstay of the excised specimens for this condition.

Methods: We present a case of a 15-year-old female with a history of escalating manic behavior and seizure activity characterized by unresponsive staring even to tactile stimulation with documented post-ictal state. Magnetic resonance imaging (MRI) of her pelvis revealed a 2 cm multilocular right ovarian cyst and she underwent laparoscopic right oophorectomy. Cerebral spinal fluid analysis demonstrated a high titre of anti-NMDA receptor antibodies.

Results: On gross examination, the ovary was smooth and intact, revealing a mature cystic teratoma composed of mature tissue of all three germ layers. The entire specimen had been sectioned and examined as neither CNS components (neuroglial or neuronal) nor lymphoid inflammation was present. Interestingly, a prominent component of the lesion was composed of small lymphovascular spaces lined by multinucleated foam cells, previously described as “sieve-like” or “pneumatosis cystoides” appearance.

Conclusion: Anti-NMDA receptor encephalitis has been associated with teratomas in about half of the cases. Another established trigger is herpesviral encephalitis, while the cause in others cases is unclear. Histologically, all cases of teratomas demonstrate central nervous tissue-like differentiation along prominent lymphocytic infiltrate arranged in tertiary lymphoid structures with distinct B and T cell patterns. Our case represents a rare case of mature cystic teratoma associated with an anti-NMDA receptor encephalitis which lacks neural and neuroglial differentiation or lymphocytic infiltration, suggesting an alternate pathological mechanism for an anti-NMDA-teratoma-associated encephalitis.

Three for the Price of One: Resolution of Celiac Disease, Aplastic Anemia, and IgA Deficiency after Bone Marrow Transplant

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Background: Exposure to gluten causes inflammation in the gut of some patients. Repeated exposure leads to gradual damage of the small intestine, which can lead to problems absorbing minerals and nutrients from food. Celiac disease affects around 1 in 100 people worldwide, and many have the condition without knowing it. There is no cure to celiac disease and the only way for someone with celiac disease to avoid the symptoms, is to keep gluten out of their diet.

Methods: We present a case of a 12-year-old girl diagnosed two years prior with celiac disease, confirmed histologically. She also had a synchronous diagnosis of aplastic anemia for which she received 10/10 HLA phenotypically matched peripheral blood stem cell transplant from her mother along conditioning regimen consisting of fludarabine, cyclophosphamide, alemtuzumab, and 200 cGy of total body irradiation. Two years after the bone marrow transplant she had no significant infections, no specific chronic graft-versus-host disease, no weight loss, no skin rashes and no symptoms related to regular gluten intake. Her TTG IgG was positive (over 250), but her TTG IgA was normal, with normal total IgA levels.

Results: From the perspective of the severe aplastic anemia post-transplant, she had a normal complete blood count and was transfusion-independent and was considered cured. From the perspective of celiac disease, her upper GI endoscopy and microscopy showed essentially normal duodenum, antral, body and oesophageal mucosae.

Conclusion: The correction of immune diseases by stem cell transplants had been previously documented. To the knowledge of the authors, this is the first child in N-America to undergo complete resolution of all her initial diseases - aplastic anemia, celiac disease and IgA deficiency - after bone marrow transplant.

The Epidemiology of Mediastinal Lesions across the Pediatric Population in the Province of Manitoba, Canada - 20 Years Experience

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Background: Mediastinal lesions (ML) in children may pose a diagnostic challenge, while being life threatening (anterior mediastinum particularly). The Pediatric Oncology Team in Manitoba has recently drafted a protocol for the management of children with anterior MLs. Specific questions had been addressed to the pathology team. The objective of this study was to address those interrogations, related to surgical approach, tissue procurement and histopathological diagnosis of ML across the pediatric age spectrum.

Methods: A retrospective review of institutional records of patients <18 years (410 cases) from 2000-2021 was conducted. We included histopathological diagnoses related to radiologically proven ML only. We extracted demographic, anatomic, surgical and pathological data from the available charts.

Results: 98 cases were included for analysis. The most common anatomic site was posterior mediastinum (35%). Neoplastic malignant (51%), developmental (17%) and neoplastic benign (10%) were the most common histopathological categories. In the anterior mediastinum, malignant neoplasms were represented by lymphoproliferative (52%); followed by germ cell tumors (9%) and developmental lesions (9%); benign neuroblastic tumors (21%), developmental (14%) and malignant neuroblastic tumors (14) for posterior mediastinum. Importantly, only children over 15 years had a predominance of neoplastic lymphoproliferative processes in the anterior mediastinum; the 0-4 years showed predominantly developmental processes, and 5-14 years predominantly non-lymphoproliferative neoplastic processes ($p=0.02$). Anterior and superior mediastinum showed a similar histopathological diagnoses distribution, while mid and posterior mediastinum had their own but different pattern ($p=0.0005$). Cases with concurrent neck/axillary adenopathy and mediastinal mass were all associated with lymphoproliferative neoplasms.

Conclusion: We identified several differences when compared to literature review. Such differences must be considered in the differential diagnosis and therapeutic approach considering the specific protocols elaborated by various Lymphoma or Pediatric Oncology Group for the pediatric patients with ML.

An Unusual Case of Extranodal Marginal Zone Lymphoma

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Background: Mature B cell non-Hodgkin lymphomas are relatively uncommon in the pediatric population, particularly in otherwise immunocompetent individuals. When they do occur, mature B cell lymphomas are often aggressive and most commonly include entities such as Burkitt lymphoma and diffuse large B cell lymphomas. Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), an indolent lymphoma, is extremely rare in the pediatric population and most commonly involves the intestinal tract or peri-orbital tissues as sequela of chronic antigenic stimulation. Here we present a very unusual case of MALT lymphoma primary to the fallopian tube in a 16-year-old female.

Methods: A 16-year-old female adolescent presented to our emergency department with left flank pain, left leg swelling, and vaginal bleeding of three weeks duration. Initial laboratory studies revealed markedly elevated creatinine and BUN. Imaging showed diffuse, slightly heterogeneous, enlargement of the uterus and bilateral ovaries with multiple locoregional soft tissue masses compressing the ureters bilaterally with hydronephrosis and bladder distension. Testing for sexually transmitted diseases and a pregnancy test were negative. At the time of diagnostic laparoscopy, the uterus was markedly enlarged and described as being "hard as a rock." The uterus and both fallopian tubes were firmly adherent to the pelvic wall. A left salpingectomy and peritoneal biopsies were performed.

Results: On histologic examination, a population of small-to-intermediate sized atypical lymphoid cells with irregular nuclear contours and slightly clumped nuclear chromatin diffusely infiltrated the sampled fallopian tube mucosa and formed a mass within the muscular wall. Immunohistochemical stains showed that the atypical cells were B cells positive for CD20 and CD79a with a low Ki-67 defined proliferation index. In-situ hybridization for EBER was negative. Molecular testing demonstrated a clonal IgH gene rearrangement, supporting the diagnostic impression of extranodal marginal zone lymphoma.

Conclusion: While the patient's age and clinical presentation are uncommon for the diagnosis of a MALT lymphoma, rare cases of primary extranodal marginal zone lymphoma of the fallopian tube have been reported. After 2 cycles of bendamustine and rituximab, the patient continues to experience ongoing kidney dysfunction but has shown excellent anatomic and metabolic responses to systemic chemotherapy on re-staging studies. This case highlights an unusual pediatric lymphoma causing an obstructive kidney injury in a patient without evidence of immunodeficiency, autoimmunity or known cancer predisposition; and demonstrates the need for experienced hematopathologists within the pediatric pathology space.

Rare Primary Renal Diffuse Large B-cell Lymphoma Mimicking Wilms Tumor: Case Report and Literature Review

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Background: Renal lymphoma, although rare, has been reported in children and should be considered in cases where the etiology of the kidney tumor is uncertain. We report a case of primary renal diffuse large B-cell lymphoma mimicking Wilms Tumor in a pediatric patient.

Methods: Clinical, radiologic, histologic, and immunohistochemical data along with cases discussed in literature were reviewed.

Results: A six-year-old male presented with recurring epigastric and abdominal pain for several weeks. CT imaging demonstrated a left renal mass measuring 14.0 x 13.0 x 9.3 cm, most likely representing a Wilms tumor, and midline lymphadenopathy. A radical nephrectomy with para-aortic lymph node sampling was performed. Grossly, the mass, confined to the kidney, was fleshy white-pink and focally hemorrhagic with multiple necrotic foci. Histologic sections showed a vaguely nodular infiltrate of large atypical lymphoid cells with round to irregularly shaped nuclei, open chromatin with occasional small multiple peripherally located nucleoli, and scant to moderate amount of clear to amphophilic cytoplasm. The atypical cells were diffusely immunoreactive for B-lymphocyte lineage markers (CD45, CD20, PAX-5) with a germinal-center type (CD10/BCL6 (+), MUM1 (-)), and non-double expressor immunophenotype (Myc (-) and BCL2 (+)). The Ki67 proliferation rate was approximately 90%. EBV ISH and FISH for BCL2, BCL6, and Myc were negative. The sampled para-aortic lymph nodes were not involved by the lymphoma. Staging bone marrow and cerebrospinal fluid studies were negative. Post-surgery PET scan showed surgical changes, but was otherwise negative. Currently, all work-up for a systemic diffuse large B-cell lymphoma has been negative, and the kidney is the only affected organ.

Conclusion: Renal diffuse large B-cell lymphoma is uncommon with most reported cases representing systemic manifestations. Adult studies show that renal lymphoma occurs more often in males with most as stage IV disease involving >2 extra-nodal sites and with an increased risk of CNS relapse. Primary renal diffuse large B-cell lymphoma is rare in the pediatric population, with few reported cases in children ranging in age from 4 to 18 years. Clinical presentation is variable from painless mass to renal failure. Primary renal lymphoma may be unilaterally or bilaterally. Pertinently, in unilateral cases, the lymphoma presented as a large mass (>12 cm) confined to the kidney, and the initial clinical suspicion was Wilms tumor based on imaging. While rare, consideration of primary renal lymphoma is essential when encountered with a renal tumor of unknown origin, especially in a child older than the typical age for Wilms tumor.

Pediatric Acute Erythroid Leukemias with Monocytic Antigen Expression and Novel Chromosomal Translocations

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Background: Acute erythroid leukemia (AEL) is a rare subtype of pediatric acute myeloid leukemia (AML). Previous genomic studies of adult and pediatric AELs identified enrichment of NUP98-rearrangement and a wide spectrum of somatic mutations. Recent study from Children Oncology Group reports recurrent NUP98-rearrangements in approximately one third of patients, but majority lack recurrent genetic abnormalities. Based on immunophenotype, they classify AEL into erythroid/myeloid and pure erythroid subtypes, with the latter subtype associated with a worse prognosis. Such that, further studies on genetic abnormalities and immunophenotypes are warranted.

Methods: Here we report two unusual AELs. The diagnosis of AEL is based on WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues 4th edition.

Results: Our first patient was a 2-month-old male with past medical history of sickle cell trait, who presented a right shoulder mass, with the biopsy showing diffuse sheets of malignant round blue tumor cells, immunostaining positive for CD43, E-cadherin and CD71, consistent with erythroid sarcoma. Peripheral blood contained approximately 13% blasts. The bone marrow biopsy showed approximately 81% blasts and no dysplasia in the background. Flow Cytometry showed blast population expressing CD13, CD43, CD71, and CD235 (partial), and variable monocytic antigen CD14. A fusion gene of CIC-NUT2MA was detected by next generation sequencing. Patient received a chemotherapy with partial response but deceased approximately 3 months after the diagnosis. Our second patient was a 2-year-old female with no significant past medical history, who presented with pancytopenia. Bone marrow biopsy identified up to 76% of blasts on aspirate smear, with a morphology compatible with erythroblasts. Flow Cytometry identified abnormal blasts with erythroid and monocytic differentiation, expressing CD71, CD117, HLA-DR, CD64 (dim), CD14 (partial), CD2 (partial/dim), CD16 (partial/dim), CD235 (partial). A fusion gene of NFIA-RUNX1T1 was detected by next generation sequencing. Patient received a chemotherapy and currently is in remission.

Conclusion: In summary, we show two pediatric AELs with erythroid and monocytic antigen expression, and unusual genetic translocations. Our data provide additional pathology on pediatric AELs and suggest that AEL is an immunophenotypically and genetically heterogeneous disease, which needs further studies.

Mucinous Cystadenoma of the Ovary with Intracystic *Enterobius vermicularis*

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Background: *Enterobius vermicularis* (E vermicularis), also known as pinworm, is a helminth that commonly causes intestinal parasitic infestation. E. vermicularis can also cause extraintestinal infestations and involve sites such as peritoneum, vagina, uterus, fallopian tubes, and ovaries. We report an E. vermicularis infestation identified in an ovarian tumor.

Methods: Clinical, radiologic, and gross and microscopic pathology of the index patient were reviewed along with reported cases of ovarian enterobiasis.

Results: A 14-year-old female presented with abdominal pain. A CT scan of showed a large 30 x 20 x 10 cm multiloculated cystic mass in the abdomen and pelvis abutting the anterior abdominal wall. The lesion displaced the bowel loops into the upper abdomen and completely effaced the right common iliac vessels. The preoperative differential was an ovarian tumor or a multiloculated cyst of the right ovary. She underwent right ovarian oophorectomy which yielded a 1,358 g, 24.3 cm flabby mass. The mass consisted of more than 30 cysts ranging in size from 0.5 to 11.5 cm and additional grape-like nodules within some of the larger cysts. The cysts contained bloody to tan-yellow mucinous fluid, and were lined by a single layer of cuboidal to columnar cells with abundant apical mucin. No features of malignancy were identified, and the diagnosis of mucinous cystadenoma was rendered. Within one of the cysts, two cross sections of a female adult nematode with a thick cuticle and lateral alae consistent with E. vermicularis were identified. Internal organs included female reproductive system with multiple eggs. The nematode was intact and "floating" within the cyst without eliciting any inflammatory response. The patient and her family members were subsequently treated for E. vermicularis infection.

Conclusion: Less than 20 cases of ovarian enterobiasis have been reported in the literature where it often causes pseudo-tumoral granulomas within which degenerated ova and adult worm are present. This report is unique in that the adult E. vermicularis identified in the extraintestinal location was relatively intact without tissue response. Mucinous secretions found within this cystadenoma, akin to lower gastrointestinal tract, may have provided more favorable environment for the pinworm to survive longer.

A Challenging Case of Unusual Presentation of “Cecal Mass” in a Morbidly Obese Adolescent Girl

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Background: Diverticulosis with fecalith has conventionally been noted in adulthood and most cases are identified pre-operatively or during surgery; we report a case of large submucosal cecal fecalith (6.3 x 4.5 x 3.6 cm) in a morbidly obese adolescent girl. This mass had clinical and radiological impression of a malignant neoplasm but the hemicolectomy specimen on pathology exam was correctly interpreted as, “Ruptured diverticulitis with fecalith”.

Methods: Patient had a broad clinical, serological, and radiological work-up. Fine needle aspirate of the cecal mass, U/S guided fine needle biopsy and ultimately right hemicolectomy were undertaken. Complete gross and histopathology exam were performed. Routine H&E staining and comprehensive immunohistochemistry panel were obtained.

Results: A previously healthy, 14-year-old girl presented with constipation and a 1-week history of severe lower abdominal pain, which was escalated during bowel movements. On physical exam, there was tenderness in the right and left lower quadrant. CBC, CMP, and UA were unremarkable without signs of infection. CT Abd/Pelvis w/ contrast showed a 6 cm well circumscribed bilobed hyperdense mass of right colon consistent with tumor, with a differential of carcinoid vs gastrointestinal stromal tumor. Given the location and size of this mass, a formal right hemicolectomy was performed, by the surgeon, to have adequate margins for potential malignancy. FNA of the mass showed “Necrotic tissue, favoring spindle cell neoplasm” and a biopsy for further characterization was recommended. Fine needle biopsy specimen revealed non-viable necrotic and calcified material. Gross examination of hemicolectomy specimen revealed a 6.3 X 4.5 x 3.6 cm yellowish brown fecalith- like mass that was impacted within a cecal diverticular outpouching. Histopathology was diagnostic for cecal diverticulitis with associated fecalith. No neoplastic process was identified. A pericolonic abscess secondary to rupture of diverticular wall was noted. Immunoprofile of mass was negative for viable tissue or neoplasm.

Conclusion: In the literature there have been occasional reports of pediatric cecal fecalith measuring up to 4 cm, with most having distinctive features on imaging studies; here we are reporting a first case in which a true cecal diverticulum with large fecalith (measuring 6.3 cm) was found in an adolescent age girl and unlike any previous case, this mass masqueraded as a malignant tumor till the time pathologic diagnosis of, “fecalith” was performed on the hemicolectomy specimen. Therefore, clinical staff, radiologists and pediatric surgeons need to keep this entity in mind in the differential diagnosis of a cecal mass. Early clarification of the etiology will enable strategic planning of the best treatment option.