handling to injury, enabling safer, personalized therapy to slow kidney damage in patients.

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Sepsis-associated PARDS is associated with an exhausted neutrophil phenotype in the lower respiratory tract

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OBJECTIVES/GOALS: This study aims to uncover immuno-endotypes in sepsis-associated pediatric acute respiratory distress syndrome (SA-PARDS) using single-cell RNA sequencing (scRNA seq) to analyze the immune cell populations of the respiratory tract of intubated pediatric subjects with SA-PARDS. METHODS/STUDY POPULATION: The inclusion criteria are an age of less than 18 years, admission to the PICU, a diagnosis of SA-PARDS, and intubation. Both sepsis and PARDS will be defined using the most recent consensus definitions. Exclusion criteria include an order of limited resuscitation and clinician discretion. A tracheal aspirate and blood sample will be obtained on days 1, 3, and 7. Both samples will be processed for single-cell RNA sequencing via the HIVE platform according to the manufacturer's protocol. cDNA libraries will be generated and undergo 150 bp sequencing, quality control, and alignment. The Seurat package in R will be used for cell-type annotation and analysis of differential gene expression. Clinical variables, labs, and outcomes will be recorded in REDCap. RESULTS/ANTICIPATED RESULTS: We evaluated five samples from two subjects. After quality control, 31,330 cells remained for analysis. UMAP projections were created, and we annotated cell types. We found that most of the cells were immune cells with very few epithelial cells. There were two groups of neutrophils, which we termed classic and migratory. Classical neutrophils expressed classic neutrophil markers, and migratory neutrophils expressed the classical markers plus increased adhesion markers. We identified a third group of cells that were neutrophil-like, and these were isolated for further analysis. These cells exhibited characteristics of exhausted neutrophils, including downregulation of CXCR1 and CXCR2, and upregulation of PI3 and SLIPI. In each subject, the proportion of exhausted neutrophils increased over time. DISCUSSION/SIGNIFICANCE OF IMPACT: We found that in our subjects with SA-PARDS, there were a subset of neutrophils characterized by down-regulation of classical neutrophil markers and up-regulation of neutrophil stress markers, suggested an exhausted phenotype.

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Structure-function-migration in the aging lymph node

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OBJECTIVES/GOALS: The aging (65+) population is rapidly growing and faces unique health issues exacerbated by immune function decline. However, the driving mechanisms behind this decline are poorly understood. We will use a mouse model to study the relationship between stiffness and cell mobility within the lymph node (LN) as one potential driving mechanism. METHODS/STUDY POPULATION: We will collect inguinal LNs from young (6–8 weeks) and aged (18+ months) female mice and section them for *ex vivo* analysis, including quantification of proteins in the fibroblastic reticular network (collagen I, collagen III, collagen VI, laminin, and fibronectin) using immunofluorescent staining and Python scripts; analysis of biomechanical properties (elastic modulus, loss modulus, and pore size) using multiple particle tracking (MPT); and migration analysis of adoptively transferred B- and T-cells using live imaging and MATLAB scripts. We will use ANOVA to compare abundance and organization of proteins, biomechanical properties, and cell migration between young and aged LNs, as well as using Pearson's correlation coefficients to identify relationships between characteristics. RESULTS/ANTICIPATED RESULTS: We hypothesize a positive correlation between fibroblastic reticular network protein deposits, especially collagens, and stiffness (elastic modulus) within murine LNs due to known mechanisms underlying age-related fibrosis. We also hypothesize that areas of increased stiffness (as revealed by MPT) will exhibit decreased cell migration due to physical hindrance to B- and T-cell mobilization. Furthermore, we hypothesize that aged murine LNs will exhibit a significant increase in stiffness and resultant decreased cell mobility when compared to young murine LN, particularly in areas with increased collagen localization. DISCUSSION/SIGNIFICANCE OF IMPACT: These studies will elucidate structure–function relationships driving age-associated LN fibrosis and stiffness, and the resultant effect on cell migration, thus clarifying some of the potential driving mechanisms behind immune aging and providing data capable of informing the development of relevant models, therapeutics, and interventions.

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Prenatal family and community exposures and associations with postnatal epigenetic signatures of child metabolic health

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OBJECTIVES/GOALS: The purpose of this study was to examine associations between prenatal family and community exposures and early childhood DNA methylation (DNAm) at genomic regions related to energy regulation or fat deposition. Results could contribute to the understanding of disparities in metabolic health risk among children from disadvantaged communities. METHODS/STUDY POPULATION: This is part of a cross-sectional study of mother–child dyads recruited from a previous RCT with pregnant women in California who had a pre-pregnancy BMI of 25–40. DNA was extracted from saliva of 49 child participants between 1 and 4 years old and is undergoing whole-genome bisulfite sequencing. Outcomes are percent DNAm, both genome-wide and at genomic regions related to energy regulation or fat deposition. Prenatal exposures were measured by the Geospatial Research, Analysis, and Services Program (GRASP) Place & Health burden percentile ranking, based on census tract of residence during pregnancy, with higher percentiles indicating greater burden. We will

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use regression to model associations of percent DNAm at gene loci of interest on GRASP percentile, controlling for relevant covariates (e.g., age, sex). RESULTS/ANTICIPATED RESULTS: Final data collection, cleaning, and analysis are in progress and on target to be completed in Fall 2025. We will present final results of these analyses, including descriptive characteristics of the sample (e.g., summary and frequency statistics) and overall DNA methylation patterns (e.g., b or M values as appropriate), and whether there is support for our hypotheses that children with greater prenatal family and community exposures will have DNAm in pertinent genomic regions related to energy regulation or fat deposition (e.g., regression parameters and confidence intervals). DISCUSSION/SIGNIFICANCE OF IMPACT: Findings will advance knowledge of early-life physiologic effects of exposures during pregnancy. Future clinicians may use this knowledge to earlier identify and treat children at high risk for adverse child metabolic health outcomes such as obesity, and health advocates can use it to promote policies to reduce adverse prenatal environments.

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Development of a pediatric obesity treatment using a Sequential Multiple Assignment Randomized Trial (SMART) design

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OBJECTIVES/GOALS: Pediatric obesity is a public health issue. Many youths in lifestyle obesity treatments do not improve their weight. Sequential Multiple Assignment Randomized Trial (SMART) study designs can support slow responders (e.g., youth who do not initially lose weight). SMARTs have limited use in pediatric obesity and lack data-driven adaptive rules. METHODS/STUDY POPULATION: This study leveraged an ongoing 6-month family-based health behavior pediatric obesity treatment for children and their parents. Parents with obesity and their children (6–11years) with overweight/obesity (body mass index >85th%ile) who lived in rural areas and were a part of the larger pediatric obesity treatment trial were invited to this study. During the treatment, parent/child dyads were asked to complete additional surveys across nine time points (weeks 1, 2, 3, 4, 6, 8, 12, 16, and 20). Surveys assessed the child's physical activity, diet, and pain, along with the family environment and parental stress. The child's anthropometrics were measured each month. RESULTS/ANTICIPATED RESULTS: Data collection will be completed in December 2025. Anticipated results will include analyses to identify responders (i.e., children who reduce their weight outcomes [body mass index (BMI) z-score, BMI percentile, or weight]) and slow responders (i.e., children who do not reduce their weight outcomes) to the pediatric obesity treatment; based on preliminary work, we expect ~50% of the sample to be responders. Once responders/slow responders are identified, survey data will be used to define the adaptive rules, including the tailoring variable (e.g., physical activity or junk foods, or early-response weight outcomes) and decision time point for second randomization. DISCUSSION/SIGNIFICANCE OF IMPACT: Data from this study will lay the groundwork for developing data-driven criteria for SMART rules (tailoring variable and decision time point for second randomization). This, in turn, will support the development of adaptive interventions to identify and support the many youths who are slow responders to lifestyle pediatric obesity treatments.

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A systematic review of medication adherence, risk factors, and implications for novel hypertension therapies

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OBJECTIVES/GOALS: This study aims to identify socioeconomic, clinical, and behavioral risk factors of non-adherence for adults with hypertension and evaluate implications for future treatment strategies. METHODS/STUDY POPULATION: We will conduct a systematic review following PRISMA guidelines. Eligible studies are peer-reviewed RCTs and observational designs assessing factors associated with antihypertensive medication adherence among adults (≥18 years) with hypertension living in North America or Europe. Studies must report adherence as the primary outcome. Studies will be excluded if they are not peer-reviewed, original research articles, published in a non-English language, reviews or meta-analyses, or studies of an intervention aimed at improving adherence. Abstract and title screening, full paper review, and data extraction will be performed in Covidence by two reviewers with conflicts resolved by consensus. RESULTS/ANTICIPATED RESULTS: A total of 5660 records were imported for screening after duplicate removal. Following title and abstract screening, 4990 studies were excluded as irrelevant, leaving 670 records for full-text review. Of 611 full texts reviewed, 491 studies were excluded and 39 are pending conflict resolution. Currently, 119 studies are marked for data extraction. Data to be extracted will include socioeconomic, demographic, clinical, and behavioral determinants of antihypertensive medication adherence. Quality assurance will involve dual independent extraction. Preliminary trends suggest wide variability in study designs and adherence definitions across North American and European populations. DISCUSSION/SIGNIFICANCE OF IMPACT: Findings will clarify key predictors of antihypertensive medication non-adherence and highlight gaps in evidence. Results will guide future adherence-improving strategies and inform design of simplified regimens, digital tools, and population-specific hypertension management policies.

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