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ABSTRACT BOOK

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Less Deformable Erythrocyte Subpopulations Biomechanically Induce Endothelial Inflammation in Sickle Cell Disease

Authors: Caruso, Christina; Cheng, Xiaopo; Michaud, Marina E; Thomas, Beena E; Szafraniec, Hannah M; Fay, Meredith E; Mannino, Robert G; Zhang, Xiao; Sakurai, Yumiko; Li, Wei; Myers, David R; Joiner, Clinton H; Wood, David K; Bhasin, Manoj; Graham, Michael D; and Lam, Wilbur A

Presenting Author: Christina Caruso, MD

Background: Sickle cell disease (SCD) is canonically characterized by reduced red blood cell (RBC) deformability leading to microvascular obstruction and inflammation. SCD vasculopathy is known to involve both hemolysis and adhesion of various blood cell populations to the endothelium, but the contribution of poor RBC deformability to endothelial dysfunction has not been explored. Leveraging interrelated in vitro and in silico approaches, we introduce a new paradigm of SCD vasculopathy in which poorly deformable sickle RBCs (sRBCs) directly cause endothelial dysfunction via mechanotransduction, where endothelial cells sense and pathophysiologically respond to aberrant physical forces independently of microvascular obstruction, adhesion, or hemolysis.

Methods: We leverage a multi-disciplinary approach involving microfluidic systems, cell immunostaining, single-cell RNA sequencing (scRNA-seq), and computational simulations with sRBCs and pharmacologically-dehydrated RBCs (pdRBCs) to address this new biophysical paradigm.

Results: Perfusion of sRBCs or pdRBCs into small venule-sized "endothelialized" microfluidics leads to pathologic physical interactions with endothelial cells that directly induce inflammatory pathways, evidenced by increased endothelial surface expression of VCAM-1 and E-selectin, biomarkers of endothelial inflammation. Using a combination of computational simulations and large venule-sized endothelialized microfluidics, we observed that perfusion of heterogeneous sRBC subpopulations of varying deformability, as well as suspensions of pdRBCs admixed with normal RBCs leads to aberrant margination of the less-deformable RBC subpopulations towards the vessel walls, causing localized, increased shear stress. Increased wall stress is dependent on the degree of subpopulation heterogeneity and oxygen tension, as sRBCs exposed to different oxygen concentrations exhibit altered rheological properties with changes in polymerized RBC number. Finally, subpopulation heterogeneity and increased wall stress leads to inflammatory endothelial gene expression via mechanotransductive pathways, as scRNA-seq reveals transcriptomic changes in endothelial cells exposed to heterogeneous RBC suspensions with a subpopulation of pdRBCs.

Conclusions: Our multifaceted approach demonstrates that the presence of sRBCs with reduced deformability leads directly to pathological physical (i.e., direct collisions and/or compressive forces) and shear-mediated interactions with endothelial cells and induces an inflammatory response, thereby elucidating the ubiquity of vascular dysfunction in SCD. This work has the potential to lead to a new paradigm of biophysical therapeutic strategies directed towards mitigating aberrant RBC margination in SCD.

Antenatal Butyrate Supplementation Improves Intestinal Barrier in Neonatal Murine Offspring

Authors: Colarelli, Andrea; and Barbian, Maria Estefania

Presenting Author: Andrea Colarelli, MD

Necrotizing enterocolitis (NEC) is a disease that primarily affects premature infants and can cause intestinal inflammation and necrosis, with mortality rate of 24%. An important driver in its pathophysiology is impaired intestinal barrier function, which may lead to bacterial translocation and excessive inflammation. Butyrate, a short-chain fatty acid, maintains colonic homeostasis, specifically intestinal barrier function, by upregulating tight junction (TJ) proteins. Our goal is to determine if antenatal butyrate supplementation (ABS) improves the intestinal barrier in neonatal offspring.

Mating pairs of C57Bl/6 mice received standard water (Control) or 90 mmol of sodium butyrate in their drinking water during pregnancy. In vivo intestinal permeability was analyzed in offspring from each treatment group at post-natal weeks 1, 2, or 3. Offspring were fasted for 4 hours, then gavaged with 20 µl/g of Fluorescein isothiocyanate-labeled 4.4-kDa dextran (FD4). After 4 hours, offspring were euthanized, and blood was collected. Serum FD4 concentration was determined by fluorescence spectroscopy. High serum FD4 suggests leaky intestinal barrier. Samples of ileum and colon were collected for future qRT-PCR analysis of TJ proteins.

2-week-old ABS offspring had lower serum FD4 compared to age-matched controls, though not statistically significant ($p=0.07$). There was a decrease in serum FD4 in control offspring as post-natal age increased ($p=0.03$). While serum FD4 was unchanged in ABS offspring as post-natal age increased, the level was similar to that of 3-week-old control offspring. The change in serum FD4 over time and between ABS and control groups was statistically significant ($p=0.02$ and $p=0.002$, respectively).

Based on this preliminary data, antenatal butyrate supplementation in mice promotes intestinal maturity in neonatal offspring. 1 and 2-week-old offspring exposed to ABS had serum FD4 concentration similar to 3-week-old control offspring. This suggests the intestinal barrier of these mice is more intact. Our sample size in each group was small; thus, we will increase our sample size to better characterize ABS' effect. Next, we will assess expression of TJ proteins in the ileum and colon of these offspring. Finally, we plan to evaluate intestinal barrier function after exposure to a model of neonatal gut inflammation to better represent the pathophysiology of NEC.

Utilizing Ribosome-directed Small Molecules and Nucleotide-based Approaches to Overcome Distinct Subclasses of CFTR Variants

Authors: Jackson, JaNise J; Foye, Catherine; Winters, Ashlyn G; Freestone, Emily; Du, Yuhong; Sasaki, Shruti; Huang, Lulu; and Oliver, Kathryn E.

Presenting Author: JaNise Jackson, PhD

Background: A substantial portion of the global CF population remains unresponsive to and/or ineligible for CFTR modulators. Our work endeavors to identify and therapeutically target genetic interactions that influence biogenesis of refractory CFTR variants encoded among such patients. We previously discovered ribosomal protein L12 (RPL12 or "uL11") as a robust modifier of mutant CFTR processing, with ~50% knockdown of RPL12 conferring improved functional expression of specific variants from different CFTR subclasses (e.g. F508del, W1282X). In the present study, novel antisense oligonucleotides (ASOs) and small molecule inhibitors of RPL12 were developed and evaluated for potential to rescue the same CFTR variants.

Methods: Ionis Pharmaceuticals generated ASOs against human RPL12, which were tested for efficacy in CF bronchial epithelia (CFBE41o-) stably expressing wild-type or F508del cDNA. CFTR mRNA expression, protein, maturation, and channel function were quantified. For high-throughput screening (HTS), Fischer rat thyroid (FRT) cells were stably transduced with W1282X-CFTR or RPL12 encoding a C-terminal, in-frame Nano-Luciferase reporter. CFTR- and RPL12-luciferase expression, as well as cell viability, were measured.

Results: In CFBE, we show two ASOs decrease RPL12 protein to similar levels achieved with siRNAs. These RPL12 ASOs significantly augment wild-type and F508del-CFTR band C maturation, in addition to F508del-dependent short-circuit currents. Early HTS results from FRT cells revealed 47 compounds at which ~50% suppression of RPL12 is attained. These agents are undergoing structure-activity relationship assessments to establish functional group modifications with correlation to improved efficacy. Preliminary data also indicate that many established inhibitors of ribosome function (e.g. G418, ELX-02, PTC-124) do not impair RPL12 production. Surprisingly however, the horse chestnut seed extract, Escin, was found to significantly reduce RPL12 expression while modestly enhancing W1282X read-through.

Conclusions: Partial depletion (~50%) of RPL12 levels represents a feasible strategy for CFTR modulation, which may be applicable to CFTR genotypes refractory to available clinical interventions. Overall, this work serves as a foundation from which future investigations may be pursued to examine efficacy and tolerability of anti-RPL12 compounds or ASOs delivered to CF animal models. This study was supported by the NIH, U.S. CFF, and Atlanta Pediatric Research Alliance.

Improving the Sickle Bone Marrow with 601

Authors: Yoo, Justin; Hernandez, Britney; Zgodny, Jordan; Priyadarshini, Anupama; Kostamo, Zachary; Zhang, Yankai; Patel, Ashwin; Shen, Huifeng; and Sheehan, Vivien

Presenting Author: Justin Yoo, MD

Background: In sickle cell disease (SCD), the abnormal sickle hemoglobin, (HbS), causes hypoxic injury to the bone marrow (BM) and pathologic angiogenesis. The BM niche is critical in stem cell maintenance and effective erythropoiesis; its health is important for the success of gene therapy (GT). Chronic transfusion reverses pathologic angiogenesis and hypoxic injury. However, years of transfusion for possible future GT is not sustainable. GBT021601 is an oral drug that increases Hb. We hypothesize that GBT021601 will reduce hypoxic injury to the BM, improve ineffective erythropoiesis, and preserve the BM vasculature similar to transfusion.

Methods: Townes HbSS mice were fed with chow containing either 0.2% or 0.4% of GBT021601, or control chow. After 12 weeks, a CBC, and flow for mitochondrial retention was obtained from peripheral blood. Spleen weight per body weight was obtained as a proxy for extramedullary erythropoiesis. Erythroblast populations and apoptosis were measured by flow cytometry of the BM. Femoral vasculature was assessed using 3D confocal microscopy staining for Sca-1 and immunoblot was performed on plasma for proangiogenic markers VCAM-1, Ang-1, Ang-2, and VEGF.

Results: GBT021601 increased Hb in a dose dependent manner (0.4%: 16.5 g/dL, $p<0.001$; 0.2%: 13.4 g/dL, $p=0.03$; vs control 8.9 g/dL), reduced mitochondrial retention (8336 MFI vs 11377 MFI, $p=0.01$) and spleen-to-whole body weight ratio (3.19 vs 5.73, $p<0.001$) compared to control mice. In the BM, GBT021601 increased percent mature RBCs while decreasing percent ortho/polychromatic erythroblasts, and percent apoptotic cells (4.4% vs 7.2%, $p=0.003$), suggesting a reduction in ineffective erythropoiesis. GBT021601 reduced markers of hypoxia and angiogenesis VCAM-1 and Ang-1 ($p<0.05$) and reduced arterial marker SCA-1 on confocal microscopy compared to controls (3.11% vs 5.13%, $p=0.04$).

Conclusions: GBT021601 effectively modifies HbS in the sickle mouse model, extending sickle RBC survival and reducing stress erythropoiesis. Our findings show the additional benefit of GBT021601 on reducing hypoxic injury to the BM, preserving the BM vasculature, and providing a more effective niche for erythropoiesis, while avoiding the risks associated with transfusions. GBT021601 is a promising oral agent that could be used to modify the sickle BM and BM niche improving stem cell health and GT outcomes.

Identifying Changes in the Lymphoma Tumor Immune Landscape in Response to the Checkpoint Molecule Siglec-15

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Presenting Author: Dailia Francis, MD, PhD

Background: While hematological malignancies such as Non-Hodgkin's lymphomas (NHL) are highly curable with multiagent chemo-immunotherapy, outcomes for pediatric patients with relapsed or refractory (r/r) remain dismal with overall survival <30%. This is despite integration of intensive salvage regimens and evolving immunotherapies. The genomic biology driving lymphomagenesis, defects in the anti-tumor immune response and/or the molecular pathways involved therapeutic resistance remains largely uncharacterized in pediatric NHL and will be crucial to improving outcomes for this population of patients. Siglec-15 (Sig-15), an immunoglobulin-like lectin, is a critical immune suppressor that is highly expressed in various human cancers and intra-tumoral myeloid cells. Importantly, inhibiting Sig-15, either through genetic knockout or knockdown, had a restorative effect on local anti-tumor immune responses and abrogated tumor progression. However, a role for Sig-15 in NHL has not yet been described.

Methods: We have evaluated Sig-15 expression in primary lymphoma patient samples as well as various lymphoma cell lines using western blot, immunohistochemistry (IHC) and immunofluorescence methods. Sig-15 expression was inhibited through genetic downregulation in the well-established murine lymphoma cell line A20 and injected into immune competent and immune deficient mice to determine the effect on tumor progression and survival. Multiparameter flow cytometry is used to analyze changes in immune subsets in murine lymphoid tissues.

Results/Conclusions: We have found higher expression of SIGLEC15 in NHL cell lines compared to normal B cells at the RNA level as well as the protein level in various lymphoma cell lines compared to healthy donor PBMCs. Further, IHC was performed on a tumor microarray consisting of 139 cases of NHL from adult patients and validation samples from 15 primary pediatric lymphoma cases (Diffuse Large B cell (DLBCL), Burkitt's lymphomas (BL)). Sig-15 was found to be highly expressed in 124 out of the 139 adult cases and all pediatric lymphoma samples with distinct staining patterns observed in the aggressive B-cell lymphomas. Specifically, Sig-15 appears to be highly expressed and associated with the cell membrane in most DLBCL and BL. Using a well-established murine lymphoma model, A20 cells were stably transduced with control, non-silencing shRNA (shNS) or shRNA against Sig-15 and injected into un-irradiated immune competent (wild type, WT) or immune deficient (Rag1-/-) BALB/C mice and monitored for signs of lymphoma development. Knockdown of Sig-15 in A20 cells abrogates disease progression in WT but not immune deficient mice (**p <0.0001, n=10 mice/group), consistent with a role for Sig-15 in immune evasion in lymphoma. Preliminary analysis suggests there is an increase in the CD8:CD4 T cell ratio in the bone marrow of mice challenged with Sig-15 knockdown lymphoma compared to control. Ongoing studies will further characterize these immune cell changes including the innate immune compartment in other lymphoid organs with knockdown of Sig-15.

Head Injury Type Influences Serum Biomarker Cyclosporin A Treatment Response in a Swine Model

Authors: Huber, Colin M; Thakore, Akshara D; Oeur, R Anna; and Margulies, Susan S

Presenting Author: Colin Huber, PhD

Background: Traumatic brain injury (TBI) is common in sports and automobile accidents with various biomechanical mechanisms; however, an effective treatment of TBI remains elusive. Previously, we found that cyclosporin A (CsA) treatment decreased axonal injury and preserved mitochondrial function in focal and diffuse TBI. Here, we hypothesized that 24 hours of CsA treatment decreased serum biomarkers in focal and diffuse TBI.

Methods: A retrospective analysis was completed to quantify biomarker concentrations in porcine serum samples collected as part of a larger study investigating CsA treatments on TBI. All protocols were approved by the University of Pennsylvania IACUC. Baseline and 1-day post serum samples were collected from 4-week-old female swine: sham (n=10), controlled cortical impact (CCI, n=35), and sagittal rapid non-impact head rotation (RNR, n=67). Injured animals received continuous intravenous saline (untreated, RNR n=39, CCI n=13) or CsA (10-60 mg/kg) for 24 hours beginning 1h or 6h after injury (RNR n=28, CCI n=22). Glial fibrillary acidic protein (GFAP) and neurofilament light (Nf-L) serum concentrations were measured using the Quanterix Simoa Human Neurology 4-Plex A assay. Two-way ANOVA determined injury group differences (injury x timepoint) in saline animals and pooled treatment effect (saline/CsA x injury group) at 1d ($p<0.05$). Mann Whitney U tests compared CsA dosage groups to saline. Fisher's exact tests compared groups (injury and CsA) to a previously established 95% healthy reference ranges (RR): GFAP (6.3–69.4 pg/mL) and Nf-L (9.5–67.2 pg/mL).

Results: Injury type altered biomarker profiles at 1d. For GFAP, RNR was similar to pre (32 to 34 pg/mL, $p>0.999$), while CCI increased significantly (21 to 1669 pg/mL, $p<0.001$) above sham and an established healthy RR ($p<0.001$). For Nf-L, CCI (23 to 89 pg/mL, $p<0.001$) and RNR (33 to 65 pg/mL, $p<0.001$) increased relative to baseline and RR. There was no pooled CsA effect at 1d post-injury. However, CsA administered at 60 mg/kg for CCI (n=4) lowered GFAP (609 pg/mL, $p=0.036$) and Nf-L (54 pg/mL, $p=0.036$) below the saline group.

Conclusions: Serum biomarkers are sensitive to injury type and CsA treatment after TBI. Serum biomarkers may provide relatively noninvasive acute treatment efficacy evidence for hemorrhagic focal TBI.

**MERTK Inhibition Induces an Anti-Leukemia Dendritic Cell – T Cell Axis While TYRO3 Inhibition Protects by a Separate Mechanism

Authors: Huelse, Justus M; Bhasin, Swati S; Jacobsen, Kristen M; Yim, Juhye; Thomas, Beena E; Branella, Gianna; Bakhtiarigheshlaghbakhtia, Mojtaba; Chimenti, Madison L; Baxter, Travon A; Wang, Xiaodong; Frye, Stephen V; Henry, Curtis J; Earp, H Shelton; Bhasin, Manoj; DeRyckere, Deborah; and Graham, Douglas K

Presenting Author: Justus Huelse, PhD

Background: TAM-family tyrosine kinases (TYRO3, AXL and MERTK) are potential cancer therapeutic targets. In previous studies MERTK inhibition in the immune microenvironment was therapeutically effective in a B-cell acute leukemia (B-ALL) model. Here, we probed anti-leukemia immune mechanisms and evaluated roles for TYRO3 and AXL in the leukemia microenvironment.

Results: Host Mertk knock-out or MERTK inhibitor MRX-2843 increased CD8α+ dendritic cells (DCs) with enhanced antigen-presentation capacity in the leukemia microenvironment and inhibited leukemogenesis. High MERTK or low DC gene expression were associated with poor prognosis in pediatric patients with ALL, indicating the clinical relevance of these findings. MRX-2843 also decreased potentially exhausted TOX[HIGH] CD8+ T-cells and combined treatment with MRX-2843 and anti-PD1 prolonged survival compared to MRX-2843 monotherapy in vivo, implicating a DC – T-cell axis. Indeed, combined depletion of CD8α+ DCs and CD8+ T-cells was required to abrogate anti-leukemia immunity in Mertk–/– mice. Tyro3–/– mice were also protected against B-ALL, implicating TYRO3 as an immunotherapeutic target. In contrast to Mertk–/– mice, Tyro3–/– did not impact CD8α+ DC frequency or antigen-presentation capacity and therapeutic activity was less dependent on DCs, indicating a different immune mechanism. Axl–/– did not impact leukemogenesis.

Conclusions: These data demonstrate differential roles for TAM kinases in the leukemia microenvironment and provide rationale for development of MERTK and/or TYRO3-targeted immunotherapies.

**Poster Finalist

Epitranscriptional Regulation of Endothelial-to-Mesenchymal Transition in CF Lung Disease

Authors: Kang, Bum-Yong, Ozuna, Hazel; Shrestha, Mahesh; Moran, John; and Kopp, Benjamin

Presenting Author: Bum-Yong Kang, PhD

Background: Cystic fibrosis (CF) is multi-factorial disease and a leading cause of pulmonary vascular impairments due to hypoxia and progressive lung damage, especially in an aging population. Evolving evidence suggests that an endothelial-to-mesenchymal transition (EndoMT) response to hypoxia contributes to endothelial dysfunction, inflammation, and vascular remodeling. However, the molecular mechanisms underlying lung damage due to CFTR impairment have not been fully elucidated. Emerging studies indicate that m6A epitranscriptomic modification regulates RNA processing and metabolism, leading to downstream biological effects. However, the functional implications of m6A epitranscriptomic modification on CFTR have not been described. Further evidence demonstrates that alterations in non-coding RNAs, such as microRNAs (miRNAs) play important roles in pulmonary disease and regulate m6A regulatory factors. Recent findings indicate that loss of CFTR function reduces PPARγ, a ligand-activated transcription factor, and stimulating PPARγ with thiazolidinedione ligands attenuates altered gene expression and reduces disease severity in a CF mouse model. Therefore, we hypothesize that defective CFTR alters PPARγ-miRNA-METTL3 axis by further feedback inhibition of CFTR, thereby perturbing the PPARγ-miRNA axis, which causes EndoMT.

Methods: To define EndoMT markers during hypoxia, HPAECs were exposed to room air (normoxia, NOR, 21% O2) or hypoxia (HYP, 1% O2) for 72 hours. To further define the functional significance of PPARγ in i-EndoMT, HPAECs were treated for 6 hours with adenovirus-mediated PPAR (AdPPARγ) (25, 50 MOI) for PPAR overexpression or green fluorescent protein (GFP) constructs (25 MOI) then incubated for an additional 72 hours in normoxia or hypoxia. Since the expression of PPARγ is decreased in pwCF and miRNAs have been implicated in CF pathogenesis, we performed in silico anlaysis (TargetScan) whether miRNAs can target the 3'UTR of mesenchymal markers such as SLUG and TWIST1. To investigate the role of inflammation on EndoMT remodeling, we refined an in vitro model to induce EndoMT (termed induced-EndoMT, i-EndoMT) in HPAEC. HPAECs were treated with inflammatory mediators (IL-1β, TNFα, and TGFβ) in dose-ranging regimens. We revisited our previous study using blood transcriptomic analyses of pwCF and non-CF (n=20 each) and showed that m6A modification factors (writers, erasers, and readers) are differentially expressed between groups.

Results: We found levels of mesenchymal markers (SLUG and TWIST1) were substantially increased whereas endothelial markers (PECAM1 and VE-Cadherin) were significantly decreased, indicating that hypoxia plays a critical role in HPAECs and vascular remodeling. PPAR overexpression mitigated expression of EndoMT markers in hypoxic cells and enhanced expression of EC markers. We also found that these inflammatory mediators (IL-1β, TNFα, and TGFβ), which are increased in CF, caused HPAEC to lose cobblestone morphology and became more elongated and spindle-shaped, indicative of EndoMT. Furthermore, the expression of PPARγ and EC markers was decreased, but EndoMT markers were increased. Interestingly, cells under i-EndoMT had increased expression of mesenchymal markers with decreased EC makers, compared to control. In silico analysis and miRNA screening assays revealed that miR-200 was significantly decreased in hypoxia-exposed HPAECs. In epitranscriptomic analysis, we found that levels of METTL3 (writer) were downregulated in pwCF, whereas levels of YTHDF3 (reader) were upregulated. We also found that miR-21 expression, which putatively binds to 3'UTR of METTL3, is upregulated in pwCF, suggesting that miR-21 regulates METTL3 in feedback inhibition of CFTR.

Conclusion: This study allows a new approach to understand CFTR's influence on the airway milieu and the impact of EndoMT in lung disease through separate but related druggable mechanisms.

**Neat1 Inhibition Alleviates the Pathology of Duchenne Muscular Dystrophy

Authors: Kim, Kyungmin; Arun, Sarang; Lee, Sangyoon; and Choo, Hyojung

Presenting Author: Kyungmin Kim, PhD

Background: Duchenne muscular dystrophy (DMD) is an X-linked genetic disease characterized by progressive muscle degeneration and weakness, primarily affecting male children with 1/5000 prevalence. Although gene therapy is promising to treat a subset of DMD patients, no effective treatment is available to cure it. Recently, long non-coding RNAs (lncRNAs) have emerged as critical regulators of gene expression and cellular processes. One such lncRNA, nuclear paraspeckle assembly transcript 1 (Neat1), has been implicated in inflammation and fibrosis in various diseases. In this study, we elucidate the role of Neat1 in muscle pathology in DMD. Ultimately, we will propose a novel therapeutic approach targeting Neat1 to alleviate the pathology of DMD.

Methods: We conducted experiments using the widely utilized mdx mouse model in DMD research. To validate the impact of Neat1, we generated Neat1 knockout (KO)/mdx mice by crossing mdx mice with Neat1 KO mice. We then compared and analyzed the pathological phenotype of Neat1 KO/mdx mice with that of mdx mice. We examined the histology of the diaphragm and limb muscles to evaluate muscle damage, immune cell infiltration, and fibrosis. Additionally, we analyzed serum samples to measure creatine kinase activity levels as a muscle membrane damage indicator. Furthermore, we elucidated the potential mechanism using qRT-PCR.

Results: Increased expression of Neat1 was observed in the diaphragm of mdx mice. Histology analysis of muscles of Neat1KO/mdx mice revealed a decrease in muscle damage along with reduced immune cell infiltration and fibrosis compared to mdx mice. The decrease in serum creatine kinase levels also suggests Neat1KO/mdx mice exhibit alleviated muscle membrane damage compared to mdx mice. The RNA analysis revealed a decreased inflammatory signaling response in Neat1KO/mdx mice, which may contribute to the improvement in the phenotype of Neat1KO/mdx mice.

Conclusions: Our study elucidates the pivotal role of Neat1 in Duchenne Muscular Dystrophy (DMD). By utilizing the mdx mouse model and generating Neat1 knockout (KO)/mdx mice, we demonstrate that targeting Neat1 leads to significant improvements in muscle pathology. These findings underscore the therapeutic potential of targeting Neat1 as a novel approach to alleviate DMD pathology.

**Poster Finalist

Ethanol Exposure During Differentiation of Human-induced Pluripotent Stem Cells Reduces Cardiomyocyte Generation and Alters Metabolism

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Presenting Author: Kun Man, PhD

Background: Prenatal alcohol exposure is known to interfere with fetal development, elevating the risk of congenital heart diseases (CHDs). Currently, there is a lack of clarity regarding the impact of alcohol exposure on early-stage cardiogenesis, and how any disruption during this critical period subsequently leads to heart defects in late stages. We hypothesized that identification of alcohol effects on early-stage cardiogenesis could potentially yield novel prevention/treatment strategies. To this end, we took advantage of in vitro cardiomyocyte (CM) differentiation from human induced pluripotent stem cells (hiPSCs), during which the gene expression patterns are similar to that of cardiogenesis in embryo, to study alcohol effects on early-stage cardiogenesis.

Methods: The hiPSC line SCVI273 was used for hiPSC-CM differentiation using Wnt signaling activator CHIR-99021 and inhibitor IWR-1. The cells were treated with ethanol at final concentrations of 17, 50, and 100mM, from day 0 to day 12 of the differentiation. At day 12, cell number and cell viability were measured using trypan blue assay. The cells were replated in 96-well plates and cell proliferation were examined by immunocytochemical analysis of Ki67, and cardiomyocyte generation was analyzed by using immunocytochemical analysis of cardiomyocyte markers NKX2-5 and GATA4.

Results: Significant decreases in both viable cell number and cell viability were observed in cultures treated with 100mM ethanol compared with untreated cultures. The proportion of Ki67-positive cells decreased in cultures treated with ethanol compared with untreated cells, and the levels of decrease were ethanol-concentration dependent. The proportion of Ki67-positive cells in NKX2-5-positive cell population also decreased concentration-dependently in ethanol-treated cells compared with untreated cells. There were no significant differences in the proportions of NKX2-5- or GATA4-positive cells among the groups. However, the number of NKX2-5-positive cells decreased in cultures treated with 100 mM ethanol and the number of GATA4-positive cells decreased in cultures treated with 50 and 100 mM ethanol compared with untreated cultures.

Conclusion: Ethanol exposure during hiPSC-CM differentiation reduced cell number and viability and caused decrease of cell proliferation in an ethanol-concentration-dependent manner, and caused a concentration-dependent decrease in the generation of cardiomyocytes.

RNA-seq Analysis of the Effects of Elexacaftor/Tezacaftor/Ivacaftor in Cystic Fibrosis

Authors: Ozuna, H; Moncada Giraldo, DM; Tirouvanziam, RM; and Kopp, BT

Presenting Author: Hazel Ozuna, PhD

Cystic Fibrosis (CF) Transmembrane Conductance Regulator (CFTR) modulator therapies have resulted in positive clinical outcomes, yet chronic inflammation and bacterial infections persists. To fill the gap of how elexacaftor/tezacaftor/ivacaftor (ETI) fails to improve bacterial clearance and pro-resolving inflammatory responses we performed an Illumina next-generation sequencing RNA-seq to identify changes in gene expression before and after ETI. Plasma was collected from 12 people with CF (pwCF) and 9 healthy controls (HC), before and 3 months after ETI therapy. Following quality control check with FastQC, STAR aligner was used to align sequences to the HG38 human reference genome. Quantification of genes was performed with HTSeq-count and adapter contamination was removed by means of data trimming using Trimmomatic. DESeq2 was used to determine differentially expressed genes (p □ 0.05). Paired analysis of CF-Pre and CF-Post showed moderate clustering with PCoA and differential gene expression analysis resulted in 111 upregulated genes and 63 downregulated genes. Differentially expressed genes includes KLC3, which has a role in ciliary trafficking; CDC42, which regulates many cellular processes including phagocytosis, and NEAT1, known to promote fibrosis and to modulate the expression of IFN-□, TNF-□ and IL-8. Pathway analysis revealed alterations in calcium-dependent phospholipid binding and ribosomal processing. These results and ongoing metadata analysis of pwCF before and after ETI will provide more information that will shed light of the effects of ETI at the gene expression level and how this correlates with clinical outcomes.

Red Cell Rheology and Blood Viscosity in Pediatric Individuals Having Received Allogenic Hematopoietic Stem Cell Transplantation or *Ex Vivo* Autologous Gene Therapy for Sickle Cell Disease

Authors: Patel, Ashwin; Kanne, Celeste; Stenger, Elizabeth; John, Tami; and Sheehan, Vivien

Presenting Author: Ashwin Patel, PhD

Background: Allogenic hematopoietic stem cell transplantation (HCT) and ex vivo autologous gene therapies (GT) are potentially curative treatments for sickle cell disease (SCD). However, there is debate around how to define cure including what degree of donor chimerism or level of functional hemoglobin constitutes a cure. Information on red cell function post-HCT/GT and how it relates to donor chimerism and resolution of SCD complications is scarce. Red cell function tests are not currently part of follow-up care post-HCT but may provide helpful information on the degree of red cell correction and risk of SCD complications. To address this knowledge gap, we report rheology and whole blood viscosity data on HbSS patients post-HCT or GT.

Methods: Blood samples were collected under an IRB-approved protocol at Baylor College of Medicine and Emory University School of Medicine. We analyzed elongation index maximum and minimum (EImax, EImin), point of sickling (PoS), hematocrit-viscosity ratio at shear rates of 45 and 225 s-1(HVR45 & HVR225), and dense red blood cell % (DRBC%). EImax, EImin, and PoS were measured using a Laser Optical Rotational Red Cell Analyzer (Lorrca, RR Mechatronics, The Netherlands). Chimerism was measured by short tandem repeat testing (STR) after cell density sorting and DRBC% was measured on ADVIA cell counter (Siemens, Germany). HCT patients were compared with controls matched to the donor's genotype, and patients post-GT were compared with HbAS controls (HCT: HbAA donor =8, HbAS donor=19, GT=2, HbAA controls=42, and HbAS controls=15). Stata 18.0 (College Station, Texas, USA) was used to perform statistical analyses. Median values and ranges were used to describe the data. Wilcoxon rank sum test was used to compare groups and a mixed model was for the longitudinal data. A p-value <0.05 was considered statistically significant.

Results: There were 29 patients with a median age of 6.6 years (range: 2.0-16.3) at HCT/GT and a median follow-up of 2.4 years (range: 0.1-7). A total of forty-three samples were analyzed. Median donor myeloid chimerism was 94% (33-100). Overall, the HbS% ranged from 28.9 - 45.2% with HbAS donors and 0% with HbAA donors.

Two HbAS HCT patients had myeloid chimerism ≤ 50% with a follow-up period of 3.3 and 1.9 years, respectively. The myeloid donor% and HbS% were 33% and 50%, and 45.2% and 41.6%, respectively. At the first post-HCT/GT time-point (median 1 year, range: 0.1-6.9), DRBC%, EImax, and EImin were lower in HbAA donor HCT patients compared to HbAA controls (0.593 vs 0.604, p=0.008; 0.581 vs 0.604, <0.001; and 0.75 vs 0.20, p=0.02, respectively). RBC function improved with a longer follow-up from HCT/GT (median 2.9 years, range 1.3-7) with EImax showing a trend of statistical significance (p=0.07).

Patients with HbAS donors had significantly higher point of sickling (PoS) values compared to the patients with HbAA donors (p=0.02). There was a trend for a lower HVR225 in patients with HbAS donors compared to the patients with HbAA donors (p=0.08).

Seven patients (24%) had one or more red cell function test values outside the genotype-matched control range despite having myeloid donor chimerism of > 25-30% - a value commonly used in clinical practice to define cure (Table & Figure).

Conclusion: A substantial number of patients had abnormal red cell function tests following HCT/GT despite achieving donor engraftment. Donor myeloid chimerism ≥ 25% has been reported protective from SCD complications while a chimerism ≥50% normalized tests for hemolysis. Higher odds of SCD-related complications have been reported with higher PoS and lower EImax/EImin. Thus, we propose that red cell function should be included in follow-up care of HCT/GT patients until values normalize and clinical relevance is further assessed.

**Role of Insulin-like Growth Factor Binding Protein-3 (IGFBP3) in Fetal Hemoglobin Induction in Primary CD34+ Hematopoietic Stem and Progenitor Cells from Patients with Sickle Cell Anemia

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Presenting Author: Anupama Priyadarshini, PhD

Background: Induction of fetal hemoglobin (HbF) is a key therapeutic strategy to treat individuals with SCD. We have identified Forkhead Box O3 (FOXO3) and Insulin-like growth factor binding protein 3 (IGFBP3) as positive regulators of gamma–globin in two independent genomic studies; they are part of the same pathway. The role of FOXO3 in HbF regulation has been confirmed with functional studies in erythroid culture (Zhang, Blood 2018). We propose to investigate the role of IGFBP3 in HbF regulation, through similar means, and determine if both IGFBP3 and FOXO3 act through the same pathway.

Method: Two phase erythroid culture was performed on three unique SCD patient samples. The effect of exogenous, IGFBP3 treatment of erythroid culture at 1µg/ml on HbF levels was determined by HPLC, flow cytometry, RT-qPCR, and western blot analysis on day 21 of culture. FOXO3 knockout CD34+ cells were generated using LentiCRISPR/Cas9 knockout strategy to test the FOXO3 dependence of IGFBP3 mediated HbF induction.

Results: Addition of purified IGFBP3 increased HbF levels by day 21 of culture. IGFBP3 did not alter the expression of known HbF regulators (BCL11A, KLF1, and MYB), and did not alter erythroid maturation as measured by flow cytometry with CD71, GPA, and Band3. Western blot analysis of transduced CD34+ cells was performed, and it was confirmed that FOXO3 was knocked down.

Conclusions: Our genomic data, in combination with functional studies, supports a role for IGFBP3 in HbF induction. Next steps include determining if FOXO3 knockdown removes IGFBP3's ability to increase HbF levels in erythroid culture, and treatment of a xenograft mouse model containing human sickle stem cells with agents like vitamin D, which increase IGFBP3 levels, to determine if the IGFBP3 induction of HbF occurs in vivo.

**Poster Finalist

Peripheral Mitochondrial Respiration in Pre-Adolescent Hispanic Children at Risk of MASLD

Authors: Ramirez Tovar, Ana; Sanchez-Torres, Cristian; Huneault, Helaina E; Beer, Rachael; Smith, M Ryan; Welsh, Jean A; and Vos, Miriam B

Presenting Author: Ana Ramirez, MD

Introduction: Mitochondria plays a significant role in the functioning of the liver. Its function and structure have been associated with diseases outside the organelle by producing reactive oxygen species (ROS), causing generalized oxidative stress, improper function to several metabolic pathways, and perpetuating a pro-inflammatory state in conditions like obesity, diabetes, and metabolic dysfunction associated steatotic liver disease (MASLD). These diseases are on the rise and increase future atherosclerotic cardiovascular disease. Little is known regarding the pathophysiology of the earliest stages of hepatic steatosis, although mitochondrial dysfunction has been shown in later stages. In young children with and without MASLD, we aimed to measure mitochondrial respiration and compare it to hepatic steatosis and other cardiometabolic parameters. The hypothesis of the sub-study was that children with MASLD will have a decreased mitochondrial function compared with healthy children.

Methods: We report data on 9 subjects, (all Latino subjects, mean age of 8.1 years, mean BMIp of 78%) who participated in screening for an ongoing clinical trial (ClinicalTrials No. NCT05292352). After consent and assent, procedures included drawing whole blood and transferring it immediately to the lab. Monocyte isolation was performed using RosetteSep™ Cocktail and measurement of fatty acid oxidation and glycolysis using the Seahorse Bioscience XFp extracellular flux analyzer. We measured the Oxygen Consumption Rate (OCR) as a proxy for mitochondrial respiration. Association with the mitochondrial parameters was evaluated with the percentage of hepatic steatosis (Hepafat), levels of ALT, insulin, and BMI percentile (BMIp). Data were analyzed using the R software program version 4.2.3

Results: Mean hepatic steatosis (HS) was 5.97% with a low of 2.4% and a high of 13%. The mean OCR for the subjects without MASLD (n=4, HS ≤5% by MRI) was 404.64 and the mean for subjects with MASLD (n=5, HS ≥5% by MRI) was 349.88. The mean OCR of subjects with a normal, overweight, and obese BMIp was 441.58, 397.45, 309.08, respectively. We found peripheral mitochondrial respiration tends to decrease as hepatic steatosis, BMIp, ALT and insulin levels increase.

Conclusion: Hepatic steatosis is associated with decreased mitochondrial respiration and perhaps perpetuates an inflammatory state at very young ages in this vulnerable population. More subjects from the NAFLD prevention study will increase the significance of the study in the future.

The Uremic Milieu Induces Aortic Cellular Reprogramming in the 5/6th Nephrectomy Mouse Model of Chronic Kidney Disease

Authors: Reyes, Loretta; Park, Christian; Villa-Roel, Nico; Li, Haiyan; Kang, Dong-Won; and Jo, Hanjoong

Presenting Author: Loretta Reyes, MD

Introduction: Chronic Kidney Disease (CKD) is a progressive condition marked by high cardiovascular morbidity and mortality. CKD has profound effects on vascular health, including the characteristic arterial uremic vasculopathy, which manifests as arterial wall calcification and pathologic vascular remodeling with resultant increased arterial stiffness and end-organ damage. The aorta contains numerous cell types that can contribute to vascular remodeling therefore we hypothesized that the uremic milieu of CKD reprograms arterial cells leading to the uremic vasculopathy phenotype.Single-cell RNA sequencing (scRNAseq) was conducted to define the transcriptomic landscape in the mouse aorta.

Methods: CKD was induced via 5/6 nephrectomy in 2 cohorts of SvJ/129X mice; non-surgical controls were included for comparison. Single cell suspensions prepared from collagenase digestion of the descending thoracic aorta at 4 weeks (cohort 1; CKD n=4, control n=4) and 8 weeks (cohort 2; CKD n=4, control n=4) were used for scRNAseq analysis. Cell clusters and differentially expressed genes were determined using Seurat R-package.

Results: Unsupervised cluster analysis from 21,721 aortic cells identified 20 distinct cell clusters representing 8 cell types (endothelial cells (ECs), smooth muscle cells (SMCs), fibroblasts (FBs), macrophages (MΦs), and other immune cells (T-cells, B-cells, neutrophils, mast cells). ECs, SMCs and FBs were the predominant cell types in both CKD and controls, however there was a relative decrease in the SMC population in CKD with a concomitant increase in the immune cell population, especially MΦs, T-cells and neutrophils. CKD resulted in EC sub-populations with overexpression of pro-inflammatory/proliferative genes, as well as endothelial-to-mesenchymal transition (EndMT) and endothelial-to-immune transition (EndIT) genes compared to controls. CKD also induced a transition from healthy, contractile phenotype to a pro-inflammatory, synthetic phenotype in SMC sub-populations. Interestingly, we identified a FB sub-population, only present under CKD conditions, that over-expressed complement/chemokine genes (C3, C4b, Cfb, Cxcl12).

Conclusion: Overall, our results demonstrated an increased proportion of immune cells in the aorta in CKD and we identified a transition to a pro-inflammatory, proliferative phenotype in several cell lines and cellular over-expression of genes involved in immune system activation. Together, our data suggests that inflammation plays a key role in the development of uremic arterial vasculopathy.

**Enhancing Oro-nasal Fistula Healing: Targeted Treatment with Probiotic Bacterial Supernatant

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Presenting Author: Keerthi Priya Chinniampalayam Sekar, PhD

Background: Oral cavity healing takes place amid persistent physical trauma, bacterial challenges, and a diverse array of microorganisms such as bacteria, fungi, parasites, and viruses. This process is particularly crucial following incidents of traumatic injury, cancer resection, and the correction of congenital anomalies, such as cleft palate. Unfortunately, adverse healing outcomes are observed in a significant proportion of cases after cleft palate repair, affecting up to 60% of children (1 in 700 births). This often results in the formation of an Oro-nasal fistula (ONF), characterized by a direct opening between the mouth and nose. Repairing such complications typically necessitates multiple surgeries. Our hypothesis revolves around the potential impact of the oral microbiome on enhancing the healing of Oro-nasal fistulas.

Methods: A critical-sized defect, representing a 1.5mm Oro-nasal fistula (ONF) injury, was replicated in the hard palate of NOD /SCID mice. A hydrogel containing L.Lactis cremoris(LLC) bacteria and ThP1 cells was then implanted at the ONF injury site. Harvesting of healing tissue and hard palate mucosa was conducted at days 1, 3, 5, and 7. Evaluation of the healed area and histological analysis aimed to explore the impact of LLC bacteria with ThP1 cells on pro-regenerative cell infiltration during the wound healing process of ONF. Additionally, multiplex assays were employed to assess cytokine production in ThP1 cells and macrophages treated with LLC bacteria.

Results: We observed that immune cell response (monocyte and macrophage) to oral wound healing with greater effects on pro-regenerative subsets following probiotic bacterial treatment along with THP1 cells. Invitro cytokine analysis of human THP-1 cells stimulated with supernatant from LLC culture induced the production of increased anti-inflammatory cytokines(IL-10 &1L-27) responsible for tissue regeneration. Endoscopic images showed 9 out of 10 (90%) of mice, compared to healing rates of between 50% and 75% in the control groups on Day 7 following PEGMAL hydrogel with bacterial supernatant.

Conclusion: This research not only advances our understanding of oral wound healing but also opens avenues for future interventions that leverage the oral microbiome to enhance regenerative processes, particularly in challenging cases such as cleft palate repair-associated Oro-nasal fistulas. The implications of our findings extend beyond the realm of experimental studies, offering promise for the development of innovative strategies to improve clinical outcomes in patients undergoing oral cavity reconstruction.

**Poster Finalist

Tumor Neurons Cooperate with Astrocytes to Uncouple Nucleotides Synthesis from DNA Methylation via Transsulfuration

Authors: Tikunov, Andrey; Bates, Dale; Fahim, Ali; Rosen, Elias; Macdonald, Jeff; and Gershon, Timothy

Presenting Author: Andrey Tikunov, PhD

Tumor cells adapt their metabolism to support uncontrolled fast growth. Current models often consider tumor's metabolism to be self-sufficient. However, we have identified a metabolic process in which tumor cells in medulloblastoma collaborate with local astrocytes - cells of non-tumor origin. This collaborative metabolism contributes to tumor robustness and may be exploited therapeutically in new combinations of novel and established chemotherapies.

We analyzed brain and tumor metabolome in mice genetically engineered to develop Sonic Hedgehog (SHH)-subgroup medulloblastoma using LC-MS, NMR and MALDESI, a spatially-resolved mass-spectrometry. Then followed up with fluxomics studies in which stable isotope labeled metabolites, including 13C,15N glycine and 13C glucose, were IP injected into tumor bearing mice. The tumors were then harvested after a short interval (30-60 minutes) and the labeled metabolites in the tumor and brain were analyzed.

We discovered that medulloblastomas accumulate cystathionine, a methionine metabolite that is in comparatively low concentration in the adjacent brain. Eukaryotic cells can only generate cystathionine through the transsulfuration pathway, where the enzyme cystathionine beta synthase (CBS) complexes homocysteine with serine. Transsulfuration requires the participation of local astrocytes because only astrocytes express CBS.

Our 13C-flux analyses show that over 30 minutes tumors made ~10% of serine de-novo; out of this serine, tumors used ~30% for folate methylation, forming glycine and mTHF and less than 10% of serine for conjugation with homocysteine to form cystathionine. Tumors showed the ability to change metabolic flow, depending on substrate availability.

Single-cell RNA sequencing and immunohistochemistry showed, as expected: astrocytes were the only cell-type in tumor tissues that express CBS. Tumor cells expressed homocysteine-generating DNA methylases, SHMT1/2 and TYMS, which are needed to methylate folate, and to channel methylated folate to thymidine synthesis.

We propose a model of intracellular collaboration: where tumor cells prioritize fueling the folate cycle using serine for nucleotides synthesis, suppressing methyl-dependent recycling of homocysteine. The methionine cycle runs as a linear pathway from dietary methionine and ends with homocysteine. Tumor cells shunt homocysteine to astrocytes, where it conjugates with serine to form cystathionine that is non-toxic and can be used for glutathione synthesis. This intercellular collaboration can be targeted therapeutically.

**Micronutrients Associated with Anemia in School-Age Children and Adolescents: BRINDA Project

Authors: Werner, Rochelle; Luo, Hanqi; Wang, Yuqing; Geng, Jiaxi; Ko, Yi-An; Suchdev, Parminder S; Addo, Yaw; and Young, Melissa F

Presenting Author: Rochelle Werner, PhD

Background: School age children and adolescents are at risk for anemia through demands on micronutrients required for growth and maturation. This multi-country analysis examined the burden of anemia in children 5-19y by sex and age category and associations with inflammation and micronutrient deficiencies.

Methods: Children 5-19y from surveys in the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project were included with measured hemoglobin, at least one micronutrient (iron, vitamin A, folate, vitamin B12, or zinc) and inflammation biomarker, and n>100 per survey. Factors with bivariate relationships with anemia (P<0.1) were selected for multivariable models. The attributable burden of anemia was examined by age category for each factor using multivariable modified Poisson regression with robust standard errors.

Results: This analysis included 54,535 children from 17 surveys. Median anemia prevalence was 16% (range: 5-59%). In most surveys, anemia did not differ by sex for children 5-14y and was lower in children 10-14y than 5-9y or 15-19y. A 1y increase in age was associated with anemia inversely in children 5-9y [prevalence ratio (PR) range: 0.6-0.9] and positively in children 10-14y but not 15-19y. Inflammation was positively associated with anemia in all age groups [5-9y (4 of 4 surveys); 10-14y (2 of 4 surveys); 15-19y (6 of 8 surveys)]. Iron deficiency was frequently associated with anemia, often with the highest PRs of examined factors [15-19y (PRs with P<0.05: 1.6-14.2; 12 of 14 surveys); 10-14y (2.1-6.5; 5 of 6 surveys); 5-9y (1.8-2.9; 4 of 7 surveys). Vitamin A deficiency was also associated with anemia [5-9y (2 of 2 surveys); 10-14y (2 of 3 surveys); 15-19y (2 of 3 surveys)]. Data for folate, vitamin B12, and zinc were sparce and often unassociated with anemia.

Conclusion: Iron deficiency was consistently associated with anemia in studies of school age children and adolescents. This research underscores the importance of examining micronutrients associated with anemia in context of factors like country, age, and sex.

**Poster Finalist

The Impact of Feeding on Intestinal Inflammation in Preterm Infants

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Presenting Author: Anand Salem, DO

Background: Fecal calprotectin is elevated in necrotizing enterocolitis (NEC), which may represent the extreme of a spectrum of intestinal inflammation. Prior studies report differing associations between diet and fecal calprotectin. Fortification of human milk is often used to enrich the nutrient content of human milk, but there is limited data on how different approaches to fortification influences intestinal inflammation. The purpose of this study was to evaluate the association between bovine or human milk-derived fortifiers on fecal calprotectin in very preterm infants.

Methods: We conducted a retrospective analysis of data from a prospective cohort study of very preterm infants with a birth weight <= 1250 grams from 2017-2021 recruited at 3 hospitals in Atlanta, Georgia. Fecal calprotectin was measured weekly in stool using an enzyme-linked immunosorbent assay. Diet information, including base diet, fortification, and volume of intake was collected daily. The analysis data set was limited to fecal calprotectin measurements in the subsequent week after an infant had fortification of human milk with either a bovine-derived or human milk-derived fortifier. Multivariable models accounting for within infant correlation, baseline calprotectin levels, total intake volume, and receipt of transfusion were used to evaluate subsequent increases in log-transformed fecal calprotectin levels. As a secondary aim, we evaluated whether red blood cell transfusion modified the relationship between human milk fortifier and calprotectin levels.

Results: We analyzed 478 separate feeding and weekly fecal calprotectin observations from 135 infants who received fortified human milk. The median birth weight of the cohort was 916 grams (25th – 75th percentile: 780 – 1086) and the median gestational age was 27.3 weeks (26.3 – 28.5). Of 478 feeding observations, 59 (12%) were with bovine-derived fortification, compared to 419 (88%) from human-milk derived fortification. On multivariable analysis with bovine-derived fortification as a reference group, infants receiving human milk fortification with a human-derived fortifier had a lower log fecal calprotectin in the subsequent week of measurement (-0.23; 95% CI, -0.46 to -0.00, P= 0.048). Red blood cell transfusion did not appear to modify this relationship (interaction P=0.27).

Conclusion: Human milk fortification with a human milk-derived fortifier is associated with decreased fecal calprotectin, compared to bovine milk-derived fortifier, in very preterm infants.

Kyotorphin: A Novel Mechanism-of-action for Arginine Therapy Targeting Pain in Children with Sickle Cell Disease (SCD)

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Presenting Author: Rawan Korman, MD

Background: Low arginine bioavailability is associated with SCD mortality and morbidity, including acute pain severity. Multiple phase-2 trials support the safety & efficacy of arginine therapy in children with SCD vasoocclusive pain episodes(VOE). As the obligate substrate for nitric oxide(NO) production, arginine's mechanism-of-action is unknown but thought to be related in part to NO, a potent vasodilator. Kyotorphin is an endogenous opioid-like analgesic composed of the amino acids arginine and tyrosine. Oral arginine given to wild-type mice increases kyotorphin levels centrally. The association between arginine supplementation as a precursor to kyotorphin in SCD is unknown.

Objective: Determine the impact of intravenous(IV) arginine on plasma arginine, kyotorphin, and NO metabolite (NOx) concentrations and their association with pain scores in children hospitalized with SCD-VOE.

Methods: A pharmacokinetics/pharmacodynamics study of hospitalized children aged 7-21 years with SCD-VOE randomized to receive one of 3 dosing arms of IV arginine: 1)100mg/kg TID(n=4); 2) 200mg/kg X1 followed by 100mg/kg(n=5); or 3) 200mg/kg followed by continuous infusion (300mg/kg/day) until discharge(n=4). Plasma arginine, kyotorphin, and NOx were measured through previously described methods. Daily numeric pain scores (0-10) were collected. Mean±SD, paired t-tests, and Pearson correlation analyses between groups were performed where appropriate.

Results: 13 patients (13±3 years, 62% males, 85% HbSS, 92% on Hydroxyurea) were enrolled. Plasma arginine and kyotorphin concentrations significantly increased and peaked at 1 hour after infusion initiation ($p<0.001$, $p=0.004$ respectively) with no significant differences in peak concentrations between study arms. Kyotorphin levels strongly correlated to plasma arginine concentration ($r=0.72$, $p<0.0001$). Plasma NOx levels also significantly increased after IV arginine infusion from pre-dose, with a mean absolute maximum change of 12.1±16.2μM ($p=0.02$). Day 2 pain scores significantly and inversely correlated with peak kyotorphin levels on Day1 of the pharmacokinetics study and with change in plasma arginine concentration(□M) from baseline to discharge in subjects receiving an arginine loading dose.

Conclusion: IV arginine therapy increased plasma arginine concentration 2-5 times above baseline, peaking within 1 hour of infusion initiation. We report for the first time the that IV arginine increases kyotorphin in SCD. This may represent a novel opioid-sparing mechanism-of-action for arginine with implications for pain syndromes beyond SCD.

Implementation of Opt-Out Human Immunodeficiency Virus Screening in Pediatric Emergency Departments

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Presenting Author: Lauren Middlebrooks, MD

The Centers for Disease Control and Prevention recommends HIV screening for all patients ≥13 years. Parts of Metro Atlanta have HIV positive rates at 8-times the national average. Adolescents are the least likely group to know their HIV status and have the lowest rate of linkage to care. Children's Healthcare of Atlanta (Children's) implemented an opt-out HIV testing program in its emergency departments (ED) for patients ≥13 years undergoing venipuncture for any chief complaint at all their sites. The objective is to increase testing in adolescents leading to earlier HIV diagnosis and linkage to care.

Children's electronic medical record EPIC and its population discovery tool were used to compare HIV testing volumes of 13–24-year-olds, 9 months pre-(October 5–July 5, 2023) and post (July 6–April 6, 2024) clinical implementation. All sites received educational promotion. Results were cross-referenced to determine newly diagnosed adolescents living with HIV (ALHIV) from known positives. The data was compared using descriptive statistics.

A total of 1052 patients were tested pre-implementation, 756 (72%) girls and 296 (28%) boys. Six new ALHIV were identified; average age was 17 and the assignment at birth was (3) male, 1 coinfected with syphilis and (3) female. Post implementation, 1864 patients were tested: 1262 (68%) girls and 602 (32%) boys. Six new ALHIV were identified; average age was 16 and the assignment at birth was (5) male, 2 coinfected with syphilis, and (1) female. This demonstrates an overall positivity rate of 0.3%; 1 in 120 boys tested positive (0.8%). At sites receiving education for 3 weeks, 5 and 9 months, testing increased by 54%, 75% and 94% respectively. All newly diagnosed cases were linked to care within 1-48 days.

Atlanta remains a hotspot for new HIV cases. Six cases in 9 months highlights the importance of universal HIV testing of adolescents and reflects a public health crisis. The new initiative significantly increased HIV screening among adolescents and will likely identify ALHIV at an earlier stage of infection, facilitating timely access to medical care. This can lead to improved clinical and immunological outcomes and a reduced risk of secondary transmission.

The Incidence, Risk Factors and Outcomes of Acute Kidney Injury in Neonates Following Cardiac Surgery

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Presenting Author: Chau Nguyen, MD

Background: The onset of acute kidney injury (AKI) following pediatric cardiac surgery has been reported previously. However, perioperative risk factors and short-term outcomes have varied significantly between reports. We aimed to describe the behavior of postoperative AKI in neonates at our institution.

Methods: It was a single center, retrospective cohort study of neonates who underwent STAT (The Society of Thoracic Surgeons-European Association for Cardio-Thoracic Surgery) category 3, 4, and 5 cardiac surgeries between 01/2016 and 12/2021. The primary outcome was to reveal the incidence of AKI, defined using the KDIGO scoring system, at our institution. We divided the cohort into patients with stage 2 or 3 AKI and those with stage 1 AKI or no AKI. Secondary outcomes were to describe postoperative morbidity and mortality between the two groups. P-values of <0.05 were considered statistically significant.

Results: Our study included 519 patients, of which 118 (22.7%) developed stage 2 or 3 AKI. Patients in the stage 2 or 3 AKI group were older (7 vs 6 days, p=0.043), had higher number of STAT category 5 surgeries (34.8% vs 25.4%, p=0.033), lower preoperative serum creatinine (0.3 vs 0.48, p<0.001), had higher rate of delayed sternal closure (57.6% vs 28.4%, p<0.001), longer duration of mechanical ventilation (MV) (118.4 vs 73.7 hours, p=0.002), higher rates of cardiac arrest (18.6% vs 8%, p=0.002), required more postoperative extracorporeal life support (ELCS) (20.3% vs 8.2%, p<0.001), and higher rate of operative mortality (23.7% vs 6.7%, p<0.001). After adjusting for confounders, patients with delayed sternal closure (aOR 4.9, 95% CI 2.7, 8.9, p<0.001) and who developed postoperative cardiac arrest (aOR 2.5, 95% CI 1.1, 4.8, p=0.026) had higher odds of developing stage 2 or 3 AKI.

Conclusion: After cardiac surgeries, neonates who developed stage 2 or 3 AKI had a higher percentage of STAT category 5 surgeries, had higher rates of cardiac arrest, postoperative ELCS requirement, and had higher operative mortality. After adjusting for confounders, patients who developed cardiac arrest had 2.5 times higher odds of development of stage 2 or 3 AKI. Future prospective, multicenter studies and randomized studies are required to further investigate these findings.

Nurse-Performed Bladder Ultrasound Prior to Bladder Catheterization in the Pediatric Emergency Department

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Presenting Author: Tal Berkowitz, MD, MPH

Background: Bladder catheterization (BC) is a common procedure in the pediatric emergency department (PED). Inadequate bladder volume during catheterization leads to repeat BCs, increasing adverse events. Point of care ultrasound (POCUS) can determine bladder volume, decreasing the chance of dry catheterization. Prior studies have evaluated doctor-performed POCUS with nurse-performed BC. This study evaluated whether nurse-performed POCUS can decrease the rate of dry catheterization.

Methods: We performed a randomized-controlled trial comparing catheterization with nurse-performed POCUS to standard catheterization. Study staff provided a 10-minute POCUS training to nurses. Patients ≤24 months in the PED requiring a BC without urogenital abnormality, indwelling urinary catheters, trauma, or family objection were randomized. Successful BC was defined as ≥2ml of urine obtained. Measurement on POCUS was considered adequate for catheterization if the bladder measured at least 2cm in any direction. If bladder measurement was <2 cm or the patient urinated during POCUS, BC was postponed and POCUS was repeated in 30-minute intervals until measurement was adequate. 4-point Likert scale satisfaction surveys were provided to caregivers after BC attempt. BC success rates were analyzed using the chi-square test and odds ratios. Time to first catheterization attempt was analyzed using the Wilcoxon rank-sum test. Satisfaction survey scores were analyzed using a two-tailed t-test.

Results: Overall catheterization failure rates were 12.9% in the POCUS group and 23.2% in the standard group p 0.08. OR 0.5 (0.2 – 1.1). Dry BC rates were 4.7% in the POCUS group and 16.8% in the standard group p<0.01 OR 0.2 (0.08 -0.8). Time to first catheterization attempt was 34 vs 32 minutes p 0.12. Caregivers in the POCUS group reported "very satisfied" more often and had better perceived time satisfaction scores.

Conclusion: A simple 10-minute training enabled nurses to perform POCUS prior to bladder catheterization in children under 2 years of age in the PED. Performance of POCUS led to a significant decrease in the rates of dry catheterization as well as an increase in caregiver satisfaction without increasing the time to performance of catheterization. We conclude that nurse-performed bladder POCUS is both practical and beneficial prior to bladder catheterization in an emergency setting.

Neonatal ECMO: Epidemiology According to Social Drivers of Health (SDoH)

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Presenting Author: Swati Chandhoke, MD

Background: SDoH impact numerous neonatal and pediatric outcome domains. ECMO is a life-sustaining therapy susceptible to SDoH influences. We studied neonates supported on ECMO leveraging the Children's Hospitals Neonatal Consortium Database of 36 US level 4 NICUs.

Methods: Neonates >34 weeks gestational age (GA) with birth weight >1.8kg supported on ECMO from 2010-2021 with a maternal zip code and race were identified. We examined clinical characteristics, outcomes, and SDoH including Child Opportunity Index (COI) quintiles, maternal race (non-Hispanic white, non-Hispanic Black, Hispanic, Asian, and other), payer, and travel distance. Univariate analyses identified associations with the primary outcome of NICU mortality. Associations between SDoH and NICU mortality were adjusted for diagnoses.

Results: Of 2254 neonates on ECMO (23% venovenous [VV]), 32% were supported for congenital diaphragmatic hernia (CDH), 27% for meconium aspiration syndrome (MAS), 13% for cardiac, and 28% for other diagnoses. Overall 29% of neonates were in the very low COI quintile (vs 14% very high), 55% had public payer and 51% were white, 25% Black, and 17% Hispanic. Small for GA and MAS were more common in low COI (versus high) and Black races (versus white). White and Hispanic races had more preterm births, while conditions such as preeclampsia were common in underrepresented races. NICU mortality was 31%. The median NICU length of stay (LOS) was 32 days [IQR 17,61] and ECMO duration was 7 [4,12]. CDH and cardiac disease had greater mortality (vs MAS, Hazard ratio [HR] 3.8 [2.8,5.0] and 2.8 [2.0,4.0]) as did preterm birth, while VV cannulation was protective (HR 0.6 [95%CI 0.4,0.7]). We found no association between COI or travel distance with mortality. Higher mortality was seen in private (HR 1.3 [1.1, 1.5]) and self-pay (HR 2.8 [1.7, 4.4]) patients vs public. Black race was associated with lower mortality (HR 0.8 [0.7, 0.9]) vs white but not when adjusted for diagnosis. Run duration and LOS were similar across SDoH.

Conclusions: We identified different epidemiological characteristics among neonates supported on ECMO according to SDoH. Relationships between social drivers, clinical variables, health access, and outcomes in the neonatal ECMO population are complex, deserving of future study.

Natural Airway Sedation for Pediatric Patients Receiving Iodine 131 MIBG Therapy in the Pediatric Intensive Care Unit

Authors: Dhuse, J; Cash, T; Elges, SM; Alazraki, A; Beer, RJ; Jergel, A; Goldsmith, K; Hall, M; and Kamat, PP

Presenting Author: Jordann Dhuse, MD

Background: Children with high-risk neuroblastoma receiving I-131 metaiodobenzylguanidine (I-131 MIBG) therapy often require sedation and analgesia prior to MIBG and adherence to strict radiation safety guidelines during MIBG infusion and clearance to ensure patient and staff safety. We evaluated the sedation-analgesia trends of patients undergoing I-131 MIBG therapy using the Pediatric Health Information System (PHIS) database and describe our own institutional sedation practice.

Methods: Retrospective data from 476 patient encounters from the PHIS from 2010-2019 as well as a case series of 13 patient encounters from Children's Healthcare of Atlanta were reviewed.

Results: Using PHIS data, we discovered considerable variability in the medications used for sedation in patients undergoing I-131 MIBG therapy at 16 PHIS institutions. Although benzodiazepines and opioids were the most used agents, there was a trend towards decreasing use of benzodiazepines and opioids in these patients after 2016. Furthermore, there has been an increasing trend in the use of dexmedetomidine and ketamine after 2016. When broken down into age groups 0-3 years and older than 3 years of age, dexmedetomidine was used more frequently in the younger age group (14.19% vs. 5.80%), while opioids were used more frequently in the older age group (36.23% vs.23.87%) (both $p < 0.05$). Our institutional case series describes the use of propofol by pediatric intensivists for the initial procedural sedation in patients undergoing I-131 MIBG therapy. We also report the use of ketamine and dexmedetomidine for prolonged sedation in these patients. Only 2/13 patients in our institutional cohort required midazolam because of the difficulty of sedation with other agents.

Conclusion: Pediatric intensivists can sedate patients undergoing I-131 MIBG therapy using propofol and fentanyl for bedside procedures without anesthesiologist or operating room resources. Ketamine and dexmedetomidine, with proper procedural monitoring, can be used for prolonged natural airway sedation without serious adverse events in the PICU.

Gun Violence Exposures Impact on Urban Youth School Performance

Authors: Fraser Doh, K; Mehta, A; Jergel, A; and Quinoy, A

Presenting Author: Kiesha Fraser-Doh, MD

Exposure to firearms has been shown to negatively impact youth's mental and behavioral health. This study's primary objective is to analyze the intersection of gun violence exposure (GVE) and school achievement (SA).

We utilized the Future of Families and Wellbeing Study (FFCWS), a longitudinal birth cohort study surveying urban youth/guardians at age 15 utilizing surveys of parents and teens. SA was measured by 4 metrics: parent report of attending summer school and repeating a grade; and student report of honor roll and failing a grade. GVE data was obtained from the Gun Violence Archive (GVA), a database that collects firearm injury data utilizing coordinates then cross-referenced data from FFCWS. Two sample t-test and chi-squared tests adapted to complex survey samples were used for p-value calculations. $P< 0.05$ indicated statistical significance

Our sample comprised 2563 students. Students who attended summer school had more cumulative total GVE (5.72 vs. 3.66, $p< 0.05$) and school GVE (2.57 vs. 1.54, $p< 0.05$) and those who repeated a grade had twice as much GVE compared to those who did not (total 7.89 vs. 3.76, $p< 0.05$; home 4.09 vs. 2.18, $p< 0.05$; school 3.80 vs. 1.57, $p< 0.05$). Students who failed a class had more GVE in both locations compared no failed class (total 4.99 vs. 3.45, $p< 0.05$; home 2.85 vs. 1.97, $p< 0.05$; school 2.13 vs. 1.48, $p< 0.05$). Those not on honor roll had more home GVE (2.77 vs. 1.87, $p< 0.05$) and total GVE (4.66 vs. 3.45, $p< 0.05$) than those on honor roll. Those on honor roll had a statistically significant higher proportion of no GVE (51% vs. 49%).

Our research reveals that an increase in cumulative exposure to gun violence is associated with metrics of student achievement and demonstrates that cumulative GVE can impact success. The level of achievement in high school may carry significant ramifications that extend into one's future career trajectory. Since academic accomplishments attained during this formative period could shape an individual's opportunities in the years to come, further research is crucial to comprehend the full impact of GVE on youth wellbeing.

**Impact of Annual $PM_{2.5}$ Exposure on Clinical and Laboratory Outcomes in Children with Sickle Cell Disease: A Retrospective Cohort Study

Authors: George, Paul E; Kalmus, Grace; Lane, Peter A; Lam, Wilbur; Howard, David; and Ebelt, Stefanie

Presenting Author: Paul George, MD

Background:Previous work demonstrated that short-term air pollution exposure is associated with poor health outcomes in children with sickle cell disease (SCD). Our objective was to examine the effects of long-term air pollution exposure in children with SCD. We hypothesized that annual air pollution exposure would be correlated with worse clinical outcomes and increased inflammatory markers. Furthermore, we hypothesized that hydroxyurea, given its anti-inflammatory properties, would partially mitigate these effects.

Methods: Patient data were collected from the Children's Healthcare of Atlanta SCD Database (Jan 2010-Dec 2019). The exposure of interest was annual ambient fine particulate matter (PM2.5) concentrations at the individual's address. Primary outcomes were clinical (e.g., hospital days/year) and inflammatory changes (e.g., white blood count (WBC), absolute neutrophil count (ANC)), across a given year. We employed a fixed effects multivariable regression, thereby adjusting for time-invariant confounding factors. We included potential time-varying confounders (i.e., age, insurance, distance from address to clinic, neighborhood socioeconomic status) in the statistical model. Hydroxyurea use was included as an interaction term.

Results: 1069 children with HbSS/HbSβ0 (age 0-17.9 years; 549 females, 520 males; 6.1 years average length of follow-up) were included in the analysis. Average annual PM2.5 was 9.5 μg/m3 (range 7.9-12.7) for the cohort. Annual PM2.5 at home address was significantly associated with hospital days per year (incidence rate ratio (IRR)=1.16, p=0.047) and abnormal stroke risk screen (OR=1.05, p<0.001); there was no significant association with annual ED visits (IRR=1.02, p=0.6). Annual WBC (β=0.19, p=0.02) and ANC (β=0.14, p=0.01) were significantly associated with annual PM2.5. Hydroxyurea use acted as a significant effect modifier by reducing the impact of annual PM2.5 on WBC and ANC; it did not significantly impact the effect of PM2.5 on hospital days or stroke screening.

Conclusions: Long-term exposure to PM2.5 was significantly associated with increased hospital days, stroke risk, and inflammatory markers in children with SCD, underscoring the harms of air pollution in this population and need for stronger health policy against air pollution-induced health effects in children with SCD. Notably, hydroxyurea usage mitigated the inflammatory effects of PM2.5 but did not impact clinical outcomes, highlighting its partial potential protective role.

**Poster Finalist

**The Association Between Peri-operative Management Strategies and Post-operative Acute Kidney Injury in Surgical Neonates

Authors: Gillen, Matthew; He, Zhulin; Gauthier, Theresa; Patel, Ravi; Greenbaum, Laurence; Poindexter, Brenda; and Shin, H Stella

Presenting Author: Matthew Gillen, MD

Background: Acute kidney injury (AKI) affects about one-third of critically ill neonates and is associated with increased morbidity and mortality. Peri-operative risk factors that are associated with post-operative AKI are uncertain.

Objective: To describe the incidence of post-operative AKI in neonates undergoing small bowel resection and to evaluate peri-operative risk factors for post-operative AKI.

Methods: We performed a retrospective observational cohort study for neonates (≤ 28 days of age) undergoing small bowel resection between 1/1/17-6/30/22 at the Children's Healthcare of Atlanta Egleston and Scottish Rite Level IV NICUs. Death within 24 hours of surgery, prior renal replacement therapy, and congenital anomalies of the kidney and urinary tract (CAKUT) were exclusion criteria. The incidence of post-operative AKI and the contribution of birth weight, binarized at 1500 g was evaluated. Unadjusted relative risks were calculated using contingency tables.

Results: We evaluated 244 neonates with small bowel resection of whom 15 were excluded (2 died; 13 had CAKUT) with 229 analyzed. One-hundred thirty-three patients (58%) were male. Median gestational age was 33 weeks (IQR 26 – 36), and median age at surgery was 5 days (IQR 2 – 14.5). Median birthweight (BW) was 1960 g (IQR 983 – 2755). Ninety-four patients (41%) had BW ≤ 1500 g. Sixty-two patients developed post-operative AKI (overall incidence 27%; 95% CI 21% - 33%). Pre-operative mechanical ventilation (RR 2.71; 95% CI 1.58 – 4.62; P<0.001), pre-operative furosemide (RR 2.42; 95% CI 1.35 – 4.33; P=0.04), pre-operative blood transfusions (RR 2.04; 95% CI 1.33 – 3.12; P=0.001), and peri-operative vasoactive medications (RR 1.65; 95% CI 1.08 – 2.53; P=0.02) were associated with post-operative AKI. Post-operative AKI was associated with in-hospital mortality (RR 4.49; 95% CI 1.70 – 11.83; P=0.002). Forty percent of patients with BW ≤ 1500g had post-operative AKI while 18% of those with BW > 1500g developed post-operative AKI (P<0.001).

Conclusions: Post-operative AKI is estimated to occur in ~1 in 4 neonates undergoing small bowel resection and is associated with increased in-hospital mortality risk. The risk for post-operative AKI is higher among infants ≤1500 g. We have identified several peri-operative risk factors that may be useful to identify infants at risk for post-operative AKI.

**Poster Finalist

Cobalamin (B12) Deficiency in Children with Sickle Cell Disease (SCD)

Authors: Hatabah, Dunia; Krieger, Rachel; Brown, Lou Ann; Harris, Frank; Korman, Rawan; Benedit, Laura; Umana, Jasmine T; Rees, Chris A; Dampier, Carlton; and Morris, Claudia

Presenting Author: Dunia Hatabah, MD

Background: B12-deficiency has been reported in 18% of adults with SCD vs 10% without SCD. There is limited and contradictory data on the prevalence of B12-deficiency in children with SCD. Diagnosis of B12-deficiency is challenging, with no gold standard; no data exists to guide B12-deficiency screening in patients with SCD. Adding to the urgency of addressing B12-deficiency risk in SCD is the increased use of nitrous oxide gas (N2O) in the United States. Pertinently, N2O is standard therapy for SCD-pain in Europe. N2O impacts B12 metabolism, with reports of neurologic sequalae in patients with SCD treated with N2O emerging.

Objective: To evaluate prevalence of B12-deficiency in children with SCD-pain in a pediatric emergency department (ED).

Methods: This is a secondary analysis of samples collected as part of ED-based clinical trials enrolling children with SCD-pain, evaluating mechanisms of hemolysis. B12-deficiency was defined as plasma MMA >318 nmol/L or urine MMA/Cr ≥2.2 mmol/mol.

Results: A total of 94 children (13±4 years, 54% female, 68% hemoglobin SS disease, and 72% on Hydroxyurea) were included. Fifty-two percent (49/94) had B12-deficiency diagnosed by either urine MMA/Cr, plasma MMA or both. Twenty-six percent (24/94) of patients were B12-deficient based on urine MMA/Cr, while 39% (37/94) were diagnosed by elevated plasma MMA. Of all subjects found to be B12-deficient by either plasma or urine, 12 children (13%) were diagnosed with B12-deficiency identified in both plasma and urine. There were no significant differences in patient demographics or clinical characteristics in B12-deficient vs non-deficient groups. Plasma MMA and urine MMA/Cr showed no correlation with hemoglobin and MCV.

Conclusion: Our data confirms that B12-deficiency is common in children with SCD, with a concerning prevalence of 52%. Given challenges with reliability of testing modalities, it is not possible to determine whether this is an over- or under-estimation of the true prevalence. Nonetheless, given the safety, affordability, and effectiveness of B12 supplementation, alongside current practices of administering folic acid to patients with SCD despite scarcity of evidence, it seems prudent to also consider incorporating B12 supplementation into our management regimen, to avoid detrimental consequences of an easily treatable cobalamin deficiency.

Characterizing Risk Factors of Pediatric Inflammatory Bowel Disease Severity

Authors: Kaba, Christine; Chatman, Kelsey; and Niklinska-Schirtz, Barbara

Presenting Author: Christine Kaba, MD

Background: The incidence of pediatric-onset Inflammatory Bowel Disease (IBD) has increased significantly during recent decades, particularly among non-White individuals. African Americans, specifically, have been shown to have higher risk of disease complications and worse disease outcomes than Caucasians. Given the tremendous impact IBD can have on the overall growth and nutritional status of a child, early recognition and risk stratification is important. While many of the earliest presenting signs and symptoms of IBD (i.e. hematochezia, weight loss or low BMI, abdominal pain) can be nonspecific and difficult to recognize, socioeconomic factors including race, zip code, and insurance status can likely aid in the early identification of patients at higher risk for severe disease. Other presenting findings such as albumin and BMI at the time of diagnosis may also vary by demographic factors including race and are often used as markers for disease severity. For example, hypoalbuminemia has specifically been shown to be associated with earlier initiation of biologic therapies. As such, we aim to describe potential indicators of IBD severity and/or poor outcomes within our pediatric population.

Methods: We are conducting a retrospective chart review on patients with IBD in the Children's Healthcare of Atlanta network. Data will be largely collected from the ImproveCareNow (ICN) registry, a learning health network for pediatric IBD utilized by multiple institutions across the country. This data set includes patients with a diagnosis of IBD for at least one year prior to October 2021. Data being collected include race, insurance type, zip code, and presenting findings, as well as longitudinal data such as BMI, albumin, and other indicators of disease outcome and severity (i.e. emergency department visits, abdominal surgeries).

Proposed Results/Conclusions: Children's Healthcare of Atlanta is the only pediatric hospital system in Atlanta and serves a diverse pediatric IBD population that includes about 36% African American patients. This provides a unique opportunity to view various presenting phenotypes of IBD and highlight how both social determinants and genetic predispositions can affect the overall presentation and severity of disease. We hope to use this data to support early risk stratification of IBD severity in pediatric patients.

**Malnutrition Diagnosis is Negatively Associated with Fat-free Mass in Critically Ill Term Newborns

Authors: McNelis, Kera; Ta, Allison; and Fu, Ting Ting

Presenting Author: Kera McNelis, MD, MS

Background: The neonatal period is a time of rapid growth, and many infants who require intensive care need extra nutritional support. The Academy of Nutrition and Dietetics (AND) published an expert consensus statement to establish criteria for the identification of neonatal malnutrition. There is limited evidence regarding outcomes associated with diagnosis from these opinion-derived criteria.

Objective: To compare anthropometric-based malnutrition indicators with direct body composition measurements in infants

Methods: Air displacement plethysmography is considered the gold standard for non-invasive body composition measurement, and this was incorporated into routine clinical care at a referral Level IV neonatal intensive care unit. Late preterm and term infants (34-42 weeks gestational age) with body composition measurement available were included in this study. Infants were categorized as having malnutrition per AND criteria. Fat mass, fat-free mass, and body fat percentage z-scores were determined per Norris body composition growth curves. Logistic regression was conducted to ascertain the effect of fat mass, fat-free mass, and body fat percentage on malnutrition diagnosis. Linear regression was performed to predict body mass index (BMI) at age 18-24 months from each body composition variable.

Results: Eighty-four infants were included, with 39% female and 96% singleton. 15% were small for gestational age and 12% were large for gestational age at birth. 45% (37/83) had a congenital intestinal anomaly, including gastroschisis and intestinal atresia. 63% of the group met at least one malnutrition criterion. Fat-free mass z-score was negatively associated with a malnutrition diagnosis, with an odds ratio 0.77 (95% CI 0.59-0.99, $p<0.05$). There was not a statistically significant association between malnutrition diagnosis and body fat percentage or fat mass. There was not a statistically significant relationship between any body composition variable and BMI at 18-24 months, even after removing outliers with a high Cook's distance.

Conclusions: Malnutrition diagnosis is associated with low fat-free mass in critically ill term infants. Body composition is not a predictor of later BMI in this small study.

**Poster Finalist

Dear Diary, Today Feels Different: Exploring Intraindividual Dynamics in Pain and Relevant Correlates for Youth with Chronic Sickle Cell Disease Pain

Authors: Mooney, Jan T; Adkins, Taylor R; and Sil, Soumitri

Presenting Author: Jan Mooney, PhD

Background: Chronic pain is prevalent and life-interfering for youth with sickle cell disease (SCD), often co-occurring with other challenges, such as sleep disturbance and fatigue. Evidence-based chronic pain management guidelines emphasize individualized treatment. Self-monitoring through daily diaries can increase awareness of symptom patterns to inform care. Prior research has observed group-level patterns between pain, sleep, and self-management techniques. This approach may obscure important person-level nuances. The goal of the present work was to represent intra-individual dynamics of pain and relevant correlates in youth with chronic SCD pain.

Method: Participants (N = 11) were enrolled in a single-arm pilot of a virtual group-based (n = 3-4) non-pharmacological interdisciplinary chronic SCD pain management treatment. Group sessions integrated neuromuscular exercise with cognitive-behavioral skills across 16 twice-weekly sessions with periodic caregiver involvement. Daily texts or e-mails prompted participants to respond on visual analog scales regarding their levels of physical activity, sleep quality, fatigue, pain, pain intensity, and soreness. Unified structural equation models (uSEM) tested relationships between all experiences individually for each participant.

Results: Teens were between age 13 and 18 (M = 16.6) and 54% identified as male. On average, participants completed 37 of 56 diary prompts (range = 14-56). For the present analyses, we included participants (n = 8) who completed at least 50% of diary prompts. Across individual network models, alternative fit indices suggested adequate fit (mean SRMR = .09, mean RMSEA = .05, mean CFI = .98, mean NNFI = .97). Concurrent and prospective relationships demonstrated both similarities and differences in strength and direction. For example, three of eight models (37.5%) contained a moderate inverse concurrent association between activity and fatigue ($-0.49 < \beta < -0.45$). In contrast, 37.5% of models estimated a relationship between soreness and future activity, though in opposing directions (β = -0.31, -0.51 vs. 0.54).

Conclusions: This innovative use of uSEM identified complex, variable interrelationships among activity, fatigue, soreness, sleep, and pain for youth participating in an SCD chronic pain management group treatment. Results suggest that insights from person-specific network analysis can inform individualized treatment. Replication of this work with larger within-person sample sizes and more consistent diary completion may help identify more stable patterns and optimize model fit.

The Clinical Significance of Non-Ebstenoid Fetal Tricuspid Valve Regurgitation

Authors: O'Meara, Daniel; Michelfelder, Erik; Jergel, Andrew; and Ro, Sanghee

Presenting Author: Daniel O'Meara, MD

Background: Physiologic tricuspid regurgitation (TR) is a common finding in fetal life. It is often difficult to discern associated findings and neonatal outcomes with the degree of TR seen by fetal echocardiography (FE). We seek to assess the spectrum of non-Ebstenoid TR in fetuses to better understand the clinical outcomes and associated FE findings.

Methods: We conducted a retrospective study on all fetuses with non-Ebstenoid TR from 1/1/2012 to 10/1/2023. Patients were divided into three groups based on the grade of TR seen on their last FE: trivial to mild, moderate, or severe. Prenatal variables and neonatal outcomes were collected. Statistical analyses included Kruskal-Wallis Rank Sum Test and Fisher's Exact Test, and values were reported as medians with interquartile ranges, or as frequencies when appropriate.

Results: There were a total of 68 fetuses with TR: 47 with trivial to mild, 16 with moderate, and 5 with severe. Those with moderate TR were more likely to have worsening TR in late gestation compared to the trivial-mild or severe groups ($p<0.001$). In contrast, the trivial-mild group was more likely to have unchanged or regression of TR (Table 1). Overall, fetuses with severe TR had a larger tricuspid valve annulus and an abnormal ductus venosus Doppler pattern ($p=0.003$). Those with severe TR were also more likely to have pulmonary regurgitation ($p=0.013$). Newborns with prenatally diagnosed severe TR were more likely to require cardiac intensive care ($p=0.002$) (Table 2). There were no differences in respiratory support, neonatal cardiac interventions, or Apgar scores.

Conclusions: Tricuspid valve annulus size and z-score, pulmonary regurgitation, and abnormal ductus venosus Doppler seen by FE were correlated with severe TR. Moderate TR was more likely to progress and worsen compared to trivial or mild TR. Fetuses with severe TR were more likely to require transfer to the cardiac intensive care unit. These findings can help guide follow-up and postnatal evaluation for prenatal findings of non-Ebstenoid TR.

**Does Risk Stratification of Fetuses with d-Transposition of the Great Arteries Shorten Time to Transport and Cardiac Intervention?

Authors: Ro, Sanghee; Nam, JoAnn; Jergel, Andrew; Clark, Shanelle; Chanani, Nikhil; Wolf, Michael; and Michelfelder, Erik

Presenting Author: Sanghee Ro, MD

Background: Predicting the need for an urgent balloon atrial septostomy (BAS) by fetal echocardiography (FE) in newborns with d-transposition of the great arteries (d-TGA) is challenging. Risk stratification and delivery planning allow for quick transport to the cardiac intensive care unit (CICU) and immediate cardiac catheterization. This is important at centers with no in-house birthing capabilities. The aim of this study is to evaluate the impact of prenatal risk stratification on transport time to the CICU and to urgent BAS for newborns prenatally diagnosed with d-TGA.

Methods: We performed a retrospective review on all fetuses with d-TGA between 1/1/2018 and 12/31/2023. All subjects were assigned a level of care-risk level (LOC-R) 3 (moderate risk for hemodynamic instability) or 4 (high risk for hemodynamic instability), based on FE findings. Primary outcomes were the times from birth to CICU arrival and to BAS along with the need for an urgent BAS. The need for a BAS within 2 hours of arrival to the CICU was based on clinical status of the newborn. A Chi-squared test or Fisher's exact test were used for continuous variables. A univariate analysis was performed to determine factors associated with an urgent BAS. A p-value of < 0.05 was considered significant.

Results: A total of 50 newborns with a prenatal diagnosis of d-TGA were analyzed, with 28 (56%) requiring BAS, and 17 (34%) undergoing BAS < 2 hours of CICU arrival. BAS was performed in 22/45 (49%) fetuses assigned an LOC-R 3, and in 4/5 (80%) assigned an LOC-R 4. Patients with a higher LOC had a significantly lower time between birth to CICU arrival ($p<0.001$) and birth to BAS ($p=0.003$) (Table 1). No prenatal FE findings were associated with need for BAS (Table 2). Need for BAS was associated with more severe clinical presentation (Table 2).

Conclusion: Formal risk stratification utilizing FE can effectively reduce the time between birth and BAS for d-TGA newborns. Prenatal FE markers are unreliable in predicting the need for BAS in this population. Therefore, appropriate delivery planning should be considered in light of these findings.

**Poster Finalist

Longitudinal Outcomes in Children with Trisomy 21 and Congenital Heart Disease after Cardiac Surgery

Authors: Saini, Ashish; Holstein, Rachel; Keane-Lerner, Kasey; McLaughlin, Sarah; Shamah, Rebecca; and Beshish, Assad

Presenting Author: Ashish Saini, MD

Background: Congenital Heart disease (CHD) occurs in 40-60% of children with Trisomy 21, the majority requiring surgical intervention. Studies have shown excellent short and long-term survival in Trisomy 21 patients after cardiac surgery. However, the literature on morbidities and reinterventions is limited.

Methods: The study includes patients with Trisomy 21 and CHD who underwent cardiac surgery between January 2008 and December 2020 at Children's Healthcare of Atlanta. Patients undergoing staged single ventricle palliation were excluded. The primary outcome of the study was to describe the epidemiology, longitudinal outcomes, and therapeutic interventions following surgical management of CHD in Trisomy 21 patients. Analysis was performed using standard statistical tests.

Results: Our study cohort comprised 261 patients, 56% being female and 13.8% being preterm. The median age was 4.9 months, and weight was 5.3 Kg at the time of surgery. The most common diagnoses were complete atrioventricular canal defect (47.1 %) and ventricular septal defect (18.4%). Twenty-nine (11%) patients underwent staged biventricular repair. The median postoperative ICU length of stay was 3 days. All but 4 patients survived until hospital discharge with a survival rate of 98.5%. A majority, 93.5% of patients, were discharged on diuretic therapy, in comparison, only 30.3% were discharged on angiotensin-converting enzyme (ACE) inhibitors. By one year, 8.4% of patients remained on diuretics and 5.7% on ACE inhibitors. Pulmonary hypertension was evident in 25.7% of the patients at hospital discharge but improved in most of the patients over time. At the time of the last follow-up, only 6.7% of patients had pulmonary hypertension. Sixteen patients (6.1%) underwent permanent pacemaker placement for postoperative complete heart block. Overall, 10-year survival was 94.5% and intervention-free survival was 75%.

Conclusion: We describe longitudinal outcomes in a contemporary cohort of Trisomy 21 patients after congenital heart surgery with excellent short and long-term survival. Most of the patients do not require long-term medical therapy. Periodic surveillance is required for prompt diagnosis of late complications and timely intervention.

Family-Centered Diet Intervention to Reduce Sugar Consumption in Latinx Children

Authors: Sanchez-Torres, Cristian; DeSantos, Karla; Ramirez, Ana; Gillespie, Scott; Huneault, Helaina; Pyo, Irene; Aguayo, Liliana; Vos, Miriam; and Welsh, Jean

Presenting Author: Cristian Sanchez-Torres, MD

Objective: While reducing added sugars is known to be beneficial for children, the most effective way to achieve this change is not known. The objective of this study was to assess the impact of a dietitian led, family-focused intervention using both food replacement and guided grocery shopping (GGS), to reduce sugar consumption and its impact on children's diet quality.

Methods: This substudy, embedded within the NAFLD Prevention trial (ClinicalTrials No. NCT05292352), includes Hispanic families with a child participant aged 6-9 years enrolled who had completed up to 3 24-hour food recalls (parent proxy) at baseline and after 12 months. The family-centered diet intervention rooted in Social Cognitive Theory, included nutrition counseling using motivational interviewing by a bilingual/bicultural dietitian, home removal of high-sugar foods, financial assistance for food replacement with family-preferred low or no-sugar alternatives (once a week in the first month) and GGS 3 times thereafter (months 3, 6 and 9). Paired t-tests compared intake changes over time between the groups.

Results: 8 subjects were included in the analysis (3 controls, 5 interventions). At the 12-month follow-up, participants in the intervention group compared to the control group reported a decrease in total calories (-270 vs +163 kcal), total sugar (-35.9 vs +20.6 g), and added sugar (-25.9 vs +7.4 g). Subjects in the intervention group also reported a slight increase in saturated fat (+0.11) and a decrease in fiber (-2.69 g). Diet quality improved in both groups with a greater increase in the Healthy Eating Index score among those in the intervention (+6.90 vs 0.94, respectively). None of the differences were statistically significant at a p-value of <0.05.

Conclusions: Interim analysis of the data suggests that a family-centered nutrition counseling intervention that uses evidence-based counseling methods, and incorporates family food replacement and GGS, results in a reduction in sugar intake in children aged 6-9 years, a critical time for nutritional improvement. Further analysis as more participants completes their follow-up assessments will be needed to confirm these initial findings.

Impact of Sickle Cell Disease Modifying Therapies on Pediatric Sleep

Authors: Yoo, Michelle; Bai, Shasha; Kalmus, Grace; Abel, Lindsey; Dampier, Carlton; Khemani, Kirshma; Leu, Roberta; Greene, Devon; Vala, Snehal; and Kopp, Benjamin

Presenting Author: Michelle Yoo, MD

Background: Children with sickle cell disease (SCD) have a higher prevalence of sleep disturbances, adenotonsillar hypertrophy, and obstructive sleep apnea (OSA) than the general population. Numerous disease-modifying therapies are now available for SCD such as hydroxyurea, voxelotor, and crizanlizumab that help to prevent pain crisis and reduce complications of SCD. The impact of these disease modifying therapies on OSA and nocturnal hypoxemia remains unclear. We hypothesized that in children with SCD, those on disease modifying therapies will have higher nocturnal oxygen saturation, improved sleep quality, decreased prevalence and degree of OSA.

Methods: This was a retrospective chart review of 1125 polysomnogram (PSG) records from January 01, 2013 to July 31, 2023 from children with SCD aged 1-18 years at Children's Healthcare of Atlanta (n=701). Data including obstructive apnea-hypopnea index (OAHI), nocturnal oxygen desaturations, sleep efficiency and arousal index were collected. Demographic and clinical data collected included age, race, sex, SCD genotype, body mass index, medications for SCD, length of time on treatment, prior airway surgeries and number of annual hospitalizations for asthma, vaso-occlusive crisis, or acute chest syndrome.

Results: Compared to those on therapy, children not on SCD disease modifying therapy were less likely to have OSA (51.8% vs. 48.3%, p=0.008), however had higher occurrence of moderate and severe OSA (10% vs 8.8% and 9.6% vs 5.7%). Mean OAHI was significantly higher in children without SCD therapy than in children on any SCD therapy (3.4 vs. 2.7, p=0.04). Children on SCD therapy had significantly lower baseline oxygen saturation, as well as higher percentage of sleep with oxygen saturation <90% compared to no therapy group (95.1 vs. 96.1, p=<0.001, 4.98 vs. 8.27, p=0.002). There was no significant difference in arousal index or sleep efficiency, with reduced sleep efficiency observed in both groups. Ongoing longitudinal analyses and multivariable modeling will assess for confounding factors and associations with clinical factors.

Conclusion: Patients on SCD disease-modifying therapies demonstrate less severe OSA but persistent abnormalities including obstructive apneas, nocturnal oxygen desaturations, and decreased sleep efficiency. Underlying factors for persistent sleep abnormalities in children with SCD remain to be determined.

**Pediatric Age-Labeling in AI/ML-Enabled Medical Devices

Authors: Zapotoczny, Grzegorz; Goyal, Ansh; Qazi, Shahida; Christmas, Madison; and Espinoza, Juan

Presenting Author: Grzegorz Zapotoczny, PhD

Background: Medical devices are imperative for the diagnosis and treatment of diseases in children and adults. Pediatric devices face greater challenges to commercialization than their adult counterparts due to numerous clinical, technical, regulatory, ethical, and financial barriers. As a result, they lag in sophistication and availability by a decade.

In 2009, the FDA created the Pediatric Device Consortia Grants Program to promote pediatric device development and funded the Consortium for Technology & Innovation in Pediatrics (CTIP). CTIP, centered at children's hospitals, helps commercialize medical devices and conducts pediatric research.

With the recent public interest in the utilization of AI/ML technologies in healthcare, we decided to investigate whether they had been tested in or approved for pediatric patients.

Objective: To evaluate marketed AI/ML-enabled medical devices for pediatric labeling.

Methods: In October 2023, FDA published a list of 691 AI/ML-enabled devices it had cleared or approved to date. We extracted the data concerning the pediatric use of these devices focusing on their labeling, safety, and effectiveness. A retrospective analysis of these regulatory submissions is presented herein.

Results: Every year since 2016 the number of AI/ML-enabled device approvals/clearances has been drastically increasing, with the majority being developed in Radiology (76.9%), Cardiovascular (10.2%), and Neurology (2.9%). Nevertheless, our preliminary analysis revealed that only 7.7% of all devices are indicated for the use in children (0-17yo), while 10% are specifically labeled not for pediatric use, and 78% are silent on age completely. In terms of clinical evidence, only 2.4% applications presented data to support pediatric labeling, while 31% presented no age data, and 61% had no clinical data at all.

Conclusions: Pediatric AI/ML-enabled medical devices are few, and even fewer present pediatric clinical data to substantiate regulatory claims. With the AI/ML space being both new (most devices were marketed with the past 8 years) and largely decentralized (302 unique companies are responsible for the 691 available products), pediatric innovators are likely facing numerous regulatory and financial barriers. Pediatric accelerators and incubators can play a vital role in lowering the barrier to entry through direct regulatory or financial support and though influencing meaningful policy changes.

**Poster Finalist

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