

Joint Plenary Session  
WAFMR, WSCI, WAP AND WSPR  
8:00 AM  
Friday, January 29, 2016

226 **DEFAULT MODE NETWORK ACTIVITY: TESTING FOR ASSOCIATION WITH EXTERNALIZING BEHAVIOR PROBLEMS WITH AND WITHOUT CALLOUS UNEMOTIONAL TRAITS**

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10.1136/jim-d-15-00013.226

**Purpose of Study** Adolescents with externalizing behavior problems (conduct disorder (CD) and substance use disorders (SUD)) are a source of large social and economic costs. Such adolescents sometimes display high levels of callous-unemotional (CU) traits and recent work supports that the presence of CU identifies a distinct subgroup of youths with CD. We sought to test whether activity of the default mode network (DMN), a functional brain network involved in self-reflective thought, empathy, and foresight, is associated with these disorders.

**Methods Used** We collected 6 minutes of resting state functional magnetic resonance imaging for 20 patients with CD/SUD and CU, 21 patients with CD/SUD without CU, and 22 controls (all males 14–18 years). We used

independent component analysis, a data-driven approach, to identify networks (i.e., clusters of voxels which activate together across time). We then utilized a standard template and spatial correlation to select the DMN. We tested: (1) whether the 3 groups differed significantly in DMN activity, (2) whether DMN activity was associated with severity of externalizing behavior problems within patients, and (3) whether DMN activity was associated with CU trait severity within patients.

**Summary of Results** Three-group comparisons revealed differences in one cluster including portions of the posterior cingulate cortex (PCC) and precuneus (Brodmann area (BA) 31). Subsequent two-group comparisons showed that both patient groups had significantly less activation in this cluster compared with controls. Our within-patient analysis showed that severity of externalizing behavior problems was negatively associated with activity of a cluster in the ventral and dorsal anterior cingulate areas (BA24/32), and positively associated with activity in a cluster within the PCC. Finally, within patients, severity of CU traits was negatively associated with activity in a cluster of the inferior parietal lobule (BA40).

**Conclusions** While both patient groups, regardless of CU, showed less activity in the DMN (BA31), higher levels of CU trait were associated with a distinct pattern of hypo-activity within patients. Further investigation may lead to better treatment of these disorders.

227 **INFLAMMATORY PROSTAGLANDIN E2 INHIBITS OLIGODENDROCYTE PROGENITOR CELL MATURATION: MECHANISM FOR NEONATAL WHITE MATTER INJURY**

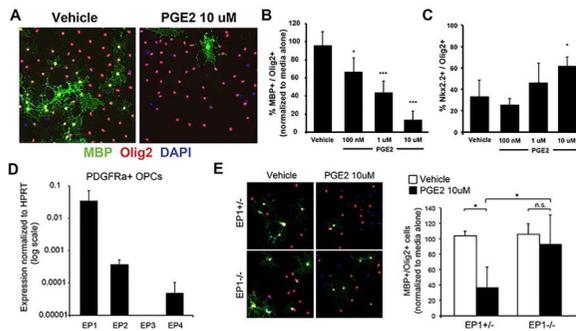
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10.1136/jim-d-15-00013.227

**Purpose of Study** White matter injury (WMI) in the extremely low birth weight (ELBW) brain predicts development of cerebral palsy (CP) and other neurodevelopmental deficits. WMI, a disturbance in myelination, features maturation arrested oligodendrocyte progenitor cells (OPCs). Risk factors for WMI and CP include systemic inflammation, but the mechanism linking systemic inflammation to OPC maturation arrest is not understood. Studies show that prolonged Indomethacin exposure protects ELBW infants from WMI. Because indomethacin is an anti-inflammatory COX inhibitor, we hypothesized that inflammatory Prostaglandin E2 (PGE2) - a major product of COX enzymes - can arrest OPC maturation.

**Methods Used** Primary OPCs were purified from mouse pups by immunopanning and differentiated in the presence of vehicle or PGE2. Cells were stained for mature marker MBP and immature marker Nk $\times$ 2.2. Cells were also collected for qPCR analysis of receptor expression. Studies were also conducted with OPCs purified from EP1-receptor-deficient pups and littermate controls.

**Summary of Results** PGE2 caused a dose-dependent decrease in MBP staining of differentiating cells (Figure 1A). Cells also had increased levels of Nk $\times$ 2.2 (Figure 1C), consistent with maturation blockade. OPCs predominantly express the EP1 receptor (Figure 1D), and



**Abstract 227 Figure 1** (A) Representative images and quantification of (B) MBP+ or (C) Nkx2.2+ cells following exposure to PGE2. (D) Expression of PGE2 receptors in OPCs. (E) Images and (F) quantification of MBP+ cells from EP1<sup>-/-</sup> and control mice exposed to PGE2.

blockade was attenuated with OPCs isolated from EP1 deficient mice (Figure 1EF).

**Conclusions** PGE2 - major product of COX enzymes - blocks *in vitro* OPC maturation via the EP1 receptor. This data suggests that COX inhibitors may provide anti-inflammatory neuroprotection against WMI and CP by blocking PGE2 effects on OPCs.

## 228 CALCIUM RELEASE PATTERNS IN MOUSE ATRIAL CARDIOMYOCYTES: ROLE OF TRANSVERSE-AXIAL TUBULES

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10.1136/jim-d-15-00013.228

**Purpose of Study** Previous reports suggest that transverse-tubules (t-tubules) are reduced in density or absent in mouse atrial myocytes, similar to rat atrial myocytes. In rats the absence of t-tubules results in specific Ca<sup>2+</sup> release patterns upon depolarization, where triggered Ca<sup>2+</sup> release by junctional sarcoplasmic reticulum (SR) at the cell periphery propagates to the center. This results in a characteristic “U”-shaped Ca<sup>2+</sup> transient in confocal line-scan images of atrial myocytes loaded with fluorescent Ca<sup>2+</sup> indicators. The purpose of our study was to test whether mouse atrial cells have similar Ca<sup>2+</sup> release patterns.

**Methods Used** We loaded enzymatically isolated mouse atrial myocytes with the membrane dye Di-4-ANEPPS, as well as the Ca<sup>2+</sup> indicator fluo-4 AM. We then recorded simultaneous membrane and Ca<sup>2+</sup> images using laser scanning confocal microscopy during field stimulation of the cells at 1 Hz.

**Summary of Results** We examined Di-4-ANEPPS signal in 217 myocytes from 7 mice, and found that 91% of myocytes possessed transverse and axial tubules (TATs). Left atrial cells had a higher percentage of TATs (96%, 134/139) than right atrial cells (80%, 63/78, p<0.001). Male mice had a higher percentage of TATs (95%, 129/136) than female (84%, 68/81, p<0.05). Out of 21 double-labeled

cells we investigated, only one cell, which completely lacked TATs, showed “U” shaped Ca<sup>2+</sup> transients. The majority of cells, with varying degrees of TATs, showed either near-synchronous “band”-shaped Ca<sup>2+</sup> transients (n=12) or “W”-shaped Ca<sup>2+</sup> transients (n=8). Rapid Ca<sup>2+</sup> release either occurred just below the surface membrane or at TATs, as marked by Di-4-ANEPPS.

**Conclusions** We conclude that mouse atrial myocytes exhibit extensive TATs. The presence of TATs can markedly influence the pattern of Ca<sup>2+</sup> release upon depolarization, thereby affecting excitation-contraction coupling and likely contractility in atrial myocytes. A higher number of Ca<sup>2+</sup> release units at t-tubule/SR junctions may also increase the possibility of spontaneous Ca<sup>2+</sup> release and atrial arrhythmia, similar to humans, making the mouse model more relevant to human atrial disease than previously considered.

## 229 IDENTIFICATION OF A NOVEL INNATE IMMUNE SIGNALING FACTOR, TNK1, THAT REGULATES VIRAL INFECTION

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10.1136/jim-d-15-00013.229

**Purpose of Study** Interferon (IFN) is the most critical cytokine in the suppression of viral infection. IFN exert its properties through the activation of Jak-STAT signaling cascade, which induces over 400 Interferon Stimulated Genes (ISGs). ISGs cooperatively restrict the infection by targeting different stages of the viral lifecycles. The degree of ISGs induction serves as a determinant of the clinical outcome by either spontaneously resolving the infection or transitioning to persistent infection. Therefore, the better understanding of how host cells regulate the IFN signaling will greatly improve our antiviral strategy. The aim of this study is to identify and characterize a novel host factor that modulate the IFN signaling with the ultimate goal to translate the insights into clinical applications.

**Methods Used** We established a reporter cell line that stably expresses luciferase regulated by a representative ISGs promoter. The reporter cells were used for a high throughput genome-wide cDNA screening in order to identify the novel host factor that governs the cellular sensitivity to IFN. In addition, we fully utilized *in vitro* and *in vivo* biochemical and genetic approaches for the characterization of the novel antiviral innate immune host factor.

**Summary of Results** The cDNA screening identified non-receptor tyrosine kinase 1 (TNK1). The function of TNK1 has been largely unknown, except for its modest tumor suppressor phenotype. Our study discovered that TNK1 is localize in the cytoplasm in resting condition. TNK1 translocates to the plasma membrane in response to IFN treatment, which promotes the tyrosine phosphorylation of TNK1. The phosphorylated TNK1 participates in the IFN receptor signaling complex by binding to Tyk2 and STAT1, wherein TNK plays a critical role in the serine phosphorylation of STAT1 at AA727. Our study found that this event governs the ISGs expression in a gene specific manner. Lastly, our *in vitro* and *in vivo* studies demonstrated that silencing or genetic deletion of TNK1 results in a severe

deficit in ISGs expression thereby enhancing viral replication.

**Conclusions** Our study identified a novel antiviral host factor that governs ISGs expression via serine phosphorylation of STAT1. Our results indicated that the modulation of TNK1 activity represents a potential therapeutic target for human pathogenic viral infectious diseases.

## Adolescent Medicine and General Pediatrics II

### Concurrent Session

12:30 PM

Friday, January 29, 2016

#### 230 A 24 WEEK PHYSICAL FITNESS PROGRAM IMPROVES BODY COMPOSITION AMONG SOUTHWESTERN NATIVE AMERICAN ADOLESCENTS AT RISK FOR DIABETES

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10.1136/jim-d-15-00013.230

**Purpose of Study** Obesity is increasing among Native American children, putting this population at risk for the metabolic syndrome, type 2 diabetes, and early cardiovascular disease. Previous studies have shown that BMI is a poor estimator of body fat and obesity status in this population, but Multi-frequency Bioelectrical Impedance Analysis (BIA) has been validated as a minimally invasive technique to analyze body composition according to total body fat, fat free mass, and body water percentages. This measurement accurately assesses different populations using population-specific means, as opposed to other methods, such as waist circumference or pediatric BMI assessment. We hypothesized that a 24 week, after-school exercise and nutrition intervention would increase fat-free mass (FFM) and reduce total body fat (TBF) composition in this population as measured by pediatric BMI and BIA.

**Methods Used** 66 Native American adolescent subjects aged 13.7±1.7 years were recruited to participate in a fitness program 3 times a week for 60 minutes of dietary instruction, aerobic exercise, and resistance training. Parents attended an instructional session on healthy eating and the preparation of nutritional lunches for the children. Body composition was assessed at baseline, 12 weeks, and 24 weeks with BIA, as were waist circumference and pediatric BMI measurement.

**Summary of Results** 32 subjects completed all three visits; 20 males and 12 females. Mean results from study weeks 0 and 24 are shown in the Data Table 1.

**Conclusions** A 24 week, standardized fitness program that incorporates nutrition education showed significant improvements in body composition among Southwestern Native American adolescents as evidenced by reduced body weight, reduced pediatric BMI percentile, and increased fat free mass as determined by BIA.

Abstract 230 Table 1

Study Week	Weight (kg)	Height (cm)	Waist Circ. (cm)	BMI-% ile	TBF (%)	FFM (%)
Week 0	81.9 ±24.6	157.7 ±7.1	104.1 ±15.7	94.8 ±10.0	28.1 ±7.7	71.8 ±7.8
Week 24	77.8 ±24.0	158.7 ±7.1	103.6 ±14.7	92.3 ±12.4	26.0 ±8.7	74.1 ±8.7
p-value	< 0.001	<0.001	0.64	0.01	<0.001	<0.001

#### 231 RELIABILITY & VALIDITY OF THE CHISPA: A NEW BRIEF ASSESSMENT TOOL FOR ADOLESCENT SUBSTANCE USE

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10.1136/jim-d-15-00013.231

**Purpose of Study** Adolescent substance use is a serious health concern in New Mexico and the US. The NM Department of Health (NMDOH) school-based health centers (SBHCs) universally administer an evidence-based screening instrument, the CRAFFT, for adolescent substance use concerns. Quality assurance efforts revealed the need for additional guidance for providers to briefly assess students after a positive screen. The CHISPA (Cocaine, Heroin, IV drugs, Synthetic Pot, Pot, Alcohol), was developed to standardize assessments following positive CRAFFT screens and provide initial guidance for SBHC providers. This study assesses the reliability and validity of the data obtained from the newly developed CHISPA.

**Methods Used** The study was conducted in early 2015 with high school-aged SBHC users in Albuquerque, NM using standard test-retest methods. We recruited 101 participants to complete the CRAFFT and CHISPA twice over 2 weeks. Using the CHISPA, students reported for the prior 3 months: types of substances used; frequency of use, signs of addiction or acute danger; grades earned; and motivation and past efforts to stop or reduce use. Total scores for number of drugs used, CRAFFT adverse events and CHISPA adverse events were computed.

**Summary of Results** Retest reliability for the CRAFFT composite measure was 0.82. CHISPA retest reliabilities were: 0.75 for alcohol use over the past 3 months; 0.86 for having used any substances; 0.92 for number of substances used; 0.81 for days per week substances used; 0.80 for student GPA; 0.60 for motivation to stop/reduce substance use; and 0.52 for frequency of trying to stop/reduce use. Composite scores from CRAFFT were used as a validity criterion. CRAFFT scores correlated with total substances used at 0.58; with days per week reported using substances at 0.58; with the total CHISPA adverse events at 0.60. GPA, motivation and efforts to stop/reduce did not correlate with CRAFFT scores. Number of substances used correlated 0.48 with days per week using substances. CHISPA adverse events correlated at 0.65 with number of drugs used and 0.38 with days using substances.

**Conclusions** CHISPA measures show strong evidence of reliability and validity. SBHC providers may benefit from use of the CHISPA to help identify and address adolescent substance use concerns.

## 232 ARTHROGRYPOSIS: LONG-TERM QUALITY OF LIFE ANALYSIS

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10.1136/jim-d-15-00013.232

**Purpose of Study** Arthrogyrosis Multiplex Congenita (AMC) is where individuals are born with two or more joint contractures. AMC is a wide spectrum that includes nearly 300 specific disorders and causes movement restriction in the limbs, jaw, neck and spine. While much focus has been given to managing the child with AMC, very little is currently known about the outcomes of adults living with AMC. The primary purpose of this study was to describe the functional long-term outcomes in adults with AMC.

**Methods Used** Recruited through international AMC support groups, participants provided information on demographics, ambulation, surgery, treatments, quality of life (SF-36) and activity (Physical Activity Scale for Individuals with Physical Disabilities - PASIPD) using an online questionnaire.

**Summary of Results** 156 individuals (42 males and 114 females) from over 10 countries participated. The majority of the participants were from the US, EU, and Canada respectively. Mean age was 40 years (range 20 to 84). 72% of the participants were living with a partner or on their own, and 60% walked independently at home. Participants completed 8% more undergraduate degrees and 17% more advanced degrees compared to the general US population. Despite their physical limitations and high level of chronic pain, the percentage of participants in full-time and part-time occupations matched those of the general US population. Individuals living with AMC had higher SF-36 scores than the general US population in all categories except vitality and physical function. The total PASIPD (activity) score for the participants was at 13.5 compared to 11 for those with other disabilities and 22 for moderately active able-bodied individuals.

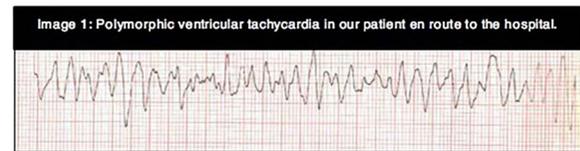
**Conclusions** People with AMC are a unique disabled population who have managed to maintain a high degree of independence despite their physical limitations. Post-secondary education has made them more competitive for careers that require little physical labor. They do remarkably well compared to the able-bodied population despite dealing with considerable musculoskeletal and other types of chronic pain.

## 233 POLYMORPHIC VENTRICULAR TACHYCARDIA (PMVT) IN AN ADOLESCENT AFTER USE OF SYNTHETIC CANNABIS: UNMASKED LONG QT SYNDROME

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**Case Report** Case Report: A previously healthy 15 year-old male with history of illicit drug use required defibrillation by EMS for polymorphic ventricular tachycardia. He had taken an unknown "synthetic drug" prior to the event. The patient was given Naloxone by EMS without



Abstract 233 Figure 1

clear response. His urine drug screen was positive for cannabinoids but negative for amphetamines, barbiturates, benzodiazepines, cocaine, methadone and opiates. He had altered mental status, was intubated and sedated and admitted to the PICU. Electrolytes were normal. Sedation included fentanyl, midazolam, and dexmedetomidine. After extubation, he experienced auditory and visual hallucinations and was combative. He was started on olanzapine and had resolution of his psychosis. Daily ECG's were done. He had initial borderline prolongation of the QTc interval, which significantly worsened with initiation of olanzapine and improved again after discontinuation. ECGs also demonstrated increased QRS amplitude and increased T wave height. A stress test was done after a 7-day washout of olanzapine showed normal baseline QTc but prolongation to 556 msec with exercise, consistent with Type I Long QTc. He was started on nadolol. Genetic testing was sent but did not reveal known LQT mutations. Two weeks after discharge, a repeat ECG showed a normal QTc interval; repeat stress testing showed persistence of abnormal QTc prolongation to 506 msec with exercise.

**Discussion** This patient presented after apparent exposure to synthetic cannabis, with a rhythm known to cause sudden death in patients with Long QT syndrome (LQTS). His initial ECG did not show obvious QT prolongation but subsequent exposure to QT prolonging medications raised that concern. His exercise test, done after those medications were allowed to wash-out, was abnormal with prolongation of his QTc in recovery to over 556 msec with large, broad-based T waves typically seen with LQTS Type 1.

**Conclusion** Synthetic cannabis can induce PMVT in patients with LQTS. It is essential for patients with Long QT syndrome to avoid use of these drugs.

## 234 PILOT STUDY ASSESSING CORRELATION OF CCL2 LEVELS, BIOMARKERS AND MODIFIABLE BEHAVIORS IN OVERWEIGHT/OBESE ADOLESCENTS

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10.1136/jim-d-15-00013.234

**Purpose of Study** Obesity is associated with chronic, low-grade systemic inflammation which can lead to several cardiometabolic derangements. For this reason, identification of obesity-dependent inflammatory markers may provide a diagnostic or prognostic indicator of inflammatory status

and enable staging of pathologic changes such as insulin resistance. We evaluated the relationship between an early inflammatory biomarker, chemokine (C-C motif) ligand 2 (CCL2), and other clinical biomarkers and lifestyle behaviors, in overweight/obese adolescents.

**Methods Used** Anthropometric measurements, biomarkers related to cardiometabolic risk, and three 24-hour dietary recalls were collected from 21 vocational high school students (91% male), 14–19 years of age, with body mass index (BMI)  $\geq 25$  kg/m<sup>2</sup>. Pearson's or Spearman's correlation coefficients were used to examine relationships.

**Summary of Results** Mean BMI was 33.2 kg/m<sup>2</sup> (range 25.7–45.6) and 38% had fasting glucose in the pre-diabetic range. Mean CCL2 was 512.9 pg/mL (range 220–917) and it was positively correlated with triglycerides ( $r=0.45$ ;  $p=0.04$ ) and TNF- $\alpha$  ( $r=0.57$ ;  $p=0.007$ ) and marginally negatively correlated with fruit/vegetable intake ( $r=-0.42$ ,  $p=0.06$ ) and omega-3 fatty acids ( $r=-0.41$ ,  $p=0.07$ ).

**Conclusions** CCL2 was positively associated with pro-inflammatory biomarkers and negatively associated with some anti-inflammatory dietary factors in overweight/obese adolescents. Future intervention studies should investigate whether higher intake of anti-inflammatory dietary factors could reduce CCL2 levels and potentially interrupt the inflammatory cascade in this adolescent population.

### 235 CAPTURING THE SUBSTANCE USE KNOWLEDGE, ATTITUDES AND PRACTICES OF NEW MEXICO'S SCHOOL-BASED HEALTH CENTER WORKFORCE

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**Purpose of Study** New Mexico has a high prevalence of adolescent substance use. In 2011, Screening, Brief Intervention and Referral to Treatment (SBIRT) was recommended by the American Academy of Pediatrics as a component of comprehensive care to address substance use among teenagers. In 2015, the University of New Mexico began SBIRT training for providers at New Mexico Department of Health (NMDOH)-funded school-based health centers (SBHCs) across the state. This study assesses baseline knowledge, attitudes and practices of the New Mexico SBHC provider workforce regarding adolescent substance use and provision of services.

**Methods Used** In early 2015, the NMDOH administered an SBHC provider workforce survey ( $n=118$ ) and achieved a 46.6% response rate. This descriptive analysis includes all survey respondents that self-identified as primary care or behavioral health providers in an SBHC serving middle or high school students ( $n=54$ ).

**Summary of Results** Among respondents, 23 (42.6%) were advanced practice nurses, 22 (40.7%) were masters level behavioral health providers, and 9 (16.7%) were other providers. Most respondents, 79.6%, agreed that risky substance use among students is common enough to warrant systematic screening and 96.2% agreed that it is the responsibility of the SBHC provider to assess for students' readiness to change. A majority reported self-efficacy

in helping students achieve change in their alcohol use, illicit drug use, and prescription drug misuse: 74.1%, 66.7%, and 64.8%, respectively. However, only 44.4% of providers reported practicing the full SBIRT model at their SBHC and 55.8% reported familiarity with SBIRT. Among respondents, 21.6% reported having received continuing education on SBIRT within the previous three years.

**Conclusions** Our results suggest that SBIRT training for New Mexico SBHC providers is timely. Results also indicate a readiness of the SBHC provider workforce to receive training. This study is the first in a series of ongoing assessments and is part of a multi-year project that seeks to improve access to substance use screening and treatment for adolescents in New Mexico.

### Cardiovascular III Concurrent Session 12:30 PM Friday, January 29, 2016

#### 236 THE IMPACT OF SEED VARIATION ON THE MEASURED ACCURACY OF MACHINE LEARNING ALGORITHMS (AUC) FOR SEVERAL COMMON MODEL VALIDATION TECHNIQUES IN NUCLEAR MEDICINE

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10.1136/jim-d-15-00013.236

**Purpose of Study** The measured accuracy and precision of predictions obtained from machine learning models can be affected by the applied model validation technique, which assesses how the results of statistical analysis will generalize to an independent data set. Variation of the seed (the separation into training and testing sets) influences the measured AUC (area under ROC curve) that is used to compare predictive models. The impact of seed variation was assessed on measured AUC for a data set (715 instances) used to predict the survival of patients who underwent nuclear medicine myocardial perfusion imaging tests.

**Methods Used** These experiments, which examined the results from models built from 100 different seeds, were done through interaction with the WEKA Java API. Validation was looped through 100 times, and the seed was accordingly changed with each iteration. The range of the AUC results was computed to find the maximum variation and a VassarStats AUC comparison between two independent ROC curves was used to assess the statistical significance of the maximum observed variation.

**Summary of Results** For the common LogitBoost algorithm, the maximum observed AUC variations were 0.1013, 0.0361, and 0.0152 for split sample, 10 fold CV, and 100 $\times$ 10 fold CV respectively, and other common algorithms followed the same pattern of results. Changing the seed when validating the model using split sample validation significantly altered the measured AUC ( $p<0.05$ ), and the 100x repeated 10 fold CV proved to be the most precise, as statistically expected.

**Conclusions** Seed selection was rendered as another independent variable when using split sample validation for this data set, capable of altering the statistical significance, which calls for extreme caution when interpreting the results in comparisons of predictive models. As branches of artificial intelligence, especially machine learning, are being incorporated more heavily in the medical field, it is important that medical professionals around the world use the best validation techniques to draw the most accurate conclusions/predictions from machine learning models, which is exactly what these experiments demonstrate.

237 **TEENAGERS AND ADULTS LIVING WITH CONGENITAL HEART DISEASE ARE BEING LOST TO FOLLOW UP: EVIDENCE FROM TETRALOGY OF FALLOT IN A DIVERSE PATIENT POPULATION**

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10.1136/jim-d-15-00013.237

**Purpose of Study** Due to improved surgical and medical management, there has been a rise in adults living with congenital heart diseases (ACHD) in recent decades. This increase in patients, along with a lack of ACHD cardiologists, creates a gap in care that may result in ACHD patients being lost to follow up (LTF) and result in increased morbidity and mortality. To date, there is no population-based tracking system to monitor the growing population of ACHD patients and their follow-up status. Using Tetralogy of Fallot (TOF) as a marker for ACHD, the purpose of this study is to develop a comprehensive population-based surveillance system to collect health utilization, demographic, and disease outcomes information on individuals in Southern Arizona with TOF who are age 13 and over.

**Methods Used** We analyzed TOF cases from a population-based clinical dataset containing visit data from 1980 to present for individuals with congenital heart defects using 2-factor MANOVA to assess the effect of insurance and residence on number of years LTF in patients over 12 years.

**Summary of Results** Of 372 confirmed diagnoses of TOF in Southern Arizona, 81% live in urban regions, 50% are female, 44% are over 12, and 9% are confirmed deceased. Both the main effect for location (urban, regional, rural) and the interaction effect between insurance (public, private) and location were significant. Specifically, public insurance reduced LTF only in urban locations. Mean lost to follow-up for urban private is 6 years as compared to less than 1 year for nonurban for all insurance types.

**Conclusions** Populations particularly at risk are individuals in urban locations on private insurance who are more likely to: 1. Suffer more serious disease and develop more significant co-morbidities 2. Succumb to death earlier 3. End up paying more for treatment than they would have for routine surveillance.

Southern Arizona is an excellent location for this type of surveillance study due to its ethnic and socioeconomic diversity. This model can therefore be used as a scale-up to

a national surveillance study, so that we may better manage adults living with TOF, and ACHDs in general.

239  **$\alpha$ -MYOSIN HEAVY CHAIN IN HEART FAILURE IS REDUCED IN A MINOR SUB-POPULATION OF RABBIT MYOCYTES EXPRESSING HETERODIMERIC  $\alpha\beta$  ISOFORMS**

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10.1136/jim-d-15-00013.239

**Purpose of Study** In mouse models of heart failure, two distinctive sub-populations of ventricular myocytes (VMCs) exist: homodimeric  $\alpha\alpha$  or heterodimeric  $\alpha\beta$  isoforms of myosin heavy chain (MyHC, Lopez *et al*, *Circ. Res.*, 2011). In failing rabbit hearts,  $\alpha$ -MyHC expression is reduced. However, the cellular distribution of MyHC isoform expression is unknown. Our objective is to identify co-existing VMC sub-populations and measure relative total (T)-MyHC protein content in control (CNTL) and heart failure (HF) rabbit hearts with single cell analysis.

**Methods Used** Non-ischemic HF in rabbits was induced with aortic valve insufficiency (volume overload) and abdominal aortic banding (pressure overload). Cardiac single cells were isolated by anterograde coronary perfusion and enzymatic digestion from CNTL and HF left ventricles (LV). VMCs median volumes were measured by Coulter Multisizer to determine hypertrophy (~7K VMCs per LV).  $\alpha$ ,  $\beta$ , and T-MyHC protein contents were measured by median fluorescence intensity (MFI) in isolated VMCs by flow cytometry. Data is presented as mean $\pm$ SD and \*\* represents a  $p < 0.01$ .

**Summary of Results** Like mice, the median volume of HF rabbit VMCs increased by 1.9 fold compared to the CNTL (54,689 $\pm$ 18,421  $\mu\text{m}^3$  from 28,192 $\pm$ 7,975  $\mu\text{m}^3$ ,  $p < 0.01$ ,  $n=8$ ). Unlike mice, rabbits have 3 VMC sub-populations based on MyHC isoform expression patterns:  $\alpha\alpha$ ,  $\beta\beta$ , and  $\alpha\beta$ -VMCs. The  $\alpha\alpha$ -VMCs fraction was similarly low in both groups. In HF rabbits the fraction of  $\alpha\beta$ -VMCs was decreased with a reciprocal increase in the  $\beta\beta$ -VMCs fraction (See Table 1). Interestingly, the  $\alpha\beta$  heterodimeric VMC had a 3-fold higher T-MyHC content per cell (MFI) when compared to the homodimeric  $\beta\beta$ -VMCs ( $p < 0.01$ ,  $n=7$ ).

**Conclusions** In this model of non-ischemic HF,  $\alpha$ -MyHC reduction is attributed to the disappearance of the minor sub-population of myocytes expressing the heterodimeric  $\alpha\beta$  isoforms. This reduction of  $\alpha$ -MyHC content in hypertrophied cardiac myocytes is a potential novel mechanism for cardiac dysfunction.

Abstract 239 Table 1

VMC Sub-population	CNTL (n=14)	Heart Failure (n=10)
$\alpha\alpha$	<3%	<3%
$\beta\beta$	61% $\pm$ 17%	83% $\pm$ 11%**
$\alpha\beta$	30% $\pm$ 19%	7% $\pm$ 5%**

## 240 REDUCED CARDIOMYOCYTE SIZE DOES NOT ACCOUNT FOR REDUCED CARDIAC WEIGHT IN GROWTH RESTRICTED FETUSES

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10.1136/jim-d-15-00013.240

**Purpose of Study** Conflicting reports have been published regarding fetal cardiomyocyte growth responses to intra-uterine growth restriction (IUGR). We sought to determine whether chronic placental insufficiency-induced IUGR (PI-IUGR) reduced size, maturation and cell cycle activity in fetal sheep cardiomyocytes.

**Methods Used** Pregnant ewes were exposed to 75 days of elevated heat (12h at 40C, 12h at 35C daily) starting at ~25% of gestation to produce PI-IUGR (n=13) and were compared to controls (n=11). At ~90% of gestation, fetal hearts were dissociated and cardiomyocytes from the left ventricle (LV), right ventricle (RV) and septum were assessed for size (length and diameter), maturation (fraction of cells binucleated), and cell cycle activity (Ki-67 positivity).

**Summary of Results** Compared to controls, PI-IUGR fetuses were 31% smaller than controls, with hearts that were 34% smaller (P<0.01). Thus, heart weight relative to fetal weight did not differ between groups. LV and septal cardiomyocytes had similar dimensions between groups, but RV cardiomyocyte lengths were 6–8% shorter than controls (P<0.02). Cardiomyocyte maturation and cell cycle activity were not different between groups at this stage of gestation.

**Conclusions** Cardiomyocyte sizes were similar or only slightly reduced in PI-IUGR fetuses, demonstrating sparing of cardiomyocyte enlargement in the PI-IUGR fetus. However, heart weights were significantly reduced. Therefore, we speculate that cardiomyocyte number was reduced by PI prior to 90% of gestation.

## 241 TIER 1: ECMO SHOULD NOT BE TIER 1 FOR HEART TRANSPLANTATION DUE TO HIGH MORTALITY

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10.1136/jim-d-15-00013.241

**Purpose of Study** Pre-operative extracorporeal membrane oxygenation (ECMO) has been correlated to poor outcome post-transplant, while MCS devices have been shown to improve survival post-transplant. Current proposals for future heart allocation policy list patients on ECMO as the highest priority for transplant (Tier 1), however, this is controversial due to the high mortality seen in this group. Opponents of this proposal suggest using ventricular assist devices (VADs) as a bridge to transplant rather than ECMO to transplant directly. Therefore, we sought to compare transplant outcomes between the two groups.

**Methods Used** Between 2010 and 2014 we evaluated 14 patients who had ECMO prior to heart transplantation. 10 of these patients were bridged with VAD to transplantations, and 4 were transplanted directly from ECMO.

## Abstract 241 Table 1

Endpoints	ECMO to VAD to OHT (n=10)	ECMO to OHT (n=4)	Log Rank P-Value
1-Year Actuarial Survival	90.0%	50.0%	0.068
1-Year Actuarial Freedom from CAV	66.7%	25.0%	0.295
1-Year Actuarial from NF-MACE	85.7%	100.0%	0.593
1-Year Actuarial Freedom from Any Treated Rejection	90.0%	100.0%	0.507
P=NS.			

Endpoints for both groups included 1-year actuarial survival, freedom from cardiac allograft vasculopathy (CAV) ≥ 30% by angiography, freedom from Non-Fatal Major Adverse Cardiac Events (NF-MACE: myocardial infarction, new congestive heart failure, percutaneous coronary intervention, implantable cardioverter defibrillator/pacemaker implant, stroke), and freedom from any treated rejection.

**Summary of Results** There was a trend for decreased 1-year survival in the ECMO vs. ECMO to VAD groups. 1-Year freedom from CAV, freedom from NF-MACE, and freedom from any treated rejection in pre-transplant ECMO patients bridged from VAD to transplant were not significantly different compared to patients transplanted from ECMO directly.

**Conclusions** While there was no significance between both groups, there appears to be a trend towards greater 1-year mortality in those patients transplanted directly from ECMO. These findings have implications for future discussions regarding tiers for heart allocation policy. Further investigation into this question is warranted.

## 242 OUTCOME OF HYPERTENSIVE HEART TRANSPLANT PATIENTS

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10.1136/jim-d-15-00013.242

**Purpose of Study** Systemic hypertension (HTN) is a known risk factor for cardiovascular disease. It has been found to be prevalent in 87% patients post heart transplantation mostly due to side effects of calcineurin inhibitor medications and previous history of essential hypertension. Hypertension post-transplant can be very difficult to control, particularly diastolic HTN. It is not known whether the severity of HTN characterized by the number of anti-hypertensive medications results in poor outcome.

**Methods Used** Between 2010 and 2013, we assessed 140 heart transplant patients. We divided patients into four groups: Group A=0 anti-HTN medications, Group B=1 anti-HTN medication, Group C=2 anti-HTN medications, and Group D=3 or more anti-HTN medications. It is assumed that blood pressure control was attained with the stated number of blood pressure medications. Three year

Abstract 242 Table 1

Endpoints	Group A No Anti-HTN Medication (n=30)	Group B 1 Anti-HTN Medication (n=30)	Group C 2 Anti-HTN Medications (n=56)	Group D ≥ 3 Anti-HTN Medications (n=24)	P-Value
3-Year Actuarial Survival	100.00%	89.80%	84.10%	91.70%	0.592
3-Year Actuarial Freedom from CAV	88.20%	95.80%	80.20%	79.30%	0.261
3-Year Actuarial Freedom from NF-MACE	100.00%	96.70%	86.80%	100.00%	0.067
1-Year Actual Freedom from Any Treated Rejection	93.30%	90.00%	77.60%	86.20%	0.398
1-Year Actual Freedom from Antibody Mediated Rejection	96.70%	93.30%	96.40%	100.00%	0.631
1-Year Actual Freedom from Acute Cellular Rejection	93.30%	96.70%	86.80%	91.00%	0.636
1-Year Actual Freedom from Biopsy Negative Rejection	100.00%	100.00%	90.70%	95.20%	0.194
Interventricular Septal Thickness (IVS) > 1.2 cm	10.0% (3/30)	13.3% (4/30)	16.1% (9/56)	16.7% (4/24)	P=NS
Left Ventricular Posterior Wall (LVPW) > 1.2 cm	3.3% (1/30)	6.7% (2/30)	7.1% (4/56)	8.3% (2/24)	P=NS

P=NS.

outcome for these groups included survival, Non-Fatal Major Adverse Cardiac Events (NF-MACE: myocardial infarction, new congestive heart failure, percutaneous coronary intervention, implantable cardioverter defibrillator/pacemaker implant, stroke), cardiac allograft vasculopathy (CAV) ≥30% by coronary angiography, first year cellular, antibody mediated, and any treated episodes of rejection.

**Summary of Results** There was no statistical difference in 3-year survival, freedom from CAV, freedom from NF-MACE, or 1-year freedom from any treated rejection between all four groups. There appears to be a trend for more hypertrophy for more anti-HTN medications required.

**Conclusions** Severity of HTN after heart transplantation does not appear to affect outcome assuming blood pressure control is attained. Left ventricular hypertrophy appears to be concordant with a need for more anti-HTN medications.

#### 243 TRANS-CATHETER CLOSURE OF PATENT DUCTUS ARTERIOSUS IN SEVERELY PREMATURE NEONATES: IS POST-LIGATION SYNDROME AN ISSUE?

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10.1136/jim-d-15-00013.243

**Purpose of Study** Patent ductus arteriosus (PDA) is the most common cardiovascular abnormality in the preterm newborn. Surgical ligation has been associated with significant morbidity including hemodynamic instability and cardiorespiratory compromise known collectively as

“post-ligation syndrome” (PLS). We have developed a new method of trans-catheter PDA closure (TC-PDA-C) applicable to this patient population. This study sought to determine if trans-catheter PDA closure results in PLS.

**Methods Used** This is a retrospective review of all premature newborns treated with TC-PDA-C at our institution between 3/13–2/15. PLS was defined as diminished left ventricular myocardial performance and/or significant alteration in cardiorespiratory stability and/or increased need for vasopressor support. Myocardial performance was assessed via transthoracic echocardiogram (left ventricular fractional shortening (SF), ejection fraction (EF)) prior to intervention, within 12 hrs of intervention, and prior to discharge. Clinical indices of cardiorespiratory stability (heart rate (HR), blood pressure (BP), systemic oxygen saturation) and the need for increased vasopressor and/or respiratory support were reviewed before, 1, 4, 8, 12, and 24 hrs following intervention.

**Summary of Results** TC-PDA-C was successfully performed in 21/24 (median weight/ age/ corrected gestational age=1152 gm/22 days/30 wks). Following PDA closure myocardial performance decreased but remained in the normal range (EF/FS: *pre*=71%/37%, *post* =63%/35%, *discharge*=66%/37%). No significant changes were noted in arterial systolic, diastolic or mean BP, HR, systemic oxygen saturation, or vasopressor or respiratory support.

**Conclusions** Transcatheter PDA closure in preterm infants is associated with a decline in myocardial performance which remained in the normal range and did not appear to be associated with any negative clinical sequelae. These findings suggest that PLS, while present using this new technique, may be less severe with catheter based PDA closure than with traditional surgical ligation in preterm newborns. Further study and longer follow-up is needed.

## Endocrinology and Metabolism II

### Concurrent Session

#### 12:30 PM

#### Friday, January 29, 2016

#### 244 EFFECT OF OBESITY AND CKD ON INSULIN SENSITIVITY INDICES

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10.1136/jim-d-15-00013.244

**Purpose of Study** The gold standard of measuring insulin sensitivity is the hyperinsulinemic-euglycemic clamp, but due to its complexity, surrogate indices have been formulated to assess insulin sensitivity. Performance of estimates across kidney function and adiposity may differ depending on the individual insulin response of different tissues.

**Methods Used** In a cross-sectional study of 59 subjects with nondiabetic CKD (estimated GFR <60 mL/min/1.73 m<sup>2</sup>) and 39 healthy controls, we quantified insulin sensitivity using hyperinsulinemic-euglycemic clamp (SI<sub>Clamp</sub>), oral glucose tolerance tests (Matsuda index), and fasting glucose and insulin (HOMA-IR). We compared the Matsuda index and 1/HOMA-IR to SI<sub>Clamp</sub> using descriptive statistics, graphical analyses, correlation coefficients, and linear regression. Multivariable modelling was done adjusting for age, sex, and race/ethnicity.

**Summary of Results** Correlations of SI<sub>Clamp</sub> with Matsuda (r=0.45) and 1/HOMA-IR (r=0.35) for patients with CKD were lower than those without CKD (Matsuda, r=0.58; 1/HOMA-IR, r=0.51) but did not differ substantially for participants with obesity (Matsuda, r=0.46; 1/HOMA-IR, r=0.38) compared to those who were not (Matsuda, r=0.53; 1/HOMA-IR, r=0.44).

Compared with nonobese participants, those with obesity had a mean Matsuda index that was 2.6 lower (95% CI -3.7- -1.5, p<0.0001) and a mean 1/HOMA-IR that was 0.5 lower (95% CI -0.7- -0.3, p<0.0001) at the same level of SI<sub>Clamp</sub>, adjusting for age, sex, and race. Similarly, compared to healthy controls, participants with CKD had a mean Matsuda index that was 1.0 lower (95% CI -2.0- -0.1, p=0.08) and a mean 1/HOMA-IR that was 0.3 lower (CI -0.5-0.0, p=0.03). Correlation coefficients of BMI to SI<sub>Clamp</sub>, Matsuda, and HOMA-IR were -0.19, -0.56, and 0.63, which suggests that Matsuda and HOMA-IR may be more influenced by adiposity than SI<sub>Clamp</sub>.

**Conclusions** Obesity and CKD alter the relationship of Matsuda index and HOMA-IR with SI<sub>Clamp</sub>; obesity biases the correlation and CKD introduces less precision. Insulin sensitivity indices should be used cautiously in patients who are obese or with chronic kidney disease. Estimates of insulin sensitivity derived from fasting measurements or the OGTT may be more strongly affected by adiposity.

#### 245 DIABETIC KETOACIDOSIS IN TYPE 2 VS. TYPE 1 DIABETES

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10.1136/jim-d-15-00013.245

**Purpose of Study** Diabetic ketoacidosis (DKA) is known to occur in type 2 diabetes (T2DM), but has not been well characterized in this condition. In the present study, we compared the clinical and biochemical features of DKA in T2DM with those in type 1 diabetes (T1DM). We also determined the effect of a DKA treatment protocol (instituted in 2009) on insulin infusion (INSINF) rate and duration, and on ICU and hospital length of stay (LOS). Finally, we characterized the role of subspecialty endocrinology consultation in the management of DKA.

**Methods Used** Diabetes and an admission  $\beta$ -hydroxybutyrate ( $\beta$ OHB)  $\geq 3.8$  mmol/l were required for inclusion. Records on 320 patients over 10.5 years were analyzed.

**Summary of Results** Individuals with T2DM (n=80) were older (60 $\pm$ 2 v 38 $\pm$ 1y, p<0.001) with higher BMIs (30 $\pm$ 1 v 25 $\pm$ 1 kg/m<sup>2</sup>, p<0.0001), slightly higher admission glucose (514 $\pm$ 31 v 447 $\pm$ 16 mg/dl, p=0.06), lower  $\beta$ OHB (7.3 $\pm$ 0.3 v 8.5 $\pm$ 0.2 mmol/l, p <0.005), longer ICU LOS (57 $\pm$ 7 v 35 $\pm$ 2h, p<0.005) and hospital LOS (153 $\pm$ 13 v 112 $\pm$ 10h, p<0.02), and slightly less documented hypoglycemia during the hospitalization (0.8 $\pm$ 0.2 v 1.3 $\pm$ 0.2 episodes, p=0.10) compared with T1DM patients (n=240). Duration of INSINF was longer in T2DM v T1DM (42 $\pm$ 6 v 26 $\pm$ 2h, p<0.01), but there were no differences in INSINF rate (0.09 $\pm$ 0.01 v 0.11 $\pm$ 0.01 U/kg/h, p=NS). Patients treated by protocol (n=178) had higher INSINF rates (0.12 $\pm$ 0.01 v 0.09 $\pm$ 0.01 U/kg/h, p=0.03) and borderline shorter duration of INSINF (27 $\pm$ 2 v 34 $\pm$ 3h, p=0.06) but no difference in ICU LOS (38 $\pm$ 3 v 44 $\pm$ 4h, p=0.18) or hospital LOS (123 $\pm$ 13 v 122 $\pm$ 10h, p=NS) compared to patients treated prior to initiation of the protocol (n=142). A minority (18%, n=59) of patients were seen by an endocrinologist; these individuals had higher  $\beta$ OHB (9.0 $\pm$ 0.4 v 8.1 $\pm$ 0.2 mmol/l, p=0.04), longer duration INSINF (42 $\pm$ 4 v 28 $\pm$ 2h, p<0.005), and longer ICU LOS (61 $\pm$ 6 v 36 $\pm$ 2h, p<0.001) and hospital LOS (182 $\pm$ 37 v 109 $\pm$ 6h, p=0.05). Although  $\beta$ OHB was similar in women and men (8.1 $\pm$ 0.2 v 8.2 $\pm$ 0.2 mmol/l, p=NS), women had lower serum bicarbonate (11 $\pm$ 0.5 v 13 $\pm$ 0.5 meq/l, p=0.01).

**Conclusions** In summary, DKA in T2DM is milder than in T1DM but is associated with longer ICU and hospital stay. A DKA protocol resulted in higher INSINF rate and shorter duration, but did not affect LOS. The endocrinologist appears to play a relatively minor role in DKA management.

## 246 EXERCISE INTERVENTION IMPROVES THE METABOLIC PROFILE OF SOUTHWESTERN NATIVE AMERICAN ADOLESCENTS

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10.1136/jim-d-15-00013.246

**Purpose of Study** Obesity is highly prevalent among Native American Youth, with prevalence as high as 37% in boys and 26% in girls. Obesity is a major risk factor for the development of Metabolic Syndrome, Type 2 Diabetes, and early cardiovascular disease. The goal of this study was to evaluate the impact of early lifestyle interventions which promote healthy eating and exercise on obesity-related risk factors among Native American children. We hypothesize that a lifestyle intervention that incorporates a tri-weekly exercise program and nutritional counseling will result in improvements in the metabolic profile and body composition characteristics of this population.

**Methods Used** Sixty-six adolescent Native American subjects with mean age  $13.7 \pm 1.7$  years were recruited to participate in an exercise program 3 times a week for 60 minutes of dietary instruction, aerobic exercise and resistance training. Parents attended an instructional session on healthy eating and preparing nutritional lunches for their children. Subjects were assessed at baseline, 12 weeks and 24 weeks for fitness outcomes using standardized testing criteria for children. Patients also had bloodwork drawn for standard metabolic parameters, and body composition was determined using bioelectrical-impedance.

**Summary of Results** Thirty-two subjects completed all three visits: 20 males and 12 females. Subjects showed significant improvements in their lipid profile, A1c, and fat free mass (FFM). Summary results are shown in the Data Table 1.

**Conclusions** A standardized fitness program among Native American children was effective at reducing A1c, fasting blood sugar, fasting lipids, and improving body composition over the course of 6 months.

Abstract 246 Table 1

Study Week	LDL Chol (mg/dl)	HDL Chol (mg/dl)	TG(mg/dl)	BMI-% ile	FFM (%)	A1c (%)
Week 0	97±20	36±8	172±89	95±10	71.8 ±7.8	5.9 ±0.7
Week 24	79±17	42±9	106±47	92±12	74.1 ±8.7	5.6 ±0.5
p-value	<0.0001	<0.001	<0.0001	<0.05	<0.001	<0.001

Abstract 247 Table 1

Statistical Data	A1C	Glycomark	Age	Diabetes Duration	BMI	Mean 24h Glucose	Mean Glucose excursion	Gender
Slope	7.77	-1.49	0.45	0.44	-2.11	0.16	0.15	NA
R-Squared	0.02	0.03	0.06	0.03	0.09	0.03	0.03	NA
p value	0.44	0.34	0.14	0.32	0.06	0.32	0.33	0.86

## 247 CAN THE DAWN PHENOMENON BE PREDICTED IN ORDER TO PROGRAM AN INSULIN PUMP?

L Ereifej, M Bouchonville, E Duran-Valdez, D Schade. *University of New Mexico, Albuquerque, NM*

10.1136/jim-d-15-00013.247

**Purpose of Study** The Dawn Phenomenon is the primary cause of hyperglycemia prior to breakfast. Diabetic patients on an insulin pump (CSII) often program an increase in insulin delivery prior to breakfast to suppress morning hyperglycemia. No studies have evaluated the benefits and risks of this approach. Since the Dawn Phenomenon occurs sporadically, our study evaluated whether the frequency of the Dawn Phenomenon can be predicted to occur in order to program CSII effectively.

**Methods Used** We studied 39 Type 1 diabetes volunteers on multiple nights (total number 376) in order to quantify and characterize the Dawn Phenomenon using Continuous Glucose Monitoring for a maximum of five continuous days per individual. We examined the relationship between the occurrence of the Dawn Phenomenon, multiple demographic features, and CGM glucose parameters as shown in the table below. Regression analysis was used for continuous variables whereas an analysis of variance was used for categorical variables. Significance was set at  $p < 0.05$ .

**Summary of Results** As documented in the table below, there were no parameters that would readily predict the occurrence of the Dawn Phenomenon. The dawn phenomenon occurred sporadically and unpredictably (median rate of 46 % of the nights).

**Conclusions** Our study demonstrates that the Dawn Phenomenon occurrence is unpredictable. Therefore, programming a CSII pump to deliver early morning insulin to counteract the hyperglycemia of the Dawn Phenomenon is not effective and potentially dangerous due to increased risk of hypoglycemia. We conclude that alternative strategies should be used to suppress early morning hyperglycemia in type 1 diabetes.

## 248 RELATIONSHIP BETWEEN GLYCEMIC VARIABILITY AND B-CELL FUNCTION IN ADULTS AT HIGH RISK OF TYPE 2 DIABETES

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10.1136/jim-d-15-00013.248

**Purpose of Study**  $\beta$ -cell dysfunction is a key feature the pathogenesis of type 2 diabetes. It has been hypothesized

that frequent glucose fluctuations, referred to as “glycemic variability,” may induce oxidative stress and thereby contribute to poor  $\beta$ -cell function. Previous studies have shown that  $\beta$ -cell function is improved following a low glycemic index (LGI) diet, but whether this is due to a decrease in glycemic variability or mediated by changes in oxidative stress is unknown.

**Methods Used** Subjects with pre-diabetes based on a screening oral glucose tolerance test (OGTT), consumed a weight stabilizing, moderate glycemic index (MGI) diet (GI: 55–58) for 2 weeks after which they underwent an insulin modified intravenous glucose tolerance test (IVGTT) and 3-days of continuous glucose monitoring (CGM). The acute insulin response to glucose adjusted for insulin sensitivity (disposition index; DI) from the IVGTT provided an assessment of  $\beta$ -cell function. The standard deviation (SD) of glucose levels by CGM provided a measure of glycemic variability. Fasting erythrocyte glutathione (GSH) and glutathione disulfide (GSSG) and thio-barbituric acid reactive substances (TBARS) were measured to assess oxidative stress. After completing the MGI diet, a subset of subjects ( $n = 10$ ) was placed on 4 weeks of a LGI diet (GI:  $<35$ ) and procedures repeated.

**Summary of Results** 25 subjects (13M/12F; age  $54.9 \pm 1.9$  y; BMI  $32.3 \pm 1.5$  kg/m<sup>2</sup>) were enrolled. Six had isolated impaired fasting glucose, 5 isolated impaired glucose tolerance, and 14 both on screening OGTT. DI trended towards a negative correlation with CGM SD ( $r = -0.40$ ,  $p = 0.06$ ). 2-hr glucose from the screening OGTT was positively correlated with CGM SD ( $r = 0.55$ ,  $p = 0.006$ ), but fasting glucose was not ( $r = 0.28$ ,  $p = 0.19$ ). There were no significant correlations between CGM SD and any of the oxidative stress markers (GSH/GSSG ratio:  $r = -0.07$ ,  $p = 0.77$ ; TBARS:  $r = 0.19$ ,  $p = 0.40$ ). The LGI diet decreased glycemic variability ( $\Delta$ SD CGM:  $-4.82$ ,  $p = 0.003$ ), but did not change  $\beta$ -cell function ( $\Delta$ DI: 690,  $p = 0.39$ ).

**Conclusions** These data suggest that glycemic variability does not induce  $\beta$ -cell dysfunction via oxidative stress in subjects with pre-diabetes.

249

#### MIXED MACRONUTRIENT MEALS NORMALIZE GLUCOSE AND INSULIN LEVELS IN IMPAIRED GLUCOSE TOLERANT OBESE TEENAGE GIRLS

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10.1136/jim-d-15-00013.249

**Purpose of Study** Obese adolescent girls with impaired glucose tolerance (IGT) are at risk for developing type 2 diabetes. The degree of postprandial hyperglycemia may be impacted by consumption of liquid carbohydrates, which leads to rapid increases in both serum glucose and insulin concentrations. It is unclear if the addition of protein and fat to a drink mitigates these responses. Thus, the goals of this study were: 1). To compare the metabolic responses to two types of drinks 2). To compare metabolic responses in IGT vs. NGT obese girls.

**Methods Used** Seven overweight (BMI  $>90$ th %ile) postmenarchal adolescent females (12–21 years old) had a 10 hour monitored overnight fast followed by an oral glucose

tolerance test with consumption of 75 g of Glucola. A carbohydrate/protein/fat shake (93.8 g. carbohydrates, 18.8 g. protein, 5 g. fat) was consumed 4 hours after the Glucola, with blood samples drawn hourly for 3 hours after each drink. Blood glucose, serum insulin and free fatty acid concentrations were measured at each time point. The area under the curve for each response was calculated. Responses to Glucola vs. shake for the entire group were compared with a students 2 tailed T-test, and responses in IGT vs. NGT subjects were also compared.

**Summary of Results** As predicted, both glucose and insulin concentrations trended higher with Glucola compared to mixed macronutrient shake ( $p$ -value 0.021, 0.126 respectively), despite Glucola's lower carbohydrate content. Glucose trended higher ( $p = 0.06$ ) for IGT vs. NGT girls with Glucola consumption. IGT girls' glucose and insulin were comparable to NGT girls ( $p = 0.23$ , 0.24, respectively) with shake consumption, suggesting that addition of protein/fat normalizes postprandial hyperglycemia in IGT girls.

**Conclusions** We found that adding protein/fat to carbohydrate drinks mitigates the peak glucose response in obese teenage girls. Further, postprandial glucose concentrations normalized for IGT girls with the addition of protein/fat, suggesting obese teenage girls could experience metabolic benefit from mixed macronutrient meals and avoidance of simple carbohydrate drinks.

## Gastroenterology Concurrent Session 12:30 PM Friday, January 29, 2016

250

#### THE PREVALENCE OF COLON ADENOMATOUS POLYPS IN ASIAN INDIANS

S Mann, J Wilson-Chiru, S Tejaswi. University of California, Davis Medical Center, Sacramento, CA

10.1136/jim-d-15-00013.250

**Purpose of Study** Most colon cancers develop via the adenoma-carcinoma sequence. This is the basis of colon cancer screening through colonoscopy with polypectomy. The National Cancer Institute advocates for individualized screening strategies but further understanding of individual risk factors including ethnicity is needed. We reviewed the California Cancer Registry for recent colorectal cancer rates and found a markedly lower incidence in South Asians compared to Caucasians. Based on this, we hypothesized that the prevalence of colon adenomatous polyps in Asian Indians would be lower than Caucasians.

**Methods Used** On retrospective chart review of average-risk screening colonoscopies at our institution between Sept 2011 and Oct 2013, and using patient-reported ethnicity data, we identified 35 Asian Indian cases. We identified 60 Caucasian controls after matching for endoscopist, date, and location of the procedure. Variables including presence, number, location (right vs left colon), size, and histology of colon polyps were recorded. Patient variables known to influence adenoma prevalence: age, sex, BMI, diabetes, smoking status, alcohol

consumption, were also documented.

**Summary of Results** Smokers were more likely to have adenomatous polyps ( $p=0.029$ ). After controlling for smoking using logistic regression analysis, Asian Indian ethnicity was not found to be a significant predictor of the presence of adenomatous polyps ( $p=0.364$ ). There was a higher proportion of tubular adenomas  $> 1$  cm in size, and sessile serrated adenomas in the Caucasian group, however this did not reach statistical significance.

**Conclusions** Contrary to the lower incidence of colorectal cancer in Asian Indians, the prevalence of precursor adenomatous polyps was higher than that of Caucasians. In addition, a majority (75%) of the adenomas were located in the proximal colon. However, there were no villous or sessile serrated colon adenomas among Asian Indians. This may imply that colon adenomas in Asian Indians have a more benign nature. This could be due to genetic, lifestyle, and dietary factors. This is promising for further research to identify protective factors to decrease the burden of colorectal cancer. Larger prospective studies are required not only to confirm/refute our findings, but also identify the most appropriate colon cancer screening strategy among Asian Indians.

251

#### ROLE OF THE LYMPHATIC SYSTEM IN THE PROGRESSION OF INFLAMMATORY BOWEL DISEASE TO COLORECTAL CANCER IN AN EXPERIMENTAL MOUSE MODEL

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10.1136/jim-d-15-00013.251

**Purpose of Study** Inflammatory bowel disease (IBD) is characterized by chronic inflammation. Longstanding inflammation in this setting can progress to colorectal cancer (CRC) (~15–20% lifetime risk in ulcerative colitis [UC]). Many factors are involved in this process, however, the role of the lymphatic system has not been fully investigated. This project uses lymphatic deficient mice (Angiopoietin 2 knockout [Ang2 KO]) to examine the lymphatic system in IBD to CRC progression. Angiopoietins are growth factors involved in blood and lymphatic vessel remodeling and maturation as well as in cancer development. Previously we found Ang2 KO mice had decreased inflammation, injury progression, and blood and lymphatic vessel remodeling in an acute model of UC. This chronic model examines whether lymphatic vascular insufficiency in the colon can protect against the progression of IBD to CRC and metastasis.

**Methods Used** C57B6 mice (Ang2 +/+, +/-, -/-) were divided into 4 groups. Group 1: 4mg/kg IP injection of azoxymethane (AOM) procarcinogen+1.5% dextran sodium sulfate (DSS) administered in drinking water. DSS was administered in 3 on-off cycles (7 days on, 14 days off). Controls included Group 2: AOM alone; Group 3: DSS alone; Group 4: untreated. Clinical severity (weight, energy, stool consistency, occult blood) was measured weekly. At sacrifice, blood was collected for biomarker/cytokine (Ang2, IL1-B, IL6, TNF, VEGF-C) ELISA analysis,

colon length measured, tumor burden, and histology assessed.

**Summary of Results** KO mice exhibited reduced survival (52%) vs +/+ (100%) and +/- (91%). Most mice developed tumors and CRC incidence did not differ among genotypes (+/+ = 89%; +/- = 95%; -/- = 93%). Tumor area was significantly increased in KOs (13.5%) vs +/- (4.6%) and +/+ (5.7%). Ang2 serum concentrations were significantly different between genotypes (+/+ = 2317; +/- = 603; -/- = 110) but were unaffected by treatment as were other serum biomarkers.

**Conclusions** Lymphatic deficiency, defective lymphangiogenesis, and impaired lymphatic-generated inflammation do not protect against clinical UC severity or progression to CRC in this experimental UC model. It is undetermined whether CRC metastasis might be inhibited.

252

#### A REMEMBRANCE OF PROCEDURES PAST: LATE HEPATIC ARTERY THROMBOSIS

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10.1136/jim-d-15-00013.252

**Case Report** Hepatic artery thrombosis (HAT) is a complication following liver transplantation. Generally there are two subsets of HAT: early HAT, which occurs within 30 days of transplant and late HAT, which occurs after 30 days.<sup>1 2</sup> The following is a case of late HAT with concurrent hepatic necrosis which is unique due to the 18 year time period between OLT and onset of late HAT.

A 63-year-old male with a history of cirrhosis with OLT 18 years prior presented to the ED with 3 days of worsening abdominal pain and markedly elevated LFT's. A contrasted CT abdomen/pelvis revealed complete occlusion of the hepatic likely secondary to hepatic artery thrombosis. Over the next few days his LFTs trended down and the expectation was that his hepatic necrosis would recover; so he was discharged without anticoagulation. The patient was soon readmitted and a CT abdomen/pelvis demonstrated unchanged HAT and hepatic necrosis with new left portal vein thrombosis. A thrombophilia workup revealed a positive lupus like inhibitor (LLI). The patient had a complicated hospital course and ultimately passed away from infectious causes.

Our patient developed late HAT 18 years after OLT, which is very rare. A review of the literature revealed one other case report of HAT 12 years post OLT.<sup>3</sup> It is unknown whether his thrombotic complications were a result of complications of OLT or the LLI or a combination.<sup>4</sup>

Late HAT is a serious vascular complication of OLT and can occur many years following the transplant and carries significant morbidity and mortality if not addressed promptly.

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**253 POST- INFECTIOUS IBS FOLLOWING RECURRENT/ RELAPSING C. DIFFICILE ASSOCIATED DIARRHEA (CDAD)**

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10.1136/jim-d-15-00013.253

**Case Report** Post infectious Irritable Bowel Syndrome (PI-IBS) is defined as the acute onset of symptoms of Irritable Bowel Syndrome (IBS) in a person who recently had an episode of infectious gastroenteritis and without a previous diagnosis of IBS.<sup>1</sup> This disease process has been described following episodes of infectious gastroenteritis from either viral, bacterial, or parasitic causes. Many studies have demonstrated a strong association between cases of bacterial gastroenteritis caused by *Campylobacter*, *Salmonella*, and *Shigella*, and the development of PI-IBS.<sup>2</sup> There remains a lack of consensus as to whether or not PI-IBS can occur following *Clostridium Difficile* (*C. Difficile*) Infection.<sup>3</sup> In view of this fact, we describe two patients who developed diarrhea-predominant IBS following recurrent/ relapsing *C. Difficile* infection. Both cases were seen in a clinic that screens patients for possible Fecal Microbiota Transplant (FMT) for the treatment of recurrent/relapsing *C. Difficile*-associated diarrhea.

The first case involves a middle aged female with recurrent CDAD for which she was ultimately treated successfully with FMT via colonoscopy. In the following months, she developed symptoms consistent with IBS, the symptoms of which had not been present prior to her *C difficile* infection. A lactulose breath test was consistent with a diagnosis of small intestinal bacterial overgrowth (SIBO). A second case involved a young adult male who was seen in clinic following CDAD. The *C. Difficile* infection resolved following a pulsed course of vancomycin. However, the patient developed symptoms consistent with diarrhea-predominant IBS and was diagnosed with SIBO. These cases support the possible association between recurrent/relapsing CDAD and PI-IBS.

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**254 COMPARISON OF TRANSCUTANEOUS RESPIRATORY MONITORING WITH CONVENTIONAL VITAL SIGNS IN THE DETECTION OF ABNORMAL RESPIRATORY EVENTS IN CHILDREN UNDERGOING ENDOSCOPY**

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10.1136/jim-d-15-00013.254

**Purpose of Study** Accurate and reliable respiratory monitoring is essential for early detection of respiratory distress during procedure related sedation. While preliminary investigations vouched for the utility of transcutaneous respiratory monitoring (TCM) for adult endoscopies and post-anesthesia care unit, it has not been evaluated in pediatric endoscopies. Accordingly, this study hypothesized that TCM would more accurately detect altered respiratory status when compared to gold standard monitoring methods.

**Methods Used** This prospective cross-sectional observational study enrolled forty-two pediatric patients, who underwent 58 colonoscopies and esophagogastroduodenoscopies. The primary aim of this study was to compare the number of altered respiratory events such as apnea, cyanosis, choking, agitation, moaning or snoring, which were detected by pulse-oximetry, micro-stream capnography (mCAP), and TCM. The secondary aim was to evaluate factors associated with developing altered respiratory events (ARE).

**Summary of Results** Results from this study suggest that the rate of detecting AREs by mCAP was more than two-fold greater, when compared in real-world conditions to TCM and other modalities including pulse-oximetry and RR by plethysmography. In this study TCM waveform was the first to change in 94% of the altered respiratory events. Using mCAP, the waveform changed first in 96.7% of the AREs. Secondly, an increased rate of developing AREs was associated with smaller size with respect to patients' weight, requirement of higher drug amounts, and an upper endoscopy procedure.

**Conclusions** Adding TCM to a patient monitoring bundle that already includes mCAP adds little benefit to monitoring pediatric patients undergoing endoscopy. Although TCM detected AREs such as apnea with high specificity, it was unable to provide the early detection of AREs that mCAP provides.

**Abstract 254 Table 1** ARE Detection Performance by TCM, mCAP, Respiratory Rate and Pulse-oximetry

<b>Total ARE</b>	<b>132</b>
Mean ARE per minute detected by each modality	
TCM	0.065/minute
Capnography	0.179/minute
Respiratory Rate	0.003/minute
Pulse-oximetry	0.019/minute

## Genetics

## Concurrent Session

12:30 PM

Friday, January 29, 2016

255 **UTILITY OF BROAD SEQUENCING TO REVEAL MTM1 GENE MUTATION CARRIERS WITH HISTORY OF RECURRENT NEONATAL DEATH**

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10.1136/jim-d-15-00013.255

**Purpose of Study** To report the utility of exome sequencing in evaluating recurrent neonatal death in a family. In many cases of history of neonatal loss, testing of affected neonates is not possible, however the availability of broad sequencing allows for directed application for family members in such situations. We present a family with history of recurrent male losses due to severe respiratory distress. The patient experienced loss of two term male infants. The first neonatal loss was due to severe respiratory distress while the subsequent pregnancy was also complicated by polyhydramnios and severe refractory respiratory distress at birth. A maternal relative also suffered three male neonatal losses from respiratory distress.

**Methods Used** Clinical whole exome sequencing was performed. Demised infant tissue was not available for testing, and sequencing of the patient via trio exome was accomplished with modifications of this application. Exome analysis was performed by filtering variants by minor allele frequency and by inheritance pattern. Inherited variants were further considered by comparison with mutation databases and using detailed phenotypic annotation.

**Summary of Results** Sequencing revealed a missense variant in the *MTM1* gene associated with X-linked myotubular myopathy, inherited maternally. The *MTM1* c.614C>T (p.Pro205Leu) mutation resides in exon 8, which contains the Rac-induced recruitment domain (RID) of myotubularin, a key muscle differentiation protein. Many previously documented pathogenic variants cluster in exon 8, and the RID domain is highly conserved region, signifying this is an important component of the protein and pathogenic changes lead to myotubular myopathy. Functionally, mutation in the RID region has been shown to lead to severe manifestations, as seen in the affected children of our patient and support the significance of this mutation.

**Conclusions** Exome sequencing can be applied to evaluate unexplained neonatal deaths in affected families. Such sequencing can be effective in patients even when genetic testing is not available on affected individuals. In this case, sequencing could only be done on asymptomatic patients with a family history. This report emphasizes the

importance of well-defined phenotyping and narrowing genomic space in evaluation.

256 **EFFICACY AND SAFETY OF DIAZOXIDE CHOLINE CONTROLLED RELEASE TABLET IN PRADER-WILLI SYNDROME: RESULTS OF THE DOUBLE-BLIND ARM OF THE STUDY**

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10.1136/jim-d-15-00013.256

**Purpose of Study** Prader-Willi syndrome (PWS) is a complex, multisystem genetic disorder characterized by multiple endocrine, neurological and behavioral abnormalities. To date, no medication has proven effective in regulating appetite in PWS. It is conceivable that Diazoxide Choline Controlled-Release tablet can be used to control appetite and possibly improve on behavioral symptoms in PWS, through the activation of a diverse set of downstream mediators.

**Methods Used** Thirteen obese PWS subjects between 10–22 yrs were enrolled in the study. Two subjects were withdrawn from the study due to a prior psychiatric illness in one and progressively compromised glycemic control at the highest dose in the other. Eleven subjects completed the open-label treatment phase, were designated as Responders (hyperphagia response rate 92%) and randomized into the double-blind phase of the study. Data was analyzed for both open-label treatment and double-blind phase.

**Summary of Results** We found significant improvements in hyperphagia in DCCR treated subjects at the end of the open-label treatment period (–31.6%, p=0.003) and in those who continued on DCCR in the double blind phase (–29.2%, p=0.006). There was significant reduction in ‘aggressive’, ‘threatening’, ‘destructive’ behavior in comparison to all other PWS associated behaviors at the end of open label treatment phase (62.5% Vs 29.8% respectively, p=0.01). The effect of DCCR on behavior seems to be independent of the effect on hyperphagia. Significant impacts were seen on fat mass (–3.8%, p=0.011), lean body mass (+5.4%, p=0.001), lean body mass/fat mass ratio (+9.8%, p=0.002) during open-label treatment. The impacts on body composition were of similar magnitude in GH treated and GH naïve subjects. DCCR treatment significantly reduced TG, Tot-C, and Non-HDL-C in both the open-label and double blind phase.

**Conclusions** DCCR significantly reduces hyperphagia, fat mass (while increasing lean body mass), aggressive, threatening, destructive behavior and improved cardiovascular risk factors. The common adverse events included peripheral edema which was responsive to dose reduction and diuretics, and transient hyperglycemia.

## 257 CLINICAL COURSE OF 4 CHILDREN WITH *GNAO1* MUTATIONS CAUSING A SEVERE AND DISTINCTIVE MOVEMENT DISORDER

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10.1136/jim-d-15-00013.257

**Purpose of Study** Mutations in *GNAO1* have been described in eight patients to date. While the majority of these patients had epileptic encephalopathy, two patients have a severe movement disorder as the prominent feature. We describe a series of patients with *de novo* *GNAO1* mutations with severe chorea, developmental delay, and hypotonia in the absence of epilepsy.

**Methods Used** Four patients with mutations in *GNAO1* as detected by whole exome sequencing were identified at 3 institutions. We report the presentation, clinical course, and response to treatment of these patients.

**Summary of Results** In all four patients, global developmental delay and hypotonia were present from infancy and onset of chorea ranged between the ages of 3–4 years. Neuroleptic treatments were most effective in the baseline management of chorea. The chorea was gradually progressive and marked with episodes of severe, refractory ballismus requiring intensive care unit admissions in 3 out of 4 patients. Exacerbations indirectly led to the death of 2 of the patients.

**Conclusions** Patients with *GNAO1* mutations can present with a severe, progressive movement disorder in the absence of epilepsy. Exacerbations may be refractory to treatment and can result in life threatening secondary complications. Early and aggressive treatment of these exacerbations with direct admission to intensive care units for treatment with anesthetic drips may prevent some secondary complications. However the chorea and ballismus can be refractory to maximum medical therapy.

## 258 ALLAN HERNDON DUDLEY SYNDROME: A CASE REPORT AND REVIEW OF THE LITERATURE

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10.1136/jim-d-15-00013.258

**Case Report** Allan Herndon Dudley syndrome (AHDS, MIM 300095) is an X-linked condition caused by mutations in *SLC16A2*, which encodes MCT8, a T3-specific transporter expressed in certain tissues. Loss-of-function mutations prevent transport of T3 into cells, leading to deficient T3 within those cells but increased T3 in the periphery. Clinically, affected males have severe hypotonia, global developmental delay or intellectual disability, and paroxysmal kinesigenic dyskinesia (a form of dystonia). Hypothyroidism is usually diagnosed but found to be refractory to typical treatments. An elevated T3 level is a hallmark of the condition.

We present a 27-month-old boy with microcephaly, hypotonia, developmental delay, failure to thrive, dystonia, and elevated T3 found to have an inherited mutation in *SLC16A2*. He had been treated for hypothyroidism from very early in life without improvement in symptoms. We will discuss clinical presentation, natural history, pathophysiology, and management of this condition, including treatment with the T3 analogue triiodothyroacetic acid, which is not clinically available in the United States but which has been successfully imported internationally in some instances. This agent can enter cells even when the MCT8 transporter is not expressed and may partially restore brain development in mouse models of the condition.

This case underscores the importance of considering hypothyroidism as a potentially important clue to diagnosis rather than a nonspecific finding. T3 level should be routinely checked in patients with unexplained developmental delays and hypotonia to screen for AHDS.

## 259 A MASSIVELY PARALLEL APPROACH TO ASSESS THE SEQUENCE-FUNCTION RELATIONSHIP OF RECOMBINASE A

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10.1136/jim-d-15-00013.259

**Purpose of Study** Recombinase A (RecA) and its homologs are crucial to DNA repair in all organisms. In bacteria, RecA also regulates the SOS response, inducing cell cycle arrest and mutagenic polymerases. Accordingly, RecA can mediate antibiotic resistance through *de novo* mutation, integration and duplication of resistance genes, and induction of a “persister” phenotype. To probe the function and evolution of RecA, we developed and validated an innovative approach linking gene sequence with function for libraries of  $>10^5$  unique *recA* mutants.

**Methods Used** Using deep mutational scanning, we generated mutant libraries containing every codon mutation at each position of the *E. coli recA* gene. Each variant was barcoded, giving each molecule a unique “ID” sequence. Using sequence subassembly, each full-length variant is reconstructed from short Illumina reads, according to its ID. The library is expressed in *ΔrecA E. coli*, which are exposed to ciprofloxacin, a DNA damaging agent requiring functional RecA for survival. The barcodes of surviving cells are sequenced to quantify each variant relative to the pre-selection library.

**Summary of Results** We made libraries in biological triplicate, each with  $\sim 6 \times 10^5$  variants. Variants contain an average of 1.5 mutations, following a Poisson distribution. We fully subassembled  $2.8 \times 10^5$  variants between two libraries, with  $\sim 10^6$  variants partially subassembled. Further sequencing will greatly increase the number of fully assembled variants. We performed selections on the libraries at 0, 4 and 10 ng/mL cipro and showed that 4 ng/mL is sufficient to inhibit *recA*<sup>-</sup> cells without affecting

*recA*<sup>+</sup> cells. Furthermore, we developed a software pipeline to analyze the results.

**Conclusions** We developed a cutting edge approach to study the sequence-function relationship of a gene involved in DNA repair and antibiotic resistance, and created software necessary to analyze such data. In addition to the insights this study will provide on the function of *recA*, we also help lay the groundwork for techniques that can be generalized to answer a multitude of questions that were previously inaccessible to experimentation.

## 260 DIAGNOSTIC OUTCOMES AND RELATIVE COST OF CLINICAL WHOLE EXOME SEQUENCING

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10.1136/jim-d-15-00013.260

**Purpose of Study** Clinical whole exome sequencing (WES) is a valuable diagnostic and prognostic tool for suspected genetic disorders, yet remains inaccessible to many due to cost. Our objective was to determine the diagnostic utility and relative cost of clinical WES for undiagnosed suspected genetic disorders in children evaluated at an academic referral center.

**Methods Used** Ours is a retrospective cohort of patients for whom clinical WES was ordered from November 2012 to August 2014. 103 patients seen in subspecialty clinics and/or the inpatient setting at UCSF Benioff Children's and UCSF Moffit/Long Hospitals were included. WES was ordered for individuals (n=11) and family trios (n=92) for a range of diagnoses as indicated by the ordering physician. The rate of clinical diagnoses made via WES and the cost relative to prior negative work-up were measured.

**Summary of Results** The three most common clinical indications for WES were global developmental delay and/or intellectual disability (GDD/ID), autism spectrum disorders (ASD), and epilepsy. A disease causing mutation was found in 45.6% of patients with GDD/ID, 33.3% of patients with ASD and 56.5% of patients with epilepsy listed as either the primary or secondary indication for testing. A diagnosis was made in 48.5% (50/103) of our total cohort. WES led to a change in the clinical impression for more than half (53.4%) of the cases and 9.7% of patients received a new treatment or supportive intervention as a result of their genetic diagnosis. With a mean prior cumulative testing cost per proband of \$16,848, exome sequencing was determined to be cost effective in our cohort up to \$8171 per family trio.

**Conclusions** Clinical WES is a powerful diagnostic and prognostic tool for patients in an academic referral center, leading to a diagnosis nearly half of the time. WES was found to be cost effective relative to prior diagnostic testing costs. Our study provides valuable evidence supporting the use of this test for patients with a presumed genetic disorder.

## 261 A DOMINANT MUTATION ASSOCIATED WITH BOHRING-OPTIZ SYNDROME REVEALED DURING WHOLE EXOME SEQUENCING ANALYSIS

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10.1136/jim-d-15-00013.261

**Case Report** Whole exome sequencing was performed on a six-year-old female patient with seizures of infantile onset, severe global developmental delay, dysmorphic features and failure to thrive. The patient is G-tube dependent and has cyclic vomiting, myopia, hypothyroidism, a submucosal cleft palate, protuberant cheeks, crooked wide-spaced teeth, tented lips, and hirsutism. MRI showed a nearly absent corpus callosum. After sequencing the proband, both parents, and two unaffected siblings, a *de novo* nonsense mutation in *ASXL1* previously implicated as causative of Bohring-Opitz Syndrome (BOS) was identified in the proband. BOS is a malformation syndrome characterized by severe intrauterine growth retardation, poor feeding, profound mental retardation, trigonocephaly, prominent metopic suture, exophthalmos, nevus flammeus of the face, upslanting palpebral fissures, hirsutism, and flexion of the elbows and wrists with deviation of the wrists and metacarpophalangeal joints (Hoischen et al., 2011). However, this particular *ASXL1* variant has also been reported seven times in the presumably unaffected individuals comprising the Exome Aggregation Consortium (ExAC) database. Although the phenotype of BOS matched the patient, the presence of this purportedly dominant variant in the ExAC database introduced ambiguity in the result interpretation. Further investigation found that acquired somatic mosaicism during hematopoietic clonal expansion for *ASXL1* variants (including truncating mutations) are known to occur with aging. Consideration of such phenomena is important when employing databases to evaluate the likelihood of pathogenicity for a genetic variant during whole exome sequencing.

## 262 EHLERS-DANLOS SYNDROME, PROGEROID TYPE, CAUSED BY A NOVEL MUTATION, P.(CYS324SER) IN B4GALT7 IN A CHILD WITH JOINT LAXITY, GROWTH RETARDATION, DYSMORPHIC FACIAL FEATURES AND NOVEL EYE FINDINGS INCLUDING BILATERAL COLOBOMAS

T Arunrut,<sup>1</sup> M Sabbadini,<sup>1</sup> M Jain,<sup>2</sup> F Scaglia,<sup>2</sup> A Slavotinek.<sup>1</sup> <sup>1</sup>University of California, San Francisco, San Francisco, CA; <sup>2</sup>Baylor College of Medicine, Houston, CA

10.1136/jim-d-15-00013.262

**Purpose of Study** To report novel ocular findings in a child with a novel missense mutation, p.(Cys324Ser) in *B4GALT7*.

**Methods Used** Retrospective clinical evaluation and chart review.

**Summary of Results** We present a 5 yo female with a distinctive phenotype comprising developmental delays, pre- and postnatal growth restriction, striking joint laxity and scoliosis. Her ocular findings included proptosis, iris and optic nerve colobomas and posterior subcapsular cataracts. Photographs showed progressive thinning of skin and adipose atrophy. She had a normal echocardiogram and brain MRI scan. There was no relevant family history or consanguinity. Whole exome sequencing revealed homozygosity for c.970T > A, predicting p.(Cys324Ser) in *B4GALT7*. The substitution was predicted to be damaging, occurred within a highly conserved region and was present at a frequency of 0.0001 in controls. Segregation of the mutation was in keeping with autosomal recessive inheritance. Mutations in *B4GALT7* cause Ehlers-Danlos syndrome (EDS), progeroid type, and Larsen syndrome. In mutation positive patients (n=27; 22 were from one family with the same mutation), commonest features were short stature (27/27), joint hypermobility/dislocations (26/26), skin hyperextensibility (26/27) and dysmorphic facial features (26/27) that were progeroid in only two patients. Learning disability (17/27) and radioulnar synostosis (14/27) were also frequent. Glaucoma (5/21) and megalocornea (1/21) were noted in one family, but the eye defects seen in the proposita have not been reported. Another mutation, p.Arg270Cys, in *B4GALT7* was associated with reduced decorin and biglycan synthesis in fibroblasts. Decorin and biglycan are small leucine-rich proteoglycans that regulate collagen fibril and matrix assembly in the cornea and mutations in decorin cause hereditary stromal corneal dystrophy.

**Conclusions** Our patient with a novel mutation in *B4GALT7* has distinctive eye findings, most likely a new phenotypic feature, although a different genetic etiology cannot be excluded.

**Neonatal Pulmonary III  
Concurrent Session  
12:30 PM  
Friday, January 29, 2016**

**263 MATERNAL RACE/ETHNICITY (RE) AND INFANT GENETIC ANCESTRY ARE ASSOCIATED WITH SURVIVAL WITHOUT BRONCHOPULMONARY DYSPLASIA (BPD) IN PRETERM NEWBORNS TREATED WITH INHALED NITRIC OXIDE (INO)**

RL Keller,<sup>1</sup> S Oh,<sup>3</sup> D Torgerson,<sup>3</sup> P Ballard,<sup>1</sup> S Huntsman,<sup>3</sup> E Burchard,<sup>3</sup> D Black,<sup>2</sup> R Ballard<sup>1</sup>. <sup>1</sup>UCSF, San Francisco, CA; <sup>2</sup>UCSF, San Francisco, CA; <sup>3</sup>Medicine, UCSF, San Francisco, CA

10.1136/jim-d-15-00013.263

**Purpose of Study** In the Trial of Late Surfactant (TOLSURF), all infants received iNO. Infants of mothers with African American (AA) RE had higher rates of survival without BPD. We sought to determine the relationship of RE to survival without BPD after adjustment for confounders, and to evaluate the effect of maternal RE and infant genetic ancestry in logistic regression (LR) models.

**Methods Used** Infants enrolled in TOLSURF [ $\leq$  28 wks' gestational age (GA), ventilated at 7–14d] were included. The primary outcome was survival without BPD (determined at 36 wks' GA by physiologic O<sub>2</sub>/flow challenge). DNA was isolated from tracheal aspirate samples following parental consent. Infants were genotyped at >800,000 single nucleotide polymorphisms on the Axiom LAT1 array (WorldArray4, Affymetrix). Global proportions of African (AFR), European (EUR) and Native American ancestry were estimated using the program ADMIXTURE. Mixed effects LR accounted for clustering by study site and infants of multiple gestation. Potential co-variables were considered for inclusion if associated with RE and a risk factor for

**Abstract 263 Table 1** Models for prediction of survival without BPD

	Full cohort (n=507)		Genetic cohort (n=414)			
	OR(95% CI)	P value	OR(95% CI)	P value	OR(95% CI)	P value
AFR ancestry					2.6(1.4, 4.8)	0.002
AA	2.0(1.3, 3.2)	0.003	2.6(1.5, 4.4)	0.0004		
Hispanic	1.2(0.6, 2.5)	0.55	1.7(0.8, 3.6)	0.19		
Other	1.8(0.7, 4.5)	0.19	2.4(0.9, 6.6)	0.08		
Birth weight	1.0(1.0, 1.0)	0.01	1.0(1.0, 1.0)	0.004	1.0(1.0, 1.0)	0.005
Birth weight centile	1.0(1.0, 1.0)	0.94	1.0(1.0, 1.0)	0.61	1.0(1.0, 1.0)	0.59
Male sex	0.4(0.3, 0.7)	0.0004	0.4(0.3, 0.7)	0.001	0.4(0.2, 0.7)	0.0003
Multiple gestation	1.1(0.7, 1.8)	0.62	1.2(0.7, 2.0)	0.41	1.2(0.7, 2.0)	0.46
RSS (MAPxFlO2) at study entry	0.7(0.6, 0.8)	<0.0001	0.7(0.6, 0.8)	<0.0001	0.7(0.6, 0.8)	<0.0001

BPD; our final model was informed by fit. For evaluation of RE and ancestry, only infants with adequate quality DNA sample were included.

**Summary of Results** See Table. White/EUR ancestry are referent groups.

**Conclusions** Maternal AA RE and infant proportion of AFR ancestry had similar effects on increased survival without BPD in infants treated with iNO. Further evaluation of the genetic basis for this relationship is warranted, particularly as response to manipulation of the NO pathway differs in AA in other settings.

#### 264 EARLY POSTNATAL HYPEROXIA INCREASES LUNG WNT3A AND MMP9 MRNA LEVELS IN GROWTH RESTRICTED RAT PUPS

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**Purpose of Study** Intrauterine growth restriction (IUGR) increases the risk for postnatal lung disease. IUGR is often accompanied by additional postnatal stressors associated with respiratory management. We previously showed, in the rat, that IUGR combined with postnatal hyperoxia, induces sex-specific structural and functional changes in the developing lung. Structural and functional changes in the developing lung are frequently associated with alterations in Wnt signaling, and subsequent transcription of Wnt output genes. However, the effects of IUGR combined with a postnatal stressor such as hyperoxia on lung mRNA levels of Wnt ligand, Wnt3a, and Wnt output gene MMP9 in the rat are unknown.

We hypothesized that the combination of hyperoxia and IUGR would sex-specifically alter lung mRNA levels of Wnt3a and MMP9 in the rat.

**Methods Used** IUGR was induced by ligation of intrauterine arteries. Control and IUGR rat pups (and dams) were exposed to hyperoxia (60% O<sub>2</sub>) or room air from postnatal days 3–6 (developing lung), then room air to postnatal day21 (mature lung), when rat pups were killed and lungs harvested. Real-time RT PCR was used to measure lung WNT 3a and MMP9 mRNA levels.

**Summary of Results** Results are mean±SD compared to RA-Con. Lung Wnt3a and MMP9 mRNA levels were not altered by IUGR alone in male or female rats. Lung Wnt3a mRNA levels were increased by hyperoxia alone in male (247±35%) and female (278±58%) rats, and further increased by the combination of IUGR and hyperoxia in male (308±64%) and in female (316±54%) rats. Lung MMP9 mRNA levels were not affected by hyperoxia alone in male or female rats, but were increased by the combination of hyperoxia and IUGR in male (200±100%) and female (275±50%) rats.

**Conclusions** The combination of hyperoxia and IUGR increases lung mRNA levels of Wnt3a and MMP9 in the rat. In contrast to our hypothesis, the alterations in Wnt3a and MMP mRNA were not different between male and female rats. We speculate that our previously observed sex-specific alterations in lung structure and function in the context of IUGR with hyperoxia may result from other sex-specific signaling pathways.

#### 265 INVASIVE MECHANICAL VENTILATION FOR 3 DAYS DOES NOT ALTER PROLIFERATION OF CARDIAC MYOCYTES COMPARED TO NON-INVASIVE NASAL VENTILATION

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10.1136/jim-d-15-00013.265

**Purpose of Study** Pulmonary hypertension (PHT) often complicates bronchopulmonary dysplasia in preterm infants. We recently showed that former premature lambs supported by invasive mechanical ventilation (IMV) for 3d after birth have indicators of PHT including right ventricular hypertrophy and persistent muscularization of pulmonary arterioles at 2 mo and 5 mo corrected postnatal age (equivalent to 2 yr and 6yr corrected postnatal age, respectively, in humans). The purpose of this study was to determine if 3d of IMV acutely increases cardiac myocyte proliferation.

**Methods Used** Premature lambs (~131d gestation (equivalent to ~29 wk gestation in humans); term ~150d), treated with antenatal steroids, postnatal surfactant and caffeine citrate were managed by IMV for 3d. The control group was premature lambs supported by non-invasive ventilation (NIV) via their nose for 3d, a respiratory support mode that leads to better lung outcomes. The heart was stopped by potassium chloride injection, then placed in iced saline followed by perfusion of the coronary arteries with iced formalin. Tissue sections were immunostained for proliferating cell nuclear antigen (PCNA) and analyzed morphometrically to quantify the number of immature cardiac myocytes proliferating at the end of 3d of respiratory support.

**Summary of Results** Percentage of PCNA-positive nuclei in immature cardiac myocytes (one nucleus per cell in longitudinal sections in situ) was the same between the two groups of premature lambs: 9±2 percent for the IMV group (n=5) and 7±2 percent for the NIV group (n=5).

**Conclusions** Three days of IMV did not acutely affect proliferation of immature cardiac myocytes compared to NIV. Morphometric measurements of myocyte volume, an index for cardiac myocyte hypertrophy, are underway. These same morphometric analyses are being performed on hearts of former premature lambs at 2 mo and 5 mo corrected postnatal age. HL110002, HL07744, LU Internship, Division of Neonatology.

266

### PERINATAL NICOTINE EXPOSURE INDUCES MESENCHYMAL MYOGENIC DIFFERENTIATION, BUT NOT EPITHELIAL-MESENCHYMAL TRANSITION IN RAT OFFSPRING LUNGS

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10.1136/jim-d-15-00013.266

**Purpose of Study** Perinatal nicotine exposure induces alterations in lung structure and function including lung fibrosis in rat offspring. Whether epithelial-mesenchymal transition (EMT), a known contributor to pulmonary fibrosis, occurs following perinatal nicotine exposure is not known.

**Methods Used** Time-mated, first-time pregnant, pair-fed Sprague Dawley rat dams received either placebo (diluent) or nicotine [1 mg/kg, s.c.] in 100  $\mu$ l volumes daily from embryonic day (e) 6 to postnatal day (PND) 21. Following delivery at term, pups breast feed ad libitum. Lungs were isolated at PND 21, and using Western analysis, q-RT-PCR and immunohistochemistry processed for evidence of EMT. To gain further supportive evidence for nicotine-induced EMT, embryonic day 19 primary rat lung alveolar type II cells (ATII) were cultured and treated with nicotine ( $10^{-6}$ M to  $10^{-9}$ M) for 24h.

**Summary of Results** By Western and qRT-PCR analysis, protein level of  $\alpha$ -smooth muscle actin, fibronectin, and calponin (markers of fibroblast differentiation) increased significantly. However, hydrophobic proteins which are in charge of stabilizing the respiratory surface of mammalian lungs (surfactant proteins B and C) and cholinephosphate-cytidyl transferase- $\alpha$  (all epithelial markers) showed no significant changes. Typical characteristics of EMT, E-cadherin, N-cadherin, and fibroblast specific protein-1, were also not significantly different between the nicotine exposed and control rats. Data from the double immunostaining of lung sections and in vitro treated ATII cells also strongly supported the Western data, indicating the absence of EMT.

**Conclusions** Enhanced myogenic profile, but unaltered specific surfactant proteins and other epithelial markers suggest a mechanistic link between nicotine exposure and myogenic differentiation, but no EMT, as evidenced by the absence of the loss of E-cadherin or gains in N-cadherin and FSP-1. These data suggest that perinatal nicotine exposure results in mesenchymal myogenic differentiation, but not EMT, as a possible contributor towards the nicotine-induced myogenic lung phenotype [Supported by NIH (HD51857, HD071731; TRDRP (17RT-0170, 23RT-0018)].

267

### FROM MOUSE DEVELOPMENT TO SHEEP LUNG INJURY

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10.1136/jim-d-15-00013.267

**Purpose of Study** Bronchopulmonary dysplasia is thought to arise from arrest of lung development. Mice & sheep models are useful for studying human BPD. Here, we examined expression of novel genes identified in a mouse model of lung development, in lambs exposed to invasive or non-invasive ventilation.

**Methods Used** Genes of interest were identified by micro-array of mouse lung tissue RNA during development. A select group with relevant purported functions included *Cyr61*, *Egr1*, *Slit2* & *Slitrk6*. Expression of genes was examined in sheep lungs delivered at gestational ages (GA) ranging from 128 to 150 days (term). We also examined expression in lungs from 2 groups of preterm sheep delivered at 130 GA. 1 group was exposed to invasive mechanical ventilation (MV) & the other was on non-invasive high frequency nasal ventilation (HFNV). Ventilation was for 3 or 21 days. RNA was isolated & gene expression assessed by quantitative PCR.

**Summary of Results** In uninjured sheep lungs *Egr1*, *Slitrk6* & *Cyr61* remained unchanged between 128 to 136 GA, but increased significantly at term. *Slit2* remained constant. In ventilated lambs, *Egr1* & *Cyr61* increased in both MV & HFNV groups on day 3. This increased adaptation was greater in HFNV compared to MV group. After 21 days, *Egr1* & *Cyr61* decreased significantly in both groups. No change in *Slit2* occurred on day 3 in either group. *Slitrk6* decreased in both MV & HFNV groups on days 3 and 21.

**Conclusions** Our study found progressive increase in *Egr1*, *Cyr61* & *Slitrk6* mRNA during fetal sheep lung development. This novel finding suggests that expression of these genes is needed for normal pulmonary maturation & adaptation at birth. *Egr1* & *Cyr61* adaptation is more robust in HFNV vs MV ventilated preemies on day 3, suggesting possible association with better outcome. *Slit2* & *Slitrk6*, which have not been described in lung development or injury were decreased in injured lungs. While the results are preliminary, they suggest adaptive changes in expression of developmentally critical genes in the lung in response to preterm birth.

Supported By: NHLBI and the Hastings Foundation.

268

### ONE YEAR PULMONARY OUTCOMES IN THE TRIAL OF LATE SURFACTANT (TOLSURF)

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**Purpose of Study** Infants in TOLSURF were randomized to late surfactant vs sham; all received inhaled nitric oxide. There was no difference in the primary outcome of survival without bronchopulmonary dysplasia (BPD) at 36 wks' post-menstrual age, determined by physiologic O<sub>2</sub>/flow reduction (Ballard RA, J Pediatr, *In Press*). We evaluated the effect of treatment on novel pulmonary outcomes at one year corrected age (CA).

**Methods Used** Infants were eligible for TOLSURF if born  $\leq 28$  0/7 wks' gestational age (GA) and ventilated at 7–14d.

Those with anomalies/syndromes or unlikely to survive 7d were excluded. Of 511 enrolled, 455 infants were discharged, 5 died after discharge. We collected data on respiratory morbidity by parental surveys at 3, 6, 9 and 12 mos CA. Morbidity was determined at each survey if parents reported medications (diuretic, bronchodilator, inhaled or systemic steroid, pulmonary vasodilator) or hospitalization for respiratory cause, or home respiratory support. Infants were classified into 2 novel outcomes, based on resource utilization over the 1st year: No Pulmonary Morbidity (No PM) if no morbidity reported vs Any PM, and Persistent Pulmonary Morbidity (PPM) if morbidity reported in  $\geq 3$  surveys vs No PPM if non-persistent or no morbidity. We analyzed the effect of treatment assignment (late surfactant vs sham) on these outcomes with GEE, to account for clustering of siblings, adjusting for imbalances in baseline characteristics.

**Summary of Results** There were no differences in GA ( $25.3 \pm 1.2$  vs  $25.3 \pm 1.2$ ,  $P=0.94$ ), male sex (57 vs 53%,  $P=0.5$ ), percent with intrauterine growth restriction (IUGR  $\leq 10$ th percentile, 19 vs 14%,  $P=0.20$ ), or maternal race/ethnicity ( $P=0.36$ ) in treatment (TRT) versus control (CTL) groups. However, infants in the TRT group were less likely to be products of multiple gestation (26 vs 36%,  $P=0.03$ ), and they had younger mothers ( $27.7 \pm 6.1$  vs  $29.8 \pm 6.6$  years,  $P=0.0004$ ) with less education ( $P=0.047$ ). 110/439 (25%) of infants were classified as No PM and 153/426 (36%) as PPM. Adjusted Relative Benefit for TRT vs CTL was 1.09 (0.58, 2.03;  $P=0.8$ ) for No PM and 1.22 (0.93, 1.58;  $P=0.15$ ) for No PPM.

**Conclusions** There was no significant benefit of late surfactant treatment on one year outcomes of No PM and No PPM in TOLSURF.

269

#### VENTILATION AFFECTS MECHANICAL PROPERTIES OF CEREBRAL ARTERIES IN PRETERM LAMBS

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10.1136/jim-d-15-00013.269

**Purpose of Study** Critical development of cerebral blood vessels occurs in the final weeks of pregnancy, making preterm infants more susceptible to both intraventricular hemorrhage and cerebral hypotension/hypertension. Ventilation of preterm has been shown to affect the development of the pulmonary vasculature; however, the effect of this treatment on the development of cerebral blood vessels remains unknown.

**Methods Used** Pregnant ewes carrying single or twin fetuses at 128–135 days (d) of gestation (term  $\sim 150$  d gestation) were used. Lambs were delivered via cesarean section and divided into three groups: a group managed by high-frequency nasal ventilation (HFNV,  $n=8$ ), a group managed by intermittent mandatory ventilation (IMV,  $n=5$ ), and a non-ventilated group ( $n=5$ ). Both HFNV and IMV lambs were ventilated for 3 days while non-ventilated lambs were euthanized at birth.

Immediately following death, middle cerebral arteries (MCAs) were collected for mechanical testing. In some cases, both left and right MCAs from the same animal were

tested. In total, 13 MCAs each were tested from the HFNV lambs, 6 MCAs from the IMV lambs, and 10 MCAs from the control group.

After being subjected to various preconditioning cycles within the physiological range (both axial and circumferential), vessels were stretched axially to failure under a constant internal pressure of 13.3 kPa.

**Summary of Results** The mean ultimate stress of both ventilated groups (HFNV, IMV) were larger than that of the control group, but only the HFNV group showed a statistically significant difference ( $p=0.006$ ). No statistical significance was found for the maximum stiffness nor any associated stretch values.

**Conclusions** These studies show that ventilation of premature lambs has a significant effect on the mechanical properties of the cerebral blood vessels. This finding has clinical relevance given the vascular pathologies associated with the brain of premature neonates. While these studies show an increase in the mechanical strength, the underlying mechanism behind these changes, and therefore any interpretation of their associated pathologies remains unclear. Future work will be done to explore changes to the vessel microstructure, with specific emphasis on changes to collagen cross-links.

270

#### RANDOMIZED CONTROLLED TRIAL OF NEBULIZED N-ACETYLCYSTEINE IN A NEWBORN PIG MODEL OF MECONIUM ASPIRATION SYNDROME

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10.1136/jim-d-15-00013.270

**Purpose of Study** Meconium aspiration syndrome (MAS) accounts for approximately 1,000 infant deaths in the United States each year. Exogenous surfactant has shown clinical benefit in both laboratory and clinical studies, however since institution into clinical practice overall mortality rates are unchanged. Agents that alter the physical properties of meconium have not been studied. We hypothesized that nebulized n-acetylcysteine (NAC), a drug with known mucolytic and anti-inflammatory properties, in addition to surfactant will improve oxygenation and ventilation and decrease short-term markers of inflammation in a piglet model of MAS.

**Methods Used** We induced MAS in thirty newborn piglets by intra-tracheal administration of human meconium. Once MAS was achieved piglets were randomized into one of three groups: Control (C), Surfactant (S), and Surfactant plus nebulized n-acetylcysteine (NAC). Short-term respiratory physiology endpoints, ventilator settings, vital signs, and arterial blood gases were monitored and recorded every 15–30 minutes for the 6-hour study period. At study end, blood was sampled for serum interleukin levels and TNF- $\alpha$ . Lungs were dissected for analysis of wet/dry ratio, histologic scoring, and analysis of interleukin levels/TNF- $\alpha$  in tissue homogenate.

**Summary of Results** Compared to controls both treatment groups showed similar increases in compliance (C vs S

$p=0.049$ , C vs NAC  $p=0.022$ ), decreased mean airway pressure (C vs S  $p=0.002$ ; C vs NAC  $p=0.003$ ), and decreased peak inspiratory pressure (C vs S  $p=0.003$ ; C vs NAC  $p=0.010$ ). Atelectasis scores were lower in both treatment groups (C vs S  $p=0.050$ ; C vs NAC  $p=0.046$ ). The surfactant group showed improved  $P_aO_2$  ( $p=0.009$ ) and oxygenation index (OI) ( $p=0.017$ ), whereas  $P_aO_2$  and OI in the surfactant+NAC group were indistinguishable from controls. Wet-dry ratios and interleukin levels/TNF- $\alpha$  were not different between any of the groups.

**Conclusions** Nebulized NAC does not appear to be a beneficial adjuvant to surfactant in this MAS model. Our data suggests use of nebulized NAC may actually be detrimental as piglets treated with nebulized NAC had worse oxygenation than those treated with surfactant alone.

## Neonatology General III Concurrent Session 12:30 PM Friday, January 29, 2016

### 271 EVIDENCE FOR MITOTIC TRANSMISSION OF NICOTINE'S EPIGENETIC EFFECTS ON MALE GONADAL GERM CELL UNDERLYING OFFSPRING LUNG MYOGENIC PHENOTYPE

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10.1136/jim-d-15-00013.271

**Purpose of Study** Perinatal nicotine exposure results in hyper-responsive lung phenotype, which is transmitted transgenerationally. By activating nicotinic acetylcholine receptors  $\alpha 7$  ( $\alpha 7nAChR$ ), perinatal nicotine exposure drives lung mesenchymal fibroblasts towards a myogenic phenotype, but the molecular mechanisms underlying the transgenerational (TG) transmission of the altered lung phenotype are not understood. We have shown that global DNA methylation is increased in testes following perinatal nicotine exposure. We hypothesize that gonadal germ cell gene-specific (PPAR $\gamma 2$ ,  $\alpha 7nAChR$ , and Wnt5A) epigenetic alterations as the underlying mechanism for the TG transmission of perinatal nicotine-induced lung phenotype.

**Methods Used** Mouse male gonadal germ cells were cultured and treated with nicotine ( $10^{-6}M$ - $10^{-12}M$ ) for 24h at passage 1 (P1); the cells were continued in culture up to P10 without any further exposure to nicotine; P1, P3, P6 and P10 cells were harvested-protein levels of PPAR $\gamma 2$ ,  $\alpha 7nAChR$  and Wnt5a were determined by Western blotting; global nuclear chromatin methylation was analyzed by ELISA and state-of-the art 3D chromatin methylation imaging (P1-P3) and promoter-specific methylation for PPAR $\gamma 2$ ,  $\alpha 7nAChR$  and Wnt5A were determined via methylation-specific PCR.

**Summary of Results** Nicotine exposure affected chromatin methylation dose-dependently with peak increase at  $10^{-9}M$ . Importantly, this effect was carried forward with mitotic cell division. Though there was some decrease in the transmission of nicotine-induced increased chromatin methylation from P1-P10, the increase was clearly retained up to P6, without any significant decrease from P2 to P6, despite the

fact that the cells were exposed to nicotine only at P1. Methylation of the PPAR $\gamma 2$  promoter increased significantly, but those of  $\alpha 7nAChR$  and Wnt5a decreased significantly. Nicotine's effect on PPAR $\gamma 2$  and  $\alpha 7nAChR$  promoter methylations, but not on Wnt 5A, carried up to P6.

**Conclusions** Nicotine exposure affected chromatin methylation dose-dependently. Nicotine's epigenetic effects on PPAR $\gamma 2$  and  $\alpha 7nAChR$ , but not Wnt5a are transmitted transgenerationally, potentially providing a novel mechanistic explanation for the TG transmission of perinatally cigarette smoke-induced lung phenotype. Grants: NIH-HD51857, HD071731; TRDRP-17RT-0170, 23RT-0018.

### 272 A 10 YEAR REVIEW OF CENTRAL LINE ASSOCIATED BLOOD STREAM INFECTION PREVENTION EFFORTS IN THE NEONATAL INTENSIVE CARE UNIT: ARE IMPROVEMENTS SUSTAINABLE?

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10.1136/jim-d-15-00013.272

**Purpose of Study** Significant reductions in Central Line Associated Blood Stream Infection (CLABSI) in the Neonatal Intensive Care Unit (NICU) have recently been reported. However, very few published reports identify key strategies associated with sustained CLABSI reduction. The purpose of this study is to review the effect of a Quality Improvement (QI) project on CLABSI rates and to identify key strategies that are associated with sustained CLABSI reduction at the NICU of the University of California, Irvine Medical Center.

**Methods Used** This is a retrospective, observational study over a 10 year period of the effect of a CLABSI reduction QI initiative. Three analysis phases are evaluated: baseline (2004–2005), active intervention phase (2006–2009) in which culture change and multiple central line care practices were implemented, and sustainment phase (2010–2014). CLABSI rates were tracked quarterly throughout the active intervention and sustainment phase along with specific practice changes.

**Summary of Results** We demonstrated a steady decline in CLABSI rate over a 10 year time span and successfully reduced CLABSI rates by 92% when compared to our baseline (8.25 vs 0.65/1000 line days,  $p<0.001$ ). Significant reductions in CLABSI rates were seen during the active intervention phase in which the CLABSI rate was reduced by 46% (4.39 vs 2.38 per 1000 line days). We have been successful in sustaining our CLABSI rate reduction during the sustainment phase with a mean CLABSI rate of 0.88/1000 line days. Key sustainment strategies identified by the project team leaders include strict compliance to insertion and maintenance practices, hand hygiene audit tools with feedback, use of a closed-medication delivery system, focused review of every CLABSI event to identify potential contributing factors or lapses in practice compliance and weekly displays of data (days between infection).

**Conclusions** Implementation of QI processes that include strict compliance to best practices and decreasing central line entry by use of a closed-medication system was

associated with a significant and sustainable reduction in CLABSI rates. This result has persisted to date.

### 273 INDUCTION OF HEME OXYGENASE-1 ATTENUATES THE SEVERITY OF SEPSIS IN A NON-SURGICAL NEONATAL MOUSE MODEL

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10.1136/jim-d-15-00013.273

**Purpose of Study** A complication of prematurity is neonatal sepsis, which is characterized by systemic bacterial invasion and multi-organ failure. Its pathogenesis is not fully known because of a lack of animal models. However, a distinct difference in inflammatory response from adults is hypothesized to be mediated by oxidative stress and apoptosis. The stress-response protein, heme oxygenase-1 (HO-1), can affect physiologic and pathologic states by its anti-inflammatory, antioxidative, and anti-apoptotic properties. Since HO-1 is developmentally regulated, we hypothesized that it plays a crucial role in the developing neonatal immune system and response to sepsis.

**Methods Used** To induce sepsis, we used the non-surgical adult sepsis model created by Starr *et al*, where a “slurry” is created by resuspending cecal contents from adult mice in 15% glycerol-PBS to 100 mg/mL. To establish the LD<sub>50</sub>, cecal slurries (CS) were given IP at various doses to 4d-old newborn wild-type (Wt) FVB mice and survival monitored for 7d. The LD<sub>40</sub> dose was given to 4d-old pups, which were then sacrificed at 24h post-CS treatment for measurements of liver and lung HO activity. To study the effect of HO-1 deficiency on survival, we treated 4d-old HO-1 heterozygote (Het, HO-1<sup>+/-</sup>) pups as described above. Finally, to study the protective role of HO-1 induction, 30- $\mu$ mol heme/kg was given SQ to 3d-old Wt mice 24h prior to sepsis induction and then monitored for survival.

**Summary of Results** A dose-dependent effect on mortality was found with an LD<sub>40</sub> of 2.0 mg/g. At this dose, HO activity significantly increased in the liver (1.7-fold) and lung (1.2-fold) 24h post-treatment. Also, treatment with 2.0 mg/g of CS caused a 75.0% mortality ( $p < 0.05$ ) in Het pups. Finally, induction of HO-1 significantly reduced mortality to 6.3% ( $p < 0.01$ ) in Wt pups.

**Conclusions** In summary, a partial deficiency in HO-1 increased the progression and mortality in a non-surgical sepsis model. Furthermore, induction of HO-1 significantly reduced the mortality in Wt pups. Thus, we conclude that

**Abstract 273 Table 1** % Mortality for Each Group as a Function of CS Dose, mg/g (LD50=2.25)

1.0	1.5	2.0		3.0		4.0
Wt	Wt	Wt	Het	Wt +Heme	Wt	Wt
0.0% (n=17)	8.3% (n=12)	40.9% (n=22)	75.0% (n=12)	6.3% (n=32)	75.0% (n=20)	100.0% (n=6)

HO-1 may confer protection against sepsis in preterm infants.

### 274 A LIPID FORMULATION OF ZINC PROTOPORPHYRIN FOR THE PREVENTION OF NEONATAL HYPERBILIRUBINEMIA DUE TO CHRONIC HEMOLYSIS

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10.1136/jim-d-15-00013.274

**Purpose of Study** Metalloporphyrins are potent competitive inhibitors of heme oxygenase (HO), the rate-limiting enzyme in bilirubin production. Infants with hemolytic disease (e.g. G6PD deficiency, ABO/Rh incompatibilities) have increased bilirubin production rates and, if left untreated, can develop severe hyperbilirubinemia. Recently, we have created a lipid-based formulation of zinc protoporphyrin (ZL) that is orally absorbable with no photo- or chemical toxicity and effectively reduces liver HO activity after a single heme-load (*Pediatr Res, in press, 2015*). Here, we extend these studies to evaluate the efficacy of ZL in newborn pups exposed to repeat heme loads as in chronic hemolysis.

**Methods Used** Vehicle (V), ZL (30  $\mu$ mol/kg), or zinc deuteroporphyrin bis glycol (ZBG, 15  $\mu$ mol/kg, shown previously to effectively inhibit HO (*Pediatr Res 70:467, 2011*)) was given intragastrically to 3d-old FVB pups. After 1.5h, pups were injected SQ with 30- $\mu$ mol heme/kg and again 24h later. 24h after the 2<sup>nd</sup> heme load, liver and spleen HO activity was measured and expressed as fold change from controls. Differences were deemed significant at  $p \leq 0.05$ .

**Summary of Results** Treatment with repeat heme loads (VHH) increased liver and spleen HO activity 1.7- and 1.5-fold over controls (VVV), respectively (see Table 1). Pre-treatment with ZL (ZL-HH) significantly decreased this heme-mediated increase in liver HO activity by 12% with no effect on the spleen. In contrast, pre-treatment with ZBG (ZBG-HH) significantly reduced heme-mediated increases in liver (15%) and spleen (30%) HO activity.

**Conclusions** In summary, ZL effectively inhibits the induction of liver HO activity following repeat heme loads after a single oral dose comparable to that of ZBG, albeit at a higher dose. Since the liver is primarily responsible for degrading heme during hemolysis, a compound that targets the liver may be the most desirable. Thus, ZL is attractive

**Abstract 274 Table 1** Fold Change in HO Activity Over Control (VVV). (\* $p < 0.05$  vs VHH)

	VVV	VHH	ZL-HH	ZBG-HH
Liver	1.0 $\pm$ 0.2 (n=8)	1.7 $\pm$ 0.3 (n=15)	1.5 $\pm$ 0.2* (n=11)	1.4 $\pm$ 0.3* (n=12)
Spleen	1.0 $\pm$ 0.3 (n=6)	1.5 $\pm$ 0.3 (n=4)	1.4 $\pm$ 0.2 (n=4)	1.1 $\pm$ 0.2* (n=4)

for use in preventing hyperbilirubinemia in infants with chronic hemolysis.

**275 SURGICAL PROCEDURES INCREASE PROGRESSION AND DURATION OF RETINOPATHY OF PREMATURITY (ROP)**

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10.1136/jim-d-15-00013.275

**Purpose of Study** ROP is a proliferative neovascular complication of preterm birth. Many preterm infants at risk for ROP undergo general surgical procedures. The effect of surgery on progression or duration of ROP is unknown. We hypothesized that infants who undergo surgical procedures will have progression and longer duration of ROP.

**Methods Used** We performed a retrospective chart review of infants born from January 2013 to December 2014. Inclusion criteria were: gestational age < 27 weeks and/or birth weight < 800g and serial ophthalmologic examinations for ROP. Infants were divided into 2 groups based on presence or absence of surgical procedures. ROP progression was defined as any advancement in stage of ROP classification documented at the 1st scheduled exam following surgery. ROP duration was defined as time from 1st description of stage 2 disease until the 1st exam documenting regression.

**Summary of Results** Seventy-six infants met inclusion criteria. Forty-nine (64%) infants had a surgical procedure and 15 (31%) had ROP progression immediately following surgery with 11 (22%) eventually requiring laser intervention for ROP. One infant at 40 weeks post menstrual age (PMA) had regressing stage 2 prior to surgery and 1 week later had pre-threshold disease and eventually required laser therapy. Median duration of ROP was 6.5 weeks for infants who had surgery compared to 3.0 weeks in those that did not ( $p = 0.03$ ). Infants that required surgery were younger (gestational age 25.2 vs 26.6 weeks) and weighed less at birth (730 g v 930 g) compared to infants who did not have surgery ( $p = 0.01$ ). For infants who had surgery, there was no difference in gestational age or birthweight between those infants who did and did not demonstrate progression of ROP.

**Conclusions** Extremely preterm infants who underwent surgery demonstrated an increased risk for progression of ROP and longer duration of the stage 2 ROP regardless of PMA. We speculate that there are risk factors related to the intra-operative procedure and/or post-operative response that, if identified, may be modifiable resulting in better visual outcomes for these high-risk infants.

**276 EFFECT OF ACUTE INTRAUTERINE ISCHEMIA ON NRF2 AND ANTIOXIDANT DEFENSES IN THE RAT PUP BRAIN**

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10.1136/jim-d-15-00013.276

**Purpose of Study** Hypoxic ischemic encephalopathy causes neurodevelopmental delay or death in up to 6 out of every 1000 live births in the United States. Oxidative stress plays a major role in tissue injury during and after hypoxia/ischemia. Nuclear factor (erythroid derived 2)-like 2 (Nrf2) is a transcription factor that upregulates the expression of antioxidant enzymes such as heme oxygenase-1 (HO-1) and superoxide dismutase (SOD), potentially increasing endogenous defenses against oxidative stress. We hypothesized that the Nrf2 pathway is upregulated in the brains of rat pups following acute *in utero* hypoxic stress.

**Methods Used** Under 2% isoflurane in O<sub>2</sub>, the uterine arteries of pregnant rats at 18 days gestation (E18) were clamped for 45 min to induce fetal hypoxia. Samples of fetal brains were collected after birth to measure SOD, HO-1 and markers of oxidative stress (nitrotyrosine, carbonylated protein). Nuclear Nrf2 levels were also measured in brains 6h after birth. To isolate the effects of surgery itself from isoflurane and O<sub>2</sub>, additional rat pups were studied with simulated anesthesia but no surgical incision.

**Summary of Results** Hypoxic exposure was effective as shown by decreased birth weights and reduced litter sizes. Contrary to our hypothesis, we found no significant differences in signs of oxidative defenses or stress in brains of pups exposed to hypoxia or sham surgery. Instead, however, both groups differed from the untreated controls, suggesting the surgical intervention itself had a greater effect than hypoxia. Compared to controls, hypoxia and sham groups both had 1) no change in nuclear Nrf2 levels, 2) increased HO-1 and SOD levels, 3) decreased nitrotyrosine levels, and 4) no changes in carbonylated protein levels. These findings indicate that any increase in oxidative stress was adequately compensated for by increases in antioxidant enzymes. Rats exposed to 100% O<sub>2</sub> on E18, with or without isoflurane, demonstrated significantly elevated HO-1 and SOD activities 96h after birth.

**Conclusions** Exposure to 100% O<sub>2</sub> during surgery, rather than the surgery, isoflurane, or hypoxic stress, induces upregulation of antioxidant defenses. Future studies should determine the effect of maternal surgery and supplemental O<sub>2</sub> on the Nrf2-antioxidant enzyme pathway.

**277 BENZYL ALCOHOL POISONING RESURFACES IN CHRONICALLY VENTILATED PRETERM LAMBS**

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10.1136/jim-d-15-00013.277

**Purpose of Study** Benzyl alcohol is commonly used as a bacteriostatic vehicle in various formulations. Examples are bacteriostatic sodium chloride and bacteriostatic water for intravenous administration. However, benzyl alcohol poisoning in neonates was reported in the 1980s (Gershanik J, *N Engl J Med* 1982; Menon PA, *Am J Perinatol* 1984; McCloskey SE, *J Pharm Sci* 1986). Human premature neonates who received infusions of formulations with benzyl alcohol developed respiratory distress, deterioration of multiple organ systems, hypotonia, and eventually died

Abstract 277 Table 1

Hours	pH	HCO <sub>3</sub> <sup>-</sup> (mEq/L)	Mean BP (mmHg)	Urine Output (mL/Kg/h)
1–12	7.290±0.081	21.7±1.3	52±3	11.1±5.4
13–24	7.399±0.134	23.9±1.3	47±7	4.4±3.1
25–36	7.267±0.074	22.9±1.4	50±8	4.9±6.0
37–48	7.199±0.129	21.7±3.1	48±8	3.6±3.2
49–60	7.248±0.048	20.0±0.5	34±4	0.9±0.8
61–72	7.018±0.010	18.6±0.1	36±1	0.6±0.1

(Gasping Syndrome). Physiological indices of deterioration included metabolic acidosis, systemic hypotension, and edema. Severity of the syndrome in human and animal neonates is greater with more immaturity. We report occurrence of this syndrome in chronically ventilated premature lambs.

**Methods Used** Six premature lambs were affected (delivered at ~130d gestation, term is ~150d; M:F 2:4; 3.08 ±1.14 Kg birth wt, mean±SD). The lambs were supported by standard methods in our laboratory for chronic ventilation of premature neonates, including antenatal steroids and perinatal surfactant replacement.

**Summary of Results** All lambs developed respiratory distress of prematurity. All lambs developed metabolic acidosis, systemic hypotension, hypotonia, and oliguria (Table 1). Bicarbonate and dopamine were infused to all lambs, beginning at 13–24h of life. Gasping became evident after 24 h. Pleural and pericardial effusions, and ascites were evident at necropsy.

**Conclusions** Benzyl alcohol poisoning occurred in the chronically ventilated preterm lambs. Formulations that contained benzyl alcohol included 30 mL vials of sterile water, sodium heparin, lorazepam, and calcium gluconate. We stopped using these formulations; no further instances of benzyl alcohol toxicity have occurred. Our results provide a reminder that formulations containing benzyl alcohol as a bacteriostatic preservative are detrimental to neonates. HL110002.

**278** **PRETERM BIRTH DECREASES PPAR $\gamma$  PROTEIN ABUNDANCE IN THE ILEUM OF CHRONICALLY VENTILATED PREMATURE LAMBS**

LA Ruybal, L Dong, M Dahl, K Albertine, L Joss-Moore. *University of Utah, Salt Lake City, UT*

10.1136/jim-d-15-00013.278

**Purpose of Study** Premature neonates supported by invasive mechanical ventilation (MV) often have feeding intolerance, and are at increased risk of developing necrotizing enterocolitis (NEC). In contrast, premature neonates supported by non-invasive nasal ventilation feed better and have lower incidence of NEC. Agonists of PPAR $\gamma$ , a nutrient-responsive transcription factor, protect against NEC. A transcriptional target of PPAR $\gamma$  that may also be important in NEC prevention is the histone methyltransferase, Setd8. The effect of preterm birth and mode of

ventilation on PPAR $\gamma$  and Setd8 protein abundance in the ileum is unknown.

We hypothesized that premature lambs supported by invasive ventilation will have lower ileal PPAR $\gamma$  and Setd8 protein abundance compared to ileum of premature lambs supported by non-invasive ventilation or unventilated term-born lambs.

**Methods Used** Premature lambs were managed by invasive MV or non-invasive nasal pulsatile Flow Ventilation® (NPFV) for 21d (term equivalent age). Both groups were fed ewe's colostrum/mature milk. Comparison group was unventilated term-born lambs. Immunoblots were used to measure PPAR $\gamma$  and Setd8 protein abundance in ileal homogenate.

**Summary of Results** Premature lambs supported by invasive MV consumed less colostrum/milk over the 21d study period (24±3% mL/Kg/d) than premature lambs supported by non-invasive NPFV. Compared to term-born lambs, preterm birth with MV or NPFV decreased ileal PPAR $\gamma$  protein abundance (MV: 31.7±0.2%; NPFV 35.2±0.2%). Compared to term-born lambs, preterm birth and invasive MV increased ileal Setd8 protein abundance (204.8 ±1.4%); however, preterm birth and non-invasive NPFV did not alter ileal Setd8 protein abundance.

**Conclusions** Preterm birth decreased ileal PPAR $\gamma$  protein abundance regardless of ventilation mode. Contrary to our hypothesis, protein abundance of Setd8 was increased in the ileum of premature lambs supported by invasive MV. We speculate that increased Setd8 protein abundance may be associated with aberrant histone methylation in ileum of premature lambs managed by invasive MV, and is the subject of further investigation.

## Neonatology Perinatal Biology I

### Concurrent Session

12:30 PM

Friday, January 29, 2016

**279** **EFFECTS OF POST-INJURY DEXAMETHASONE THERAPY ON BRAIN INFARCT SIZE IN A RAT MODEL OF NEONATAL HYPOXIC-ISCHEMIC ENCEPHALOPATHY**

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10.1136/jim-d-15-00013.279

**Purpose of Study** Hypoxic-ischemic encephalopathy (HIE) is the most common cause of neonatal brain damage resulting from asphyxia in the peripartum period and causes significant neurologic sequelae including intellectual disability and cerebral palsy. Currently therapeutic hypothermia is the only accepted treatment in addition to supportive care for infants with HIE. It has been previously shown that pre-insult dexamethasone treatment via intracerebroventricular injection (ICV) in a rat model of neonatal HIE provides neuroprotection and decreases cell death. Therefore, this study examined if post-injury local dexamethasone treatment would provide similar neuroprotective effects.

**Methods Used** A modified Rice-Vannucci model was used with 7 day old rat pups to represent term neonatal HIE.

Pups were subjected to right common carotid artery ligation, followed by hypoxic treatment with 8% FiO<sub>2</sub> for 2 hours. After 2 hours of recovery they were treated with 0.1 μg dexamethasone or normal saline (NS) via ICV injection. 48 hours following the treatment the pups were sacrificed, their brains were sectioned and stained with 2,3,5-triphenyltetrazolium chloride monohydrate (TTC), then photographed and analyzed to determine the overall percentage of brain infarct size.

**Summary of Results** A total of 38 pups were treated with dexamethasone (n=18) and NS (n=20) after the hypoxic-ischemic insult. There was not a significant difference in the pups initial weight (14.4±0.3 g vs 13.9±0.3 g, p>0.05) or weight gain after injury (1.5±0.3 g vs 1.8±0.2 g, p>0.05) between the dexamethasone and control groups respectively. However, there was a significant reduction in the overall percentage of brain infarct size seen 48 hours after insult. The dexamethasone treated pups had an overall infarct percentage of 12.1%±1.7% vs 19.1%±1.7% in the placebo treated pups (p<0.01).

**Conclusions** This study suggests that local cerebral treatment with dexamethasone following hypoxic-ischemic injury can provide neuroprotective effects and significantly decrease local cell death. Future studies are planned to evaluate the mechanism of neuroprotection as well as different delivery methods such as intranasal application for easier non-invasive delivery.

280

#### FETAL LEUCINE INFUSION INCREASES LACTATE CONCENTRATIONS IN LATE GESTATION FETAL SHEEP

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10.1136/jim-d-15-00013.280

**Purpose of Study** Leucine is an essential amino acid with significant contributions to oxidative metabolism and growth. The effect of increased leucine supply on fetal metabolism has not been studied. We aimed to determine effect of an intravenous fetal leucine infusion (Leu) on umbilical and fetal hindlimb blood flow and metabolism.

**Methods Used** The umbilical and hindlimb circulations were catheterized in late gestation fetal lambs. Fetal blood gas, hormone concentrations, umbilical and hindlimb blood flow rates, and fetal and hindlimb net substrate uptake rates were measured at baseline (BL, n=7) and after 4-hr Leu (n=4) and 24-hr Leu (n=4) at ~200 μmol/kg/hr. Time periods were compared using a repeated measures ANOVA.

**Summary of Results** Fetal plasma leucine concentrations increased with Leu (BL: 152.8±7.9; 4-hr: 207.9±20.2; 24-hr: 256.7±14.3 μM, P<0.01). Essential amino acids valine and threonine decreased by 15% and 25%, respectively (P<0.01), and phenylalanine increased by 50% (P<0.01). Fetal arterial blood pH, pCO<sub>2</sub>, plasma IGF-1, and glucose concentrations did not change. Fetal lactate concentration increased (BL: 1.99±0.11; 4-hr: 2.50±0.13; 24-hr: 2.77±0.13 μmol/ml, P<0.01). Insulin increased at 4-hr but returned to BL by 24-hr (BL: 0.22±0.02; 4-hr: 0.31±0.03; 24-hr: 0.15±0.03 ng/ml, P<0.05). Fetal

arterial blood pO<sub>2</sub> decreased by 8% (P<0.01). O<sub>2</sub> content decreased by 35% at 24-hr (P<0.01), however fetal hematocrit similarly decreased, likely the result of insufficient maternal blood transfusion to replace fetal blood removed. Umbilical blood flow did not change, but hindlimb blood flow increased at 24-hr (BL: 14.4±1.8; 24-hr: 18.8±1.9 ml/min/100g hindlimb, P<0.01). Fetal and hindlimb glucose, oxygen, and amino acid uptake rates did not change; however, hindlimb lactate output increased at 24-hr (BL: 2.42±0.69; 4-hr: 2.41±0.42; 24-hr: 4.16±0.84 μmol/min/100g, P<0.01).

**Conclusions** Leu for 4 and 24 hrs, which increased fetal plasma leucine concentrations 35% and 65%, respectively, had minimal effect on fetal substrate and oxygen utilization. Fetal lactate concentrations increased by 24-hr, likely the result of fetal hindlimb production. Further studies are needed to determine if Leu reduced glucose oxidation, thus leading to increased lactate production.

281

#### SKELETAL MUSCLE GROWTH IS RESTRICTED DESPITE MAINTAINED HINDLIMB BLOOD FLOW AND OXYGEN CONSUMPTION IN THE LATE GESTATION IUGR FETUS

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10.1136/jim-d-15-00013.281

**Purpose of Study** Acute fetal hypoxemia redistributes cardiac output to vital organs such as the brain and away from the musculature. In situations characterized by chronic fetal hypoxemia such as IUGR, reduced blood flow to skeletal muscle might decrease muscle growth and contribute to lifelong reductions in muscle mass. Therefore, our objective was to determine the effect of chronic placental insufficiency on hindlimb blood flow, metabolism and growth in the late gestation IUGR fetus.

**Methods Used** The hindlimb was catheterized with aortic and femoral venous sampling catheters in IUGR (n=10) and control (CON, n=10) fetal sheep at 90% gestation. Hindlimb blood flow was measured using an ultrasonic transducer and normalized to 100g hindlimb weight. Fetal hindlimb blood flow, substrate uptake rates and skeletal muscle weights were compared between CON and IUGR groups.

**Summary of Results** IUGR fetal lambs weighed 34% less and had lower arterial blood oxygen (-39%), plasma glucose (-33%), insulin (-50%), and IGF1 (-55%) concentrations with higher lactate concentrations (34%) compared to CON (P<0.05). IUGR fetal hindlimb weight relative to fetal weight was 10% lower (P<0.005) and hindlimb skeletal muscle weights normalized to lower extremity limb length were ~35% lower (P<0.01). Hindlimb blood flow was maintained in IUGR (CON 14.8±1.4, IUGR 18.9±1.8 ml/min/100g; P=0.09). Despite lower fetal glucose and oxygen concentrations, the hindlimb uptake rates for glucose (CON 2.7±0.2, IUGR 2.4±0.3 μmol/min/100g) and oxygen (CON 14.7±1.3, IUGR 12.1±1.1 μmol/min/100g) were similar in IUGR and CON and hindlimb lactate output rates tended to be higher (CON 1.5±0.4, IUGR 2.3±0.3 μmol/min/100g; P=0.07).

**Conclusions** Despite lower glucose and oxygen concentrations, glucose and oxygen uptake rates are maintained indicating increased extraction efficiency by hindlimb muscle in the IUGR fetus. These results support previous findings of maintained whole-body glucose uptake and increased skeletal muscle Glut-1 transporter expression. We speculate that skeletal muscle adapts to chronic hypoxemia by maintaining oxygen metabolism to support basal energy requirements, possibly by increased glycolysis and lactate production, at the expense of skeletal muscle growth.

## 282 DEVELOPMENTAL CHANGES IN ANTIOXIDANT DEFENSES IN THE PERINATAL BRAIN

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10.1136/jim-d-15-00013.282

**Purpose of Study** Approximately 6 out of every 1000 newborns are affected by perinatal cerebral hypoxia/ischemia. Tissue hypoxia/ischemia results in a great increase in reactive oxygen species that overwhelms antioxidant defenses and leads to tissue damage. Endogenous antioxidant defenses include: 1) heme-oxygenase 1 (HO-1), 2) superoxide dismutase (SOD), and 3) glutathione (GSH). A determination of the role of these enzymes in perinatal hypoxic/ischemic injury first requires a more complete understanding of their role in the transition from fetus to newborn, when brain tissue oxygen concentrations increase by more than four-fold. Therefore, the purpose of this study was to characterize the levels of these antioxidant defenses during the perinatal period.

**Methods Used** We collected brain and liver samples from rat pups at embryonic days E19 and E21, and postnatal hours P6h, P48h, and P96h. We also took adult samples for comparison. Samples were homogenized by mincing in ice-cold lysis buffer. Finally, we measured concentrations of HO-1 (by ELISA) and GSH (by colorimetric metabolite assay), and activity of SOD using kit assays (Sigma-Aldrich). Brain and liver samples were also assayed for total antioxidant capacity.

**Summary of Results** Within 6 hours after birth, we measured a significant increase (~40%) in brain HO-1 levels. SOD activity was increased significantly at E21 compared to E19 and remained elevated until P96h when concentrations were lower than all other timepoints. There were no significant changes in GSH concentrations at any time point. Total antioxidant capacity of the brain tissue decreased significantly within 6 hours after birth and remained low for at least 96 hours. Liver total antioxidant capacity also decreased significantly within 6 hours after birth.

**Conclusions** These results indicate there is a perinatal upregulation of the antioxidant enzymes HO-1 and SOD in the brain, but a decrease in total antioxidant capacity. We speculate that the decrease in total antioxidant capacity may be due to increased consumption of small molecule antioxidants, other than GSH, associated with the rapid increase in brain tissue PO<sub>2</sub> that occurs at birth. Future studies will compare brain tissue to other organs, and measure levels of other specific antioxidants.

## 283 HYDROCORTISONE PROTECTS THE BRAIN FROM HYPOXIC-ISCHEMIC INJURY IN NEONATAL RATS

KR Salcedo-Concepcion, Y Li, L Zhang. Loma Linda University SOM, Loma Linda, CA

10.1136/jim-d-15-00013.283

**Purpose of Study** Hypoxic-ischemic encephalopathy (HIE) is a major cause of neonatal disability and mortality. Infants that acquire HIE are at risk for developing neuronal diseases such as severe cerebral palsy, mental retardation, seizures, and other neurodevelopmental disabilities. HIE brain damage is largely due to perinatal asphyxia and hypoxia prior to, during or after birth. Inflammation has been shown to play a critical role in neonatal brain damage and is an important contributor to the pathogenic cascade. Inflammation can both sensitize, as seen in intrauterine infections, and participate in the injury response to a hypoxic insult. In the present study, we modified a Rice-Vannucci model in rat pups to better understand the consequences of inflammation and hypoxic-ischemic (HI) brain injury and possible therapeutic interventions. Previous studies have shown that pretreatment with dexamethasone, an anti-inflammatory steroid medication, in neonatal HI brain injury demonstrates a neuroprotective effect and decreases HI-induced infarct size. Because dexamethasone may have detrimental side effects, we explored the potential therapeutic use of hydrocortisone. The aim of this project was to develop a model to study the post-HI treatment effects of hydrocortisone.

**Methods Used** We used a modified Rice-Vannucci model in rat pups.

**Summary of Results** Our results demonstrated that rat pups treated with hydrocortisone post the HI insult decreased brain infarct size. We further demonstrated that LPS treatment prior to the HI insult significantly increased HI-induced brain infarction in neonatal rats. Of importance, our study revealed that intracerebroventricular injection of hydrocortisone 4 hours after the HI insult significantly reduced brain infarction in the pups that received LPS. We also tested whether intranasal delivery of hydrocortisone had an effect in decreasing brain infarct size. Although our initial result showed that post-HI intranasal delivery of hydrocortisone did not have a significant effect, this was likely due to dose being too low.

**Conclusions** Ongoing studies are to increase the doses of intranasal delivery of hydrocortisone. Our results suggest a potential therapeutic effect of hydrocortisone for HIE in neonates. Future studies are needed to investigate the mechanisms by which hydrocortisone acts in neuroprotection in the developing brain.

## 284 MATERNAL TOBACCO SMOKE EXPOSURE IMPAIRS PLACENTAL FUNCTION AND INDUCES SEX-SPECIFIC ALTERATIONS IN FETAL FATTY ACIDS

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10.1136/jim-d-15-00013.284

**Purpose of Study** Maternal tobacco smoke (MTS) exposure sex-specifically programs adult-onset disease in humans

and rat models. The programming of many adult-diseases involves alterations in circulating fatty acid (FA) levels in the offspring. Maternal-fetal FA transfer is regulated by placental FA transporters. We previously showed that MTS exposure sex-specifically alters FA transporter expression in the rat. However, the effect of MTS on rat placental insufficiency, placental histology and fetal FA levels is unknown. We hypothesize that MTS exposure causes placental insufficiency, changes in placental histology, and sex-specific changes circulating fetal FA levels in the rat.

**Methods Used** Pregnant rats were exposed to tobacco smoke (MTS) or room air (Control) from E11 to term (E21). Fetal and placental weights were measured at birth. Umbilical arterial blood flow was measured via Doppler ultrasound on E15 and E21. Placental sections were used for histological examination. Fetal serum FA levels were measured using direct transesterification and gas chromatography.

**Summary of Results** MTS decreased placental and pup weight of female and male offspring compared to sex-matched control. MTS reduced umbilical arterial flow at E15 and E21 in female, and male placenta compared to sex-matched controls. Female and male MTS placentas had smaller labyrinth layer size, retention of glycogen-rich cells and the presence of larger, immature appearing, trophoblastic giant cells. MTS did not significantly affect circulating fetal FA levels in female rat pups. However, MTS altered circulating fetal FA levels in male rat pups (decreased palmitoleic, oleic and docosapentaenoic acid, and increased docosatetraenoic acid).

**Conclusions** MTS exposure causes placental insufficiency, and changes in placental histology in female and male rat pups. Despite similar placental changes in both female and male rat pups, MTS sex-specifically alters circulating fetal FA levels in rat pups. We speculate that previously observed sex-specific differences in FA transporter expression may reflect sex-specific alterations in transcriptional regulation of transporters.

285

#### EPITHELIAL MEMBRANE PROTEIN 2 (EMP2) DEFICIENCY ALTERS PLACENTAL ANGIOGENESIS MIMICKING FEATURES OF INTRAUTERINE GROWTH RESTRICTION

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10.1136/jim-d-15-00013.285

**Purpose of Study** Intrauterine growth restriction (IUGR) is a poorly understood complication of pregnancy, affecting up to 10% of pregnancies, and is associated with disordered placentation. The objective of our study is to understand the role of epithelial membrane protein-2 (EMP2) in human placentation and IUGR.

**Methods Used** 1. Human placentas from normal and IUGR-associated pregnancies were obtained. Protein lysates for immunoblot analysis or whole sections fixed for immunohistochemistry were obtained. Staining was performed for EMP2, CD34 for endothelial cells, HIF1a, and *Dolichos biflorus* agglutinin (DBA) for uNK cells.

2. Cell culture using human choriocarcinoma cells were created to overexpress an EMP2-GFP fusion protein or knock out EMP2 expression via a specific shRNA. Cell migration and capillary tube formation assays were conducted. mRNA and supernatant was isolated from cells for VEGF PCR and ELISA.

3. EMP2-null mice were generated using a conditional knockout allele for the *Emp2* gene. Mice were bred; litters counted and weighed at birth. Uterine tissues were also obtained at E9.5, E13.5, and E16.5, sectioned, and stained for EMP2, cytokeratin (trophoblasts), tomato lectin and CD34 (vascular structures), and DBA.

**Summary of Results** EMP2 is expressed in placental villi and extravillous trophoblast cells, and expression is significantly reduced in human IUGR placentas compared to normal pregnancies. In cultured human choriocarcinoma cells, EMP2 levels produce a reciprocal regulation in HIF1 $\alpha$  and VEGF, sufficient to induce HUVEC tube formation. EMP2-null mice demonstrate reduced litter sizes and placentas are hypovascular, with increased fibrin deposition, and increased number and persistence of uNK cells.

**Conclusions** This study defines an important relationship between angiogenesis and uNK regulation at the maternal-fetal interface via the tetraspan protein epithelial membrane protein-2 (EMP2). We provide the first evidence of altered EMP2 expression in human placentas with IUGR. Functional studies using cell lines suggest that EMP2 regulates HIF1a and VEGF levels within these cell populations. Deletion of EMP2 in mice reduces litter size by 20%, and also results in changes in placental vascularity, potentially via altered NK cell presence early in gestation.

286

#### EFFECT OF PERINATAL DEVELOPMENT ON NRF2 AND ANTIOXIDANT DEFENSES IN THE RAT PUP BRAIN

T Nguyen,<sup>1</sup> E Calma,<sup>1</sup> L Kim,<sup>1</sup> M Zhang,<sup>1</sup> T Liu,<sup>1</sup> G Power,<sup>2</sup> AB Blood<sup>2</sup>. <sup>1</sup>Loma Linda University, Loma Linda, CA; <sup>2</sup>Loma Linda University, Loma Linda, CA

10.1136/jim-d-15-00013.286

**Purpose of Study** At birth infants are exposed to a rapid 4 to 5-fold increase in arterial and tissue oxygen tensions and the increase may be greater yet in infants who receive supplemental oxygen. Thus there is risk of oxidative stress, but little is known about how antioxidant defenses change during the transition from fetus to newborn. Many antioxidant enzymes are under the influence of a transcription factor called nuclear erythroid 2-related factor (Nrf2). Currently, Nrf2 signaling in the regulation of antioxidant capacity in the fetal and newborn brain has not been studied. This research was undertaken to characterize the changes in Nrf2 and antioxidant capacity in the brain before and after birth.

**Methods Used** Perfused rat pup brains were collected at fetal days E19 and E21, and postnatal hours P6h, P48h and P96h. Samples were homogenized in ice-cold lysis buffer. Western blot analysis was used to determine nuclear and cytosolic Nrf2 protein concentrations. Total antioxidant capacity, a measure of both small molecule and enzyme-mediated antioxidants, was determined in whole

brain homogenates from each of the time points above. Nitrotyrosine and carbonylated proteins, both byproducts of oxidative stress, were also determined in whole brain homogenates.

**Summary of Results** Nrf2 levels in the nucleus increased with development from fetus to newborn with concentrations at P96h significantly greater than E19, E21 and P6h. There were no significant changes in carbonylated protein levels during development, but nitrotyrosine concentrations were significantly decreased at P48h and P96h compared to E21. Total antioxidant capacity decreased significantly within 6 hours after birth and remained low for at least 96 hours.

**Conclusions** Results showed pronounced increase in tissue PO<sub>2</sub> at birth is not associated with an increase in tissue oxidative stress. The decrease in total antioxidant capacity may reflect the consumption of small molecule antioxidants necessary for prevention of oxidative stress at birth. The increase in nuclear Nrf2 levels suggests an upregulation of antioxidant enzyme transcription takes place following birth. Future studies will examine corresponding levels of specific Nrf2 target enzymes and responses to hypoxic/ishemic stress.

## Surgery II Concurrent Session 12:30 PM Friday, January 29, 2016

### 287 ENHANCED RECOVERY IN PLASTIC SURGERY: A REVIEW OF THE LITERATURE AND A PROPOSAL OF ACTIONABLE MEASURES

CV Vu, S Gupta. Loma Linda University, Westminster, CA

10.1136/jim-d-15-00013.287

**Purpose of Study** In 2007, the World Health Organization (WHO) introduced a 19-item safe-surgery checklist that when implemented in general, non-cardiac surgeries saw striking reductions in morbidity and mortality. Yet its effectiveness in many surgical sub-specialties was less definitive, suggesting a need for evidence-based interventions that target the entire surgical experience. Coincidentally, many surgical specialties have sought to achieve enhanced recovery after surgery (ERAS) for patients through evidence-based changes in perioperative care that aim to reduce surgical stress and hasten recovery. The purpose of this study was to explore the effectiveness of ERAS protocol across multiple specialties and to draft a preliminary evidence-based ERAS protocol for patients in plastic surgery.

**Abstract 287 Table 1** Statistically significant outcomes upon ERAS application in 11 studies

	Length of Stay	Complications	Readmits	Cost
Improved	11	3	1	1
Not Improved/reported	0	8	10	10
Total	11	11	11	11

### Abstract 287 Table 2 Proposed ERAS pathway for plastic surgery

Preoperative	Intraoperative	Postoperative
-Pre-admission patient counseling & education	-Opioid-sparing anesthetic regimen	-Postoperative warming
-Minimize starvation/dehydration	-Best surgical techniques	-Early mobilization
-DVT and antibiotic prophylaxis	-Euvolemia	-Wound care
-Preoperative warming	-Antiemetics	-Early resumption of oral hydration and diet
-Multimodal prophylactic analgesia	-Minimize drains	-Multimodal analgesia with opioids for breakthrough pain
	-Intraoperative warming	

**Methods Used** An analysis of 11 ERAS pathways across 10 surgical specialties described in peer-reviewed literature was conducted to identify core multimodal components of ERAS protocols and to assess the effectiveness of these pathways in achieving four endpoints: reductions in stay, decreased post-surgical morbidity, and decreased costs.

**Summary of Results** Table 1 and Table 2.

**Conclusions** Integration of ERAS protocols yields definitive reductions in hospital stay. However, current pathways fail to reliably improve morbidity and readmits. The emotional toll exerted on patients by complications and readmissions, along with the burdens of added costs, oblige a need for fine-tuning ERAS pathways to address all aspects of perioperative care to minimize surgical stress and wound-related complications. A call to action in the field of plastic surgery, where an optimal ERAS protocol has yet to be developed, is appropriate and necessary. Development of an ERAS, in conjunction with a best practices approach, is crucial in the face of changing healthcare system where cost-containment and optimizing patient outcomes are more important than ever.

### 288 GROWING DISPARITY IN COLLECTION RATES: THE EFFECT OF THE AFFORDABLE CARE ACT IN PLASTIC SURGERY

I Campwala, N Biskup, S Motakef, S Gupta. Loma Linda University, Loma Linda, CA

10.1136/jim-d-15-00013.288

**Purpose of Study** On March 23, 2010, the Patient Protection and Affordable Care Act (ACA) was signed into law. It required all Americans to have health insurance by 2014 or pay a monthly fine. The uninsured rate has remained 13.4% through the second and third quarter of 2014. The previous low point was 14.4% in the third quarter of 2008. As of September 2014, the ACA had increased the number of insured by 9 million. By 2019, the number of uninsured is expected to go from the current 57 million to 23 million. There have been no studies that detail the effects of the ACA on providers' revenue. Previous to the ACA, our eight-plastic-surgeon practice had seen approximately 14–16% of patients uninsured. In a preliminary study, we found that the ACA has led to a significant decrease in the percentage of self-pay plastic surgery patients by an average of 64.22%. Theoretically,

**Abstract 288 Table 1**

Months	IEHP Charge Capture	All Other Charge Capture	P-Values
Jan 2013-Jun 2013	11.07%	25.95%	0.001114
Jul 2013-Dec 2013	13.66%	26.65%	0.0003675
Jan 2014-Jun 2014	14.52%	27.77%	7.89E-06
Jul 2014-Dec 2014	15.17%	29.24%	3.69E-05

this decrease should have increased revenue. This study seeks to quantitatively analyze the difference in revenue for our plastic surgery service over the time period impacted by the ACA and the Medicaid Expansion.

**Methods Used** Plastic surgery billing information for January 2013 to December 2014 (before and after the start of the ACA) was collected. The collection rates of the local managed Medicaid ACA provider, IEHP, and the total of all other insurance payors were calculated by dividing matched payments by charges. Collection rates were compared side-by-side in 6-month intervals using 1-tailed paired t-tests.

**Summary of Results** Table 1 contrasts IEHP collection rates and other payors. This study revealed that IEHP collection rates are significantly lower than that of all other insurance payments (p-value<0.05).

**Conclusions** The ACA has decreased the number of uninsured patients, but the growing division between the collection rates of Medicaid and other insurances will ultimately cause provider bias and changes in the finances of plastic surgery practices and healthcare services as a whole.

289

**LACK OF INPUT SPECIFICITY, GREAT OUTCOME VARIABILITY, AND IMPRECISE RISK CALCULATIONS: PITFALLS OF THE ACS-NSQIP RISK CALCULATOR IN PLASTIC SURGERY**

C Johnson, I Campwala, S Gupta. *Loma Linda University, Loma Linda, CA*

10.1136/jim-d-15-00013.289

**Purpose of Study** Surgeons have internally measured and monitored morbidity and mortality as metrics of quality for over 90 years. American College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP) created the Surgical Risk Calculator to allow a risk-adjusted 30-day surgical outcome prediction. While ACS-NSQIP offers the only multidisciplinary surgical care predictor, it has not been validated in plastic surgery.

**Methods Used** A retrospective analysis of all plastic surgery intradepartmental complications from a review of a quality assurance database from September 2013 through July 2015 was performed. Preoperative risk factors were entered into the Surgical Risk Calculator, and predicted outcomes were compared to actual morbidities. The difference in average predicted complication rate versus the actual rate of complication was examined to assess the validity of the calculator in plastic surgery.

**Summary of Results** Within the study population of patients with complications (n=104), the calculator

**Abstract 289 Table 1 Results**

Outcomes	Correctly Predicted Complications	Average Predicted Risk in Study Population (n=104)	Actual Incidence in Practice (n=4924)
Serious Complication	14 (20.9)	6.3%	67 (1.4)
Any Complication	73 (78.5)	8.7%	93 (1.9)
Surgical Site Infection	10 (24.4)	3.3%	10 (0.2)
Venous Thromboembolism	0	0.4%	1 (0.0)
Return to OR	14 (24.1)	4.9%	58 (1.2)

accurately predicted an above average risk for 20.9% of serious complications. For surgical site infections, the average predicted risk for the study population was 3.3%; this prediction was proven only 24.4% accurate. The actual incidence of any complication in our plastic surgery practice from September 2013 through June 2015 was 1.89%.

**Conclusions** The most common complications in plastic surgery include seroma, hematoma, dehiscence, and flap-related complications. The ACS Risk Calculator does not present rates for these risks. The calculator's surgery input does not include many plastic surgery-specific procedures, which induces great variability to risk predictions. The difference in predicted versus actual complication rates indicates that this tool does not accurately predict outcomes in plastic surgery. To facilitate adequate patient care, further research is needed to develop accurate risk stratification tools in plastic surgery.

290

**PRELIMINARY CLINICAL EXPERIENCE WITH DEOXYCHOLIC ACID IN CONTOURING SUBMENTAL FAT**

DH Lee, J Chidester, S Gupta. *Loma Linda University, Loma Linda, CA*

10.1136/jim-d-15-00013.290

**Purpose of Study** "Tech Neck" is defined here as a maldistribution of submental fat, increased neck skin laxity, and loss of a youthful neck contour after repetitive downward screen viewing. With the addition of deoxycholic acid to a surgeon's armamentarium, we now have the ability to non-surgically subtract as well as add volume from the whole face aesthetic, offering patients dynamic control over facial harmony with rapid recovery and minimal down time. We present our pilot experience using deoxycholic acid and offer a few technical pearls for whole face volume manipulation.

**Methods Used** Three female patients were injected with deoxycholic acid [ATX-101, Kybella (Kythera Biopharmaceuticals Inc., CA, USA)] in the submental region. Immediately before injection, each patient's whole neck aesthetics was assessed using the Cervicofacial Classification Scale (min-max: 0-4). Anatomic boundaries were marked using sternocleidomastoid muscles laterally,

**Abstract 290 Table 1** Patient Demographics

Patient	Age	Cervicomeatal Classification Scale Score	Dose in mL (10 mg / 1 mL)	Pain Score	Adjunctive Treatments
1	48	2.25	3.4	3	Cheek volume augmentation*
2	39	1.75	3.6	2	Fat grafting**
3	41	2.75	5.6	4	None

\*Patient 1 underwent treatment with Juvéderm Voluma XC (Allergan, CA USA) in the malar region three weeks prior to injection.

\*\*Patient 2 underwent autologous fat grafting to her mid-face five months prior to injection.

the hyoid bone inferiorly, and the inferior border of the mandible superiorly. Deoxycholic acid was then administered in 0.2 mL aliquots (10 mg/1 mL) into the preplatysmal fat. After completion of injection, a compression wrap was placed and removed the following day.

**Summary of Results** Patient demographics are summarized in Table 1. Pain was reported on a 10-point pain verbal descriptor scale. One patient developed a 60-minute transient left-sided marginal mandibular nerve paresis following lidocaine injection. Three days post-procedure, all patients denied any complications and were satisfied with the overall experience.

**Conclusions** Deoxycholic acid offers a novel, minimally invasive approach to volume manipulation of the submental region. Reducing pre-platysmal fat will allow plastic surgeons to extend facial contouring to include the suprahyoid neck.

291

### MANAGEMENT AND OUTCOMES OF INCIDENTAL DONOR DERIVED PULMONARY EMBOLISM

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10.1136/jim-d-15-00013.291

**Purpose of Study** Pulmonary embolism (PE) may go undetected in donor lungs used for lung transplantation (LT). Approximately 30% of donor lungs may have incidental PE. PE might be associated with either early graft failure or late complications in LT recipients. This report reviews our experience with detection of significant incidental donor PEs and their treatment.

**Methods Used** This is a single center retrospective cohort study of LTs performed between 9/2012–7/2015. Our standard practice for lung recovery and preservation included in-vivo 3 liters (L) antegrade flush with cold low-potassium dextran preservative followed by a 2L retrograde flush after cardiectomy. In 2012 we added an additional 2L *ex-vivo* retrograde flush prior to implantation. We defined significant PE as either large single and/or heavy embolus burden.

**Summary of Results** In 115 double LTs performed, 4 of the donor lungs had significant PE (incidence: 3.5%). These 4 had median intubation time of 4 days (range: 3–6). After PE/s were noted, 3/4 lungs were flushed retrograde with an additional 1–4L preservative *ex-vivo* beyond

the standard 2L to clear the emboli. The 1st pair of lungs had fat emboli; therefore, the recipient was not anticoagulated. The remaining 3 received heparin infusion post-LT and had a CT scan with PE protocol 2 weeks later. 1 of these 3 had a lower lobe (RLL) infarct requiring a necrosectomy 3 months later; this pair was not flushed *ex-vivo* beyond the 2L standard. The 2nd underwent an *ex-vivo* donor lobectomy prophylactically of the lobe with the most PEs and this subject showed a persistent RUL PE for which anticoagulation was continued; and the 3rd, with the largest volume of flush, showed no evidence of PE at 2 weeks. Due to fluctuating PaO<sub>2</sub>/FIO<sub>2</sub> ratio we elected to support the latter 2 on ECMO. Median length of stay post-LT was 16 days (range: 12–30). All patients survived to discharge.

**Conclusions** While the incidence of significant PE in donor lungs in our study was low, the short and mid term effects were detrimental. Our cohort supports the use of additional flush *ex-vivo*. If PE is detected during flush a post-LT CT scan to look for PE and anticoagulation should be considered. These changes may improve outcomes in LT. Institutions may need to adopt new strategies for donor lung management to successfully identify and treat incidental donor PE.

292

### A RETROSPECTIVE STUDY ON DEMOGRAPHIC, CLINICAL, AND OUTCOME DATA OF WOMEN UNDERGOING SACROSPINOUS LIGAMENT FIXATION

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10.1136/jim-d-15-00013.292

**Purpose of Study** Pelvic organ prolapse (POP) occurs when muscles and ligaments of the pelvic cavity weaken. POP affects 40% of women over the age of 40, and 200,000 women annually receive some form of POP surgery. We evaluated differences in demographics, clinical characteristics, and intraoperative and short-term post-operative outcomes for patients with sacrospinous ligament fixation surgeries (SSLF) versus any other POP surgery.

**Methods Used** A retrospective chart review was performed among 31 patients at Banner University Medical Center who required surgical treatment for POP from 2013 to 2014. Patients were divided into two groups: SSLF surgery and any other POP surgery. Data was obtained from patient charts in Epic electronic medical records.

**Summary of Results** Patients were categorized into 2 groups: SSLF procedure group (n=17) and other POP surgery group (n=14). Women with SSLF procedures were on average older (68.2 years old versus 60.4 years old) and more likely to be smokers (17.6% versus 7.1%) and diabetics (29.4% versus 0%) compared to the non-SSLF group. Among intraoperative outcomes, the group without SSLF surgery had a higher proportion of vaginal hysterectomy (50.0% versus 41.2%) and urethral sling incontinence procedure (78.6% versus 23.5%). However, the SSLF surgery group had a higher proportion of anterior repairs (64.7% versus 57.1%), posterior repairs (70.6% versus 42.9%), and vaginal apex suspensions (100.0% versus 35.7%). The largest difference was observed between the estimated blood loss in individuals with SSLF surgery and

those without SSLF surgery ( $259.7 \pm 269.6$  mL versus  $84.6 \pm 75.3$  mL). Finally, for short-term outcomes data, the patients without SSLF surgery had a shorter post-operative stay ( $1.3 \pm 0.9$  days versus  $2.1 \pm 2.3$  days) and shorter catheter draining days ( $1.6 \pm 1.4$  days versus  $4.7 \pm 6.8$  days).

**Conclusions** The short-term data indicated a longer post-operative length of stay, greater number of catheter draining days, and greater blood loss in those women who underwent SSLF surgery compared to those who underwent other POP surgeries. Therefore, more data needs to be obtained to verify these results for future POP surgical management.

293

### DO CONVERSION TOTAL HIP ARTHROPLASTY YIELD COMPARABLE RESULTS TO PRIMARY TOTAL HIP ARTHROPLASTY?

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10.1136/jim-d-15-00013.293

**Purpose of Study** Increasing hip fracture incidence in the United States is leading to higher occurrences of total hip arthroplasty (THA) including primary THA, revision THA, and conversion THA for failed ORIF of the hip joint. In spite of studies showing higher complication rates in conversion THA, the Centers for Medicare & Medicaid services currently bundle conversion and primary THA under the same diagnosis related grouping (DRG). DRG groupings are used to determine reimbursements and performance evaluations. We compared the cost of treatment, implant characteristics, and perioperative characteristics of conversion and primary THA. The purpose of this study is to determine the degree of difference between conversion and primary THA. Our hypothesis is that conversion THA will have higher cost and resource utilization than primary THA.

**Methods Used** Fifty-one consecutive conversion THA patients (CPT 27132) and 105 matched primary THA patients (CPT 27130) were included in this study. Perioperative and implant characteristics for conversion THA and primary THA were compared using regression, chi-square, and ANOVA analysis. The natural log transformed costs for conversion and primary THA were compared using regression analysis. Conversion THA subgroups formed based on etiology were compared using ANOVA analysis. Age, gender, BMI, ASA, Charlson comorbidity score, and smoker status were controlled in the analysis.

**Summary of Results** For perioperative and implant characteristics, Conversion THA were determined to be significantly different ( $p < 0.05$ ) in length of stay (LOS) (0.999 days longer), operative time (34.9 minutes longer), and odds of requiring revision type hardware (at least 5 times more likely). Conversion THA were also determined to be significantly different and greater than primary THA in the following costs: hospital operating direct cost (29.2% greater), hospital operating total cost (28.8% greater), direct hospital cost (24.7% greater), and total hospital cost (26.4% greater).

**Conclusions** Based on greater LOS, operative time, odds of requiring revision type hardware, and cost of treatment, conversion THAs have greater cost and resource utilization than primary THAs. In order to prevent disincentives for treating these complex surgical patients, reclassification of conversion THA is needed as they do not fit together with primary THA.

294

### CAPITONNAGE FOR SURGICAL TREATMENT OF PULMONARY CYSTIC ECHINOCOCCOSIS: SYSTEMATIC REVIEW AND META-ANALYSIS

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10.1136/jim-d-15-00013.294

**Purpose of Study** Cystic echinococcosis is a neglected zoonotic disease that affects over one million people in predominately rural populations of underdeveloped countries in Africa, South America, Southwest Asia, and Central Asia. Surgical management is most common for cysts in the lungs, but there is controversy surrounding which techniques are preferred. Capitonage is a widely used but criticized technique for closure of the residual cavity in lung parenchyma after pulmonary cyst removal. This review evaluated available literature for the efficacy of capitonage.

**Methods Used** PubMed and PubMed Clinical Queries were searched for studies reporting the use of capitonage, either alone or head-to-head with non-capitonage, for the treatment of pulmonary cystic echinococcosis. Case series, cohort studies, and randomized or non-randomized clinical trials were considered.

**Summary of Results** Sixteen studies published between 1999–2014 detailing surgical management of 2221 patients met criteria for inclusion. The studies included one randomized clinical trial, eleven retrospective cohort studies, three case series, and one prospective cohort study. Four studies restricted their patient population to pediatrics, two to adults, and the remainder were a mix of children and adults. Twelve studies compared capitonage head-to-head with non-capitonage. Outcomes considered were duration of hospitalization (10), morbidity (15), and prolonged air leak (15). Most studies found capitonage to be superior (9), some found there to be no advantage (5), and a few offered no opinion (2). An evaluation of head-to-head studies found capitonage superior to non-capitonage for prolonged air leak (odds ratio=0.27, 95% CI, 0.18–0.39,  $P < 0.0001$ ,  $n=2016$ ), and total morbidity (odds ratio=0.42, 95% CI, 0.32–0.55,  $P < 0.0001$ ,  $n=1818$ ).

**Conclusions** This meta-analysis of available evidence suggests that post-operative outcomes associated with capitonage are as good or superior to those associated with non-capitonage in most patients. There may be specific patient factors or long-term outcomes that favor non-capitonage over capitonage, but thus far they have not been elucidated. For now, this meta-analysis is the most up to date and comprehensive review of this question of which we are aware.

Behavior and Development  
Concurrent Session  
3:30 PM  
Friday, January 29, 2016

295 CAPSASSOCIATIONS AMONG MOTHER-TODDLER  
PLAY AND DEVELOPMENT IN PRETERM TODDLERS

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10.1136/jim-d-15-00013.295

**Purpose of Study** Preterm children are at risk for cognitive, language, and self-regulation delays. Responsive, non-controlling parenting fosters the development of self-regulation, cognition, and behavior. We investigated the associations between parenting quality and neurocognitive outcomes in English and Spanish speaking preterm and term toddlers.

**Methods Used** This study included 50 term and 49 preterm toddlers (18–24 months) and their mothers. Participants were English (n=75) and Spanish (n=24) speaking. Parental behavior was coded for videotaped play using the Dyadic Parent-child Coding System that measures parent's behaviors including commands, questions, praise, and statements. The Bayley Scales-III measured child cognitive and language abilities and the Snack Delay tested child impulsivity.

**Summary of Results** For preterm children, increased use of indirect commands (p=0.012) and unlabeled praise (p=0.036) positively correlated with cognitive scores. Increased use of unlabeled praise was positively correlated with the snack delay (p<0.001), and increased use of descriptive questions was positively correlated with the snack delay (p<0.001). For the term children, increased use of descriptive questions was also positively correlated with the snack delay (p=0.026). Increased use of direct commands (p=0.045), descriptive questions (p=0.007), and reflective statements (p=0.014) positively correlated with language scores. Increased use of descriptive questions was positively correlated with cognitive scores (p=0.016). Finally, increased use of information questions positively correlated with the snack delay (p=0.047). For English speaking children, descriptive questions (p=0.028) and reflective statements (p=0.027) positively correlated with language scores; and descriptive questions (p=0.003) positively correlated with cognitive scores. Descriptive questions (p=0.037) and information questions (p=0.048) positively correlated with the snack delay.

**Conclusions** Different types of parent verbal behaviors correlated with cognition, language, and impulsivity for preterm compared to term toddlers. Parent behaviors correlated with testing scores only for English speaking children, due to small Spanish speaking subsample. This has

implications for parental education in early intervention programs.

296 EXPLORING SEX DIFFERENCES IN AUTISM  
SPECTRUM DISORDERS USING THE CHARGE STUDY

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10.1136/jim-d-15-00013.296

**Purpose of Study** Autism spectrum disorders are much more prevalent in males when compared to females. In 2010, the CDC estimated that males with autism outnumbered females with the disorder 4.5 times to 1. Many theories have emerged as to why this is, including the protective effect of the additional X chromosome in females, the potential deleterious effect of the Y chromosome in males, hormone mediated pathways that are influenced by the environment, among many others. A recent review article found that females with autism are more likely to have intellectual disability and seizure disorders. Males may exhibit more severe restricted/repetitive behaviors. Understanding the phenotypic differences between males and females may allow insight into the etiology of autism spectrum disorders. Once this is better understood, sex specific diagnostic and treatment pathways may be developed.

**Methods Used** The Childhood Autism Risks from Genetics and the Environment (CHARGE) study is a population-based case-control study that is ongoing at the UC Davis MIND (Medical Investigations of Neurodevelopmental Disorders) Institute. Starting in 2003, participants with autism spectrum disorders and developmental delay were recruited to participate. Age and location matched typically developing controls are then selected at random. Children must be age 2–5 years, have been born in California, speak English or Spanish and live with at least one biological parent. The visit includes a comprehensive medical history, physical exam, epidemiologic information regarding in utero and early life exposures, blood draw, parental assessment of the child's behavior and adaptive skills, as well as developmental functioning and autism severity (if applicable).

Males and females with autism from the CHARGE study will be compared to examine differences in development (Mullen Scales of Early Learning), behavior (Aberrant Behavior Checklist and Vineland Adaptive Behavior Scales) and autism severity (Autism Diagnostic Observation Schedule). I plan to correct for age, socioeconomic status, time in treatment and cognitive functioning (for ADOS severity score).

**Summary of Results** Preliminary results will be presented at the 2016 meeting.

**Conclusions** Preliminary results will be presented at the 2016 meeting.

297

### WHAT MIGHT EXPLAIN SOCIAL IMPAIRMENTS IN CHILDREN WITH CHROMOSOME 22Q11.2 DELETION SYNDROME?

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10.1136/jim-d-15-00013.297

**Purpose of Study** Autism spectrum disorders (ASD) are frequently reported in children with chromosome 22q11.2 deletion syndrome (22q), although no studies have used gold-standard evaluations to diagnose ASD. It is unclear if the social impairments in 22q are better explained by underlying cognitive challenges. This study investigates whether specific cognitive abilities are related to the social impairments in 22q that frequently lead to ASD diagnoses. **Methods Used** We performed a retrospective analysis of relevant data from 114 children, collected as part of a study designed to study the neurocognitive bases of spatio-temporal impairments in children ages 7–14 with 22q. The Social Communication Questionnaire (SCQ), a screening tool for autism, was used to quantify social impairments. These were related to subtest scores from the Wechsler Intelligence Test for Children, 4th edition (WISC-IV) to test our hypothesis that conceptual and linguistic delays contribute strongly to social impairments. Pearson's correlation coefficient was calculated to determine the strength of relationship between variables.

**Summary of Results** Mean age was 11.2±2.5 years. 50% were male. SCQ score correlated negatively with WISC-IV Verbal Comprehension Index (VCI;  $r=-0.2$ ,  $p=0.03$ ) and WISC-IV VCI subscales Vocabulary ( $r=-0.27$ ,  $p=0.004$ ) and Comprehension ( $r=-0.23$ ,  $p=0.02$ ), but not Similarities ( $r=0.09$ ,  $p=0.33$ ). However, Perceptual Reasoning ( $r=-0.07$ ,  $p=0.5$ ) and Processing Speed ( $r=-0.1$ ,  $p=0.27$ ) composites were not related to SCQ scores.

**Conclusions** In children with 22q, social impairments (as measured by the SCQ) were related to communication and language abilities but not other WISC-IV domains. This suggests a specific role for cognitive abilities underlying communication in partially explaining the level of social functioning in children with 22q. Future study should compare a broad array of cognitive domains in 22q and idiopathic ASD to determine whether distinct intermediate phenotypes explain observable behavior and thus provide different targets for treatment.

298

### THE EFFECTS OF ANIMAL ASSISTED INTERVENTION ON THE SOCIAL INITIATION BEHAVIORS OF CHILDREN WITH AN AUTISM SPECTRUM DISORDER

H Albasha, M Kelly, J Andrews, S Rice. *The University of Arizona College of Medicine—Tucson, Oro Valley, AZ*

10.1136/jim-d-15-00013.298

**Purpose of Study** Animal Assisted Intervention (AAI) is a therapy tool used to improve social behaviors in children with an Autism Spectrum disorder (ASD). During AAI therapy sessions children are encouraged to participate in activities with a pet therapy animal. There are few studies

on AAI therapy for ASDs, and these studies lack robust study designs, merely showing proof of concept and requiring further investigation. The following study investigates the effects of AAI therapy sessions with a dog on social initiation by children with ASD.

**Methods Used** Six males and three females (ages 6–9) participated in the study – 6 are verbal and 3 are nonverbal and the average SRS2 total score was 81.78 (71–90). Each child attended one, 25-minute session per week for 8 weeks. The children were randomly assigned to have their first 4 weeks with the dog and the next 4 with the human proxy, or vice versa. The proxy served as the control and participated in the activities without speaking. Sessions were recorded with consent.

During the sessions, a facilitator instructed the child through a series of play activities, with a minimum of two minutes and a maximum of 5 minutes for each. The instances of when the child initiated a social interaction with the facilitator or parent through starting a conversation or physical touch (SI) were tallied by an in-person and video coder. We used repeated measures ANOVA to determine the differences in SI over the 4 weeks with the dogs and without the dogs.

**Summary of Results** There was a main effect of time on SI,  $F(3, 15)=8.29$   $p<.01$  with SI toward parent or facilitator reducing over the 4 weeks. There was no significant effect of dog/proxy or an interaction effect between intervention and time; however the trend differed between the two groups with the proxy group decreasing from 17 to 10 and the dog group from 31 to 8 SI events.

**Conclusions** The decline in the instances of SI toward the facilitator or parent was surprising and may be the result of increased SI with the dog or proxy, which we did not account for. Further investigation should be performed with expanded measures accounting for social interactions and expressions between the children and dog or proxy.

299

### IMPACT OF ERYTHROPOIESIS STIMULATING AGENTS ON BEHAVIORAL MEASURES IN INFANTS BORN PRETERM AND TERM

R Rieger,<sup>1</sup> RA Yeo,<sup>1</sup> S Winter,<sup>2</sup> J Phillips,<sup>3</sup> N Moss,<sup>1</sup> RK Ohls,<sup>3</sup> JR Lowe<sup>3</sup>. <sup>1</sup>University of New Mexico, Albuquerque, NM; <sup>2</sup>University of Utah Health Care, Salt Lake City, UT; <sup>3</sup>University of New Mexico Hospital, Albuquerque, NM

10.1136/jim-d-15-00013.299

**Purpose of Study** Erythropoietin (Epo) receptors are present in the brain of the developing fetus, which may explain the benefit it has for the premature brain. As children born preterm are at-risk for difficulties in academic achievement, behavior and executive functioning. Erythropoiesis stimulating agents (ESAs) may prove beneficial to development.

**Methods Used** Preterm infants born 500–1250 grams were randomized to receive Epo or Darbe (ESAs group  $n=35$ ). Preterm placebo ( $n=14$ ) and term group ( $n=22$ ) were recruited. Children were evaluated at 3 to 4 years with intelligence testing and parents completed the Behavioral Assessment Scale of Children Scale 2 (BASC-II).

**Summary of Results** Demographic variables were grouped into 2 factors: Socioeconomic Status (SES; maternal education, ethnicity, income) and Stress (number of children under 6 in the home and number of family moves). Using multivariate modeling, with all three groups, the effect of group was significant ( $F(8, 112)=3.91, p<0.001$ ), as was the effect for SES ( $F(4,55)=4.81, p=0.002$ ) and the interaction of Group with SES ( $F(8, 112)=3.63, p=0.001$ ). The same multivariate model for ESAs and Placebo resulted in a significant effect of SES ( $F(4, 38)=5.67, p=0.001$ ), and interaction of SES with Group ( $F(4, 38)=5.92, p=0.001$ ). At a univariate level, the interaction was significant for Adaptive Skills ( $p=0.008$ ), Behavior Symptoms ( $p<0.001$ ), and Externalizing problems ( $p=0.026$ ).

**Conclusions** Preterm children treated with ESAs performed significantly better on BASC-II variables compared to preterm children with no treatment, most notably on the Adaptive Skills, Behavior Symptoms, and Externalizing Problems composites. Overall, ESAs seemed to “protect” children born preterm from the adverse impact of low socioeconomic status on behavioral functioning. Children treated with ESAs were more similar to the Term group on SES factors than the Placebo group, indicating that ESAs mediated the impact of socioeconomic variables on preterm children. These findings have implications for future use of ESAs as part of medical treatment of children born preterm.

### 300 DEVELOPMENTAL MILESTONES BOOKLETS AID IN EARLY DIAGNOSIS/INTERVENTION

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10.1136/jim-d-15-00013.300

**Purpose of Study** The CDC spends a significant amount of money yearly to make the “Milestone Moments” booklets. To date, there has not been a study that shows whether or not these booklets are helpful to parents. This study aims to investigate the utility of these booklets in helping parents identify possible developmental delays in their children, which could lead to earlier diagnosis and intervention.

**Methods Used** CDC booklets are given to parents during a Lied Clinic visit. A pediatric resident explains the booklet’s contents, confirms comprehension with parents, and parents complete a signature form indicating the date the family received the booklet. At 2, 6, 12 and 18 months parents are asked to fill out an age appropriate questionnaire targeted at each area of development (motor, language, social) and complete a survey about the usefulness of the booklet. Signature forms and questionnaires are collected and tracked.

**Summary of Results** Currently our clinics have given out 78 books with completed signature forms. 37/78 patients have completed forms for age appropriate visits. 14/36 are 2 month visit, 13/36 are 6 month visit, 5/36 are 12 month visit, 4/36 are 18 month visit. One patient has completed both 2 and 6 month visit follow-up forms and has found the booklet useful. The remaining 41 patients have not returned for 2/6/12/18 month visit. Of those parents who completed a follow-up form after receiving a booklet at an

earlier visit, 75% (12/16) indicated that they used the booklet and found it useful while 25% (4/16) did not use the book and therefore did not find it useful. 50% (6/12) of respondents who found it useful are parenting their first child; 25% (3/12) have parenting experience; 25% (3/12) of respondents did not indicate parenting experience. Of the four respondents who did not use the booklet, participants did not respond regarding prior parenting experience.

**Conclusions** While data collection is still in progress as patients complete follow up visits, those who have completed surveys and utilized the book feel that it is helpful. It also seems to be more beneficial for first time parents, though conclusions are hard to make when sibling status is not known for all respondents. Preliminary findings indicate that the “Milestone Moments” booklet is an important resource to provide to parents with a particular emphasis for first time parents.

### 301 ADHERENCE TO A DEPRESSIVE DISORDERS CLINICAL PATHWAY IS ASSOCIATED WITH LONGER LENGTH OF STAY

B Lifland,<sup>1</sup> A Desai,<sup>2,3</sup> D Wright,<sup>2</sup> R Mangione-Smith,<sup>2,3</sup> K Schloredt.<sup>2</sup> <sup>1</sup>University of Washington School of Medicine, Seattle, WA; <sup>2</sup>Seattle Children’s Hospital, Seattle, WA; <sup>3</sup>University of Washington, Seattle, WA

10.1136/jim-d-15-00013.301

**Purpose of Study** Clinical pathways are used in the inpatient setting to improve the efficiency of care and reduce costs. Evidence regarding the effectiveness of inpatient psychiatric clinical pathways is scarce. The objective of this study was to examine the association between level of adherence to an adolescent depressive disorders clinical pathway and length of stay (LOS), cost, and readmissions.

**Methods Used** Retrospective cohort study of 558 patients 10–18 years old placed on the Adolescent Depressive Disorders Clinical Pathway at Seattle Children’s Hospital from 1/14–5/15. Seven pathway processes of care were tracked by psychiatric unit staff and recorded in an internal database. An adherence score (0–100 scale) was generated for each patient by computing the sum of completed processes of care divided by the number of processes for which a patient was eligible. Patients were categorized as follows based on their adherence score: low= $<80$  (N=53), medium=80–99 (N=190), high=100 (n=315). We used multivariable linear and logistic regression models to examine the association between adherence category and (1) LOS ( $\leq 7$  days versus  $>7$  days), (2) cost adjusted for LOS, (3) 30-day return emergency department (ED) visits (0/1), and (4) 30-day readmissions (0/1). Models were adjusted for patient gender, level of medical complexity, and insurance status.

**Summary of Results** The mean adherence score was 92.6 (SD=11.0). Patients in the highest adherence category had a higher odds of having a LOS  $>7$  days (OR 2.16, 95% CI 1.13, 4.12); however, there was no significant difference in costs adjusted for LOS between categories. Additionally, there were no significant differences in 30-day return ED

visits or readmissions between categories.

**Conclusions** Higher adherence to an adolescent depressive disorders clinical pathway was associated with longer LOS, which is likely a reflection of the time needed to complete all of the recommended processes of care. Further research is needed to improve the efficiency of inpatient care for adolescent patients admitted for depressive disorders and understand the effectiveness of these pathways for improving patient outcomes.

**302 PARENTAL PERCEPTIONS ABOUT EXERCISE AND ADHD**

SC Cohen,<sup>1</sup> S Taylor,<sup>2</sup> R Rashedi,<sup>3</sup> A Ramakrishnan,<sup>4</sup> R Hansen,<sup>1</sup> J Schweitzer<sup>4</sup>. <sup>1</sup>UC Davis MIND Institute, Sacramento, CA; <sup>2</sup>UC Davis, Sacramento, CA; <sup>3</sup>UC Davis, Davis, CA; <sup>4</sup>UC Davis MIND Institute, Sacramento, CA

10.1136/jim-d-15-00013.302

**Purpose of Study** There is emerging evidence that exercise, including yoga, helps decrease ADHD symptoms in children, but we do not know which types of exercise parents perceive as the most beneficial and if parents are open to having their children participate in yoga. The purpose of this study is to survey parents about their perceptions regarding the effects of exercise on their children's ADHD symptoms, and evaluate parental receptiveness to yoga as a form of exercise for children with ADHD. Through this study we hope to learn which types of exercise children with ADHD are most likely to participate in, and look for trends in the types of exercise that parents perceive as the most beneficial for their children's ADHD symptoms.

**Methods Used** Parents of children 3–17 years of age with ADHD are being recruited to participate in this study, which involves completing an online survey, via REDCap (Research Electronic Data Capture), consisting of 18 questions with sub-questions to elicit parent responses regarding effects of different types of exercise on their children's ADHD symptoms. Study subjects are recruited from the UC Davis MIND Institute Subject Tracking System (STS) database which has 500 subjects with an ADHD willing to be contacted. Additional subjects are being recruited by fliers in pediatric waiting rooms, as well as online via the CHADD newsletter, UC Davis ADHD Facebook group, and MIND Institute website. The REDCap survey link on emails, fliers and web posts direct participants to the informed consent and survey through REDCap. Survey data will be analyzed to estimate proportions, such as the proportion of children with ADHD reported to have improved behavioral responses after various types of exercise as well as the proportion of parents willing to consider yoga as an ADHD intervention. With a sample size of 150 to 200, proportions can be estimated with a margin of error of no greater than 0.08 to 0.07, which provides sufficient precision to characterize the responses elicited through the survey.

**Summary of Results** Preliminary study results will be analyzed and presented at the meeting.

**Conclusions** Preliminary study conclusions will be presented at the meeting.

**Community Health II  
Concurrent Session  
3:30 PM  
Friday, January 29, 2016**

**303 A PROGRESSIVE AND INNOVATIVE APPROACH TO INCREASE THE VARIETY OF RESIDENT EXPOSURE TO UNDERSERVED COMMUNITIES**

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10.1136/jim-d-15-00013.303

**Purpose of Study** To describe an innovative method which one training program implemented to increase the variety of resident exposure to low income and underserved communities. This exposure was targeted to improve residents' understanding of issues surrounding medical care for underserved patients.

**Methods Used** The pediatric residency program leadership took several measures to increase the exposure of residents to the underserved community. The first step was to reach an understanding with our community. REACH, associated with the Ventanilla de Salud, is an organization whose mission is providing medical education and linkage to medical care of the underserved Hispanic population in our community. The second step was to reconfigure the "Community" and "Underserved" rotations to incorporate new experiences. Trainees were asked to do bi-monthly home visits in the underserved segment of our community with "Promotores" who are a group of very committed, highly skilled health educators and community leaders trained by REACH. Advertisements were placed at the Ventanilla de Salud by REACH announcing a monthly trainee provided education topic for a large group format.

**Summary of Results** Residents have been exposed to the underserved community in a manner which is mutually beneficial. Residents are doing basic health assessment of underserved patients at home visits and in a large group format at the Ventanilla de Salud. They are referring patient to clinics as needed and providing counseling. They are also doing home safety assessment and providing counseling. They are receiving first hand exposure to the living conditions of the underserved population, enhancing their ability to provide empathic and achievable healthcare. Resident feedback about this exposure has been extremely positive. Promotores input has also been supportive with mutually beneficial recommendations to improve the resident experience by encouraging residents to be proactive educators during home visits.

**Conclusions** Resident exposure to low income and underserved population has been established in a manner which is highly rewarding for both residents and the community. This was possible due to a well-organized systematic approach involving community collaboration.

304 **BEFORE IT'S TOO LATE ⇒ VACCINATE: SCHOOL BASED VACCINATION CLINIC IN CONRAD, MT**

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10.1136/jim-d-15-00013.304

**Purpose of Study** This project aims to increase vaccination coverage rates in Conrad, MT through student and parent education in conjunction with a school-based clinic. Conrad (pop 2,581) is the county seat of Pondera County, located in central Montana. Pondera County's vaccination rates are below state and national averages, particularly for Varicella, HPV & MCV4 vaccines. Additionally, there are no school nurses in the region, so compliance monitoring of student immunization records is poor. As of October 2015, Montana will be the 50th state to require pertussis and two varicella vaccinations for school entry.

**Methods Used** Discussions about health issues and community needs with the Pondera County Health Department (PCHD) Health Nurse revealed that access and education about vaccinations are insufficient. Many students, parents and healthcare providers agreed that these were the biggest challenges to increasing vaccination rates. A literature review of immunization strategies in rural areas demonstrated that providing vaccines outside the traditional medical home, specifically in schools, is the most effective way to increase immunization rates. Combining student and parent education to encourage support of school-based vaccination clinics and increase knowledge about vaccines is needed as well. Meetings were organized with the Conrad HS Principal to help form a partnership with the PCHD and to address the need for vaccination education. Additionally, Care Van, a BlueCross BlueShield service, was contacted to ensure coverage of the vaccination costs for un/underinsured students.

**Summary of Results** Through the coordination of the PCHD, Conrad HS & Care Van, a vaccination clinic will be piloted at Conrad High School on 9/15/15. An action plan was created to manage the deliverables needed to initiate this intervention. An education curriculum was designed for students and provided to the partners. A packet was developed for parents including the creation of: vaccination timelines, consent forms, and insurance/billing forms.

**Conclusions** This intervention will increase vaccination knowledge and provide an accessible solution. Montana tracks immunization data in the imMTrax system and PCHD can track coverage rates after the clinic to demonstrate program efficacy. Long-term, this program will serve as a model for other schools in Pondera County and nearby counties.

305 **ENCOURAGING EARLY LITERACY IN THE SWINOMISH INDIAN TRIBAL COMMUNITY THROUGH REACH OUT AND READ**

LC Thornquist. *University of Washington, Seattle, WA*

10.1136/jim-d-15-00013.305

**Purpose of Study** This project aims to foster positive attitudes about shared reading, improve book ownership, and improve early literacy skills among young patients at the Swinomish Indian Health Clinic. The clinic serves residents of the local Swinomish tribe and members of other federally recognized tribes. Based on conversations with the providers in the clinic and observations made in appointments, some parents in this community have limited preparation for or experience with a home environment that supports early literacy.

**Methods Used** To address this issue, an evidence-based intervention (Reach Out and Read) appropriate for this setting was researched, identified, and proposed to the clinic. A community partner (La Conner Regional Library) was identified and a proposal for a long-term mutual relationship was developed. A plan to advertise the new program to the community was also developed.

**Summary of Results** The application for ROR was completed, submitted, and is now under review by the Washington ROR director. Sustaining funding for ROR was secured through the clinic itself. Training will begin for 3 pediatric providers next week, and a site visit by the ROR logistics liaison will be scheduled once training is complete. A community partnership with the La Conner library has been forged, and donations collected. An article about the benefits of early reading, details of the new ROR program, and an invitation to La Conner library story time will appear in *quyuks*, the tribal newsletter. A basket of nearly 50 donated children's books is now in the waiting room. A flash drive with passwords, ROR contacts, La Conner Library contacts, collected research, and digital copies of all documents generated for the project will be left at the clinic.

**Conclusions** The next steps for ROR at Swinomish are to finish required training, schedule an ROR site coordinator visit, and purchase books. Provider and staff support for the program is strong, as was patient response to the proposal. Last year, 167 qualifying pediatric patients were seen, with nearly 320 well-child visits in which to implement ROR. Similar or greater numbers are projected for this upcoming year. ROR Swinomish is expected to start in full by early September.

306 **INCREASING INFANT VACCINATION RATES IN PARK COUNTY, MONTANA BY PRENATAL VACCINATION EDUCATION**

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10.1136/jim-d-15-00013.306

**Purpose of Study** To increase vaccination rates for infants in Park County, MT by educating parents on vaccine safety by means of a prenatal class and a binder given to expectant mothers at their first prenatal visit. 38% of children in MT and 46% of children in Park County have not received all of the recommended vaccinations. According to the local public health nurse, one reason rates are low in Park County is the lack of vaccine education among parents. Currently, in Livingston Healthcare, the first time that vaccinations are discussed is during their child's 2-month well check. This is the ideal time to begin vaccinations and does

not give parents time to research and understand them.

**Methods Used** Research from a literature review of primary sources led to the conclusion that parent education of vaccine safety before the pediatric visit with vaccinations can increase vaccination rates. Sharing this research with the obstetrics physician in Livingston who leads a prenatal class series at the community health center, a short presentation was added to the current prenatal classes about vaccine safety and importance. In order to educate the maximum number of mothers, vaccination information was also added to a prenatal binder that all mothers receive during their first prenatal visit in Livingston.

**Summary of Results** In collaboration with the OB physician leading the prenatal class, education focused on the vaccine schedule and clarifying safety myths about ingredients, immune system harm, and the link with autism. The parents receiving the education described themselves as being more informed about immunizations and felt more comfortable making a decision regarding whether or not to vaccinate their child.

**Conclusions** The project was successful, in that parents were appreciative of the information and felt more informed and comfortable making vaccination decisions regarding their child. The clinic will continue to include vaccine information in its prenatal binder and classes. The education of parents in Livingston will, hopefully, lead to an increase in vaccination rates. From the literature reviewed, vaccine education can be beneficial both before and after birth, but should occur before vaccinations begin. This education should focus on clarifying myths about vaccination safety, as that is a main reason parents do not vaccinate their children.

307

### 'NO SMOKING' SIGNS IN THE CITY PARKS OF EPHRATA, WASHINGTON

DE Marriott. *University of Washington School of Medicine, Seattle, WA*

10.1136/jim-d-15-00013.307

**Purpose of Study** Ephrata is a rural town in central Washington State with a large youth population: 35% under the age of 18 compared to the state's 29%. Although the 16% adult smoking rate in Ephrata is the same as the State's, Healthy People 2020 proposes a goal of 12%. Additionally, 17% of high-school seniors in Ephrata report smoking in the last 30-days (compared to 15.6% in WA). 'No smoking' signs in city parks contributes to a comprehensive tobacco control program. Reducing second-hand smoke exposure to park goers, combating youth/adult smoking rates and altering the social norms regarding smoking are all important and feasible goals.

**Methods Used** Meetings were held with a public health nurse from the Grant County Health District (GCHD) and the Ephrata Parks and Recreation director. It was decided that of the currently pending projects, a smoke-free parks policy was the most tangible to research and pursue. A review of literature was commissioned to evaluate the cost and efficacy of the project. The research revealed that smoking bans in parks effectively reduces the number of smokers in parks. It also confirmed the legitimate health concerns of second-hand smoke in outdoor settings. The

costs were determined to be low with minimal enforcement required. Other proven effects from a smoke-free parks policy include positive behavior changes in current smokers and changes in citizens' perceived social norms regarding smoking.

**Summary of Results** The review of literature was presented to the GCHD and the Parks and Recreation Department. Additional supporting documents were requested and submitted: photographs of other municipality's outdoor smoking signs and stakeholder interviews of impacted parties. The delivered materials will be used in a presentation to the City Council to obtain funding approval, generate ideas for signage as well as prompt a discussion about legislation creation.

**Conclusions** Installation of 'No Smoking' signs in Ephrata parks is an effective proposal because of its current acceptance and readiness to be acted upon by community partners. The funding and staff required for sign creation/installation are already available making the implementation very likely. A challenge is the city's hesitancy to pass legislation to enforce the signage; it is unclear if an un-enforceable smoking policy will have the desired impact.

308

### QUALITY IMPROVEMENT INTERVENTION TO IMPROVE ANNUAL PAPANICOLAOU SMEAR RATES AMONG HUMAN IMMUNODEFICIENCY VIRUS-INFECTED WOMEN

V Ganta,<sup>2</sup> D Patel,<sup>1</sup> S Moonie,<sup>3</sup> A Hunt,<sup>3</sup> J Richardson,<sup>2</sup> D Di John,<sup>1</sup> E Ezeanolue<sup>3</sup>. <sup>1</sup>Univ. of Nevada School of Medicine, Las Vegas, NV; <sup>2</sup>University Medical Center, Las Vegas, NV; <sup>3</sup>Univ. of Nevada Las Vegas, Las Vegas, NV

10.1136/jim-d-15-00013.308

**Purpose of Study** Current guidelines recommend annual Papanicolaou (Pap) smears for human immunodeficiency virus (HIV)-infected women for cervical cancer screening. Rates for such screening in Nevada are below the national rate. We postulated that reminders to HIV-infected adult women by text or phone messages could improve rates of annual Pap smear screening.

**Methods Used** We identified a cohort of 485 HIV-infected adult women at an outpatient center affiliated with University Medical Center (UMC). Approval was obtained from the UMC Institutional Review Board. Demographic and HIV care-related data were obtained from medical records. Review of records showed that 12 women had obtained a Pap smear in the past year. A questionnaire previously administered to the cohort identified a need for reminders in scheduling a Pap smear. We undertook a quality improvement intervention from June - Sept. 2015, in which reminders to schedule a Pap smear were sent to the remaining cohort of 473 women, first via 3 sequential text messages, followed by 3 attempts at phone calls. An incentive of a ten-dollar gift card was offered for those who obtained a Pap smear. Reports of completed Pap smears after the intervention were tabulated on a weekly basis. Data were analyzed using McNemar's test for marginal homogeneity.

**Summary of Results** Among our cohort of 485 HIV-infected women (average age: 46.7 years; race/ethnicity: 54.6% African-American, 27.2 % white, 13.0 % Hispanic), 335/485 (69.1%) had an HIV viral load <40 copies/ml, 418/485 (86.2%) had CD4 counts > 200/mm<sup>3</sup>, and 429/477 (89.9%) were receiving anti-retroviral therapy. There was an increase in the rate of completed Pap smears from 2.5% at baseline to 11.5% (56/285) after intervention ( $p < 0.0001$ ). Of all 68 Pap smear results, 20 (29.4%) were abnormal.

**Conclusions** Rates for annual Pap smears continue to be low in Nevada and in the United States, especially among HIV-infected women. The need for reminders has been identified as a barrier to fulfilling this recommendation. Our intervention, utilizing methods of communication like text messaging and phone calls, markedly increased the rate of completed Pap smear screening in our population.

### 309 ADDICTION BEFORE BIRTH: DRUG ABUSE DURING PREGNANCY

RC Braunberger. *University of Washington School of Medicine, Seattle, WA*

10.1136/jim-d-15-00013.309

**Purpose of Study** This project aims to raise community awareness of Neonatal Abstinence Syndrome (NAS) to show those dealing with drug abuse during pregnancy that they are not alone; and that help is available before, during, and after pregnancy. St. Joseph Hospital (SJH) is located in Polson, Montana on the Confederated Salish and Kootenai Reservation. The community of Lake County (LC) is dealing with an increase in drug abuse. A recent article stated that 50% of recent vacant housing tested positive for methamphetamines. The percentage of infants born at SJH with exposure to licit and illicit drugs is over 100% the national average, and shows the need to raise awareness. The national average of NAS births in 2009 was 0.339%, compared to 34% in the first quarter of 2015 locally. NAS infants have increased risks including birth defects, seizures, and bipolar disorders.

**Methods Used** Clinical observation showed an increased number of infants with exposures to licit and illicit drugs listed in their medical histories. Meetings with the Director of Nursing, LC Health Department, OB Nursing staff, and physicians showed the large scale of the problem in LC. A literature review showed how storytelling was used in Latino and Native American populations to address health concerns. Research also showed the effectiveness of storytelling in addiction, utilized heavily by Alcoholics Anonymous. Following meetings with the staff, a partnership was made with Best Beginnings (BB) of LC.

**Summary of Results** A Facebook (FB) page was created linking an anonymous survey to collect and share personal stories from those affected by NAS. A flyer was created using one of the stories, and distributed at the county health department, WIC offices, and SJH. The BB program was given control over the FB page and survey to continue to gather stories for a future larger work containing multiple stories. A presentation was given to the staff at a neighboring hospital about the project, and to increase the network for story gathering.

**Conclusions** This project had an initial challenge gathering stories. The creation of a survey allowing anonymous submissions removed this initial hurdle. This project is going to be continued by BB of LC in the hope of establishing future support groups, and a larger collection of stories. The effectiveness of the project depends on the usage of the materials, and the ability to establish support groups.

### 310 SEXUAL HEALTH AWARENESS COURSE AT FORT PECK COMMUNITY COLLEGE: PEER-EDUCATION, PREVENTION, AND TREATMENT OF SEXUALLY TRANSMITTED INFECTIONS

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10.1136/jim-d-15-00013.310

**Purpose of Study** The Sexual Health Awareness Course developed at Fork Peck Community College (FPCC) aims to reduce the prevalence and incidence of sexually transmitted infections (STI's) in Roosevelt County (RC), Montana. RC has a population of over 11,000 people with the second highest rate of gonorrhea in Montana at 418/100,000. Public health officials are concerned about the rise in gonorrhea, as well as the potential increase of other STI rates. There is a community need for a sustainable source of sexual health information and treatment options.

**Methods Used** Initial conversations were held with the Roosevelt County Health Department (RCHD). The Director of the FPCC Wellness Center presented the idea of a sexual health awareness course offered at FPCC with college students having a primary role in community outreach initiatives. A literature review was conducted for studies with community college students as peer-educators for STIs. As the population of RC is 58% American Indian (AI), a focus was placed on studies with both rural and AI perspectives. A rural, 28-week peer-educator training program demonstrated a 16% self-efficacy increase in condom use, and a 30% decrease in unprotected intercourse. STI education at an AI basketball camp reduced the potential of risky sexual behavior up to 12 months. Other studies of AI populations noted the benefits of media technologies of obtaining sexual health information.

**Summary of Results** The FPCC Wellness Center Director helped develop a course outline, approved informally by the FPCC President, and the VP of Student Affairs for formal approval. The proposal includes the following topics: epidemiology of common STIs; psychological and cultural-specific focus groups; a peer-education model for public school outreach; mobile screening & treatment units; and media technology interventions. The course will initially be offered in the Fall 2015 semester.

**Conclusions** The development of the Sexual Health Awareness Course at FPCC has broad initial support for its successful implementation at FPCC. The RCHD will also introduce the course to the RC Epidemiology Team. Risky sexual behaviors are also heavily interrelated to other issues in RC, including drug and alcohol abuse, which are significantly higher than the state average.

**311** **COMPARING THE EDUCATIONAL OUTCOME OF UNDERREPRESENTED IN MEDICINE (URM) AND NON-URM STUDENT PARTICIPANTS IN A HEALTH ENRICHMENT PROGRAM**

N Rezakahn Khajeh,<sup>1</sup> MJ Vennat,<sup>1</sup> B Afghani<sup>1,2</sup>. <sup>1</sup>University of California, Irvine, Irvine, CA; <sup>2</sup>CHOC Hospital, Orange, CA

10.1136/jim-d-15-00013.311

**Purpose of Study** The purpose of this follow-up study is to compare the educational outcome of the URM and non-URM participants of the high school students who participated in the Health and Science Enrichment Program at UC Irvine School of Medicine.

**Methods Used** The University of California, Irvine School of Medicine implemented the Summer Premedical Program in 2010. The program allows high school students to complete workshops that are not typically accessible until medical school while also providing a unique exposure to medical education. 20–25% of students enrolled in the program are URM and receive scholarship funds for program completion. All URM and non-URM students who have completed the program between 2010 and 2014 were contacted to obtain their educational outcome.

**Summary of Results** Of 515 high school students who participated in our program between 2010 and 2014, 462 (90%) responded. Of 462 respondents, 107 (23%) were URM and 355 (77%) were non-URM. 18 (17%) of URM and 80 (23%) of non-URM were still attending high school at the time of our follow-up. Of 89 URM and 275 non-URM who completed high school, 88 (99%) and 275 (100%) were enrolled in a college. The breakdown of colleges is shown in the table.

**Conclusions** Enrollment in UC schools had the least percent difference (1.67%) between URM and non-URM students. Ivy League and junior college enrollment had the highest percent differences (117.36% and 158.97%, respectively). Non-URM students had over three times more enrollments in Ivy League schools than URM students. URM students had over fifteen times more enrollments in junior colleges than non-URM students. In conclusion, while both categories possessed higher educational pursuits, non-URM students had more enrollments

**Abstract 311 Table 1** Breakdown of Colleges

Colleges Attending	URM (n=88)	Non-URM (n=275)	P Value
UC	46.00%	45.80%	0.90
Ivy League	1.10%	4.40%	0.56
Top 20	4.60%	6.90%	0.62
Cal State	8.00%	4.00%	0.15
2 Year College	16.00%	1.80%	0.0001
Other	23.90%	37.10%	N/A

\*Top 20\* was obtained from USA news' nationwide university ranking.

in prestigious private colleges while URM students had more enrollments in junior colleges.

**312** **AN EXCHANGE FOR HEALTH: IMPLEMENTING A SYRINGE EXCHANGE PROGRAM IN BROWNING, MT**

JL Ebner. University of Washington, Seattle, WA

10.1136/jim-d-15-00013.312

**Purpose of Study** The goal of starting a Syringe Exchange Program (SEP) in Browning, MT is to reduce transmission of Hepatitis C Virus (HCV) and prevent Human Immunodeficiency Virus (HIV) among people who inject drugs (PWID). Browning is the largest community on the Blackfeet Reservation and the seat of tribal government. Browning is located in Glacier County which has a population that is 63.3% American Indian/Alaskan Native (AI/AN). In 2013, the rate of chronic Hepatitis C cases reported in Glacier County was 376/100,000, more than 3 times the rate for Montana. However, HIV rates in Glacier County and Montana are low.

**Methods Used** Through community conversations and clinical observation the HCV frequency among PWID in Browning became apparent. A group was already meeting with interest in starting a SEP. This group, termed the Blackfeet Action Committee (BAC), consisted of staff from the hospital, the local rehab program, Community Health Representatives, and Tribal Health. After a meeting with BAC, a literature review was conducted to evaluate the effectiveness of SEPs in reducing HCV and HIV transmission. This revealed that SEPs are evidence based interventions for decreasing injection risk behavior and in some studies SEP presence correlated with reductions in HCV and HIV transmission rates.

**Summary of Results** Established SEPs, were contacted for support and information. A Facebook Page was created to announce information about time and place the SEP will occur. Supporting documents were made including: an intake form, a system for monitoring syringes out/in per client, and a handout to provide basic information about syringe safety, HCV and HIV, overdose prevention, and syringe disposal. Also, flyers advertising for the exchange were made and given out during a local parade. Research was conducted on funding options for the SEP. All research results and documents were presented to BAC at a meeting or by email.

**Conclusions** The strong community involvement is a promising indication for the future success of a SEP. The biggest challenge that exists will be building trust with and establishing a sense of confidentiality for PWID, which may take time. However, this is a meaningful project because there are few options available to rural reservations for distributing clean syringes. The next steps for BAC will be to apply for funding and to develop a protocol for the SEP.

Health Care Research II  
 Concurrent Session  
 3:30 PM  
 Friday, January 29, 2016

313 PATIENT WRITTEN NOTES AND THEIR USE IN  
 PRIMARY CARE: "I FELT LIKE I WAS ABLE TO BE  
 HEARD"

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10.1136/jim-d-15-00013.313

**Purpose of Study** Setting agendas at the beginning of a clinic visit is recommended, but can be challenging in primary care. We assessed the feasibility, acceptability, and utility of having patients type their own visit agendas in an outpatient clinical setting.

**Methods Used** We recruited patients from a large academic teaching clinic at Harborview Medical Center, a safety-net county hospital in Seattle, WA. Participating patients typed their own visit agenda into their electronic medical record before being seen by their physician (e.g., a list of questions and/or concerns they wanted to address with their physician). Patients and providers were surveyed after the clinic visit to assess their perceptions of the patient-typed agenda and patient-provider communication.

**Summary of Results** 101 patients of 28 primary care providers typed an agenda and completed the survey. The response rate was 70.1% of invited and eligible patients (e.g., English speaking and able to read and type on a computer). Agendas averaged 27 words in length (range 1 to 420) taking less than 5 minutes to type. Using a 5-point Likert scale, the majority of providers (75%) and patients (75%) agreed or strongly agreed that the patient-typed agendas improved the provider's understanding of the patient's priorities for the visit. Providers were significantly more likely than patients to agree that patient-typed agendas helped prioritize the visit (84% vs 79%,  $p < .05$ ) and a desire to use them in the future (79% vs. 72%,  $p < .05$ ). Compared to providers, patients were more likely to report that the provider seemed more prepared because of the patient agenda (82% vs 75%) and made the visits as more efficient (80% vs 67%,  $p < .05$ ). Providers (75%) and patients (79%) were equally likely to report improved patient-provider communication. Qualitative review of survey comments supported these findings.

**Conclusions** Patients from a safety-net population demonstrated a willingness and ability to type their own agendas before clinic visits into the electronic health records. Both patients and providers agreed that patient-typed agendas improved patient-provider communication during visits.

Enabling patients to type visit agendas may enhance care by engaging the patient and giving providers a useful and efficient way to prioritize patients' concerns.

314 PROVIDER/STAFF PERCEPTIONS OF ONLINE PORTAL  
 BARRIERS AND BENEFITS IN A SAFETY NET  
 SETTING

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**Purpose of Study** Online health portals, which provide patients access to personal health records, have been shown to improve overall health outcomes, and are increasingly becoming adopted across health systems. While a large amount of research has been conducted on patient barriers to portal use, little research has been done on provider perceptions, especially in safety-net settings where there are a high number of competing demands. This study examined providers' beliefs about the barriers and benefits to portal use in the SF Health Network.

**Methods Used** In January 2015, we conducted a 25-question online survey with primary care providers (PCPs) and non-medical provider staff. The survey focused on perceived barriers and benefits to portal use. In addition, providers and staff were asked for their age, gender, specialty/role, practice setting, and previous experience with patient portals. It included both closed-ended questions to assess the prevalence of specific barriers/facilitators to portal use as well as open-ended questions to openly assess perceptions.

**Summary of Results** A total of 77 recipients responded to the survey. 46 were physicians, 15 were other healthcare providers, and 14 were other staff. When asked about portal use among their patient population, providers listed major barriers of English proficiency and access to technology, and the most important benefit of increased access to PCP. However, in regards to their own work, providers/staff felt the biggest barrier was lack of time in a workday to manage patient portals, answer secure messages, and teach patients how to use the portal.

**Conclusions** PCPs in a safety net setting reported more barriers than benefits to patient portal use before portal implementation. Overall, the concerns centered on English proficiency, lack of internet access, and lack of provider time. As portals improve and become ubiquitous, PCPs have the potential to facilitate patient-provider communication and increase patient engagement. However, our findings suggest that PCPs, particularly those working in safety net settings, may need additional support in using portal websites within their existing workflows.

### 315 LISTENING BEYOND AUSCULTATING: A QUALITY INITIATIVE TO IMPROVE HCAHPS COMMUNICATION SCORES

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**Purpose of Study** Use of the Plan-Do-Study-Act (PDSA) model to test interventions that could enhance patients' perception of listening by physicians as measured by the Hospital Consumer Assessment of Healthcare Providers and Systems Survey (HCAHPS).

**Methods Used** Eight third-year medical students conducted a literature review of communication strategies and observed hospitalists' bedside interactions at Kaiser Oakland Medical Center. Factors with potential to affect patients' perceptions of physician listening yielded 41 potential interventions – 24 were tested and 4 selected for a larger pilot based on feasibility and perceived impact. Qualitative feedback produced one top intervention: use of an open-ended question to solicit a specific patient concern. A reminder was embedded in the hospitalist progress note template in the electronic health record (EHR), modifying the traditional SOAP (Subjective, Objective, Assessment, Plan) to "ScOAP," with the "c" designating patient concerns. A 3-day pilot was assessed by chart review and interviews.

**Summary of Results** Of the 4 interventions tested, the open-ended question had the greatest participation and perceived impact. Hospitalists' feedback indicated that eliciting patient concerns yielded relevant information and that the EHR prompt was helpful. Of 150 eligible charts in the ScOAP pilot, 67% included documentation of patient concerns, most commonly pain and discharge planning. The hospitalist group has since formally adopted the ScOAP template.

**Conclusions** The PDSA method allowed for testing of 24 interventions to improve patients' perceptions of physician listening and refinement of one intervention, which was implemented by a large medicine service. Studies suggest such patient-centered approaches can increase satisfaction without compromising efficiency. Further, addressing a specific concern can help shift an encounter from being task-oriented to care-oriented. The structured ScOAP reminder in the EHR facilitated behavioral change without being overly burdensome. Correlation with HCAHPS scores may elucidate the impact of the ScOAP note on patients' satisfaction.

### 316 PREVALENCE OF HOT THYROID NODULES SUSPICIOUS FOR MALIGNANCY

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10.1136/jim-d-15-00013.316

**Purpose of Study** To estimate the prevalence of thyroid nodules suspicious for malignancy in hyperthyroid patients.

While contemporary guidelines emphasize that "hot," or toxic nodules are unlikely to be malignant, case reports suggest that these nodules may be malignant in a small, but not insignificant number of individuals. The prevalence of malignancy in "hot nodules" is unknown.

**Methods Used** This retrospective study analyzed data from the VA Northern California Health Care System for patients enrolled between January 2010 and December 2014. Veterans were identified by ICD-9 codes for hyperthyroidism, and either thyroid nodules, thyroiditis, thyroid adenoma, or goiter. Veterans who underwent radioiodine or ultrasound thyroid scanning were identified. These records were subsequently reviewed manually for suspicious ultrasonographic findings (size, calcification, or geometry).

**Summary of Results** 760 Veterans were identified by ICD-9 coding. Of these, 230 had thyroid ultrasounds, and 113 had radioiodine thyroid scans. There were 70 patients that had both ultrasound and radioiodine thyroid scans of which 84.3% were male and the average age was 62.9. Twenty-five had hyperthyroid nodules or areas on radioiodine studies and 31 had suspicious ultrasound scans (we excluded 7 individuals with "cold" thyroid scans). 18 had both and of these, 6 underwent fine needle aspirate (FNA) and 1 is planned for FNA.

**Conclusions** While most Veterans identified as hyperthyroid did not undergo imaging studies, of those who did, a remarkable number had heretofore unexpected ultrasonographically-suspicious nodules. This preliminary observation suggests that ultrasound scans, a noninvasive and relatively inexpensive diagnostic modality, may have a role in the evaluation of the hyperthyroid patient in identifying those who might benefit from FNA.

### 317 REMOVING BARRIERS TO MEDICATION NON-COMPLIANCE: PATIENTS' USE OF A DRUG PRICE COMPARISON WEBSITE TO REDUCE COSTS AND IMPROVE ADHERENCE

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**Purpose of Study** Increasing out-of-pocket medication costs is associated with a decrease in medication adherence. The inability to afford medications is the most stated reason by patients for medication non-adherence. Rates of medication non-adherence have remained high in the last three decades and are expected to rise as the burden of chronic disease increases. However, price transparency, through websites such as GoodRx, has improved. GoodRx is an aggregator of medication cost information including cash prices, prices from pharmacy savings' programs, and coupons. It allows users to compare prescription drug prices at pharmacies in their area by entering their zip codes and medication names.

Little is known about the effects of such online price transparency on medication adherence. This study examines patients' reasons for seeking medication prices on GoodRx and assesses the impact of cost savings on medication adherence.

**Methods Used** Our sample was survey respondents recruited from GoodRx (www.GoodRx.com). Data were collected from a varied demographic group over a four day period in August-September 2014. Questions asked included, "Did you find your prescription for less on GoodRx?" and "Are you more likely to fill your prescription after using GoodRx?". Descriptive statistics were used to characterize survey responses.

**Summary of Results** Overall, 675 people completed the survey. Nearly 45% of the sample was 60 years of age or older, with 61% having insurance. Results showed that 74% of respondents found their prescription for less than they'd been paying, and those savings translated into more than 60% of respondents reporting increased likelihood of filling their prescription. The average savings across 450 prescriptions was \$82.09 per prescription.

**Conclusions** This study highlights the pressing need to find new ways to ensure lower out-of-pocket medication costs and presents a compelling argument for cost-savings websites. By providing medication cost transparency and significant savings, such sites may play an important role in promoting medication adherence.

318

#### THE IMPACT OF FIRST RESPONDER TURNOUT SEGMENT ON SURVIVAL FROM OUT-OF-HOSPITAL CARDIAC ARREST

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10.1136/jim-d-15-00013.318

**Purpose of Study** Patients with out-of-hospital cardiac arrest (OHCA) are more likely to survive when emergency medical services (EMS) arrive quickly. The turnout segment (turnout) is between time at notification of an incident and when the assigned vehicle begins moving. A 60 second turnout standard has been proposed. We hypothesize that as turnout increases, survival to hospital discharge from OHCA decreases.

**Methods Used** We retrospectively evaluated responses to adult non-traumatic OHCA on EMS arrival from 2009 to 2014 in an urban, fire department based system. Turnout was calculated from the computerized time stamps for dispatch activation until first responding fire personnel pressed the "en route" button on the mobile data terminal. We categorized turnout, in seconds, into two groups: 0–60 (short) and >60 (long). Cases in each category were compared with OHCA survival to hospital discharge. We used logistic regression modeling to ascertain whether turnout independently predicted OHCA survival to discharge.

**Summary of Results** Of 2,485 treated OHCA responses, 653 (26%) were excluded, leaving 1,832 for analysis. The median turnout was 59 seconds, and 50% were between 45 and 78 seconds. About 53% met the 60 second standard, and 47% were longer. Turnout accounted for 16% of the interval from 9–1–1 call receipt to fire personnel beginning chest compressions. Turnout was shorter when initial dispatch indicated OHCA (61±28 vs. 65±30; mean difference 4, 95% CI 1–7) and when OHCA occurred during the day vs. night (56±27 vs. 78±29; mean difference 22,

95% CI 19–25). Turnout decreased from 2009 to 2014 (59±31 vs. 68±29; mean difference 10, 95% CI 5–14). No differences in survival to discharge, return of spontaneous circulation, or finding an initial shockable rhythm were noted between the two turnout groups. Logistic regression was unable to demonstrate a statistical relationship between turnout and OHCA survival (p=0.28).

**Conclusions** Turnout segment represents about a sixth of the overall call-to-patient interval. A possible explanation for failure to detect an effect on long term survival is the relative consistency in turnout segment in this system.

319

#### PROSPECTIVE COHORT STUDY OF REMOTE EAR DISEASE DIAGNOSIS USING A SMARTPHONE OTOSCOPE

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10.1136/jim-d-15-00013.319

**Purpose of Study** Ear disorders result in over 12 million office visits per year. Smartphone attachments, like the CellScope Oto™ (CSO), enable a video of the ear canal and tympanic membrane to be transmitted wirelessly to a remote physician for cost-effective diagnosis and treatment. The device has been shown to be effective for remote diagnosis in a pediatric population and among emergency physicians. This study aimed to determine whether the device could be used effectively in patients seeking otolaryngology evaluation. We hypothesized that at least 25% of clinic visits could be avoided with use of the device.

**Methods Used** A prospective pilot study was conducted. Voluntary English-speaking patient-caregiver pairs presenting to an otolaryngology clinic were recruited. Caregivers were trained on use of the device, and then performed videotoscopic examinations without assistance. CSO videos were transmitted to a blinded physician for remote diagnosis. Patients attended their scheduled office visit with a provider who recorded an independent diagnosis without access to the CSO video. Data on patient and caregiver experience as well as remote provider perception and comfort with diagnosis were collected. Descriptive statistics were applied.

**Summary of Results** Videotoscopies were collected from 28 ears from 14 patient-care-giver pairs. Caregivers found the device to be very user friendly, reporting a mean ease of use score of 4.54/5 [2–5], and comfort 4.61/5 [2–5]. Patients reported low discomfort scores of 1.57/10 [1–4]. Remote providers found the image quality to be comparable to clinic otoscopy equipment in 60.7% of cases. They were comfortable making a diagnosis in 32.1% of cases, and willing to initiate treatment in 32.1% of cases.

**Conclusions** The CSO appears to be an effective tool for remote diagnosis and treatment of ear conditions for many patients who might otherwise require travel to an otolaryngology clinic. The device has the potential to save significant time and travel expenses associated with medical visits.

320 HIGH-FLUENCE LIGHT EMITTING DIODE-GENERATED RED LIGHT MODULATES KEY CELLULAR CHARACTERISTICS OF SKIN FIBROSIS IN HUMAN SKIN FIBROBLASTS

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10.1136/jim-d-15-00013.320

**Purpose of Study** Skin fibrosis, often referred to as scarring, is a significant international health problem, with an estimated incidence of greater than 100 million persons per year world-wide. Skin fibrosis is a disabling clinical problem with limited cost-effective, non-invasive therapeutic modalities. The unifying hallmarks of skin fibrosis are increased collagen production, an increased number of fibroblasts, and increased migration. Light-emitting diode red light (LED-RL) may represent a potential safe, portable, and cost-effective treatment for skin fibrosis. We previously found that (LED-RL) decreases the proliferation and migration speed of human skin fibroblasts. We hypothesized 633-nm LED-RL may increase reactive oxygen species generation and decrease collagen production in human skin fibroblasts.

**Methods Used** To test our hypothesis we irradiated human skin fibroblasts with commercially-available LED-RL units (Photomedex). To ensure the measured effects were due to LED-RL alone, each LED-RL treated sample was matched to a light-protected control sample subjected to the same environmental conditions. Intracellular ROS was measured with dihydrorhodamine by flow cytometry. We quantitated total collagen levels following LED-RL irradiation using a micro-sirius red (PSR) colorimetric assay. We confirmed that LED-RL reduces procollagen 1A1 production using western blot.

**Summary of Results** LED-RL at fluences of 320J/cm<sup>2</sup> and 640J/cm<sup>2</sup> resulted in a significant dose-dependent increase in intracellular ROS for up to 4 hours following irradiation. LED-RL at fluences of 320J/cm<sup>2</sup> and 640J/cm<sup>2</sup> resulted in decreased collagen levels, as measured by PSR, in a dose-dependent manner at 48-hours post-irradiation (83.2% and 54.1%, respectively,  $p < 0.05$ ). Furthermore, we found that 320J/cm<sup>2</sup> and 640J/cm<sup>2</sup> LED-RL reduced production of procollagen 1A1 2 hours post-irradiation (55% and 51%, respectively) as measured by western blot.

**Conclusions** We conclude that LED-RL increases ROS production and decreases collagen production that is associated with fibrosis. We envision that our findings will serve as the foundation for future translational studies that contribute to the management of fibrotic skin disease.

Hematology and Oncology II  
Concurrent Session  
3:30 PM  
Friday, January 29, 2016

321 PRETREATMENT CLINICAL PROGNOSTIC FACTORS FOR BRAIN METASTASES FROM BREAST CANCER TREATED WITH GAMMA KNIFE RADIOSURGERY

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10.1136/jim-d-15-00013.321

**Purpose of Study** Brain metastases significantly affects morbidity and mortality rates for patients with metastatic breast cancer. Treatment for brain metastases lengthens survival, and options such as stereotactic radiosurgery (SRS) can increase survival to 12 months or longer. This study retrospectively analyzes the prognostic factors for overall survival (OS) for patients with one or multiple brain metastases from breast cancer treated with SRS.

**Methods Used** Between December 2001 and May 2015, 111 patients with brain metastases from breast cancer were grouped by potential prognostic factors including age at diagnosis, KPS score, number of brain metastases, and whether or not they received adjuvant treatments such as whole brain radiotherapy (WBRT) or surgical resection. Survival rates were determined for all groups, and hazard ratios were calculated using univariate and multivariate analyses to compare differences in OS.

**Summary of Results** Median overall survival was 16.8 ± 4.22 months. Univariate analysis of patients with a KPS ≤ 60 and multivariate analysis of KPS 70–80 showed significantly shorter survival than those with KPS 90–100 (5.9 ± 1.22 months, 21.3 ± 11.69 months, 22.00 ± 12.56 months,  $P = 0.024$  and  $< 0.001$ ). Other results such as age ≥ 65 years and higher number of brain metastases trended towards shorter survival but were not statistically significant. No difference in survival was found for patients who had received WBRT in addition to SRS ( $P = 0.779$ ).

**Conclusions** Stereotactic radiosurgery has been shown to be safe and effective in treating brain metastases from breast cancer. We found our median survival to be 16.8 ± 4.22 months, an increase from other clinical reports. Also, 38.4% of our population was alive at 2 years and 15.6% survived 5 years. Significant prognostic factors can

help inform clinical treatment decisions. This study found that KPS was a significant prognostic indicator of overall survival in these patients.

### 322 EVALUATION OF MICRONUCLEI FREQUENCY IN CULTURED PERIPHERAL BLOOD LYMPHOCYTES OF PROSTATE CANCER PATIENTS BEFORE AND AFTER PROTON RADIOTHERAPY

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10.1136/jim-d-15-00013.322

**Purpose of Study** The purpose of this study is to evaluate whether peripheral blood lymphocyte (PBL) biomarkers can be used to characterize individual radiosensitivity and predict the severity of acute/late effects from proton radiotherapy (RT). We aimed to characterize dose and time response relationships for the *in vitro* and *in vivo* radiation induction of two biomarkers: Micronuclei (MNI) and 53BPI (p53 binding protein) foci in PBLs.

**Methods Used** Forty patients with prostate cancer were treated with proton RT. Depending on staging (T1A to T3A), one of four proton protocols was used: 1) 81Gy to prostate, 2) 60Gy hypofractionated to prostate, 3) 50.4Gy to prostate and proximal seminal vesicles followed by 30.6Gy to prostate; and 4) 36Gy to prostate and seminal vesicles followed by 45Gy to whole pelvis with photons. Fractions were 1.8Gy except protocol 2, which received 3.0Gy/fraction.

*In vitro*: Prior to RT each patient's blood was drawn and irradiated (0, 0.5, 1, 2, and 4Gy) with protons and gamma radiation. 10 healthy donors also had samples irradiated to establish a control population. Two assays were used to evaluate cytogenetic damage: MNI frequency via the cytokinesis-block micronucleus method and 53BPI foci induction via immunocytochemistry. Dose response curves were generated for each patient.

*In Vivo*: MNI and 53BPI were assessed for each patient during the middle of treatment and at the end of treatment in order to correlate *in vivo* assay results with those of *in vitro* PBLs.

**Summary of Results** A dose- and time-dependent increase in the frequencies of MNI and 53BPI foci was observed for both *in vitro* and *in vivo* irradiated samples. Proton-induced MNI increased linearly at low doses and saturated at high doses. Gamma-induced MNI also increased linearly at low doses but without saturation. At mid-treatment, an approximate two-fold increase was observed in the MNI frequency compared to baseline. At treatment completion, MNI frequency continued to increase, and was significantly higher than that of pre- and mid-treatment samples.

**Conclusions** MNI formation in PBLs can be a biomarker of *in vivo* and *in vitro* radiation exposure and individual sensitivity. Also, our findings suggest that the individual differences between patients in MNI yield for the prescribed doses appear to be significant.

### 323 ASSESSMENT OF RASSF1C PI-RNA-TARGET GENES IN LUNG TUMOR TISSUES

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10.1136/jim-d-15-00013.323

**Purpose of Study** The Ras association domain family 1 (RASSF1) gene encodes two major isoforms that play a role in carcinogenesis: RASSF1A, an established tumor suppressor, and RASSF1C, an emerging oncoprotein. Our lab has discovered that RASSF1C promotes lung cancer cell proliferation and migration while attenuating apoptosis. RASSF1C also up-regulates important genes in lung cancer cell growth including a stem cell self-renewal gene, PIWIL1. PIWI-like proteins interact with small PIWI-interacting RNA molecules (piRNAs) that are 24–32 nucleotides long to form complexes that regulate transcriptional and translational repression leading to inhibition of apoptosis, stimulation of cell division and proliferation, and down-regulation of cyclin inhibitors and tumor suppressors. To further investigate the RASSF1C-PIWIL1-piRNA axis in lung cancer, we carried out a global piRNA microarray screen to identify piRNAs that are regulated by RASSF1C in non-small cell lung cancer (NSCLC). In this study, we assessed the expression of the selected piRNAs in 8 NSCLC tissues.

**Methods Used** Real Time PCR: PCR was performed on RNA isolated from tumor and matched normal lung tissues obtained from eight patients with NSCLC using KAPA SYBER<sup>R</sup> FAST qPCR Kit with a forward primer consisting of the first 21 nucleotides of each piRNA sequence coupled with a universal reverse primer. The Real Time PCR reactions were carried out in triplicates and the fold change was calculated using the  $2^{-\Delta\Delta CT}$  method.

**Summary of Results** piR-34871 and piR-52200 were found to be upregulated in 62% of the lung tumor tissues when compared with corresponding normal tissue from the same patient. piR-35127, piR-46545, and piR-50485 were downregulated in 62%, 50%, and 60% of NSCLC tissues, respectively. In addition, there was an inverse correlation between piR-35127 and RASSF1C expression levels in all of the NSCLC tissues.

**Conclusions** Variable levels of piRNAs found in tumors suggest that these may be useful tools as biomarkers for early detection. piR-35127 holds particular interest due to its negative correlation with RASSF1C. Further investigation will be required to determine if piR-35127 plays a role in some of the oncogenic functions of RASSF1C.

### 324 MINIBEAM THERAPY AND TISSUE SPARING POTENTIAL

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10.1136/jim-d-15-00013.324

**Purpose of Study** Excessive radiation to tissue surrounding a target area is a common concern in radiation therapy,

especially when treating brain and superficial tumors. In this study orthovoltage X-rays at 250 kV will be made into 0.3-mm planar minibeam, using a collimator with 1-mm tungsten leaves, to determine if toxicity to proximal tissue can be spared. In addition the radiation target will be set in a linear motion at multiple angles to simulate physiological motion, such as breathing, to detect the extent of smearing and further enhance this method's clinical relevance.

**Methods Used** A collimator is placed in the 250 kV beam path to create 0.3-mm planar beams with 1-mm spacings. The divergence of the planar minibeam was measured by placing radiochromic film vertically beneath the collimator for six minutes. The radiation was then repeated with the film moving 8-mm along the sagittal plane at 0, 5, 10, 15, 20, 40, 80, and 90 degrees, parallel to the planar beams. A dose map was created from the films and the peak-to-valley ratios were found by dividing the average of the highest intensity beam by the lowest intensity spared region at each angle.

**Summary of Results** At zero degrees the proximal end of the film showed clear, discrete lines but as the angle of movement increased, the lines became smeared and the merge depth occurred closer to the source, showing less skin sparing. Proximal sparing was seen based on the high center beam dose and low dose where the tungsten leaves blocked the beam. The peak-to-valley ratio declined as the angle increased, with a ratio of 18.6 at zero degrees, 6.9 at five degrees, 5.4 at 10 degrees, 2.6 at 15 degrees and 1.3 at twenty degrees.

**Conclusions** Smearing was minimal within a 15 degree range, which supports use of this method in a clinical setting and demonstrates that movements such as breathing will not negate minibeam therapy benefits. Proximal tissue sparing patterns observed after radiating with orthovoltage X-rays are analogous to previous studies using a proton beam. Further studies may be conducted to see if this lower cost method can be implemented in underprivileged locations because it can be operated at 1/100ths of the cost of proton beams.

325

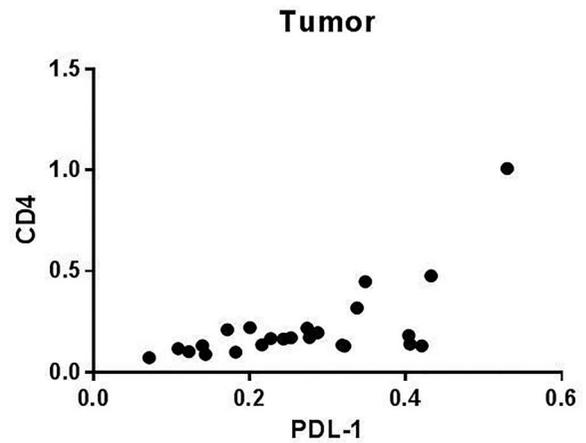
#### IMMUNOLOGIC PROFILE OF HEAD AND NECK SQUAMOUS CELL CARCINOMA CANCER STEM CELLS

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10.1136/jim-d-15-00013.325

**Purpose of Study** Head and neck squamous cell carcinoma (HNSCC) frequently recurs and metastasizes via cancer stem cells (CSCs) through signaling mechanisms such as reliance on the PI3K pathway. However, immune evasion is a critical component of cancer progression and the basis of the interaction between tissue stroma and HNSCC tumor. The present study aims to elucidate the immunologic profile of HNSCC in tissue stroma both in primary tumor sites and metastatic lymph nodes, and to correlate to clinical data.

**Methods Used** We are comparing ALDH1, CD44, SOX2, S6K, PD-L1, PD-1, CD3, CD4, CD8, CD19, CD25, CD45, CD56, CD68, and CD151 expression between the



**Abstract 325 Figure 1** Scatter plot showing relationship of PDL1 expression (percent of image pixels positive) and CD4 lymphocyte infiltration (as measured by percent image pixels positive) in HNSCC.

primary cancer and lymph node metastasis sites in 50 HNSCC cases. Each stained tissue section is stacked into a layered image array for each case and quantified by software simultaneously, allowing for direct comparison of relative expression. Results are correlated to clinical data.

**Summary of Results** There is a significant correlation between PDL1 and CD4 expression (Spearman coefficient 0.598,  $p=0.002$ ) in the first 10 samples. This relationship was only seen in the HNSCCs that had high expression of SOX2 (Spearman coefficient 0.929,  $p=0.0009$ ,  $N=8$ ), but not those with low SOX2 expression (Spearman coefficient 0.143,  $p=0.6$ ,  $N=16$ ).

**Conclusions** Our early data suggests that SOX2 expression is related to PDL1 expression, and increased T-regulatory (CD4) expression. PDL1 expression is likely related to HNSCC immune evasion.

326

#### EVALUATION OF MLN0264 AS A NOVEL THERAPY FOR PANCREATIC CANCER

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10.1136/jim-d-15-00013.326

**Purpose of Study** Pancreatic adenocarcinoma (PDAC) is the fourth leading cause of cancer deaths annually. Current therapies only minimally improve overall survival, indicating that newer treatments for this devastating disease are urgently needed. An emerging class of targeted cell based biotherapeutics known as antibody drug conjugates (ADC) are currently being developed for the treatment of cancer. MLN0264, a novel investigational ADC that targets guanylyl cyclase C (GCC), consists of a fully human anti-GCC monoclonal antibody conjugated to the cytotoxic microtubule disrupting agent monomethyl auristatin E (MMAE) via a protease cleavable linker (linker/toxin technology licensed from Seattle Genetics). The objective of this study was to determine the efficacy of MLN0264 as a potential

target specific agent for the treatment of pancreatic cancer. **Methods Used** Five unique pancreas cancer explants were treated with MLN0264 and treatment responses were determined after 28 days. Tumor size was evaluated twice per week by caliper measurements. Sensitivity to MLN0264 was defined as having a tumor growth inhibition index (TGII) of  $\leq$  to 20%. The activation of p53 and CHK2 were evaluated by immunoblotting at day 28 after MLN0264 treatment. GCC expression was compared between matched normal and tumor tissue.

**Summary of Results** Two of the five pancreas tumor explants showed sensitivity to MLN0264 with Panc 122 having a TGII of 15.78% and Panc 150 having a TGII of 10.5%. The three remaining tumors Panc 193, Panc 129 and Panc 137, displayed intermediate sensitivity to MLN0264 having a TGII of 27.4%, 38.9% and 62% respectively. Evaluation of the pharmacodynamic effects of MLN0264 revealed a significant increase in p53 and CHK2 activation following treatment in the Panc 150 sensitive tumor. In addition, GCC protein expression was analyzed by western blot in normal versus tumor tissue; a marked increase in GCC expression was observed in tumor tissue when compared to matching normal tissue.

**Conclusions** Preliminary results show that MLN0264 has good activity in pancreas tumor explants. These findings support further investigation of MLN0264 for the treatment of pancreatic cancer.

### 327 PRECLINICAL EVALUATION OF THE TRANSLATIONAL INHIBITOR SVC112 IN COLORECTAL CANCER

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10.1136/jim-d-15-00013.327

**Purpose of Study** Colorectal cancer (CRC) ranks third in new cases and cancer deaths in the U.S. annually. The current frontline treatments for metastatic CRC (mCRC) are ineffective for an appreciable proportion of patients and cause significant toxicities. Thus, there is an urgent need for the development of new therapeutic strategies. Eukaryotic elongation factor 2 (eEF2) is often overexpressed in CRC causing upregulation of translation, upon which CRC may be dependent. Therefore, it may be possible to differentially target CRC cells vs normal cells by inhibiting translation. SVC112 is a novel inhibitor of translation through its ability to lock eEF2 on the ribosome. As such, this agent may be effective against mCRC by blocking translation of key oncogenes such as c-myc which is overexpressed in mCRC.

**Methods Used** 44 CRC cell lines were exposed to SVC112 in vitro and CellTiter Glo ATP quantification was used to determine sensitivity or resistance based on IC50 values. Immunoblotting was performed to assess levels of c-myc, p-S6RP and cyclin D1 in response to treatment with SVC112. Amino acid incorporation was assessed using the Click-IT AHA kit protocol. Identification of genes and pathways associated with responsiveness to SVC112 in vitro was determined by KEGG pathway analysis. Two cell line xenografts determined to be sensitive based on IC50 values were treated in vivo with SVC112.

**Summary of Results** A subset of CRC cell lines were determined to be sensitive to SVC112 in vitro. In these cell lines, c-myc is downregulated by SVC112 in a dose-dependent manner. In resistant cell lines c-myc levels are not affected. Moreover, SVC112 causes a reduction of amino acid incorporation in sensitive cell lines and does not change amino acid incorporation in resistant cell lines. However, in the models tested thus far, SVC112 has not shown efficacy in vivo with the experimental dosing regimen.

**Conclusions** SVC112 shows anti-cancer effects in a subset of CRC cell lines. Resistance to SVC112 in vitro is due to the inability of SVC112 to bind to the drug target as it does not downregulate translation in resistant cell lines. Further analysis of SVC112 binding to the ribosome will be conducted. Additionally, different dosing regimens in vivo may be warranted.

### 328 CENTRAL LINE ASSOCIATED BLOOD STREAM INFECTIONS IN PEDIATRIC HEMATOLOGY/ONCOLOGY INPATIENTS AND OUTPATIENTS

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**Purpose of Study** Central venous access devices (CVADs) are required for care in pediatric hematology/oncology (ph/o) but may become infected. Central line associated blood stream infections (CLABSI) are a major source of unreimbursed costs and a serious cause of harm to patients. Typical inpatient CLABSI rates in ph/o are 2–3/1000 line days, but combined in/outpatient rates are not known. In a retrospective review we measured the incidence of in/outpatient CLABSIs and describe the first CLABSI.

**Methods Used** After IRB approval, charts of patients treated in the ph/o center from 1/1/2011 to 12/31/2013 who were 6mos–21yrs with CVAD placed and seen at least twice were reviewed. Data collected included demographics, diagnosis, type of line placed, dates of placement and removal, and positive blood cultures. Each culture was evaluated using CDC CLABSI criteria and only those meeting criteria were considered. The total number of CLABSIs was divided by the total number of line days to determine the overall incidence rate of CLABSIs. The frequency of demographics, timing, CVAD type, and cancer category were also analyzed for the first CLABSI.

**Summary of Results** During the study period, of 1783 unique patients seen during this 3-yr period, 312 had CVADs placed. There were 477 new CVADs with complete data and 113,717 central line days during the study period. There were 140 CLABSIs, and the overall incidence was 1.23/1000 central line days. The mean length of time a CVAD was in place was 236 days (95% CI 214, 262). The mean time to first CLABSI was 117 days (95% CI 89, 146). Patients with acute myeloid leukemia (AML), stem cell transplants (SCT) and those patients who had a double lumen (DL) broviac, peripherally inserted central catheter (PICC) had the highest incidence of an initial CLABSI (2.6, 2.4, 3.5, and 3.2/1000 central line days  $p < 0.0001$  using chi-square). 35 patients had more than one CLABSI.

**Conclusions** To our knowledge, this is the first analysis of CLABSI in a combined in/outpatient ph/o population. DL broviacs, PICCs, patients with AML and SCT had the highest incidence of CLABSIs. Using this data, targeted efforts can be studied to prevent CLABSI in these high-risk populations.

**329 CORRELATION BETWEEN EPITHELIAL-MESENCHYMAL TRANSITION AND INVASIVENESS IN OVARIAN CANCER CELL LINES AND ORTHOTOPIC XENOGRAFT**

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**Purpose of Study** Within United States women, ovarian cancer (OC) is the 5th most common cancer and the 4th most common cause of cancer death. In women greater than 50 years old, median 5 year survival is still below 50%. Approximately 90% of malignant OC tumors are of epithelial origin. Epithelial-mesenchymal transition (EMT) is the process by which relatively sedentary epithelial cells assume a more migratory and invasive mesenchymal phenotype. While EMT occurs normally during gastrulation and embryo development, its reoccurrence in adulthood is associated with pathological processes such as cancer metastasis. EMT causes the downregulation of intercellular adhesion proteins and the upregulation of matrix metalloproteases, both of which facilitate mobility and invasive capacity. The transcription factors Snail (Sn) and Twist (TW) are known to induce this transition. Though EMT has been demonstrated to occur in early OC tumorigenesis, much remains to be elucidated in relation to OC pathophysiology and how it relates to EMT. We wish to examine the effects of induced EMT on OC invasiveness, and hypothesize that induction of EMT via Sn and TW will increase OC invasiveness.

**Methods Used** The human OC derived cell line OVASHO was subjected to knockdown and overexpression of Sn and TW. Migratory capacity, invasiveness and anchorage independent growth were assessed using three assays: cell wound closure assay, transwell invasion assay and soft agar colony formation assay respectively. Characterization of migratory capacity and anchorage independent growth of other OC derived cell lines (OVCAR8 and COV318) are currently being assessed. The OC line OVCAR8 and patient derived cells were injected into ovarian bursa of nude mice to create an orthotopic xenograft model of OC. Tumors were tracked with bioluminescent imaging.

**Summary of Results** Overexpression of Tw and Sn in OVSAHO was accompanied by increased migratory capabilities but no observable effect on anchorage independent growth. Initial bioluminescent assessment of OC injected mice suggests a correlation between Sn knockdown and reduced tumor growth.

**Conclusions** Overexpression of Tw and Sn may increase migratory capacity or rate of proliferation, and the knockdown of Sn may reduce rate of tumor growth.

**330 QUALITY OF ONLINE RESOURCES FOR PANCREATIC CANCER PATIENTS**

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10.1136/jim-d-15-00013.330

**Purpose of Study** The internet is a growing source of information for pancreatic cancer patients. Given that the disease is usually diagnosed at an advanced stage, access to quality information in a timely manner is critical. Our aim is to systematically assess the information available to primary pancreatic cancer patients on the internet.

**Methods Used** An internet search using the term “pancreatic cancer” was performed, with the meta-search engines “Dogpile”, “Yippy” and “Google.” The top 100 websites returned by the search engines were evaluated using a validated structured rating tool to assess their currency, disclosure, attribution, interactivity and content. Inter-rater reliability was confirmed via kappa statistics and results were analyzed via descriptive statistics.

**Summary of Results** The results of 100 pancreatic cancer websites revealed both strengths and notable gaps. While 90% of websites disclosed ownership, sponsorship and advertising, only 40% identified an author. 87% of websites identified a date of creation, but 61% of websites were last updated over 4 years ago or had no date of last modification identified. With respect to coverage, etiology/risk factors and symptoms were almost always accurate and most likely to contain all required information (63 and 62% of websites). Conversely, prevention and prognosis were the least accurate and least completely covered sections (28 and 27%). 22% of websites were at a university reading level.

**Conclusions** Since the majority of patients use a search engine to access health information rather than direct links from reputable sources, it is important to inform patients about the strengths and weaknesses of information. With respect to pancreatic cancer, this study shows the quality of online pancreatic cancer information is variable. Many websites are outdated and lack author information. While the majority of information presented is factually correct, sites may lack information on prognosis. This knowledge can inform the dialogue between healthcare providers and patients addressing the gaps in the available information.

**Infectious Diseases II  
Concurrent Session  
3:30 PM  
Friday, January 29, 2016**

**331 IDENTIFYING MEN WHO HAVE SEX WITH MEN AND TRANSGENDER WOMEN WHO HAVE POOR LINKAGE TO HIV CARE IN LIMA, PERU**

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10.1136/jim-d-15-00013.331

**Purpose of Study** HIV is a growing public health concern in Peru, especially among Men Who Have Sex with Men

(MSM) and Transgender Women (TW). The Peruvian Ministry of Health Antiretroviral Therapy (TARGA) program provides accessible HIV care at most hospitals in the country. Few studies have evaluated the HIV care cascade and barriers to linkage to care in the Peruvian health system, where antiretroviral therapy is provided for free.

**Methods Used** MSM and TW in Lima, Peru were screened for HIV at enrollment into a treatment as prevention study between May 2013 and May 2015. Participants who were HIV-positive at baseline were referred for HIV care. Study data from the enrollment visit for these participants were linked to TARGA program data. Potential predictors of linkage to care were gathered from questionnaire data on demographics, sexual identity, alcohol use, and history of sex work collected prior to HIV diagnosis. We used univariate and multivariate logistic regression and survival analysis to model care linkage within 3 months.

**Summary of Results** Of 3394 participants, 487 were newly diagnosed with HIV. Approximately 45% (218 participants) linked to care within 3 months. In logistic regression, Alcohol Use Disorder (AUD; hazardous or harmful drinking, or alcohol dependence) was a strong negative predictor of linking to care (OR=0.63,  $p=0.02$ ). Additional risk factors included previous history of sex work (OR=0.47,  $p<0.01$ ) and bisexual identity (OR=0.57,  $p=0.02$ ). A trend of lower linkage was observed for TW (OR=0.62,  $p=0.13$ ). Participants 25 years or older were more likely to link to care (OR=1.86,  $p<0.01$ ). Cox regression analysis gave similar results. Income and education level were not statistically significant in predicting linkage.

**Conclusions** Vulnerable populations (sex workers, high risk drinkers, youth, and bisexuals) were significantly less likely to link to care within the overall MSM population. AUD is common – MSM and TW in Peru are five times more likely to have AUD than the general male Peruvian population. Thus, alcohol use disorder provides an opportunity for intervention that is currently not being addressed during patient referral. Lower rates of linkage among sex workers, youth and bisexuals also warrant further study.

### 332 EFFECTS OF INTERFERON GAMMA ON THE RESPIRATORY BURST OF VARIANT X-LINKED CHRONIC GRANULOMATOUS DISEASE PATIENTS VERSUS CLASSIC X-LINKED DISEASE

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10.1136/jim-d-15-00013.332

**Purpose of Study** The purpose of this study was to evaluate the effect of recombinant interferon gamma (IFN- $\gamma$ ) on the respiratory burst (and in some instances nitric oxide production) by polymorphonuclear leukocytes (PMN) and monocytes of patients (n=4) with variant X-linked chronic granulomatous disease (CGD) in comparison to patients (n=2) with classic X-linked CGD, 4 carrier X-linked mothers, and controls.

**Methods Used** We employed phorbol myristate (PMA) stimulated dihydrorhodamine fluorescence (DHR) as well as the determination of superoxide production induced ferricytochrome c reduction after incubation of the cells in culture media containing recombinant human IFN- $\gamma$  (Actimmune; Horizon Pharma plc, Dublin, Ireland) in varying concentrations for varying periods of time. All CGD patients had been confirmed to be either variant, or severe X-linked CGD cases by DHR fluorescence and the exact mutation determined by high resolution melting and targeted exon sequencing.

**Summary of Results** Two of the four variant CGD patients and one of the two classic X-linked patients demonstrated increases in PMN superoxide production after incubation with IFN- $\gamma$ . One of three variant X-linked female carriers had an increase in PMN superoxide induced by IFN- $\gamma$ . Two variant CGD patients and their carrier mothers and two X-linked severe CGD patient and their carrier mothers showed increases in monocyte superoxide production after incubation with IFN- $\gamma$ . In contrast, we seldom saw significant enhancement of DHR reduction to rhodamine following incubation with IFN- $\gamma$  as recently reported.

**Conclusions** In conclusion, incubation of PMNs and monocytes from patients with variant and severe X-linked CGD and their carrier mothers showed enhanced superoxide production in both severe X-linked patients and 2 of 4 variant patients and their mothers indicating that it is likely that the specific mutation may determine subsequent responses to IFN- $\gamma$ .

### 333 DISSEMINATED COCCIDIOIDOMYCOSIS IN CHILDREN

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10.1136/jim-d-15-00013.333

**Purpose of Study** To describe the clinical presentation, treatment and outcome of coccidioidomycosis in children admitted to a tertiary Pediatric Intensive Care Unit (PICU) located in the San Joaquin Central Valley.

**Methods Used** Retrospective review of charts, laboratory and imaging records of all coccidioidomycosis patients admitted to the PICU between January 1st 2010-December 31st 2014.

**Summary of Results** Seven patients (4 males, 3 females) were admitted to the PICU, mean age 6 years (.5–18 years). All patients had at least one previous medical visit, four previously hospitalized for pneumonia and one for “viral meningitis”. Disseminated disease (central nervous system, cardiac, mediastinal, and/or bone disease) was present in all cases. Diagnosis was made by enzyme linked immunoassay, immunodiffusion, complement fixation, fungal culture and/or histopathology. All patients received intravenous amphotericin. Six patients received both amphotericin and an azole antifungal. Three patients, refractory to amphotericin, received intravenous caspofungin and voriconazole. One patient had fungal meningitis and also received intrathecal amphotericin and interferon gamma. Treatment continued until the

normalization of titers and clinical and radiologic resolution of the disease. Mean length of treatment was 16 months (3–29 months). Patients underwent surgical debridement and excision of fungal lesions. Mean PICU length of stay was 19 days (2–78 days) and mean hospital length of stay was 94 days (7–196 days). Two patients died. **Conclusions** Despite being in an endemic area, coccidioidomycosis diagnosis and subsequent treatment is delayed due to lack of recognition. Risk factors for disseminated disease in previously healthy children are not known. Further studies elucidating immune factors and effectiveness of combination antifungals are needed.

334

#### IMPACT OF RAPID ORGANISM IDENTIFICATION WITH MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT ON OUTCOMES IN HOSPITALIZED PEDIATRIC PATIENTS

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10.1136/jim-d-15-00013.334

**Purpose of Study** Identification of causative agents of bacteremia traditionally relies upon time-consuming microbiological techniques that may require several days. However, use of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectroscopy has demonstrated more rapid and accurate identification of blood pathogens in adult populations, which in turn has improved clinical outcomes with concurrent use of an antimicrobial stewardship team (AST). We present the first data on the use of MALDI-TOF in hospitalized children without use of an AST.

**Methods Used** We retrospectively assessed time to organism identification (after growth on culture) and length of stay in all hospitalized children with bacteremia in 2011 utilizing the Phoenix automated microbiology system (PHX), and in 2014 utilizing both MALDI-TOF and the PHX system. A Welch's t-test was used to assess for statistical significance in mean differences.

**Summary of Results** 221 total blood cultures (134 in 2011 and 87 in 2014) were collected from 170 patients (95 in 2011 and 75 in 2014). The most frequently identified organisms included coagulase negative Staphylococci (51.4%), *S. aureus* (12.2%), Enterococcal species (10.4%) and Viridans group Streptococci (68%). In 2014, MALDI-TOF identified 30 of the 57 organisms the PHX system was unable to identify. MALDI-TOF identified organisms a mean of 15.9 hours faster than the PHX system in 2014 ( $p < 0.001$ ). However, there was no significant difference in the duration of hospitalization between 2011 and 2014 (1011.3 vs. 1010.2 hours respectively,  $p = 0.498$ ). No significant differences were seen when only shorter hospitalizations were considered ( $> 24$  hours and  $< 240$  hours in length,  $p = 0.101$ ).

**Conclusions** MALDI-TOF identified organisms from blood cultures nearly 16 hours faster than traditional

microbiologic methodology among hospitalized children. Despite these timesavings, the duration of hospitalization was not significantly reduced without concurrent use of an AST. These are novel findings which argue for the importance of providing real-time feedback to providers in the context of an antimicrobial stewardship program.

335

#### SALMONELLA OSTEOMYELITIS IN IMMUNOCOMPETENT CHILDREN: A CASE SERIES AND REVIEW OF THE LITERATURE

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10.1136/jim-d-15-00013.335

**Purpose of Study** *Salmonella typhi* osteomyelitis is frequently encountered in children with sickle cell anemia (SCA), particularly in resource-limited settings. However, the frequency and clinical course of non-typhoidal *Salmonella* osteomyelitis in otherwise healthy children is poorly described. We present a case series of such patients and systematic review of the literature.

**Methods Used** Children  $< 18$  years of age discharged between March 1st 2003 and March 1st 2014 from our institution were retrospectively assessed for acute osteomyelitis utilizing 40 different ICD-9 codes. Abnormal imaging and a compatible history were required. A faculty librarian conducted a PubMed search with the following strategy to produce 48 references: “Salmonella”[Mesh] NOT “Salmonella typhi”[Mesh] AND “Osteomyelitis”[MeSH] Filters: Humans; English; Child: birth-18 years. References from relevant studies were also searched.

**Summary of Results** One case of osteomyelitis was identified in our clinical review. Additionally, we identified 3 cases at our institution during 2015. All patients were male, immunocompetent and without SCA. The fibula, talus, femur, tibia and T10 vertebral body were involved. *S. enterica* serotype Durban, *S. enterica* serotype Montevideo, *S. enterica* serotype Oranienburg and *Salmonella enterica* serotype San Diego were isolated. Reptile exposure was present in 3 subjects. One subject was bacteremic. Our literature review produced 37 cases between 1960 and 2014, with 14 from the U.S. Ages ranged from 3 months to 18 years (mean 7.7 years), with 21 boys. *S. enterica* serotype Enteritidis was the most common serotype (8 cases). No risk factors were reported in 78%, and only 2 were associated with reptile exposure. Only 9 patients (24.3%) had antecedent gastrointestinal illnesses. Vertebral involvement was most commonly reported (9 patients). Only 37.5% of blood cultures demonstrated growth of *Salmonella*. Suppurative complications were reported in 10 (27.0%) subjects, with relapse in 4 (10.8%).

**Conclusions** *Salmonella* osteomyelitis is a rare but serious infection in immunocompetent children without SCA. Risk factors for *Salmonella* infection may be absent and blood cultures may be sterile.

### 336 EFFECTIVENESS OF POWERPOINT PRESENTATIONS IN TEACHING EMERGENT DERMATOSIS TO PEDIATRIC RESIDENTS

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10.1136/jim-d-15-00013.336

**Purpose of Study:** Background Identification of skin disorders is vital in the repertoire of outpatient, ER, and inpatient pediatric physicians, but many physicians and even dermatologists report deficiencies in their knowledge and comfort with pediatric dermatology while in residency. **Objective** To determine if furthering the education of pediatric residents through interactive lectures during residency training will increase their ability to identify, diagnose and treat pediatric emergent dermatosis.

**Methods Used** Resident physicians were given a 10-question pretest following by a series of post-tests based on content specifications from American Academy of Pediatrics (AAP). The post-tests were provided after intervention with PowerPoint lectures.

**Summary of Results** Over five months, from November 2014 to April 2015, a total of 30 pediatric residents (10 pediatric year I (PGY1), 10 pediatric year II (PGY2), and 10 pediatric year III (PGY3)) were tested on emergent dermatological conditions. Total possible participants included: 30 pediatric residents; 30 actual participants (100% of the possible participants). Assessment of long-term memory showed that the phase 1 mean score for PGY1 (0.4200) is significantly different than Phase 4 mean score for PGY1 (0.8727), ( $p < 0.001$ ). There were no significant differences in the scores from second or third year residents.

**Conclusions** This study's results depict that overall, there is a statistically significant improvement noted from the pre-intervention test and post-intervention test, primarily in the first-year residents. Although the second and third year residents did not produce statistically significant improvements in their scores, they did generate educationally significant results. Their scores and their level of comfort improved overall after the intervention.

### 337 EXPRESSION PATTERNS OF HEME OXYGENASE-1 IN IMMUNE CELLS DURING LATE GESTATIONAL INFLAMMATION

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10.1136/jim-d-15-00013.337

**Purpose of study** Heme oxygenase-1 (HO-1) is a major regulatory enzyme in innate and adaptive immunity. Induction of HO-1 inhibits phenotypic maturation of dendritic cells (DCs) and regulates regulatory T-cell (Treg) responses. We have established a late gestational inflammation model, which results in maternal immune cell changes, placental pathology, and pup loss. Here, we extend these

### Abstract 337 Table 1 Intracellular HO-1-Expression in Blood and Spleen Immune Cells (MFI±SEM)

Blood HO-1	NPR	PR	LPS-PR	p
Neutrophils	7048±1614	5276±260#	7777±434#	#0.0006
Monocytes	5996±527	5256±243#	10383±602#	#< 0.0001
MHC II+ DCs	8999±1329*	5393±281*#	11360±552#	*0.0241, #0.0001
Spleen HO-1				
Neutrophils	3347±1062	2843±205	3115±138	ns
Monocytes	3387±981	2968±211#	0.0±0.0#	#< 0.0001
DCs	8089±1100	6488±708	6992±180	ns

findings to determine the expression of intracellular HO-1 in maternal inflammatory immune cells using this model.

**Methods used** Pregnant wildtype (WT) dams (n=6) were injected IP with lipopolysaccharide (LPS, *E. coli*, 90 µg/kg) at E15.5 and monitored for signs of systemic infection. At 24h post-LPS (LPS-PR), blood and spleens were collected. Untreated age-matched pregnant (PR) (n=6) and non-pregnant (NPR, n=6) females served as controls. HO-1 expression patterns in immune cells were identified using multicolor flow cytometry.

**Summary of Results** Compared to PR dams, exposure to LPS significantly increased HO-1 expression in blood neutrophils, monocytes, and DCs. HO-1 in blood MHC II+ mature DCs was highest among all immune cell populations for all groups. Most interestingly, splenic monocytes were totally absent in LPS-PR dams compared to PR and NPR controls (see Table 1).

**Conclusions** In summary, the effect of LPS on intracellular HO-1 expression in inflammatory immune cells appears to be tissue specific. In addition, the LPS-induced phenotypic maturation of DCs appears to coincide with an increased expression of HO-1 in blood DCs. We conclude that since mature DCs activate effector adaptive responses, they may disrupt immune homeostasis and then lead to adverse changes as a result of inflammation during pregnancy.

## Morphogenesis and Malformations Concurrent Session

3:30 PM

Friday, January 29, 2016

### 338 MICROPHthalmIA WITH LIMB ANOMALIES (MIM #206920). A RECOGNIZABLE SYNDROME INVOLVING THE DEVELOPMENT OF THE EYES, BRAIN AND LIMBS

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10.1136/jim-d-15-00013.338

**Case Report** Microphthalmia with limb anomalies (MIM #206920) was initially reported by Waardenburg in 1961. This autosomal recessive condition has also been named Waardenburg anophthalmia syndrome, Acromesomelic syndrome and Anophthalmia syndactyly. The condition has been reported in 35 cases since then and is known to be

caused by mutations in the secreted modular calcium-binding 1 protein SMOC1 (MIM #608488) since 2011.

A 9 year old girl was referred for Genetics/Dysmorphology because of lack of vision due to severe microphthalmia, abnormal hands and feet, severe intellectual disability with lack of speech but reporting hyperacusis, prolonged G-tube feeding and constipation. She was adopted from India, had suffered neglect and malnutrition, but nothing is known about her family history. Her physical exam showed short stature, microcephaly, very short downslanting palpebral fissures, a depressed midface, a prominent philtrum, mild scoliosis and an anterior anus, syndactyly of hands (3–5) with marked ulnar deviation of the 5th finger, a sandal gap with syndactyly 2–4 and oligodactyly of feet missing the 5th toe, a linear crease over the tibia, mild scoliosis and hypermobile joints. A skeletal X-ray survey identified hypoplastic femoral heads and absent proximal fibulae, bony syndactyly 4–5 in both hands, and a missing 5th toe.

A SNP array identified several large fragments of homozygosity, the largest 50 Mb in size including 14q23, where SMOC1 is located. Close consanguinity is suspected. Sequencing of SMOC1 is pending.

The review of all previously reported cases shows consistent diagnostic findings, allowing for a firm clinical diagnosis, where the severe microphthalmia and the limb radiologic anomalies are specific. Zebrafish and mouse model studies have shown the implication of the gene, a member of the SMARC (osteonectin) family, in the development of the optic vesicles that undergo arrest in development, as well as in several areas of the brain and the limbs. Reduced apoptosis through dysregulation of the bone morphogenetic proteins (BMPs) is well proven in SMOC 1 null mice, and underlies the syndactyly phenotype. All reported SMOC1 human mutations predict complete loss of function of the protein, owing for this consistent recognizable phenotype.

### 339 CHEST WALL ASYMMETRY AND ABSENT LUNG IN UNIPARENTAL DISOMY 16

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10.1136/jim-d-15-00013.339

**Case Report** Trisomy 16 is the most common chromosome defect found in spontaneous abortions. A small fraction of pregnancies affected by trisomy 16 can survive to term by trisomy rescue resulting in uniparental disomy of chromosome 16 and occult trisomy 16 mosaicism. There is a well-described mosaic trisomy 16 syndrome with the following features; symmetric IUGR, slow growth, imperforate anus, hypospadias, and normal cognition. Most interestingly if these cases survive past delivery the prognosis then becomes quite favorable. This report adds chest wall defects and pulmonary artery stenosis to that phenotype. This female infant presented with symmetric IUGR, complete agenesis of the left lung, complete absence of the left pulmonary artery and significant hypoplasia of the left breast bud. She also presented with congenital scoliosis and underdevelopment of her left chest wall which were thought to be a secondary to her absent lung. She was

otherwise symmetric in her extremities and facial features. A microarray was ordered as part of a work up for multiple congenital abnormalities which showed stretches of homozygosity of chromosome 16 consistent with trisomy 16 rescue. To demonstrate that her thoracic abnormalities were due to trisomy 16 rescue we reviewed 29 ‘parent stories’ of children that had been made public online by the Disorders of Trisomy 16 Foundation. Out of those cases we found similar asymmetric findings in 5/29. One had absence of the left breast which became noticeable at puberty. One had absence of the right nipple. One had unspecified hemihypertrophy. One had fused ribs and hemivertebra. And the last had partial absence of the left lung, congenital pulmonic stenosis and died due to complications from pulmonary hypertension. A major limitation of this study is that personal narratives were reviewed instead of original medical records. Further work will need to be done to formally phenotype these cases. Importantly, these results suggest that trisomy 16 rescue should be considered as a diagnosis in patients with IUGR and chest asymmetry. It also suggests that for patients with trisomy 16 rescue screening recommendations should include echocardiograms with the pulmonary arteries as congenital pulmonary artery stenosis may be more frequent than otherwise expected.

### 340 CLASSIFYING CONGENITAL DEFECTS BY CAUSE AND CLINICAL PRESENTATION: A POPULATION-BASED STUDY IN UTAH

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10.1136/jim-d-15-00013.340

**Purpose of Study** Understanding the causes of congenital defects is a clinical, research, and public health priority. Epidemiologic studies report many associations with genetic or environmental factors; however, only 2 studies have directly explored the applicability of these findings in clinically well-defined population-based cohorts. To this end, we developed a multidimensional classification system that considers etiology (known, unknown), morphology (isolated, multiple major, minors only), and pathogenesis (sequence, development field, pattern).

**Methods Used** We used this classification system to assess a cohort with major defects among all pregnancy outcomes (livebirths, stillbirths, pregnancy terminations) from Utah’s population-based birth defect surveillance system. Excluding selected mild conditions (e.g., muscular septal defect), we generated a final cohort of 5,500 cases (birth prevalence, 2.1%).

**Summary of Results** Using strict and systematic criteria for causal assignment, 20.3% were assigned a known etiology (n=1,114). Most cases with a known etiology were chromosomal or single genetic conditions (1054, 94.4%) with a small proportion due to a known human teratogen (46 or 4.1%) such as uncontrolled pregestational diabetes or abnormalities of twinning (e.g., conjoined or acardiac twins; 16 or 1.4%). Overall, 79.7% (n=4,386) could not be assigned a clear etiology, with the majority being isolated defects (3,870 or 88.2%).

**Conclusions** These findings underscore the current gaps in causal knowledge that hinder the translation of epidemiologic associations to specific cases of congenital defects, even for risk factors deemed to be well known (e.g., smoking). This etiological classification of congenital defect cases is a sensitive tool for assessing our current knowledge about causation. The minimum estimates of cases directly attributable to specific factors underscore the need to dramatically improve our understanding of congenital defects in order to reduce their occurrence.

341 **MESODERM LOSS OF SONIC HEDGEHOG AND TGF-BETA SIGNALING CAUSE TRACHEAL MALFORMATIONS**

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10.1136/jim-d-15-00013.341

**Purpose of Study** The trachea is the major structure that connects the upper airways to the gas exchange units of the lungs. In neonates, abnormalities in development of trachea cause life threatening malformations such as tracheal stenosis or tracheomalacia. We have been interested in the molecular and cellular signaling mechanisms that govern tracheal and lung morphogenesis. Two major signaling pathways in vertebrate development are TGF-beta and Sonic Hedgehog.

**Methods Used** The boundaries of the mesodermal compartments within the trachea were examined by assessing expression of SOX9,  $\alpha$ -smooth muscle actin ( $\alpha$ SMA), and Platelet-derived growth factor receptor  $\alpha$  (PDGFR $\alpha$ ). The epithelial compartment was identified by immunostaining for NKX2.1. The potential role played by TGF $\beta$  or SHH signaling in the establishment of compartments and differentiation of their mesodermal cells was examined by targeted inactivation of Alk5 or Smoothed in early mesodermal progenitors.

**Summary of Results** At E9.5, PDGFR $\alpha$  is broadly expressed in the ventral mesoderm surrounding the anterior foregut, the site of specification of the lung endodermal primordium, identified as NKX2.1<sup>positive</sup>. Sox9 is expressed in both mesoderm and foregut endoderm, co-localized with NKX2.1, the origin of all pulmonary epithelial cell types. At E10.5, PDGFR $\alpha$ , Sox9 and  $\alpha$ SMA are confined to three mesodermal compartments with delineated boundaries surrounding the NKX2.1<sup>positive</sup> cell domain. Subsequently, Sox9 expression becomes exclusively restricted to the mesoderm, and epithelial expression ceases. Inactivation of either Alk5 or Smoothed in the mesodermal progenitors disrupts all three compartments.

**Conclusions** TGF $\beta$  signaling via Alk5 and SHH via Smoothed play critical roles in early patterning and organization of the normal mesodermal and endodermal compartments. This research is supported by NHLBI and the Hastings Foundation.

342 **FUNCTIONAL ENGRAFTMENT OF MURINE MC3T3-E1 PRE-OSTEOBLASTIC CELLS IN A ZEBRAFISH MODEL OF EPIMORPHIC REGENERATION**

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**Purpose of Study** It has long been established that urodeles such as newts and salamanders have the capacity to regenerate limbs following amputation. This occurs through epimorphic regeneration, a process characterized by the formation of a proliferative mass of partially dedifferentiated cells called the blastema. The engraftment of mammalian cells into the blastema would represent a powerful strategy to identify novel factors regulating bone regenerative processes. However, such xenograft models have yet to be developed due to incompatibilities in amphibian and mammalian biology (e.g., differences in body temperature).

**Methods Used** To address this challenge, our goal was to develop a xenograft model of epimorphic regeneration by introducing mammalian cells into the zebrafish tail fin. For cell injections, we employed the MC3T3-E1 murine pre-osteoblastic cell line, a standard and well-characterized model of mammalian osteoblastic differentiation. Two days post amputation (dpa) of the caudal fin, zebrafish were injected with CM-Dil-labeled MC3T3-E1 cells either intramuscularly, proximal to the caudal fin (to examine homing), or directly into the blastema. All studies were carried out in fish housed in ~33°C water, a temperature which we discovered was permissive to both fish and cell survival.

**Summary of Results** Cells directly injected into the blastema exhibited clear cellular engraftment and stability. Cellular activity was variable from fish-to-fish, with some cells remaining in stable clusters, and others showing migration and/or proliferation. At 5dpa, decreased fluorescence was observed, potentially occurring due to the dye fading and/or immune system activity. No injected cells were observed in the blastema following intramuscular injection.

**Conclusions** Collectively, our studies suggest that the regenerating zebrafish tail fin may serve as a viable system for xenograft models of bone regeneration, and further indicate that mammalian cells are fundamentally capable of surviving in an environment undergoing epimorphic regeneration. Provided the correct conditions, mammalian cells may be capable of engrafting and proliferating in an epimorphic regeneration process.

343 **CRANIOSYNOSTOSIS: VARIANT SEQUENCE ANALYSIS OF THE THYROID SIGNALING PATHWAY**

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10.1136/jim-d-15-00013.343

**Purpose of Study** Craniosynostosis (CS), the pathologic premature fusion of cranial sutures, may have many underlying causes including genetic components and potentially

alterable environmental factors. Several observational studies indicated a link between thyroid dysregulation and CS. The purpose of this study was to identify sequence variation of genes in the thyroid pathway with the hypothesis that sequence variants predispose children to CS.

**Methods Used** Within the previously completed RNA sequence data of osteoblast cell lines derived from 200 CS and 50 control patients, the variant expressions of 100 thyroid genes were filtered limiting the candidate gene list to those most likely resulting in disease. The list was further narrowed using Combined Annotation-Dependent Depletion (CADD-Phred) scores above 12, indicating a likelihood of being deleterious, and Genomic Evolutionary Rate Profiling (GERP) scores above 3, demonstrating evolutionary conservation. From this analysis, the two most promising candidate genes, GLIS3  $n=9$ , and NLRP1  $n=15$ , including 24 distinct variants associated with CS, were identified in 20 individuals. To validate each variant, primers were designed, and Polymerase Chain Reaction (PCR) was used to amplify the regions of interest in patient and control genomic DNA. After optimization, each of these PCR products was Sanger Sequenced to provide visual confirmation of variants.

**Summary of Results** The DNA samples were amplified via PCR and separated using Gel Electrophoresis. Afterward, 24 products each from CS cases and controls were sent for sequencing. 100% of the control sequences were homozygous at the desired locus, while 100% of the CS sequences confirmed the variants identified by RNA sequencing.

**Conclusions** The validation of the variants suggests these patients do have potentially disease-causing variants in thyroid pathway genes associated with bone formation, while none of the non-CS control samples showed variants in these pathways. These variants, rarely seen in large control cohorts, may predispose individuals to CS via genetic or an environmental and genetic interaction. Additionally, many of the patients share variants in other genes indicating a potential multigenic inheritance interaction.

#### 344 GASTROINTESTINAL FEATURES IN A NEWBORN WITH CURRY-JONES SYNDROME

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**Case Report** Curry-Jones syndrome (CJS) is a pattern of malformation that includes craniosynostosis, pre-axial poly-syndactyly, agenesis of the corpus callosum, cutaneous and gastrointestinal abnormalities. Nine cases have been reported in the literature. This report describes the gastrointestinal and surgical findings in a baby with CJS who presented with abdominal obstruction.

A 41 week 4165 gm, female presented with craniosynostosis, pre-axial polysyndactyly, and patchy, atrophic areas of depigmentation on the unilateral trunk and lower extremity consistent with a clinical diagnosis of CJS. On the second day of life she developed abdominal distension and

underwent a rectal suction biopsy to exclude Hirschsprung disease. The biopsy revealed ganglion cells and rare minimally hypertrophied nerve bundles. An upper GI was suggestive of malrotation without volvulus. Exploration revealed an intestinal malrotation for which she underwent a Ladd procedure. Additional findings were multiple small nodules on the surface of the small and large bowel, and an apparent intestinal duplication that seemed to originate posterior to the pancreas. Histopathology of serosal nodules revealed bundles of smooth muscle with associated ganglion cells. Post-operatively, the infant had a prolonged ileus. The infant was discharged home on full oral feedings on hospital day number forty-five.

Gastrointestinal smooth muscle hamartomas are a recognized feature of children with CJS typically presenting with abdominal obstruction requiring surgical intervention. Two of the nine cases described in the literature had similar involvement. Pseudo-obstruction and congenital short bowel have also been described. Gastrointestinal findings in these patients likely relate to aberrant development of the enteric nervous system with impaired development of peristalsis and obstruction from bowel hamartomas. All cases of CJS have been sporadic. As a germline mutation would likely be lethal, this disorder is probably caused by mosaicism.

#### 345 A NOVEL *DE NOVO* MUTATION IN *CACNA1A* IS ASSOCIATED WITH ATYPICAL NEUROLOGICAL FEATURES AND MITOCHONDRIAL DYSFUNCTION

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10.1136/jim-d-15-00013.345

**Purpose of Study** Mutations in *CACNA1A*, a gene that encodes the pore-forming alpha1 subunit of human voltage-gated CaV2.1 (P/Q-type) Ca<sup>2+</sup> channel, cause several autosomal dominant neurologic disorders characterized by paroxysmal symptoms, including episodic ataxia type 2, familial hemiplegic migraine type 1, and spinocerebellar ataxia type 6. Brain abnormalities, including cerebral edema and cerebellar vermian atrophy, may be present. To increase understanding of the phenotypic spectrum associated with calcium ion channel defects, we report a boy with a novel *de novo* p.F1367I (c.4099T>A) variant in *CACNA1A*.

**Methods Used** Whole exome sequencing (WES) and retrospective chart review.

**Summary of Results** We report a 14-year-old boy with developmental disability primarily in motor skills. He can work a computer, follow commands and communicate well with his mother. He has diffuse hypotonia with myopathic weakness, oculomotor and speech apraxia. Although he had short episodes of tonic upward gaze appearing at 2 months, these paroxysms decreased after 4 years with no additional significant paroxysmal symptoms. Muscle electron transport chain analysis at 18 months showed complex I deficiency at 28% of the control mean (66 nmol/min/mg protein; nl 188–361 nmol/min/mg protein), and he was initially considered to have a mitochondrial disorder. At 7 years, electron transport chain activity was performed on fibroblasts and results were normal. WES

detected a novel *de novo* p.F1367I (c.4099T>A) variant in *CACNA1A* that was predicted to be deleterious by *in silico* modeling.

**Conclusions** Our case illustrates that *CACNA1A* variants may be associated with significant developmental disability and hypotonia in the absence of more classical paroxysmal symptoms, such as ataxia or migraine. Mitochondrial complex I deficiency was also present in our patient. Because an abnormality in complex I genes was not identified on WES, it appears that the biochemical complex I deficiency observed in this case may be a secondary phenomenon. It is tempting to speculate that there may be mitochondrial involvement in channelopathies related to CaV2.1 abnormalities, but further studies are needed to elucidate the possible role of mitochondrial dysfunction in disease pathogenesis.

## Neonatology General IV Concurrent Session 3:30 PM Friday, January 29, 2016

### 346 DELAYED EPO THERAPY IMPROVES BEHAVIORAL AND HISTOLOGICAL OUTCOMES AFTER NEONATAL RODENT STROKE

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**Purpose of Study** Stroke is a major contributor to neonatal morbidity and mortality, and diagnosis is often delayed. There are also no therapeutic options specific for neonatal stroke. The purpose of this study is to investigate the efficacy of delayed initiation of multiple dose erythropoietin (EPO) therapy to repair the immature brain after stroke.

**Methods Used** Postnatal day 10 (P10) Sprague-Dawley rats underwent sham surgery or transient middle cerebral artery occlusion (MCAO) for three hours, resulting in moderate injury. EPO (1000 U/kg) or vehicle was administered intraperitoneally at age P17, 20, and 23 (three doses total). At four weeks after MCAO sensorimotor function was assessed in these four groups (vehicle-sham, EPO-sham, vehicle-MCAO and EPO-MCAO) with forepaw preference in cylinder rearing trials. Animals were then sacrificed and brains sectioned for hemispheric volume analysis.

**Summary of Results** A total of 32 rats underwent transient MCAO or sham surgery. EPO-MCAO animals performed significantly better in forepaw use compared to vehicle-MCAO animals at four weeks after stroke ( $p < 0.0001$ ), with more symmetric use of their forelimbs, and did not differ from shams ( $p = 0.1936$ ). There was also a significant increase in hemispheric brain volume in EPO-MCAO compared to vehicle-MCAO animals ( $p < 0.0001$ ), which correlated with functional performance ( $p = 0.0031$ ).

**Conclusions** These results suggest that delayed EPO therapy improves both behavioral and histological outcomes at one month following neonatal stroke, and may provide a late treatment alternative for early brain injury.

### 347 A PHASE II RANDOMIZED CONTROLLED TRIAL OF ERYTHROPOIETIN AND HYPOTHERMIA FOR NEONATAL NEUROPROTECTION IN HYPOXIC-ISCHEMIC ENCEPHALOPATHY

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10.1136/jim-d-15-00013.347

**Purpose of Study** Among infants with hypoxic-ischemic encephalopathy (HIE), to evaluate safety and biomarkers of brain injury in those treated with erythropoietin+hypothermia, compared to those treated with hypothermia alone.

**Methods Used** In a double-blind placebo-controlled trial, we enrolled 50 newborns with moderate (N=43) or severe (N=7) HIE. All patients had encephalopathy; perinatal depression (10-minute Apgar  $\leq 5$ , pH  $< 7.00$  or base deficit  $\geq 15$ , or need for resuscitation at 10 minutes); and received hypothermia. We randomized newborns to receive Epo 1000 U/kg IV or placebo at 1, 2, 3, 5 and 7 days. Two independent observers determined MRI brain injury severity using the Washington University scoring system. 6- and 12-month developmental outcomes were assessed by Warner Initial Developmental Evaluation (WIDEA) questionnaire.

**Summary of Results** Of 154 newborns with HIE at 7 hospitals, 81 (53%) met study eligibility, and 50 were enrolled (consent rate = 74%). Mean consent age was 12.6 (SD 7.2) hours. Mean age at 1st study drug was 16.5 (SD 5.9) hours. There were no safety concerns. In-hospital mortality was 14%. Mean WIDEA score at 6 months was higher in Epo-treated babies (Table 1). 12 month evaluations are on-going. Brain MRI at mean 5.1 (SD 2.3) days showed lower brain injury score (3.9 vs. 16.4) and less moderate/severe brain injury (5% vs. 44%) in Epo vs. placebo groups. Subcortical injury (i.e., basal ganglia, thalamus or posterior limb of the internal capsule) was less common in the Epo than placebo group (36% vs. 68%).

**Conclusions** In this small RCT, infants with HIE who received high-dose Epo+HT had less brain injury on neonatal MRI compared to those who received HT alone. A large efficacy trial is needed to determine whether Epo further improves long-term neurodevelopmental outcomes in HIE

Abstract 347 Table 1

	Epo (N=24)	Placebo (N=26)	P value
Severe encephalopathy	21%	15%	0.72
In hospital mortality	2 (8%)	5 (19%)	0.47
6 month WIDEA	75.3 (SD 9.0)	69.0 (SD 11.0)	0.04
MRI-normal	8 (36%)	3 (12%)	0.01
MRI-mild injury	13 (59%)	11 (44%)	
MRI-moderate injury	1 (5%)	6 (24%)	
MRI-severe injury	0 (0%)	5 (20%)	

**348** **DECREASED RISK OF CEREBELLAR HEMORRHAGE IN PREMATURE NEWBORNS EXPOSED TO ANTENATAL MAGNESIUM SULFATE**

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10.1136/jim-d-15-00013.348

**Purpose of Study** Cerebellar hemorrhage (CbH) is now gaining recognition as a common form of brain injury in premature newborns. Our objective was to determine the clinical predictors of CbH in a prospective cohort of premature newborns evaluated with magnetic resonance imaging (MRI).

**Methods Used** A cohort of 68 preterm newborns (<33 wks gestation) imaged with 3T MRI soon after birth (2011–2015) was studied. Exclusion criteria included clinical evidence of a congenital syndrome, congenital infection, or clinical status too unstable for transport to MRI. A pediatric neuroradiologist scored axial T2-weighted and iron-susceptibility sequences for the presence of CbH blinded to the clinical history. Medical records were reviewed for clinical predictors of CbH blinded to MRI findings. Clinical predictors were compared between newborns with CbH and those without using descriptive statistics. Predictors associated with CbH ( $P < 0.1$ ) were evaluated in a multivariable logistic regression model.

**Summary of Results** CbH was present in 26/68 (38.2%) infants imaged with MRI soon after birth. Newborns with CbH were younger at birth (mean  $27.6 \pm 2.3$  wks vs.  $28.7 \pm 1.8$  wks,  $P = 0.03$ ), and of lower birth weight (median 919 grams, IQR 760–1140 vs. 1200, IQR 1000–1450,  $P = 0.002$ ). CbH was significantly associated with antenatal magnesium sulfate exposure (RR 0.42, 95% CI 0.24–0.73,  $P = 0.003$ ), hypotension (RR 2.54, 95% CI 1.17–5.5,  $P = 0.008$ ), patent ductus arteriosus (RR 2.14, 95% CI 1.2–3.8,  $P = 0.02$ ) and mechanical ventilation  $\geq 7$  days (RR 2.05, 95% CI 1.13–3.73,  $P = 0.02$ ). Maternal factors and mode of delivery were not associated with CbH. Adjusting for predictors associated with CbH and postnatal age at MRI, antenatal magnesium sulfate was independently associated with decreased CbH (OR 0.11, 95% CI 0.029–0.44,  $P = 0.002$ ).

**Conclusions** The rate of CbH in our cohort is higher compared to other studies, suggesting 3T MRI may be more sensitive for the detection of CbH in premature newborns. Antenatal magnesium sulfate exposure is independently associated with a decreased risk of MRI-detected CbH, which may help explain the reason underlying the neuroprotective effects of magnesium sulfate in premature newborns.

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**349** **MEASURING CALPROTECTIN IN STOOL AS PART OF THE “RULE OUT NECROTIZING ENTEROCOLITIS” EVALUATION**

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10.1136/jim-d-15-00013.349

**Purpose of Study** Calprotectin is an antimicrobial protein complex constituting about 60% of the soluble cytosolic proteins of granulocytes. An elevated level of calprotectin in stool is a marker of inflammatory bowel disease. Several reports show elevated stool levels of calprotectin from neonates with necrotizing enterocolitis (NEC). Our present study is the first to determine whether a fecal calprotectin level at the onset of signs concerning for NEC differentiates between those who have NEC *vs.* those who have a benign feeding intolerance. We also sought to define the source of calprotectin in the intestine of neonates with NEC.

**Methods Used** Neonates were eligible for this prospective study when an X-ray was ordered to “rule out NEC”. Calprotectin was quantified in stool at that time and in a follow-up stool. About one week later, each “rule out NEC” episode was categorized as having been due to NEC or not due to NEC. Immunohistochemistry of bowel excised from neonates with NEC was used to determine the microscopic location of calprotectin.

**Summary of Results** Of 30 episodes of “rule-out NEC”, 15 turned out to be NEC and 15 were not NEC. The 15 with NEC had much higher first-stool calprotectin levels (median 516  $\mu\text{g/g}$  stool vs. 110  $\mu\text{g/g}$  stool,  $p < 0.00001$ ). Those with NEC also had higher second-stool calprotectin (372  $\mu\text{g/g}$  stool vs. 54  $\mu\text{g/g}$ ,  $p < 0.0001$ ). As an early identifier of NEC, calprotectin performed better than an abnormal neutrophil count, immature to total neutrophil ratio, or platelet count. Calprotectin in bowel excised from neonates with NEC was associated with neutrophil extracellular traps (NETs) from activated neutrophils.

**Conclusions** At the onset of suspicion for NEC, a high stool calprotectin may help distinguish NEC from less pernicious forms of feeding intolerance. Calprotectin in stools of neonates with NEC is derived from activated neutrophils migrating to the bowel mucosa and lumen and exporting antimicrobial calprotectin by way of NETosis.

**350** **OUTCOMES OF EXTREMELY LOW BIRTH WEIGHT INFANTS WITH TRANSIENT HYPERGLYCEMIA TREATED WITH INSULIN**

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10.1136/jim-d-15-00013.350

**Purpose of Study** Transient hyperglycemia is common (40–80%) among extremely birth weight (ELBW) infants in the first few weeks of life. Increased risks for death and morbidities have been reported in ELBW neonates who develop hyperglycemia. The purpose of this study was to assess the short-term outcomes of ELBW neonates treated with insulin for transient hyperglycemia.

**Methods Used** Retrospective review of electronic medical records of ELBW neonates admitted to the neonatal intensive care unit at LAC+USC Medical Center from July 2009 to October 2014 without hyperglycemia and with hyperglycemia treated with insulin. Maternal and neonatal demographics and short-term outcomes were extracted from the database.

**Abstract 350 Table 1** Maternal and Neonatal Demographics and Outcomes

	Insulin N=61	Non-insulin N=69	P- value
Birth weight, g*	613±150	786±149	< 0.001
Gestational age, wks.*	25.1±1.6	27±2.7	< 0.001
Chorioamnionitis–N (%)	26 (42.6)	20 (29)	0.11
PIH–N (%)	27 (44.3)	22 (31.9)	0.15
Antenatal Steroids–N. (%)	56 (91.8)	64 (92.8)	0.61
Advanced resuscitation at birth–N. (%)	29 (47.5)	17 (24.6)	0.005
Vaginal delivery–N (%)	12 (19.7)	7 (10.2)	0.13
Mortality–N (%)	11 (18.3)	5 (7.3)	0.062
Severe IVH–N (%)	6 (9.8)	3 (4.4)	0.21
Severe ROP–N (%)	23 (37.7)	7 (10.2)	< 0.001
BPD–N (%)	40 (65.6)	38 (55.1)	0.014

\*Mean±SD.

**Summary of Results** A total of 130 infants were included in the study. There were 69 infants with hyperglycemia treated with insulin and 61 without hyperglycemia. There was a statistically significant difference in birth weight (BW), gestational age (GA) and need for advance resuscitation at birth in ELBW neonates treated with insulin. These infants had a higher rate of severe Retinopathy of Prematurity (ROP) and Bronchopulmonary Dysplasia (BPD) (Table 1). There were four patients (6.6%) who developed hypoglycemia during insulin infusion and were given dextrose boluses.

**Conclusions** ELBW neonates who developed hyperglycemia and were treated with insulin were smaller and more immature. Despite a difference in GA and BW, ELBW infants treated with insulin for hyperglycemia had the same rate of mortality and severe IVH but had a higher rate of BPD and severe ROP than preterm infants without hyperglycemia.

### 351 IDENTIFICATION OF NEONATAL HEMOLYSIS IN THE WELL BABY NURSERY

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10.1136/jim-d-15-00013.351

**Purpose of Study** Covert hemolysis can increase total bilirubin (TB) levels in otherwise healthy newborns. Studies have shown that pre-discharge measurements of TB together with end tidal breath carbon monoxide (CO), corrected for inhaled CO (ETCOc), can be used as an index of increased bilirubin loads due to hemolysis. We recently reported that a newly developed, point-of-care CO monitor (CoSense, Capnia, CA) detects ETCOc (precision ±0.1 ppm), which strongly correlated to measurements of carboxyhemoglobin. Here, our aim was to establish ETCOc ranges adjusted to risk for hyperbilirubinemia in healthy infants.

**Methods Used** Term and late-preterm healthy infants (n=103) from the well baby nursery were enrolled between

**Abstract 351 Table 1**

Risk on TB Nomogram ETCOc (ppm; median; IQR)	ETCOc <1.0	1.0 ≤ ETCOc ≤1.5	1.5 < ETCOc <2.5	ETCOc >2.5
TB<40th %tile (n=18) ETCOc (1.3; 1.1–1.6)	4 (22%)	9 (50%)	5 (28%)	0 (0%)
TB=40th to 75th %tile (n=34) ETCOc (1.2; 1.1–1.7)	3 (9%)	19 (56%)	12 (35%)	0 (0%)
TB>75 ≤ 95th %tile (n=25) ETCOc (1.8; 1.4–2.0)	0 (0%)	9 (36%)	14 (56%)	2 (8%)
TB>95th %tile (n=4) ETCOc (2.7; 2.4–3.0)	0 (0%)	0 (0%)	1 (25%)	3 (75%)
Total (n=82)	7 (9%)	38 (47%)	32 (40%)	5 (6%)

6 h and 6d of age. Serial ETCOc (≤3 timepoints) were made concurrently with clinical TB determinations from each subject until discharge. ETCOc values were stratified by postnatal age (PA) epochs to establish ETCOc ranges. Hyperbilirubinemia risk by ETCOc was then assessed by plotting associated TB on the Bhutani nomogram.

**Summary of Results** Stratification of ETCOc by PA epochs (0–24, 24–48, 48–72, >72h) were: (1.7, 1.6, 1.3–2.0), (1.6, 1.6, 1.2–2.0), (1.7, 1.6, 1.3–2.1), and (1.6, 1.3, 1.2–1.5), respectively. ETCOc stratified to TB risk groups showed that infants with ETCOc: ≥2.5 are at the highest risk; between 1.5 and 2.5 are at moderate risk, and ≤1.5 are at the lowest risk (Table 1). Risk due to hemolysis as detected by ETCOc alone is not independent (chi square=47.2, p<0.00001) and, by deduction, may be due to delayed bilirubin elimination.

**Conclusions** Combined measures of ETCOc and TB can accurately identify the pathologic basis of hyperbilirubinemia and guide clinical management. Bilirubin production clearly influences the degree of hyperbilirubinemia. Thus, infants with high ETCOc are at most risk and closely monitored. Infants with high TB but low ETCOc should be evaluated for delayed bilirubin elimination. The remainder should be followed clinically for other unpredictable risks, such as G6PD deficiency.

### 352 NON-INVASIVE VENTILATION AND THE EFFECT ON INTUBATION IN NEWBORNS WITH RESPIRATORY DISTRESS SYNDROME: A POPULATION BASED STUDY

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**Purpose of Study** The use of non-invasive ventilation (NIV) has increased in newborns with respiratory distress syndrome (RDS). However, there is limited information about the association between NIV and subsequent

intubation and resource utilization. Therefore, the objectives of this study are to: (1) To describe the frequency of NIV and intubation in newborns with RDS from 2006 to 2010; (2) To identify predictors of intubation, length of stay (LOS) and charges.

**Methods Used** Data was obtained from the California Office of Statewide Health Planning and Development (OSHDP) birth cohort file for the years 2006 to 2010. Procedure and diagnosis codes were identified using ICD-9-CM codes. Bivariate analysis was used to describe differences between cohorts. Multivariable (MV) analysis was used to determine predictors of intubation and LOS.

**Summary of Results** A total of 2,523,368 newborns were identified from 2006 to 2010. Of these, 5,138 patients were identified with RDS; 16.2% of patients received NIV; 27.4% were intubated. On MV analysis, after adjusting for insurance, sex, gestational age (GA), and ethnicity, use of NIV was not associated with an increase in intubation for infants > 28 weeks GA, aOR (95% CI), 1.3 (0.9–1.6). Use of NIV was associated with shorter LOS (95% CI): 4 (2.5, 6.9) vs. 18 (15.9, 19.6) days for intubated infants. Use of NIV decreased charges (\$/1k) (95% CI) by 96.8 (59.8, 134.4) vs. 267.1 (236.6, 298.0) for intubated infants. Comparing NIV patients to intubated patients, there was a statistically significantly lower frequency (%) of sepsis (46.6 vs 33.5), patent ductus arteriosus (50.8 vs 34.9), hypotension (12.0 vs 8.6), and chest tube placement (5.8 vs 2.0).

**Conclusions** Non-invasive ventilation was effectively used without an increased need for intubation among infants >28 weeks GA. NIV use was associated with less morbidities. Furthermore, NIV was associated with shorter LOS and charges.

353

#### IMPACT OF EXTRACORPOREAL MEMBRANE OXYGENATION ON CEREBRAL METABOLISM IN THE NEWBORN BRAIN

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10.1136/jim-d-15-00013.353

**Purpose of Study** Extracorporeal membrane oxygenation (ECMO) is an effective therapy for supporting infants with reversible cardiopulmonary failure. Still, survivors are at risk for long-term neurodevelopmental impairments, the cause of which is not fully understood. To address this, we used magnetic resonance spectroscopy (<sup>1</sup>H-MRS) to investigate the effects of venoarterial (VA) and venovenous (VV) ECMO on cerebral metabolism.

**Methods Used** Neonates who received ECMO between January 2004 and December 2014 were screened from medical records. We excluded neonates with comorbid conditions that could independently affect the developing brain. 42 neonates treated with ECMO for meconium aspiration syndrome, congenital diaphragmatic hernia or

persistent pulmonary hypertension were identified and contrasted with 38 age-matched neonates from our <sup>1</sup>H-MRS database. All <sup>1</sup>H-MRS data were acquired from standardized grey matter (GM) and white matter (WM) regions of interest using a short-echo (TE=35 ms) point-resolved spectroscopy (PRESS) sequence and quantitated using LCModel. Metabolite concentrations (mmol/kg) were compared across groups using multivariate analysis of covariance (MANCOVA).

**Summary of Results** Elevated creatine ( $p=0.004$ ) and choline ( $p=0.009$ ) concentrations were observed in the GM among neonates treated with ECMO relative to the reference group. Likewise, choline concentrations were elevated in the WM ( $p=0.004$ ) while glutamate was reduced ( $p=0.033$ ). Contrasts between ECMO groups revealed lower osmolite concentrations (e.g. myoinositol) among the VV ECMO group.

**Conclusions** Neonates who underwent ECMO were found to have an abnormal cerebral metabolic profile, with the pattern of abnormalities suggestive of an underlying inflammatory process. Additionally, neonates who underwent VV ECMO had low osmolite concentrations of cerebral osmolites, as seen in vasogenic edema.

## Nephrology and Hypertension Concurrent Session 3:30 PM Friday, January 29, 2016

354

#### ASSESSMENT OF URINE PROTEIN DIPSTICK AND PROTEIN/CREATININE RATIO AS DIAGNOSTIC TESTS IN CHILDHOOD NEPHROTIC SYNDROME

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10.1136/jim-d-15-00013.354

**Purpose of Study** Current guidelines recommend the use of urine protein dipstick (UPD) or protein creatinine ratio (PCR) as diagnostic tools in childhood nephrotic syndrome (NS). Our recent clinical observations and previous evidence in adults suggests that PCR may overestimate protein excretion in dilute urine samples. This study aimed to assess the agreement between UPD and PCR in defining NS diagnosis/relapse and remission considering urine concentration.

**Methods Used** Data was retrospectively obtained from electronic health records of NS patients followed at British Columbia Children's Hospital. Paired laboratory UPD and PCR results from 77 patients between March 2013 & August 2015 were analyzed. Recommended cut-offs for NS diagnosis/relapse and remission were tested in dilute and concentrated urine samples as measured by urine specific gravity (<1.015 vs >1.025). Kappa statistic was used to measure agreement between UPD and PCR along with 95% confidence intervals (95% CI).

**Summary of Results** A total of 406 urine samples from 77 children were obtained. Using PCR  $\geq 200$  mg/mmol and UPD  $\geq 3+$  as cut-offs for NS diagnosis/relapse, there was a substantial discordance in the most dilute (Kappa 0.574,

95% CI: 0.422–0.726) compared to most concentrated samples (Kappa 0.848, 95% CI: 0.747–0.949). Using PCR <20 mg/mmol and urine dipstick <1+ as cut-offs for NS remission, there was also a difference in the agreement between the most dilute (Kappa 0.618, 95% CI: 0.467–0.878) and concentrated (Kappa 0.976, 95% CI: 0.930–1.000) samples. Mean urine creatinine concentrations were significantly lower in the most dilute vs concentrated samples (3.28 vs 14.96 mmol/L respectively,  $p=0$ ).

**Conclusions** Considerably lower agreement of UPD and PCR in dilute urine samples was observed in defining NS diagnosis/relapse and remission. PCR may overestimate protein excretion due to low urine creatinine concentrations seen in dilute urine. Overestimation of proteinuria in children with NS can have several diagnostic, treatment, and prognostic implications. We suggest that urine PCR should be performed in concentrated urine, such as first AM samples, in order to avoid some of these issues.

### 355 PHYSICAL ACTIVITY AND METABOLIC HEALTH IN CHRONIC KIDNEY DISEASE

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10.1136/jim-d-15-00013.355

**Purpose of Study** People with chronic kidney disease (CKD) are at high risks of progressing to end stage renal disease and cardiovascular disease. Poor metabolic health may be a modifiable risk factor for these adverse outcomes. We examined the associations physical activity has on key metabolic health factors in subjects with moderate-severe non-diabetic CKD by using gold standards of measurement.

**Methods Used** We performed a cross-sectional study of 76 people, 47 with CKD (eGFR <60 ml/min/1.73m<sup>2</sup>) and 29 healthy control subjects. Physical activity was measured by accelerometry over a seven day period, insulin sensitivity by the hyperinsulinemic-euglycemic clamp method, and fat mass by DXA. We also measured blood pressure, serum lipids, and serum high sensitivity C-reactive protein. We tested associations of physical activity with these metabolic outcomes using multivariable linear regression, adjusting for possible confounding factors including demographics, comorbidities, and medication use.

**Summary of Results** Subjects with CKD were less active than control subjects, had lower insulin sensitivity 4.1 (2.1) versus 5.2, (2.0 (mg/min)/(μU/mL)), higher fat mass 32 (11.4) versus 29.4 (14.8 kg), and higher triglycerides 153.2 (91.6) versus 99.6 (66.8 mg/dL). Physical activity was positively correlated with insulin sensitivity and negatively correlated with fat mass and triglycerides. With adjustment, each two-fold higher level of physical activity was associated with a 0.9 (mg/min)/(μU/mL) higher insulin sensitivity (95% CI 0.2, 1.5,  $p=0.006$ ), an 8.0 kg lower fat mass (–12.9, –3.1,  $p=0.001$ ), and a 37.9 mg/dL lower triglyceride concentration (–71.9, –3.9,  $p=0.03$ ). Associations of physical activity with insulin sensitivity and triglycerides did not differ significantly among subjects with and without CKD ( $p$ -values for interaction >0.3), while the association of

physical activity with fat mass appeared weaker with CKD ( $p$ -value for interaction = 0.045).

**Conclusions** Greater physical activity may promote metabolic health for patients with moderate-severe CKD. Insulin sensitivity, adiposity, and dyslipidemia are logical intermediate targets for short-term physical activity trials that assess what types of physical activity may best promote metabolic health in CKD.

### 356 RENAL OUTCOMES IN PEDIATRIC ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODY ASSOCIATED VASCULITIS – A PEDIATRIC VASCULITIS INITIATIVE (PEDVAS) STUDY

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**Purpose of Study** Renal disease is the most common complication affecting patients with ANCA-associated vasculitis (AAV). However, renal outcomes have not been well described in affected children with AAV. We aimed to describe the 12 month renal disease course and outcome of pediatric AAV patients presenting with renal disease.

**Methods Used** Patients extracted from A Registry of Childhood Vasculitis (ARCHiVE) - with contributions from 29 international sites - were included if diagnosed with AAV <18 years of age and had either biopsy-proven pauci-immune glomerulonephritis or decreased renal function requiring dialysis. Patients' renal findings and investigations were examined at presentation and 12 months. Glomerular filtration rate (GFR), estimated using the Schwartz equation, was used to stratify renal outcomes according to the proportion of children with normal GFR (>90 ml/min/1.73m<sup>2</sup>), moderately reduced (MR) GFR (30–60 ml/min/1.73m<sup>2</sup>), severely reduced (SR) GFR (15–30 ml/min/1.73m<sup>2</sup>) and renal failure (<15 ml/min/1.73m<sup>2</sup>).

**Summary of Results** Of the 56 included patients, 71% were female, 57% were Caucasian, and 79% had Granulomatosis with Polyangiitis. Initial GFR of the 36 patients with creatinine data at presentation were: renal failure (36%, n=13), SR-GFR (19%, n=7), MR-GFR (25%, n=9) and normal GFR (19%, n=7). At presentation, renal findings of the 56 patients include hypertension (38%), oliguria (27%), nephrotic syndrome (14%) and; 25% required acute dialysis. At 12 months, 64% of those requiring dialysis at presentation either remained on chronic dialysis (n=7) or received a kidney transplant (n=2); 38%, 15% and 4% had normal, moderately reduced or severely reduced GFR respectively.

**Conclusions** Early renal disease in pediatric AAV is often severe with over 80% of patients presenting with a GFR <60 ml/min/1.73m<sup>2</sup> and 25% of patients requiring acute dialysis. Even though a number of patients improved after induction therapy, the 12-month renal outcomes are still generally poor with only 38% of patients achieving a normal GFR, and 18% with renal failure at 12 months. Future analysis from ARCHiVE will allow us to better explore predictors of long term outcomes including demographics, treatment regimens, and biopsy classifications.

### 357 COMPLEMENT INHIBITION FOR PREVENTION AND TREATMENT OF ANTIBODY-MEDIATED REJECTION

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10.1136/jim-d-15-00013.357

**Purpose of Study** Antibody-mediated rejection (ABMR) of renal allografts represents a significant and often serious complication of transplantation in sensitized patients. Recent data suggest that antibody-mediated activation of the complement system plays a significant role in mediation of ABMR. Here we report on the utility of two novel complement inhibitors (Eculizumab, anti-C5) and C1 esterase inhibitor (C1INH) for prevention and treatment of ABMR. **Methods Used** Therapeutic interventions aimed at the human complement system are recognized as potentially important strategies for the treatment of inflammatory and autoimmune diseases as there is often evidence of complement-mediated injury by pathologic assessments. Currently, there are two approved drugs aimed at inhibition of complement activation. Here we review data from controlled clinical trials that assess the importance of complement inhibition in prevention and treatment of ABMR.

**Summary of Results** Eculizumab (anti-C5, Alexion, Cheshire CT) which is approved for the treatment of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome. Eculizumab has also been studied in human transplantation, for treatment and prevention of antibody-mediated rejection. Initial data from uncontrolled studies suggested a significant benefit of Eculizumab for prevention of antibody-mediated rejection, but a subsequent randomized placebo controlled trial failed to meet its primary end point. Anecdotal data, primarily from case studies does show benefits in treating complement-mediated ABMR. A second approved complement-inhibiting therapy is C1-INH, which is approved for use in patients with hereditary angioedema, a condition caused by mutations in the gene which codes for C1-INH. A recent placebo controlled trial of C1-INH for prevention of ABMR in HLA sensitized patients showed the drug to be safe with evidence for inhibition of systemic complement activation and complement-activating donor-specific antibodies (DSAs).

**Conclusions** Complement inhibition appears to be a useful approach for prevention and treatment of ABMR. Early aggressive ABMR is always associated with complement activation as detected by C4d deposition and has a very poor prognosis. Here, complement inhibition has offered hope for reversal of ABMR episodes not seen with any other agents.

### 358 NF-KB TRANSCRIPTIONAL INHIBITION AMELIORATES CISPLATIN-INDUCED ACUTE KIDNEY INJURY (AKI)

C Edelstein,<sup>1</sup> A Ozkok,<sup>1</sup> Q Wang,<sup>1</sup> D Ljubanovic,<sup>2</sup> K Ravichandran.<sup>1</sup> <sup>1</sup>Univ Colorado Denver, Aurora, CO; <sup>2</sup>Univ Hospital Dubrava, Zagreb, Croatia

10.1136/jim-d-15-00013.358

**Purpose of Study** The NF-KB signaling pathway is important in inflammation and cell survival. Inflammation and

cell death in the kidney are features of cisplatin-induced AKI. It is known that cisplatin induces NF-KB signaling in the kidney. The purpose of the study was to determine the NF-KB responsive genes and the effect of direct NF-KB transcriptional inhibition in cisplatin-induced AKI.

**Methods Used** Mice injected with cisplatin, 25 mg/kg, developed AKI, acute tubular necrosis (ATN) and apoptosis on day 3. Mice were treated with JSH-23 (20 or 40 mg/kg) which directly affects NF-κB transcriptional activity.

**Summary of Results** Kidney function, tubular injury (ATN, serum neutrophil gelatinase-associated lipocalin [NGAL], but not apoptosis) and myeloperoxidase (MPO) activity were significantly improved by JSH-23 (40 mg/kg). Sixty one NF-KB responsive genes were increased by cisplatin of which 21 genes were decreased by JSH-23. Genes that were decreased by JSH-23 that are known to play a role in cisplatin-induced AKI were IL-10, IFN-γ, the chemokine CCL2 and caspase-1. Another gene, caspase recruitment domain family, member 11 (CARD11), not previously known to play a role in AKI, was increased more than 20-fold and completely inhibited by JSH-23. CXCL1 and TNF-α, known mediators of cisplatin-induced AKI, were decreased by JSH-23. Necroptosis, a form of programmed necrosis, mediated by receptor interacting-protein kinases (RIPKs), has been shown to be a mechanism of proximal tubular cell death. There was an increase in RIPK1 and 3, receptor-interacting serine/threonine-protein kinases, that play an important role in necroptosis, in cisplatin-induced AKI. In novel data, it is demonstrated that NF-KB transcriptional inhibition with JSH-23 results in a significant decrease in RIPK1 and RIPK3 suggesting that NF-KB activates RIPK1 and RIPK3 in cisplatin-induced AKI. In mouse proximal tubule cells in culture, JSH-23 resulted in an increase in apoptosis suggesting that the mechanism of protection against AKI by JSH-23 is not due to a direct effect on proximal tubules.

**Conclusions** NF-KB transcriptional inhibition in cisplatin-induced AKI ameliorates kidney function and ATN without a significant effect on apoptosis and is associated with a decrease pro-inflammatory mediators, CARD11 and RIPKs.

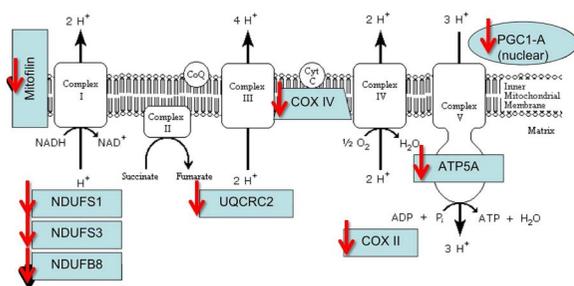
### 359 DYING MITOCHONDRIA: REDUCTION OF MITOCHONDRIAL PROTEINS IN PROGRESSIVE KIDNEY DISEASE

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10.1136/jim-d-15-00013.359

**Purpose of Study** Chronic kidney disease affects more than 10% of people in developed nations. Our lab has studied the metabolomics of diabetic chronic kidney disease and found that of 13 differentially expressed urine metabolites, 12 were linked to mitochondrial function and indicated reduction of mitochondrial function. We hypothesized that mitochondrial proteins were reduced in a model of progressive renal failure, the Alport *Col4a3*<sup>-/-</sup> mouse.

**Methods Used** Total protein was isolated from kidney cortex. To evaluate regulation of mitochondria I measured the levels of the following proteins that are linked to



Abstract 359 Figure 1

mitochondrial biogenesis or the electron transport chain via Western Blot: PGC1-a, Complex I subunits NDUFS1, NDUFS3, and NDUFB8, Complex III subunit UQCRC2, Complex IV COX II, and Complex V subunit alpha (ATP synthase), Mitofilin, and COX IV. Western Blot results were quantified by ImageJ software and standardized against B-Actin loading control. The ratios of protein of interest to B-Actin were statistically analyzed via unpaired T-test comparing 58 day old wild-type to 58 day old Alport mice.

**Summary of Results** All of the proteins measured were significantly reduced ( $p < 0.05$ ) in the diseased kidney of the Alport mouse ( $n=3$ ). These data suggest that there is a profound loss of mitochondrial proteins with progressive renal failure.

**Conclusions** We saw a dramatic reduction in mitochondrial proteins in the Alport model of progressive kidney disease. These findings indicate increased mitochondrial death, or decreased mitochondrial biogenesis. There is a strong association between progressive renal failure and mitochondrial dysfunction.

## Neuroscience II Concurrent Session 3:30 PM Friday, January 29, 2016

### 360 THE ROLE OF DIACYLGLYCEROL LIPASE IN THE TESTOSTERONE-INDUCED ENHANCEMENT OF RETROGRADE ENDOCANNABINOID SIGNALING IN EXCITATORY INPUTS TO ANOREXIGENIC PRO-OPIOMELANOCORTIN NEURONS

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10.1136/jim-d-15-00013.360

**Purpose of Study** Anorexigenic pro-opiomelanocortin (POMC) neurons in the hypothalamic arcuate nucleus play an integral role in eliciting satiety. Testosterone augments feeding behavior by enhancing endocannabinoid tone onto POMC neurons. This results in inhibition of neural transmission via depolarization-induced suppression of excitation (DSE). 2-Arachidonylglycerol (2-AG) is an endogenous agonist of cannabinoid CB1 receptors synthesized by the diacylglycerol lipase (DAGL) enzyme. We therefore tested the hypothesis that testosterone upregulates DAGL activity in POMC neurons to enhance endocannabinoid-mediated DSE.

**Methods Used** Male guinea pigs were orchidectomized 5–7 days prior to experimentation and injected with testosterone propionate (TP; 400  $\mu$ g; s.c.) or its sesame oil vehicle (0.1 ml; s.c.) 24 hours prior. Subjects were decapitated, the brain rapidly dissected, and 300  $\mu$ m-thick slices through the hypothalamus were cut using a vibratome. Whole cell patch clamp recordings were performed using biocytin-filled electrodes. Perfusion of the DAGL inhibitor, Orlistat (3  $\mu$ M), or its DMSO vehicle began 3 minutes prior to recording baseline spontaneous excitatory post-synaptic current (sEPSC) activity, and continued throughout recordings. After baseline recording, 60-mV depolarizing pulses (3 seconds in duration) were delivered from a holding potential of  $-75$  mV every 30 seconds for 15 trials. The data were analyzed by organizing the post-stimulation amplitude and frequency into 5-second bins over 20 seconds. DSE measurements were normalized as a percentage of baseline frequency and amplitude. After electrophysiological recording, slices were processed for immunohistochemistry to confirm POMC phenotype.

**Summary of Results** TP significantly potentiated endocannabinoid-mediated DSE, as manifested by prolonged increases in sEPSC frequency and amplitude compared to recordings from vehicle-treated controls. Pretreatment with the DAGL inhibitor blocked both the DSE observed in vehicle-treated animals, and the more pervasive DSE in TP-treated animals.

**Conclusions** These results demonstrate that testosterone-induced enhancement of DSE in anorexigenic POMC neurons is dependent upon upregulated biosynthesis of the endocannabinoid 2-AG.

### 361 ANDROGENS RAPIDLY POTENTIATE RETROGRADE ENDOCANNABINOID-MEDIATED INHIBITION OF ANOREXIGENIC POMC NEURONS

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10.1136/jim-d-15-00013.361

**Purpose of Study** The purpose of our study was to determine whether androgens could rapidly augment endocannabinoid tone and thus retrograde inhibition of excitatory input onto anorexigenic proopiomelanocortin (POMC) neurons by a process known as depolarization-induced suppression of excitation (DSE). In doing so, we hoped to identify the mechanisms by which androgens and endocannabinoids interact to regulate the hypothalamic feeding circuitry in order to further understand the pathology of obesity and related chronic disease. We hypothesized that androgens increase energy intake in male guinea pigs by rapidly enhancing the inhibitory effect of endocannabinoids on appetite-suppressing POMC neurons.

**Methods Used** We used male Topeka guinea pigs castrated 6–8 days prior to experimentation as subjects, and prepared 300  $\mu$ m-thick coronal slices through the hypothalamus. Using whole-cell patch clamp recordings, we first measured baseline levels of spontaneous excitatory post-synaptic currents (sEPSCs) from a holding potential of  $-75$  mV in slices treated with the dihydrotestosterone mimetic, CI-4AS-1 (100nM), or its ethanol vehicle prior to delivering a 60-mV depolarizing stimulus (3 seconds in

duration) every 30 seconds over 15 trials. We then quantified the DSE-induced changes in sEPSC frequency and amplitude observed over four consecutive, 5-second bins beginning immediately after termination of the depolarizing stimulus. Post-hoc cell identification was accomplished using various phenotypic markers for POMC neurons (i.e.,  $\beta$ -endorphin,  $\alpha$ -melanocyte-stimulating hormone, cocaine-amphetamine-regulated transcript) and fluorescence immunohistochemistry.

**Summary of Results** We found that CI-4AS-1 (100 nM) significantly potentiated endocannabinoid-mediated DSE in POMC neurons, as manifested by more pronounced and prolonged reductions in sEPSC frequency and amplitude compared to those seen in vehicle-treated controls.

**Conclusions** Our results support our hypothesis that androgens rapidly enhance retrograde, endocannabinoid-mediated inhibition of excitatory input impinging on anorexigenic POMC neurons. As such, they provide meaningful insight into the androgenic regulation of energy balance.

### 362 THE ROLE OF DIACYLGLYCEROL LIPASE IN THE ANDROGENIC REGULATION OF ENERGY HOMEOSTASIS

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10.1136/jim-d-15-00013.362

**Purpose of Study** Energy balance is regulated in large part through hormone-controlled mechanisms occurring within the hypothalamic feeding circuitry. Both synthetic and naturally-occurring cannabinoids have been demonstrated to initiate hyperphagia via inhibition of presynaptic neurotransmitter release on anorexigenic pro-opiomelanocortin (POMC) neurons in the hypothalamic arcuate nucleus. Testosterone has been shown to activate AMP-dependent kinase (AMPK), which then augments retrograde endocannabinoid signaling at excitatory inputs impinging on the POMC neuron. This could be attributed to increased biosynthesis due to up-regulated enzymes like diacylglycerol lipase (DAGL) which produces the appetite-stimulating endocannabinoid 2-arachidonoylglycerol (2-AG) from diacylglycerol. The purpose of this study was to elucidate if testosterone up-regulates DAGL in the ARC, thereby increasing energy intake and expenditure.

**Methods Used** Male Topeka guinea pigs underwent two surgical procedures. The first involved a stereotaxic guide cannula implantation into the third ventricle 14 days prior to experimentation. After a five-day recovery, orchidectomies were performed seven days prior to experimentation. After a three-day acclimation period, the behavioral study was initiated in which energy intake, meal pattern, O<sub>2</sub> consumption, CO<sub>2</sub> output, and metabolic heat were measured around the clock over a five-days. During this period the animals were treated every day at 8:00 with either the DAGL inhibitor Orlistat (3  $\mu$ g;i.3.v) or its cremephor/ethanol/0.9% saline vehicle (1/1/18; v/v/v; 2  $\mu$ l; i.3.v), and every other day with testosterone propionate (TP; 400  $\mu$ g; s.c.) or its sesame oil vehicle (0.1 ml; s.c.). The energy intake and expenditure data were collected via OxyMax software for later analysis.

**Summary of Results** increased energy intake, meal size, as well as O<sub>2</sub> consumption, CO<sub>2</sub> output, and metabolic heat production. These effects were markedly diminished by Orlistat.

**Conclusions** These data show that the androgen-induced changes in energy homeostasis and meal pattern and involve increased expression of DAGL, and hence augmented endocannabinoid tone within the hypothalamic feeding circuitry.

### 363 CALPAIN-2 AND PROTEIN TYROSINE PHOSPHATASE NON-RECEPTOR TYPE 13 COLOCALIZATION IN HIPPOCAMPUS OF ADULT WILDTYPE MICE

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10.1136/jim-d-15-00013.363

**Purpose of Study** This study looked at interactions between calcium-dependent thiol-protease calpain-2 and protein tyrosine phosphatase non-receptor type 13 (PTPN13). A previous study found that PTPN13's PDZ domain had a high binding activity with calpain-2's c-terminus, which has its own PDZ binding domain. The CA1 region of the hippocampus was assessed, where both proteins are expressed. PTPN13 and calpain-2 regulate cell growth, differentiation, and apoptosis, but whether they interact together to carry out these functions remains unknown. Such interaction could explain neurodegenerative disease mechanism, and thus elucidate new target therapies.

**Methods Used** Whole brain dissected from adult C57BL/6 J mice were fixed following cardiac perfusion of PFA, and prepared as frozen sections. Sections were washed, blocked, and separated into two groups: the experimental group (calpain-2/PTPN13) and the control group (calpain-1/PTPN13). Calpain-1 was used as a control because its c-terminal does not interact with PTPN13's PDZ binding domain. Sections were incubated in their respective primary and secondary antibodies, and then mounted onto slides with DAPI and visualized with a confocal microscope. ImageJ JaCOP plugin was used to quantify the co-localization using Pearson's correlation coefficient. Prism unpaired t-test tested for significance of the Pearson's coefficient averages between calpain-1/PTPN13 and calpain-2/PTPN13 co-staining.

**Summary of Results** Calpain-2 and PTPN13 were found to have a significantly higher colocalization in the CA1 region of adult wildtype mice hippocampi than calpain-1 and PTPN13. This was evident from calpain-2 and PTPN13's higher average Pearson's coefficient versus the average Pearson's coefficient of Calpain-1 and PTPN13.

**Conclusions** There was a strong correlation of colocalization and thus interaction found between PTPN13 and Calpain-2 in the CA1 hippocampal region. Co-IPs, western blots, and hippocampal neuronal staining are being done to confirm this. Hopefully this potential interaction will be better understood to optimize possible therapies for neurodegenerative diseases.

364

### IN UTERO HYPOXIA-ISCHEMIA AND LIPOPOLYSACCHARIDE ADMINISTRATION IN RATS INDUCES DEFICITS IN COGNITION EVALUATED USING A NOVEL TOUCHSCREEN PLATFORM

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10.1136/jim-d-15-00013.364

**Purpose of Study** Preterm infants are prone to numerous neurological deficits including cerebral palsy, epilepsy and intellectual disability. Among the most impactful, cognitive deficits pose a significant barrier to achieving independent adult living. Touchscreen testing is similar to the Cambridge Neuropsychological Test Automated Battery (CANTAB) in humans, and is a novel paradigm for assessing cognition in rodents following brain injury. We hypothesized that our established model of in utero injury would induce deficits in learning and memory in young adult rats as measured on a touchscreen platform.

**Methods Used** On embryonic day 18 (E18) a laparotomy was performed on Sprague-Dawley rats under anesthesia, with transient (1hr) uterine artery occlusion (TSHI). Lipopolysaccharide (LPS, 0.4g/sac) was then injected into each amniotic sac and laparotomy was closed. Sham animals received a laparotomy only. Rats were born at term, and began operant conditioning training on postnatal day 35 after mild food restriction. Animals were then trained on the touchscreen platform and following successful completion of training, performed a visual discrimination (VD) task (n=8/group).

**Summary of Results** TSHI+LPS rats are capable of successfully completing training and VD paradigms. Notably, TSHI+LPS rats have significantly longer latency to paradigm learning (Fisher's exact test, p=0.06) and log significantly fewer correct responses in the first five days of VD than sham rats. To more specifically examine the rate of learning, trajectories of mean correct response were plotted and revealed that during testing days 3 to 5, TSHI+LPS rats show a markedly lower proportion of correct responses than shams (mixed ANOVA p=0.01).

**Conclusions** Using a touchscreen operant platform to assess specific cognitive domains, we show that TSHI+LPS rats have significant impairment in visual discrimination, the first time to our knowledge this deficit has been reported using touchscreen platforms in a model of perinatal brain injury. Further testing with this platform will allow dissection of deficits in distinct pillars of cognition following in utero injury and in response to emergency therapeutic strategies for preterm infants.

365

### INFANTILE TRAUMATIC BRAIN INJURY IS MITIGATED BY ERYTHROPOIETIN TREATMENT IN A PRECLINICAL RODENT MODEL

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10.1136/jim-d-15-00013.365

**Purpose of Study** Traumatic brain injury (TBI) is the leading cause of death and severe morbidity for infants born healthy. However, despite the pervasiveness of severe, chronic diffuse brain injury, no targeted treatment currently exists to actively promote recovery. We developed a preclinical model of infantile TBI in rats to examine neuroradiological outcome following injury and putative efficacy of the neuro-repairative agent erythropoietin (EPO) for this vulnerable patient population.

**Methods Used** Anesthetized postnatal day 12 (P12) rats underwent left parietal craniectomy and 0.6mm controlled cortical impact (CCI). Sham animals underwent anesthesia with scalp incision only. Injured animals received 6 doses of erythropoietin (EPO) 3000U/kg/dose or vehicle over post-injury days 1–8. Brains and serum were collected at P35 for magnetic resonance imaging (MRI), and serum biomarker analysis using multi-array electrochemiluminescence (MECI).

**Summary of Results** Diffusion tensor MRI of the ipsilateral and contralateral cortex and white matter in CCI-veh rats showed widespread injury and significant abnormalities of functional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). Treatment with EPO reversed changes in MD, AD and RD. Serum analyses reveal a peripheral pro-inflammatory signature of CCI injury defined by anomalous increases in CXCL1. Notably, in addition to mitigating injury observed on MRI, treatment with EPO normalized CXCL1 in CCI animals.

**Conclusions** In our model of infantile moderate to severe TBI, injured animals demonstrated diffuse white matter and gray matter injury that was repaired with EPO treatment in a clinically relevant dosing regimen, concomitant with improved serum biomarker profiles. Further analyses of cognitive and behavioral outcomes of injury and treatment have the potential to inform the design of clinical trials for this at-risk patient population.

366

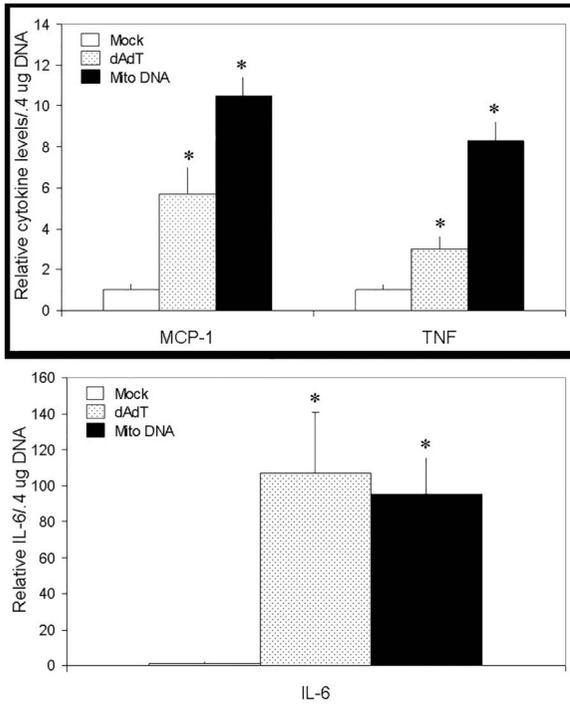
### OXIDATIVELY DEGRADED MITOCHONDRIAL DNA IS A NEWLY IDENTIFIED DAMP FAMILY MEMBER IN ASTROCYTES, AND POTENTIAL TRIGGER OF ALZHEIMER'S DISEASE

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10.1136/jim-d-15-00013.366

**Purpose of Study** It has been shown that mitochondrial DNA can stimulate immune cells as a DAMP (damage associated molecular patterns) family member. Here we extend our original studies to determine whether oxidant-initiated degraded mitochondrial polynucleotides (DeMPs), are more likely to be pathophysiologically relevant than whole intact mitochondrial DNA, and are able to stimulate an immune response in mouse primary astrocytes.

**Methods Used** Hamster fibroblast cells were cultured the Mitochondrial DNA purified and degraded and was transfected into mouse primary astrocytes using Lifectamine 2000. The cytokine analysis was performed using a Cytometric Bead array. Then a southern blot was performed for hybridization of mitochondrial DNA to labeled probes.



Abstract 366 Figure 1

**Summary of Results** Induction of proinflammatory cytokines IL-6 (95-fold), MCP-1 (11.2-fold), and TNF $\alpha$  (8.4-fold) was observed in mouse primary astrocytes transfected with degraded mitochondrial DNA. Proinflammatory IL1 $\beta$  was induced 7.2-fold, implicating inflammasome activation. CSF and plasma was found to contain detectable DeMP signal indicating release of mitochondrial DNA.

**Conclusions** These studies demonstrate that degraded mitochondrial DNA can elicit a proinflammatory cytokine induction in astrocytes which includes the inflammasome, and that DeMPs are detectable in CSF and plasma. These are supportive of our hypothesis that DeMPs are a trigger of neurodegenerative diseases associated with inflammation and oxidation such as Alzheimer's.

**367 LEARNING AND MEMORY TESTING IN LAMBS**

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10.1136/jim-d-15-00013.367

**Purpose of Study** Neurodevelopmental impairment may be a long-term outcome for survivors of premature birth and invasive respiratory support. Premature lambs supported similarly develop diffuse brain damage. Damage is evident as increased apoptosis, and decreased proliferation, of neurons and glia compared to premature lambs supported by non-invasive ventilation and unventilated term lambs. The hippocampus also is damaged. Because the hippocampus is damaged, learning and memory may be affected. However, before assessing learning and memory

in former premature lambs, we optimized learning and memory tests for lambs.

**Methods Used** Ten term control lambs were tested (8 group-raised; 2 solitary-raised). Each lamb had two 10-min trials, with rest between. The routine was repeated the next day. Different tests were separated by a rest day. The first test was habituation to the room. The second test used novel objects, using 3 conditions (two dissimilar objects; a new novel object replaced a previous object; added a third novel object). The third test used a mirror and a non-reflective surface. For these 3 tests, the number of vocalizations, time exploring, and time standing were recorded. For the novel object and reflection tests, the time to first-notice of an object, number of times the object was approached, and total time spent with an object were recorded. The fourth test was a maze, for which the reward was ewe's milk. Time to locate the reward was recorded.

**Summary of Results** Individually-raised lambs had markedly different responses to all tests and were excluded. The number of vocalizations did not decrease during habituation by the group-raised lambs. Time spent standing still or exploring was not different over time. Lambs approached a novel object faster than a familiar object. Lambs spent more time looking at the mirror than the non-reflective surface ( $p < 0.05$ ). Lambs approached the mirror slower on the first trial than the second trial ( $p < 0.05$ ). Lambs completed the first maze trial slower than the second trial ( $p < 0.05$ ).

**Conclusions** Lambs are amenable to neurodevelopmental tests of learning and memory. Our data also suggest that lambs should be raised in similar conditions to ensure validity of test results. Our next step is to test former premature lambs. HL110002, HL062875.

**Surgery III  
Concurrent Session  
3:30 PM  
Friday, January 29, 2016**

**368 SAFETY AND EFFICACY OF LIPOSOMAL BUPIVACAINE ACROSS A SPECTRUM OF PLASTIC SURGERY PROCEDURES**

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10.1136/jim-d-15-00013.368

**Purpose of Study** Postoperative pain management presents significant challenges to plastic surgeons. A trial found that patients receiving postoperative anesthesia via continuous infusion pump systems used less patient-controlled anesthesia (PCA) and transitioned earlier to oral narcotics. These pump systems are costly and involve complications such as infection, sepsis, and pump malfunction. Opioids, the mainstay of pain management, are associated with nausea, vomiting, ileus, pruritus, sedation, respiratory depression, drug-dependence, and opioid-induced hyperalgesia. Liposomal bupivacaine (Exparel; Pacira Pharmaceuticals, Parsippany, N.J.) is a liposomal formulation of a commonly used anesthetic agent (bupivacaine); it provides up to 72

Abstract 368 Table 1

Procedure	Number
Delayed breast reconstruction with tissue expanders, bilateral serratus anterior muscle flap	5
Delayed reconstruction of bilateral breasts with breast implants under TRAM	1
Breast reduction	4
Cheek fasciocutaneous flap for skin cancer reconstruction	2
Finger flexor tendon tenolysis	3
Thumb CMC joint arthroplasty	1
Split thickness skin graft	5
Harvest of skin graft from right leg and skin graft to right and left axillae measuring 430 cm <sup>2</sup>	1

hours of relief with a single injection in a more controlled fashion. It has not been evaluated widely in plastic surgery. **Methods Used** We piloted local tissue infiltration in 22 adult patients. The liposomal bupivacaine is prepared in a 1.33% solution in 20 mL vials, diluted per manufacture recommendations with an equivalent volume of normal saline. A total dose of 266 mg was infiltrated into the operative field using a 22g needle.

**Summary of Results** Table 1 outlines the procedures supplemented with liposomal bupivacaine infiltration. The infiltration of the drug provides no technical hurdles, and we did not note any intraoperative nor post-anesthesia care unit morbidity. Anecdotally, we have noted significant subjective positive pain relief for patients post-operatively.

**Conclusions** We noted improved postoperative pain management and the potential to reduce the length of patients' hospital stays, early postoperative morbidity, and healthcare costs. With better pain control, post-operative complications and the psychological burden of surgery decrease.

### 369 AN EVALUATION OF ANTIHISTAMINE PRE-TREATMENT IN FACIAL INJECTABLE TREATMENTS

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10.1136/jim-d-15-00013.369

**Purpose of Study** Aesthetic soft tissue facial augmentation using neuromodulators and dermal fillers has become a popular minimally invasive procedure in the United States. Even though satisfaction rates are high, complications such as pain, edema, and erythema are encountered. Anecdotal evidence suggests that oral antihistamines are effective in reducing these complications. The purpose of this pilot study was to evaluate the effectiveness of pre-treatment with topical antihistamines in reducing pain, edema, and erythema following injection of dermal filler.

**Methods Used** A pilot study was performed with six women receiving bilateral dermal filler injections of the malar region, nasolabial folds, and peri-oral region. A topical cream containing benzocaine 20%, lidocaine 6%, tetracaine 4% (BLT) compounded with diphenhydramine 2% was applied 30 minutes prior to injection. To test the efficacy of the topical diphenhydramine, a split-face

Abstract 369 Table 1 Facial Edema and Redness Rating Scale

Pigmentation (Bruising)	Redness	Turgor (from edema)	Swelling (Height)
0, no bruising	0, none	0, normal	0, normal
1, slight bruising	1, light to pink	1, supple	1, <2 mm
2, moderate bruising	2, pink to red	2, yielding	2, 2–5 mm
3, heavy bruising	3, red 4, vivid red	3, firm 4, hard	3, >5 mm

application was employed, with one half of the face receiving the topical BLT+diphenhydramine cream and the other half of the face receiving a topical cream containing only BLT. Photographs were taken pre-injection, 15 minutes post-injection, and 72 hours post-injection.

**Summary of Results** Both patient and the injecting clinician were asked to fill out separate versions of the facial edema and redness rating scale (seen below), adapted from the Vancouver scar scale. A scale rating at 72-hours post-injection was also obtained from each patient. Early results are promising but inconclusive regarding the efficacy of the topical anti-histamine cream.

**Conclusions** Although anecdotal evidence suggests oral antihistamines are effective in the pre-treatment of dermal filler injections, the pilot study data is inconclusive regarding the efficacy of topical antihistamine cream in reducing dermal filler related redness and swelling. In order to further elucidate the efficacy of oral and topical antihistamines and its role in soft tissue facial augmentation, additional studies are needed.

### 370 INTEGRA FLOWABLE WOUND MATRIX AND THE LOWER EXTREMITY: IMPLICATIONS FOR THE RECONSTRUCTIVE LADDER

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10.1136/jim-d-15-00013.370

**Introduction** We report the use of Integra Flowable Wound Matrix (FWM) in the treatment of large trauma wounds to the lower extremity. Integra FWM is composed of cross-linked collagen and glycosaminoglycan, a semi-liquid indicated for use in tunneling wounds. We propose the use of a "sandwich" of Integra's FWM between sheets of Bilayer and Monolayer Wound Matrix Dressing with Negative Pressure Wound Therapy (NPWT). To our knowledge, FWM has not been tested as the primary source of healing to large trauma wounds in the lower extremity. We propose the use of Integra, without flaps or grafts, facilitating a "reconstructive elevator", enhancing both recovery times and outcomes through the use of more efficient methods.

**Case** A 68-year old female presented with traumatic diabetic degloving, with a wound measuring 14 cm×11 cm. There was not sufficient tissue to allow for local flaps, and

her risk for perioperative complications was high enough to warrant other treatment. The wound was first irrigated and debrided, followed by treatment with an Integra “sandwich” consisting of Integra FWM between sheets of Monolayer and Bilayer Wound Matrix Dressings. NPWT was administered. Two weeks after application, the volume of injury had regressed. Six months later, the injury remained healed with the use of an Integra “sandwich” without skin flap or graft procedures.

**Discussion** Options for reconstruction have grown and are no longer linear in patient morbidity, risk factors, and complexity. It is not adequate to consider technical virtuosity in the absence of patient factors. The strategy for selecting the best option for the defect requires a deeper understanding of tissue biology and wound physiology. It requires clinical acumen derived from evidence-based review of patient factors that impact healing and patient safety factors that have increasingly been measured. Edits to the initial reconstructive ladder have included evolving techniques, providing comprehensive descriptions of technologies. The correlation of indications tempered by diagnoses and method-specific morbidities will add the needed context to techniques to guide the strategy for reconstruction of individual wounds. The optimization of patient status, wound factors and perioperative safety form the framework for the treatment goal.

### 371 SPIDER SILK AS A NEXT GENERATION SUTURE

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10.1136/jim-d-15-00013.371

**Purpose of Study** Through the development of finer, stronger sutures, new medical procedures have arisen. Spiders have evolved to create a silk with incredible mechanical properties. Due to their cannibalistic nature, spider silk cannot be farmed at an industrial scale. As such, methods are being developed to produce spider silk fibers synthetically. This study explored whether an aqueous system using recombinant spider silk proteins could be applied to create a stronger, finer suture. Due to current innovations, spider silk has the potential to create the next generation of sutures.

**Methods Used** MaSp1 and MaSp2 spider silk proteins were solubilized in water to make a dope. The dope was drawn into a 5mL plastic syringe and mounted on a custom wet fiber spinning system. Fiber diameters were measured using a Motic BA310 microscope with 10 MP Motic camera and analyzed on Motic's Image plus 2.0 ML software. Samples were mechanically tested using a MTS Synergie 100 with a custom 10 g load cell. Various methods were implemented to braid fibers together. To assess knot strength, spider silk thread was looped around two metal pins 7mm apart and knotted with a surgeon's knot using an instrument tie technique.

**Summary of Results** Using a 24% (weight/volume), 90/10 ratio MaSp1/MaSp2 dope spun with a 0.01 inch inner diameter needle into a 100% IPA coagulation bath, 80/20 IPA/H<sub>2</sub>O first stretch bath, and 50/50 IPA/H<sub>2</sub>O second stretch bath, with a 2.25× stretch ratio in both baths, fibers were produced with mechanical properties most suited for

### Abstract 371 Table 1 Spider Silk vs. Suture Stress Comparison

Sample	Fiber Stress (St. Dev.)	Knot Stress (St. Dev.)
MaSp1/MaSp2 12 Fiber Thread	147.43 MPa (29.64)	166.93 MPa (12.21)
6-0 Vicryl Suture	368.33 MPa (37.77)	180.88 MPa (11.14)

braiding and tying knots. Such fibers were braided and knotted as 3 sets of 4 with average stress values of 166.93 MPa. These mechanical properties rival 6-0 Vicryl sutures, with an average knotted stress of 180.88MPa.

**Conclusions** Through numerous trials, parameters were developed to create fibers with mechanical properties most suited for braiding and tying. Throughout this process, it was observed that spider silk threads did not demonstrate the marked decrease in stress which the 6-0 Vicryl sutures had when tied into a knot. When knotted, spider silk threads rival current sutures on the market, and with innovative developments in protein manufacturing, further improvements are anticipated.

### 372 VAGINAL WALL SLING: PILOT STUDY OUTCOMES AND VIDEO DEMONSTRATION OF TECHNIQUE

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10.1136/jim-d-15-00013.372

**Purpose of Study** Synthetic graft material has been a staple of treatment for stress urinary incontinence (SUI). Benefits of mesh include its unlimited supply, consistent quality, and customizable shape and size. However, drawbacks include graft placement into the urinary tract, infection, and erosion into the urethra, bladder, or vagina. In 2011, FDA released a safety update on transvaginal mesh for treatment of pelvic organ prolapse and SUI, encouraging providers and patients to discuss non-mesh options. We aim to evaluate patient satisfaction in patients who received an autologous suburethral sling using vaginal wall tissue for the treatment of SUI, as well as to demonstrate this technique with a video.

**Methods Used** A retrospective review was performed on autologous vaginal wall sling procedures from May 2011 to July 2015. A telephone survey was performed post-operatively to assess patient's current voiding symptoms and satisfaction, measured using a Likert Scale (1=very dissatisfied, 5=very satisfied). Mann-Whitney U test and Fisher's Exact test were used for statistical analysis, with a P<0.05 for significance.

**Summary of Results** Vaginal wall sling placement was performed in 53 patients. Mean age was 51 years (29–86). Mean BMI is 28.9 (21–45). Mean number of 5 vaginal deliveries (range 0–8). All patients concurrently underwent cystocele repair (n=52). Mean sling length was 8.5 cm (range 5.5–11 cm).

Average preoperative pad usage was 1.03/day (range 0–10), which decreased to 0.6/day (range 0–3;  $p=0.019$ ). 21 patients (68%) agreed to participate in a phone survey at a mean of 308 days postoperatively (24–657 days), where 14 (67%) reported zero pad usage, while 5 (24%) used 1–2 pads/day and 2 (10%) used >2 pads/day.

Patient's mean satisfaction postop was 4.0 on the Likert scale. Patients with 0 pad usage were significantly more satisfied than those who continued to require pads (4.2 vs. 1.7;  $p=0.001$ ).

**Conclusions** Autologous suburethral sling using vaginal wall tissue can be an effective and well tolerated technique in patients with SUI. In our cohort, patients were satisfied with the procedure, with 67% reporting no pad use postoperatively. Our video demonstrates the technique of harvesting and placing a suburethral sling using vaginal wall tissue.

### 373 DEVELOPMENT OF AN IMPLANTABLE CHITOSAN HEMOSTAT

M Dopp. *Loma Linda University, Loma Linda, CA*

10.1136/jim-d-15-00013.373

**Purpose of Study** We wanted to develop a novel hemostatic agent that could be used to control intraoperative hemorrhages without the need for removal. There is a great need for novel hemostats because the agents used at present are costly, contain thrombin, or have significant performance issues. Chitosan, a natural biopolymer is known to be an excellent hemostatic agent. However, it is only approved for using in achieving topical hemostasis due to issues with reducing pyrogen contamination. Thus, we hypothesized that non-thermal nitrogen gas plasma would effectively decontaminate chitosan while preserving its hemostatic properties. We tested our hypothesis in a porcine laparoscopic partial nephrectomy model.

**Methods Used** 4×4 cm pads composed of 100% chitosan acetate (Scion Cardio-Vascular, Inc.) were used for the study. Prior to initiating the animal work, non-thermal nitrogen gas plasma was optimized for decontaminating chitosan. Pads were then treated with either the optimized nitrogen gas plasma, or a standard electron beam sterilization procedure. The hemostatic effectiveness of the chitosan hemostat material was tested in six pigs, with three pigs in each sterilization group. In each pig, the lower pole of the left kidney was resected and 1–2 pads were placed on the cut surface. Two pigs were given a bolus of epinephrine after initial hemostasis, to challenge the hemostatic properties of the chitosan. At 14 weeks all pigs were sacrificed in order to determine the reaction to the implanted chitosan and the degree of reabsorption.

**Summary of Results** We were able to achieve hemostasis rapidly by placing 1–2 pads on the surgical field. In the two pigs that received a bolus of Epinephrine, we found that it took 2–3 additional pads, but hemostasis was achieved. Upon autopsy we found that all six pigs had healed normally without an inflammatory response. The Chitosan itself appeared to be in the process of being reabsorbed. We theorize that had the experiment continued

another few weeks there would have been no identifiable chitosan at the surgical site.

**Conclusions** Chitosan is an acceptable alternative to the current surgical hemostats. It would be far cheaper to produce than alternatives such as surgical. Also it is safer for human use than current hemostats that utilize human thrombin. In future studies we plan to do head to head tests to compare the efficacy of different hemostatic agents.

### 374 EFFICACY OF ANTEGRADE AND RETROGRADE WARM SALINE PERFUSION DURING RENAL CRYOABLATION FOR URETERAL PRESERVATION

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10.1136/jim-d-15-00013.374

**Purpose of Study** Percutaneous renal cryoablation of tumors adjacent to the ureter or pelvicalyceal system risk collecting system injury due to freezing. Although cold antegrade perfusion has been described for microwave and radiofrequency ablation, antegrade and retrograde warm saline perfusion for renal cryoablation has not been well characterized. The purpose of this study was to describe the safety and feasibility of antegrade and retrograde warm saline perfusion to protect the collecting system during renal cryoablation.

**Methods Used** A retrospective review was performed of 136 patients treated with percutaneous renal cryoablation at a single academic institution between 2009 and 2015. From this series, six patients undergoing antegrade ( $n=3$ ) or retrograde ( $n=3$ ) warm saline perfusion for protection of the collecting system were identified. The antegrade technique was performed by perfusion of warm saline through a 3 French catheter under continuous gravity drainage. The retrograde technique was performed using an open-ended ureteral catheter inserted into the ureter, and used to instill warm saline. Follow-up consisted of contrast enhanced cross-sectional imaging performed at 3–12 month intervals depending on tumor pathology. Primary outcomes were success of urothelial preservation and tumor ablation. Secondary outcomes included hospital stay, blood loss, operating time, and complication rate.

**Summary of Results** Four tumors were renal cell carcinoma and two were benign. The mean distance from tumor to ureter was 0.68 cm (0.08–1.15 cm). There were two complications including one patient who suffered a urine leak at the site of the antegrade perfusion (Clavien 1). The second complication was a patient who suffered a pulmonary embolism the day following surgery (Clavien 2). There was no recurrence of renal tumors at a mean follow-up of 15.6 months (1–27). The mean operative time was 3 hours and 9 minutes. There was no statistical difference in complication rate in those who underwent antegrade or retrograde perfusion ( $p>0.05$ ).

**Conclusions** This study demonstrates the feasibility of both methods for ureteral preservation during cryoablation. Future studies could help identify the relative merits of each approach and the appropriate indications.

### 375 AN ANALYSIS OF INFECTIOUS RISK FROM ENDOSCOPIC VALVE BACKFLOW DURING URETEROSCOPY

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10.1136/jim-d-15-00013.375

**Purpose of Study** Visualization of the urinary tract is improved by high-pressure irrigation with saline. Such irrigation has potential to overcome the resistance of the endoscopic valve resulting in backflow. This backflow may expose surrounding personnel to blood products within the effluent. The purpose of this study is to evaluate the effluent from ureteroscopic backflow to characterize its risk for infectious transfer.

**Methods Used** Using a bench-top ureteroscopy model, effluent from backflow was collected following a variety of ureteroscopic tasks including normal saline irrigation alone and saline irrigation following guidewire passage, laser fiber passage, basket passage and barbotage collection. The renal model was charged with a standardized concentration (1:100) of blood to simulate bleeding encountered during routine ureteroscopy procedures. In each scenario high pressures (mean: 34 mm Hg) were generated using hand irrigation and the backflow effluent was analyzed for erythrocyte and protein content. Ten trials were performed for each of the five conditions and a positive confirmation for blood or protein was equated with risk for transmission of infectious diseases.

**Summary of Results** Hemocytometric analysis revealed the presence of erythrocytes in no trials (0/10; 0%) using routine saline irrigation, irrigation following guidewire passage, irrigation following laser insertion and removal, and irrigation following basket insertion and removal. However, erythrocytes were present in 8/10 samples collected following barbotage. Similarly, serum protein was not detected in any (0/10; 0%) trials of effluent collected following routine saline irrigation, irrigation following guidewire passage, and irrigation following laser fiber insertion and removal. However, protein was detected in a single trial (1/10; 10%) following basket passage and removal. Protein was detected in 10 of 10 (100%) samples collected following simulated barbotage.

**Conclusions** The results suggest that backflow during ureteroscopic lithotripsy including irrigation alone, irrigation following guidewire, laser or basket insertion is at very low risk for transmission of infectious diseases. In contrast backflow following barbotage collection contains both blood and protein, placing operating room personnel at risk for transmission of infectious disease.

### 376 DAMAGE TO POLYTETRAFLUOROETHYLENE-COATED GUIDEWIRES: A POTENTIAL FOREIGN BODY RISK

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10.1136/jim-d-15-00013.376

**Purpose of Study** The polytetrafluoroethylene (PTFE)-coating of guidewires used during endourologic

procedures was recently modified to avoid the bonding agent PFOA (Perfluorooctanoic Acid) which was felt to be carcinogenic. Without this agent there is concern that the PTFE coating may become damaged during endoscopic surgical procedures running a risk of leaving a foreign body inside the patient. The purpose of this study was to examine the damage caused to guidewires following ureteroscopy and percutaneous nephrostolithotomy to determine if there was a risk of leaving PTFE flakes in the urinary collecting system.

**Methods Used** Changes to PTFE guidewires were observed endoscopically and then evaluated ex vivo following ureteroscopy and percutaneous nephrostolithotomy procedures. The used wires were then photographed with a Nikon D3200. Wires were categorized using a semiquantitative scoring system to determine the extent of loss of the PTFE coating.

**Summary of Results** PTFE wires employed during ureteroscopy and percutaneous nephrostolithotomy were associated with damage and loss of the coating inside the patient. Endoscopic video footage demonstrated abundant bright blue PTFE flakes inside the urinary collecting system. The photos of the used wire shows extensive damage to the PTFE coating, at times leaving the PTFE coating almost completely bare.

**Conclusions** The use of PTFE coated wires has the potential to leave small flakes within the collecting system. Further studies will be required to determine the risk that these retained flakes cause to the patient.

L: Loss of coating inside urinary collecting system. R: Damaged coating on wire.



Abstract 376 Figure 1

### 377 INCIDENCE AND RISKS IN THE DEVELOPMENT OF PIN-SITE INFECTIONS AFTER PELVIC EXTERNAL FIXATION

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10.1136/jim-d-15-00013.377

**Purpose of Study** External fixation is an essential tool in the treatment of unstable pelvic fractures. This procedure carries risks including superficial and deep pin-site infections as well as injury to the lateral femoral cutaneous nerve (LFCN). Little data exists surrounding infection rates, with no data found on LFCN damage. The purpose of this study was to identify the incidence of superficial and deep infections after pelvic external fixation and

examine which risk factors may increase the risk of post-surgical pin-site infections. Secondly the study aimed to describe the incidence of LFCN damage.

**Methods Used** We retrospectively reviewed 68 consecutive patients from a prospective database who underwent pelvic external fixation between January 2010 and December 2014 to identify factors associated with the development of infection or permanent damage to the LFCN over the course of fixation. Risk factors examined included patient age, length of stay, ASA classification, ISS, BMI, gender, diabetes status, tobacco use, and requirement of ICU placement. Logistical regression was used for statistical analysis to account for possible confounding.

**Summary of Results** Seventeen of 68 patients (25%) developed superficial pin-site infections, with 2 patients (2.9%) developing deep infections. Fifteen were treated with oral antibiotics, 1 was treated with IV antibiotics, and 8 required surgical debridement. Eight required early removal of their external fixation and 2 of 68 patients (2.9%) had symptoms consistent with damage to their LFCN after one-year follow-up. Utilizing logistical regression to account for possible confounding, no specific factors were associated with an increased risk of infection. Adjusted logistical regression models identified no specific factors which were associated with increased risk of infection.

**Conclusions** While the incidence of superficial infections related to external fixation is high, the technique remains a valuable tool in the definitive fixation of unstable anterior pelvic fractures. While there is a low risk of LFCN injury, further work is needed to mitigate the risk of infection. Surgeons should be aware of these concerns and counsel patients of risks and benefits in the perioperative period.