

American Thoracic Society
EDUCATION

Minority Trainee Development Scholarship

ATS2019

AWARD WINNING ABSTRACTS



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PROGRAM OVERVIEW

The American Thoracic Society (ATS) would like to congratulate the 40 Minority Trainee Development Scholarship (MTDS) awardees who are being honored at the 2019 Diversity Forum for their outstanding abstracts and excellent contribution to the Society!!

The MTDS program provides an opportunity for individuals of minority status in science and medicine to travel to the ATS International Conference yearly. A unique aspect of the MTDS is that a trainee at any level (high school and beyond) who submits an abstract is eligible to be considered for this award. On average each year, the ATS receives 60+ applications and a sub-committee chaired by Dr. Yolanda Mageto, ranks applications to select the top applicants.

History and Results:

The MTDS program aims to address the lack of underrepresented minorities in respiratory medicine. The program was created in 2002 under the leadership of Membership and Training Committees spearheaded by Dr. Estelle Gauda in an effort to recruit the best and brightest underrepresented minorities to the field of pulmonary, critical care and sleep medicine. Each year the MTDS recipients are honored at the Diversity Forum and presented with a \$1000 scholarship and a certificate of achievement.

- Over the past **18 years**, the ATS have given a total of 407 MTDS scholarships to attend the ATS International Conference.
- Out of those **18 years**, ATS has received educational support from Merck for **12 consecutive years** to provide scholarships for the MTDS.
- This is the fifth (5) consecutive year that the ATS funds the MTDS scholarships.
- Of the **447** past awardees **195 participants** are pursuing a career in pulmonary, critical care and /or sleep medicine and **159 participants** have remained members of the ATS.
- MTDS Awardees have stated in surveys done from 2011 – 2018 that they would not have attended the ATS International Conference if it were not for the MTDS scholarship.

Criteria:

Each MTDS recipient is an author of an abstract accepted for presentation at the ATS 2019 International Conference. The awards are based on the quality of science, the contribution of the trainee to the project and the potential impact of the award on the trainee's career development. Additional award criteria includes:

- Must be a member of an underrepresented minority group as defined by the NIH (African American, Hispanic, Native American, Alaskan Native or Pacific Islander)
- Must not be a recipient of another abstract award to the 2019 ATS International Conference
- Must be a trainee (high school through post-doctoral fellow) at a US Institution.
- Must be an author (preferably first author) of an abstract accepted for presentation at the ATS IC.

The Minority Trainee Development Scholarship (MTDS) would like to thank the American Thoracic Society for their generosity in supporting this program!



CONGRATULATIONS
to all Minority Trainee
Development
Scholarship (MTDS)
AWARDEES

Graft vs Host Disease Is Not Associated with HIV-Mediated Pulmonary Smooth Muscle Hypertrophy in Humanized Mice

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Rationale: Individuals infected with Human Immunodeficiency Virus (HIV) and receiving antiretroviral therapy still succumb to non-infectious pulmonary diseases. How HIV contributes to pulmonary pathophysiology remains uncertain; therefore, dissecting molecular mechanisms in animal models remains a priority. Non-human primates recapitulate HIV disease well, however, studies in monkeys are expensive and difficult to justify. Several studies have established vascular remodeling in HIV-transgenic rodents though, these non-infectious models may not recapitulate HIV-mediated pulmonary diseases. Humanization of the mouse immune system via engraftments with human hematopoietic stem cells (CD34+) and surgical implantation of human fetal thymic and liver tissue (BLT hu-mice) have greatly advanced the field of HIV immunopathogenesis. One limitation with the BLT model is the development of graft-versus-host disease (GvHD). Thus, GvHD may be a confounding factor when investigating pulmonary diseases that compromise vascular compliance in HIV-infected hu-mice. Here we compared the histopathology of HIV disease and GvHD in the pulmonary vasculature of hu-mice.

Methods: NSG mice (NOD.Cg-PrkdcscidIl2rgtm1Wjl/Szj) were engrafted with human CD34+, thymus and liver at 6-8 weeks. Only mice with >70% human CD45+ peripheral cells, as assessed by flow cytometry 12 weeks post-engraftment, were analyzed. BLT mice were infected with HIV and remained naïve to antiretrovirals for the duration of the study. Lung tissues were formalin-fixed, paraffin-embedded, and stained with hematoxylin & eosin. Pulmonary vascular histopathology was compared to that on NSG mice that developed full-blown xenogeneic GvHD after injection with human PBMC. All mice were monitored for signs of GvHD, including mild/severe hair loss, blepharitis, weight loss, and ruffled fur.

Results: Histopathological analyses of pulmonary vascular tissue of HIV-infected hu-mice demonstrated diffuse inflammatory infiltrates in pulmonary vessels, with significant thickening of the tunica media consistent with smooth muscle hypertrophy, as reported in HIV transgenic rats and non-human primates. Conversely, mice from the GvHD model exhibited distinct patchy inflammatory infiltrates resembling lung granulomas. Moreover, GvHD mice showed prominent tunica media thinning, consistent with engulfment of the smooth muscle layers, as reported by others.

Conclusions. This study demonstrates that the pulmonary vascular pathologies associated with GVHD and HIV infection are substantially different based on histopathology. These preliminary studies rule out GvHD as confounding factor in HIV-associated pulmonary complications that increase vascular pressures, like pulmonary hypertension (PH) because the thin pulmonary vessels observed in GvHD are less likely to hold the high hemodynamic pressures featured in PH. In fact, GvHD is a well-known complication of bone marrow transplantation but PH is extremely rare in GvHD.

Expression of a Novel Surfactant Protein C BRICHOS Mutation Causes Epithelial ER Stress and Spontaneous Lung Injury and Fibrotic Remodeling.

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Rationale: Mutations in the BRICHOS domain of the alveolar type 2 (AT2) cell restricted Surfactant Protein C gene [*SFTPC*] are associated with adult interstitial lung disease (ILD) and pediatric (ChILD). This class of *SFTPC* mutation produces misfolded and aggregation prone proSP-C isoforms inducing an AT2 ER stress response in vitro. In vivo modeling of SFTPC BRICHOS mutations can provide proof of concept studies linking epithelial ER stress response and clinical ILD.

Approach: A targeted ES cell approach was used to knock-in a cysteine to glycine substitution at position 185 [C185G] into the BRICHOS domain of the endogenous mouse *sftpc* locus. The founder line had purposeful hypomorphic *sftpc* gene expression due to retention of a *LoxP* flanked intronic PGK-neomycin [PGK-neo] selection cassette. PGK-neo excision and full allelic expression was achieved by crossing the founder line to a CMV-Cre constitutive deleter line or tamoxifen inducible Rosa26-ERT2-Cre line (I-SftpcC185G)

Results: Heterozygous expression of the SftpcC185G during fetal development resulted in a dominant negative proSP-C mutant isoform with ER retention of both the mutant and wild type isoforms. The lung phenotype from constitutive heterozygous SftpcC185G expression was a severe toxic gain of function with early post-natal (P2-3) lethality from respiratory failure. Intraperitoneal tamoxifen injections of 8-12 week old I-SftpcC185G mice rapidly increased the mutant proSP-C isoform producing an ER retained SP-C pro-protein and resultant activation of the IRE1 and ATF4/CHOP ER stress pathways. The resultant lung phenotype from adult inducible SftpcC185G expression was an early (7 days post-tamoxifen) severe mixed granulocytosis and lung injury. Following resolution of the alveolitis, I-SftpcC185G mice developed spatially heterogeneous regions of fibrotic remodeling and septal thickening.

Conclusions: This *in vivo Sftpc* model provides direct evidence for the role of an ER stress based AT2 cell phenotype in the pathogenesis of ILD. The model will provide a platform for further studies on the role of dysfunctional epithelium as a driver of adult and pediatric ILD.

The Smoking Gun: A Multi-variate Discriminant Analysis Model for Predicting Smoking Status

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Purpose: Smoking exposure is a major prognostic factor in pulmonary disease and an important consideration in management. However, in several critical contexts, patient self-report has proven unreliable. Developing reliable assessment tools will advance clinical care.

Methods: The dataset consisted of 711 patients undergoing pulmonary function testing from 2011 to 2017. Discriminant analysis was used to test the hypothesis that non-smokers, former smokers, and current smokers would differ significantly on a combination of carboxyhemoglobin (COHb), difference between pre and post actual Force Expiratory Volume 1 (FEV1) values in milliliter, and difference between pre and post percent predicted FEV1 values.

Results: Preliminary analysis of 589 patients in the cohort has been completed. Of these, 196 self-identified as nonsmokers, 133 as current smokers and 260 as former smokers. The overall multivariate test was significant, Wilk's $\Lambda = 0.544$, $X^2 = 356.43$, $df = 6$, $p = 0.000$. The linear function extracted accounted for 91.0% of the variance explained by the discriminating variables. The measure of CoHb was the greater discriminating factor that separated the groups followed by the difference between pre and post percent predicted FEV1 values and lastly the difference between pre and post actual FEV1 values in milliliter. 72.3% of the non-smokers, 46.7% of former smokers, and 68.4% of current smokers were correctly reclassified into their original categories using this model.

Conclusions: Correct identification of smoking status is important for effective patient counselling and joint decision-making. Our model correctly classifies smoking status in a significant number of subjects, outperforming clinician judgment alone. The fact that it fails to identify correctly a greater percentage of current smokers could be due to under-representation in the dataset, or the broadness of category encompassed by "former" smokers.

Clinical Implications: Current alternatives for verifying smoking status are methodologically unreliable or impractical for broad application. Employing this model as one piece of data in a multi-modal assessment of a patient's true smoking status could improve clinical efficiency, improve decision-making, and reduce bias.

Pneumoconiosis Mortality: Progress towards Healthy People 2020 Goal

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Rationale: Pneumoconioses are preventable occupational lung diseases caused by the inhalation of mineral or organic dust particles. Pneumoconioses are associated with working in a variety of dusty occupations and industries such as mining, construction and manufacturing. Each decade, the Department of Health and Human Services updates the Healthy People (HP) initiative, developing a new set of goals and objectives to improve the health of all Americans. The goal for HP 2020 Occupational Safety and Health (OSH) is to promote the health and safety of US workers through injury and illness prevention, and early disease intervention.

Specifically, objective 4 (OSH-4) was set to reduce pneumoconiosis deaths by 10% from the baseline of 2,430 deaths in 2005 to 2,187 deaths in 2020.

Methods: To assess progress towards the HP 2020 objective OSH-4, deaths were analyzed for decedents aged 15 years and older, using the 2005–2016 multiple cause-of-death data from the National Center for Health Statistics. Death certificates that listed the following International Classification of Diseases, 10th revision codes as the underlying or contributing cause of death were identified using the entity axis code field: J60 (coal workers' pneumoconiosis; CWP), J61 (pneumoconiosis due to asbestos and other mineral fibers), J62 (pneumoconiosis due to dust containing silica), J63 (pneumoconiosis due to other inorganic dusts [including berylliosis]), J64 (unspecified pneumoconiosis), J66 (airway diseases due to specific organic dust [including byssinosis]). JoinPoint regression software was used to analyze disease time-trends.

Results: During 2005–2016, 23,966 pneumoconiosis deaths were identified. Most deaths were associated with asbestosis (15,377, 64.2%) followed by CWP (5,425, 22.6%). The number of pneumoconiosis deaths decreased 31% (p-value for time trend <.05) from 2,430 in 2005 to 1,676 in 2016. The greatest decline of 353 deaths (54.1%) was for CWP. If the current trend continues, approximately 1,494 pneumoconiosis deaths could be expected in 2020, corresponding to a 38.5% reduction from the 2005 baseline (Table).

Conclusions: The HP 2020 OSH-4 goal has been achieved, likely reflecting changes in industry and workforce characteristics (e.g., decline in number of workers in dusty trades), adherence to permissible exposure limits (PELs), and various occupational respiratory health strategies. Continued surveillance is warranted to monitor trends over time.

The “Two Bag” System for Treatment of Adults with Diabetic Ketoacidosis: a Prospective Randomized Trial

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Rationale: The “two bag” system (2BAG) for intravenous (IV) fluid administration is efficacious in treating diabetic ketoacidosis (DKA) among children. It involves two bags of saline: one with 10% dextrose and the other without dextrose. The two fluid bags are administered simultaneously at a total constant rate, and rates of individual fluid bags are adjusted by a nurse-driven protocol per patients’ glucose levels. To our knowledge, there have been no prospective trials studying 2BAG in adults with DKA. The aim of this prospective randomized controlled trial is to determine whether 2BAG closes the anion gap faster than usual care using a one bag system recommended by the American Diabetes Association (ADA).

Methods: Adult patients admitted with DKA to the medical stepdown or intensive care units at an academic, urban, safety net hospital were randomized to either 2BAG (intervention) or the usual care (control) group. Exclusion criteria included pregnancy, septic shock, heart failure, and renal failure requiring dialysis. Subjects in both groups initially received insulin infusions at 0.1 units/kg/hour. The control group was treated with IV fluids according to ADA guidelines for DKA management. The primary endpoint was time from initial laboratory studies confirming DKA to anion gap closure. This study was approved by the Human Subjects Division of the MetroHealth System (IRB #18-00025).

Results: Fourteen subjects were recruited over the first 4 months (8 were randomized to the 2BAG arm). Mean age was 34.4 ± 11.5 years and 9 (64%) were female. Ten (71%) of subjects had Type 1 diabetes mellitus (DM). The mean pH at admission was 7.16 ± 0.15 , with no significant difference between the two groups. In the preliminary analysis, there was no difference in time to anion gap closure between the control and 2BAG groups (median: 12.8 (interquartile range (IQR): 7.5-17.9) vs. median: 13.3 (IQR: 12.3-14.6) hours, Wilcoxon rank-sum test $p=1.00$). The IQR was significantly wider in the control group, as compared to the 2BAG group (see Figure 1). No significant adverse events were noted among any subjects.

Conclusion: In this preliminary analysis there was no difference in time to anion gap closure between the two groups. However, even at this early stage of subject recruitment, the 2BAG system shows more consistency in the time required to correct the metabolic derangements associated with DKA. These findings suggest that with increased recruitment, 2BAG may be a safe, efficient, and more efficacious method of treating adults with DKA.

Application of Neuromuscular Electrical Stimulation of the Abdominal Wall Muscles in Combination with Pressure Support Ventilation to Assist Respiration

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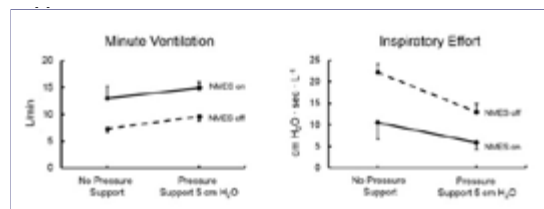
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Rationale: We recently reported that it is possible to provide inspiratory assistance through the use of transcutaneous neuromuscular electrical stimulation of abdominal muscles in healthy subjects who breathe spontaneously (McLachlan AJRCCM 191:A2681). Whether stimulations on expiration can provide inspiratory assistance during pressure support ventilation is unknown.

Aim: To investigate the effect of combining neuromuscular electrical stimulation of the abdominal wall muscles with pressure support ventilation on minute ventilation and inspiratory effort.

Methods: Six healthy subjects were studied while semi-recumbent. Two minutes of breathing without stimulation followed by two minutes of breathing with stimulation were measured under two conditions: breathing while not connected to the ventilator (unsupported breathing) and breathing while connected to the ventilator set at pressure support of 5 cm H₂O. Flow, gastric pressure and esophageal pressure were recorded throughout the experiment. Intensity of electrical current was set at 90% of maximum tolerable current: 53 ± 3.8 mA (mean \pm standard error). Data was analyzed using a two factor repeated measures ANOVA with the presence of stimulation and presence of pressure support specified as within subject effects.

Results: All subjects tolerated neuromuscular stimulation without difficulty. In all instances, stimulation was successfully delivered during exhalation. Minute ventilation and inspiratory effort (pressure time product of the diaphragm per liter of minute ventilation) during the experiment are shown in the figure. As expected, breathing with stimulation, compared to breathing without stimulation, increased minute ventilation ($p < 0.01$) and reduced inspiratory effort ($p < 0.01$). A similar effect on minute ventilation ($p = 0.04$) and inspiratory effort ($p = 0.03$) was found when breathing with pressure support compared to unsupported breathing. The increases in minute ventilation elicited by neuromuscular stimulation while subjects were concurrently on pressure support and when they were not on pressure support were similar ($p = 0.84$). Likewise, the decreases in inspiratory effort elicited by neuromuscular stimulation while subjects were concurrently on pressure support and when they were not on pressure support were similar ($p = 0.30$). These results suggest that the effects of the two adjuncts to respiration were additive.



Conclusions: In healthy subjects, inspiratory assistance provided by neuromuscular stimulation of the abdominal-wall muscles and by pressure support are additive.

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Model-dependent effects of taurine supplementation in experimental pulmonary hypertension

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Rationale: Taurine (2-aminoethanesulfonic acid) is an abundant nutrient in meat, fish, and energy drinks that is considered to have critical osmoregulatory, anti-inflammatory, and anti-oxidant effects. Previous studies have shown that taurine supplementation decreases right ventricular hypertrophy and pulmonary artery muscularization in the monocrotaline and chronic hypoxia rat models of pulmonary hypertension, respectively; however, the effects of taurine supplementation on cardiopulmonary hemodynamics in pulmonary hypertension remain unknown. In this study, we sought to measure the effects of taurine supplementation on the development of experimental pulmonary hypertension in the monocrotaline and Sugen/hypoxia (SuHx) models.

Methods: In separate experiments, male Sprague-Dawley rats (~ 250 g) were treated with either monocrotaline (60 mg/kg IP) or Sugen 5416 (20 mg/kg SC) plus 10% oxygen for three weeks prior to hemodynamic assessment with right and left (SuHx only) heart catheterization and organ harvest. Taurine was supplemented in drinking water at 3% w/v and was provided *ad libitum*. Taurine content was measured utilizing a targeted liquid chromatography-mass spectrometry assay. Data are presented as mean \pm SD with N = 4 animals per group and were analyzed using 2-way ANOVA with Sidak *post hoc* test.

Results: Rats consumed approximately 4 mg/kg/d of taurine. Taurine supplementation increased plasma and lung taurine content by 4- and 2-fold, respectively. In the monocrotaline model, taurine decreased right ventricular systolic pressure (RVSP) (59 ± 9 v. 80 ± 13 mmHg, $p = 0.008$) in association with decreased right ventricular hypertrophy by Fulton's index (0.29 ± 0.05 v. 0.40 ± 0.04 , $p = 0.005$). In the SuHx model, taurine increased pulmonary vascular resistance (79 ± 8 v. 46 ± 8 kdyn \times s \times cm $^{-5}$, $p < 0.001$) owing to modest increases in RVSP (61 ± 7 v. 54 ± 10 mmHg, $p = 0.26$) and modest decreases in cardiac output (18 ± 5 v. 21 ± 4 mL/min, $p = 0.7$) and left ventricular end-diastolic pressure (5.0 ± 0.3 v. 5.9 ± 1.9 mmHg, $p = 0.7$).

Conclusions: Taurine administered at the time of disease inception ameliorates monocrotaline-induced pulmonary hypertension but exacerbates SuHx-induced disease. These data raise important concerns related to dietary contributors to pulmonary vascular remodeling in patients. Further, mechanistic studies of these model-dependent effects will provide important insights into targeted therapies for human disease subtypes.

Impulse Oscillometry Pulmonary Function Abnormalities in Sickle Cell Disease

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Introduction: Sickle Cell Disease (SCD) is characterized by an inherited point mutation in the hemoglobin (Hgb) *HBB* gene, which results in hemoglobin polymerization and red blood cell sickling when not fully oxygenated. Inflammatory responses to intravascular hemolysis may be the cause of abnormal pulmonary function found in sickle cell disease. Our goal was to determine if Impulse oscillometry (IOS) is a sensitive tool to identify abnormal pulmonary function in SCD patients with increased hemolysis, a marker of more severe SCD related morbidity.

Methods: We conducted a case control study of subjects with SCD and Hgb AA/AS (Controls). We collected medical history and blood samples to measure markers of hemolysis for all patients. IOS was performed to measure total airway resistance at an oscillation frequency of 5-20Hz (R5-R20) and airway reactance (AX). Nowowiejska (<19yo) and Vogel & Smidt (≥19yo) reference equations were used depending on age. R5 values greater than 150% were considered abnormal.

Results: 22 SCD and 20 Control subjects were studied. There was no significant difference between the two groups in the demographics and oscillometry data studied (Table 1). There was no association between abnormal R5 and markers of hemolysis. The percentage of subjects with abnormal R5 values was high in both populations (37% SCD and 40% Controls). Post hoc analysis revealed that the abnormal R5 population had a higher BMI of 25.5 kg/m² (IQR 20.9-30.8) vs 21.2 kg/m² (IQR 19.3-23.7) (p=0.007).

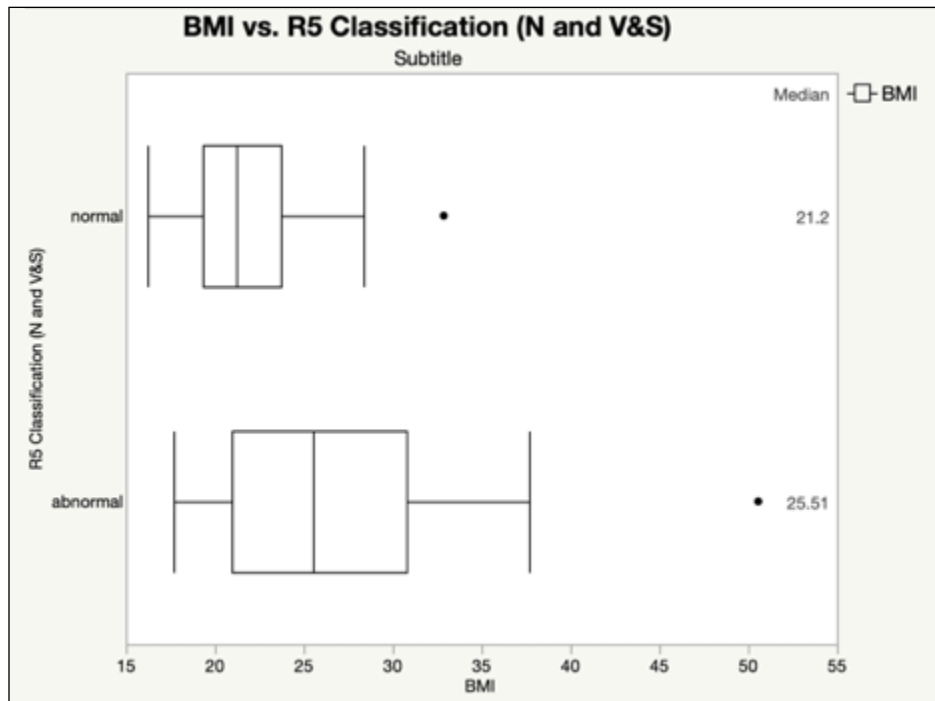
Conclusion: Our study demonstrates a higher than expected rate of oscillometry abnormalities in SCD subjects and the controls by asthma prevalence. The abnormal R5 SCD population did not have increased hemolysis. Higher BMI was associated with abnormal pulmonary function measured by oscillometry suggesting the role of obesity in airway obstruction.

Table 1: Population Characteristics

	SCD (n=22)	Controls (n=20)	p-value
Demographics			
Age, years*	21 (17-29.8)	22.5 (15.3-24)	>0.05
Male/Female, N (%)	13/9 (59/41)	11/9 (55/45)	>0.05
Ethnicity, N (%)			>0.05
African American	19 (86)	19 (95)	
Hispanic	2(9)	0	
Middle Eastern	1(5)	1(5)	
BMI kg/m ² *	23 (19-25)	23 (20-30)	>0.05
Ongoing Asthma, N (%)	3 (14)	1 (5)	>0.05
Hemolysis Parameters			
Hemoglobin g/dL*	9 (8-11)	13 (12-14)	<0.0001
Free heme μM*	1.6 (0.7-4.6)	0.08 (0.04-0.17)	<0.0001
Reticulocyte %*	10 (5-17)	1.4 (0.9-2.1)	<0.0001
LDH U/L*	890 (726-1230)	485 (398-589)	<0.0001
IOS Parameters			
R5 % Predicted*	137 (113-168)	140 (122-179)	>0.05
R10 % Predicted*	126 (112-167)	137 (113-155)	>0.05
R15 % Predicted*	126 (113-163)	138 (117-158)	>0.05
R20 % Predicted*	132 (117-162)	139 (116-171)	>0.05
R5-R20 %*	20 (12-27)	17 (13-30)	>0.05
X5 % Predicted*	-405 (-864 - -23)	-136 (-2541 - -21)	>0.05
AX cmH ₂ O/L*	7 (4-12)	7 (4-13)	>0.05
Fres % Predicted*	177 (161-230)	178 (138-198)	>0.05
CO5*	0.81 (0.73-0.88)	0.80 (0.78-0.84)	>0.05
CO10*	0.92 (0.88-0.94)	0.92 (0.91-0.94)	>0.05

* median (IQR)

Impulse Oscillometry Pulmonary Function Abnormalities in Sickle Cell Disease *continued*



Simvastatin Inhibits Airway Epithelial H1N1 Influenza Viral Replication

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Rationale: Respiratory viral infections such as influenza, cause severe morbidity and mortality worldwide despite the availability of drug treatments and vaccines. Novel treatment modalities that target mechanisms regulating viral replication are urgently needed. Using the rhesus macaque monkey model, we previously reported that pandemic influenza A/H1N1 virus infection results in distal airway inflammation and airway epithelial cell viral replication in vitro. The statin drugs which inhibit the mevalonate (MA) pathway and downstream isoprenoid lipid and cholesterol biosynthesis, may also possess anti-microbial and anti-viral properties. We also previously showed that statins can be safely delivered via inhalation in rhesus macaques achieving high airway drug distribution. Given this therapeutic potential for inhaled statins in our monkey model, we hypothesized that statins inhibit H1N1 influenza viral replication in primary airway epithelial cells in vitro.

Methods: Fully differentiated primary tracheobronchial epithelial cells derived from rhesus monkey tracheas were grown in bi-phasic, air-liquid interface conditions to full confluency. Cells were then pre-treated with Simvastatin (5 μ M) for 24 hours. followed by in vitro infection with A/California/04/2009 H1N1 at MOI=10 for 1 hour. Simvastatin was then continued as post-treatment for an additional 48 hours. Viral replication and antiviral genes were assessed by qRT-PCR. **RESULTS:** Treatment with Simvastatin significantly inhibited H1N1-induced M gene expression in primary airway epithelial cells (>99% reduction; *p<0.05). Treatment with Simvastatin also significantly reduced H1N1-induced IL-8, IL1 β , and IFN β gene expression. There was no gross evidence of cytotoxicity, cell death, or changes in cell morphology as determined by brightfield microscopy.

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Conclusions: Simvastatin inhibits H1N1 influenza viral replication in primary airway epithelial cells, suggesting that depletion of MA and its downstream lipid metabolites may play a role in the prevention and/or treatment of influenza infection. Simvastatin's decrease in the expression of antiviral genes indicates potential regulation of airway mucosal anti-viral innate immunity. Limiting the duration and severity of influenza infection is clinically important to avoid epithelial denudation, subsequent bacterial superinfection, and pneumonia. Delivering statins as an anti-viral therapy directly to the airway epithelium via inhalation should be investigated further.

Mice Fed a High DHA Diet Exhibit Increased Resolvin D1 and Altered Inflammatory Outcomes Following a Single Inhalational Exposure to Organic Dust Extract

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Rationale: Individuals exposed to agricultural organic dusts exhibit increased inflammation-associated chronic lung disease. Omega-3 fatty acids are utilized as substrates for the biosynthesis of specialized pro-resolving lipid mediators (SPM) that orchestrate the resolution of inflammation. Our previous studies have demonstrated that mice fed a docosahexaenoic acid (DHA) supplement by oral gavage for seven days prior to receiving a single intranasal challenge with extracts of swine confinement facility dust (DE) experience lower levels of airway inflammation compared to control fed mice. This study aims to further examine how increased DHA dietary intake in mice alters recovery following a single DE exposure, including alterations in SPM levels.

Methods: C57Bl/6 mice were fed a control diet containing no DHA, or a high DHA diet for four weeks prior to receiving a single intranasal challenge to DE. Five hours following DE challenge, mice were euthanized, bronchoalveolar lavage was performed, and lung tissues were collected for RNA and protein analyses. The lung tissues were homogenized and prepared for assessment of transcript-level gene expression changes using the NanoString mouse immunology panel.

Results: DHA diet-fed mice exhibited significantly reduced neutrophils ($p = 0.03$) and increased macrophages ($p = 0.04$) in their bronchoalveolar lavage fluid compared to control diet-fed mice challenged with DE. When comparing saline versus DE-instilled mice, using the NanoString platform, we identified 119 differentially regulated transcripts ($p \leq 0.05$). Of the DE-exposed animals, 21 transcripts were differentially regulated ($p \leq 0.05$) between DHA and control diet-fed mice, including significant upregulation of CXCL13, CCL2, MyD88, and TLR5. In addition, DHA diet-fed mice challenged with DE had significantly increased levels of DHA-derived SPM RvD1 ($p < 0.05$) in lavage fluid.

Conclusions: These studies indicate that a DHA-based diet can provide increased substrate for the biosynthesis of SPM during recovery following a single DE exposure. Furthermore, DHA-diet fed mice exhibit alterations in TLR signaling and leukocyte chemo-attractive factors which may promote lung resolution and repair processes following organic dust exposure.

Club Cell Specific Heme Oxygenase-1 Deletion Does Not Alter Hyperoxic Sensitivity in Adult Mice

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Thioredoxin reductase-1 (TXNRD1) inhibition activates nuclear factor (erythroid-derived 2)-like 2 (Nrf2) responses and attenuates acute lung injury (ALI). Heme oxygenase-1 (HO-1) induction following TXNRD1 inhibition is Nrf2-dependent in airway epithelial (club) cells. Whole body HO-1 deletion dramatically alters lung development. To overcome this obstacle, we developed club cell specific HO-1 knockout (KO) mice with the ultimate goal of evaluating the contribution of HO-1 toward the protective effects of TXNRD1 inhibitors *in vivo*. The present studies characterized club cell specific HO-1 KO mice generated by crossing *Hmox1^{flx/flx}* mice with transgenic mice with constitutive cre recombinase expression under control of the club cell 10 kDa protein (CC10) promoter. Characterization of CC10/*Rosa^{mt/mg}* mice identified that recombination was limited to the bronchiolar and alveolar epithelia with >95% recombination by 3 weeks' postnatal age. Twelve-week old wildtype (WT) and KO mice were continuously exposed to >95% hyperoxia (HYP) or room air (RA) for 72h. Following exposure, mice were sacrificed and weighed, the left lung inflation fixed, and the right lung collected and weighed. Lung morphometry was assessed in RA exposed WT and KO mice by calculating radial alveolar counts (RAC) and mean linear intercepts (MLI; 6 images/animal; n=6-8). Injury was quantitatively assessed by calculating right lung/body weight ratios (g/kg; n=7-8). Data (mean±SEM) were analyzed by unpaired t-test or 2-way ANOVA followed by Tukey's post hoc, with significance noted at p<0.05. In RA, body weights and RAC were not different between WT and KO mice. RAC in WT/RA mice were 6.6±0.6 vs 5.4±0.3 in KO/RA (p=0.1). In contrast, MLI was significantly greater in KO/RA mice (70.1±1.8) than in WT/RA (60.1±3.1; p=0.04). Two-way ANOVA indicated an independent effect of hyperoxia, but not genotype, on right lung/body weight ratios. Specifically, ratios were 2.9-fold greater in WT/HYP (5.6±0.7) when compared to WT/RA mice (2.7±0.7, p<0.001) and 3.2-fold greater in KO/HYP (5.6±0.67) than in KO/RA mice (2.4±0.6, p<0.001). In conclusion, club cell specific HO-1 deletion results in slightly altered lung morphology in adulthood as reflected by an increase in MLI. The impact of whole body HO-1 KO deletion on lung morphology was, by comparison, more dramatic. The physiological relevance of increased MLI in CC10/*Hmox1^{-/-}* mice is currently under investigation. The lack of differences in hyperoxic susceptibility between WT and KO mice indicates that club cell HO-1 does not significantly contribute to hyperoxic lung injury. Future studies will utilize this unique transgenic model to evaluate the contribution HO-1 toward the protective effects of TXNRD1 inhibitors *in vivo*.

Trauma Patients With Sepsis: A National Inpatient Analysis

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Introduction: Sepsis is the leading cause of in-hospital mortality and is the most expensive condition treated in the United States (US). However, the epidemiology of sepsis in trauma patients is poorly described. We aim to evaluate outcomes and cost related to injured patients who develop in-hospital sepsis.

Methods: We performed an analysis using the National Inpatient Sample, the largest all-payer inpatient care database in the US. The study population comprised of adult patients with primary International Classification of Diseases, Ninth Revision, codes for trauma at the time of discharge between 2012 and 2014. Sepsis diagnosis was identified by Angus Criteria, and severe injury was defined by an Injury Severity Score (ISS) of ≥ 15 . Baseline demographics were presented as weighted counts and means using discharge weights to provide national estimates. The outcomes of interest were in-hospital mortality and cost of care between sepsis and non-sepsis trauma patients which was risk-adjusted in multivariable analyses.

Results: In the US inpatient population, there were 2.8 million patients admitted for trauma during the study period. The incidence of sepsis in trauma patients was 6.0% (n=167,460). Injured patients with sepsis were older (65 vs. 58; $p < .01$), had higher Elixhauser scores (4 vs. 2; $p < .01$), higher ISS (13 vs. 8; $p < .01$) and a greater proportion of severe head/neck (39% vs. 19%; $p < .01$) and chest (23% vs. 12%; $p < .01$) injuries compared to non-septic patients. The sites of infection with the greatest frequencies in the sepsis group were genitourinary (45%) and lower respiratory (37%). Trauma patients with sepsis had a longer length of stay (16 vs. 4; $p < .01$) and a greater proportion with in-hospital mortality (9% vs. 2%; $p < .01$; Table 1) compared to non-septic trauma patients.

In multivariable analysis, severely and non-severely injured septic patients had a odds ratio of 1.40 (95% CI: 1.22, 1.58) and 4.62 (95% CI: 4.10, 5.22), respectively, for in-hospital mortality compared to non-septic patients. The marginal cost of caring for all septic trauma patients compared to non-septic patients was \$23,000 (95% CI: \$22,728, \$24,138).

Conclusion: Sepsis is a significant risk factor for in-hospital mortality in trauma patients, and the septic patients with non-severe injuries had a four-fold increased odds risk. Trauma patients with genitourinary and lower respiratory infections comprised the majority of the infectious sites in trauma sepsis. These findings highlight a priority to further examine sepsis in non-severe traumas and the reasons for pulmonary and urinary infections for preventive efforts.

Trauma Patients With Sepsis: A National Inpatient Analysis *continued***Table 1. National estimates of trauma patient characteristics with and without sepsis**

	Sepsis		No Sepsis		Total		p
Patient, n (%)	167,460	5.8%	2,702,320	94%	2,869,780	100%	
Age, mean (SE)	65.3	0.2	58.1	0.1	58.5	0.2	<.01
Female, n (%)	79,770	47.6%	1,271,755	47.1%	1,351,525	47.1%	0.07
Elixhauser, mean (SE)	4	0.02	2	0.01	2	0.01	<.01
Race/Ethnicity, n (%)							
White	118,965	71.0%	1,850,150	68.5%	1,969,115	68.6%	<.01
Black	15,860	9.5%	274,925	10.2%	290,785	10.1%	
Hispanic	14,010	8.4%	267,850	9.9%	281,860	9.8%	
Other	8,885	5.3%	149,560	5.5%	158,445	5.5%	
Insurance, n (%)							
Medicare	95,975	57.3%	1,148,325	42.5%	1,244,300	43.4%	<.01
Private	19,150	11.4%	266,220	9.9%	285,370	9.9%	
Medicaid	33,915	20.3%	768,459	28.4%	802,374	28.0%	
self-pay	9,130	5.5%	271,175	10.0%	280,305	9.8%	
no charge	840	0.5%	22,420	0.8%	23,260	0.8%	
Other	8,015	4.8%	217,990	8.1%	226,005	7.9%	
AIS >2, n (%)							
Head and Neck	64,480	38.5%	522,775	19.3%	587,255	20.5%	<.01
Face	570	0.3%	3,950	0.1%	4,520	0.2%	<.01
Chest	38,050	22.7%	319,415	11.8%	357,465	12.5%	<.01
Abdomen	1,480	0.9%	6,415	0.2%	7,895	0.3%	<.01
Extremities	22,315	13.3%	274,155	10.1%	296,470	10.3%	<.01
External	270	0.2%	2,330	0.1%	2,600	0.1%	<.01
ISS, mean (SE)	13	0.12	8	0.04	9	0.0	<.01
The site of Infection, n (%)							
Bone and Joint	2,435	1.5%	7,175	0.3%	9,610	0.3%	<.01
Central Nervous System	1,985	1.2%	1,565	0.1%	3,550	0.1%	<.01
Cardiovascular	42,000	25.3%	95,000	3.5%	140,000	4.8%	<.01
Device	3,220	1.9%	3,805	0.1%	7,025	0.2%	<.01
Gastrointestinal	14,000	8.2%	14,000	0.5%	27,000	1.0%	<.01
Genitourinary	76,000	45.3%	150,000	5.6%	230,000	7.9%	<.01
Lower Respiratory Infection	62,000	37.1%	51,000	1.9%	110,000	4.0%	<.01
Upper Respiratory Infection	2,400	1.4%	5,300	0.2%	7,700	0.3%	<.01
Skin & Soft Tissue	12,000	7.0%	37,000	1.4%	49,000	1.7%	<.01
Other	49,000	29.5%	83,000	3.1%	130,000	4.6%	<.01
Hospital Characteristics, n (%)							
Rural	8,580	5.1%	215,385	8.0%	223,965	7.8%	<.01
Urban nonteaching	41,255	24.6%	748,025	27.7%	789,280	27.5%	
Urban teaching	117,625	70.2%	1,738,910	64.3%	1,856,536	64.7%	
LOS, mean (SE)	16	0.18	4	0.02	5	2.7%	<.01
in-hospital mortality, n (%)	15,411	9.2%	61,105	2.3%	76,516	2.7%	<.01
Cost, mean (SE)	\$53,259	\$801	\$14,352	\$112	\$16,625	\$149	<.01

*Elixhauser risk score for mortality. Sepsis criteria adapted from Angus et. al. Injury Severity Score (ISS); Abbreviated Injury Score (AIS); length of stay (LOS); cost inflated to 2014

Risk Factors For Infection And Evaluation Of Sepsis-3 In Patients With Trauma

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Introduction: Sepsis is a common complication in patients with trauma and discriminating between infectious and non-infectious organ dysfunction is challenging. We aim to evaluate the Sepsis-3 definition and examine risk factors for infection in trauma patients.

Methods: This was a retrospective cohort study of consecutive patients with primary trauma at a Level I trauma center between January 2014 and January 2016. We examined patients with suspected infection, as defined by culture and antibiotic orders. Detailed retrospective chart review was used to determine the likelihood of infection, and risk factors, including injury severity score (ISS). Associations between patient baseline risk factors and the development of infection were assessed in multivariable logistic regression analysis.

Results : A total 958 trauma patients met the inclusion criteria for suspected infection and 25% (n=242) of the suspected infection cohort were identified as having “proven” or “probable” infection after chart review. The median time to infection was 4.5 days (IQR 2.4-8.1 days). The most common mechanisms of injury were blunt (81.8%, n = 784) and penetrating (17.6%, n = 169) trauma. Patients with infection had a higher Elixhauser comorbidity score and a greater proportion with severe injury than the non-infected cohort (p<0.01).

The median SOFA score at the time of admission was higher for infected (6; IQR 2-9) patients compared to non-infected patients (2; IQR 0-5, p<0.01). The median SOFA score at the time of infection was 5 (IQR 2-7). The admission SOFA score was greater than the SOFA score at the time of infection in 57% (n =137) of infected cases. Of those with infection, 11.3% (n = 27) met the Sepsis-3 criteria. None of the patients meeting Sepsis-3 died, whereas 10.4% (n=22) of the infected patients who did not meet Sepsis-3 died during their hospitalization.

In multivariable analysis, the risk factors associated with the development of infection were age (OR 1.08 per 10-year increase; 95% CI: 1.00-1.18), female sex (OR 1.76; 95% CI: 1.23-2.52), admission SOFA (OR 1.14% per 1-unit score increase; 95% CI: 1.09-1.19), Elixhauser comorbidity score (OR 1.05 per 1-unit score increase; 95% CI: 1.03-1.07), ISS \geq 15 (OR 1.52, 95% CI: 1.07-2.18) and AIS > 2 of face (OR 1.73, 95% CI: 1.26-2.39).

Conclusion : Patients with trauma often arrive with organ dysfunction, which adds complexity and inaccuracy to the Sepsis-3 definition. Trauma patients at risk for infection are better identified when characteristics unique to trauma patients are used.

Risk Factors For Infection And Evaluation Of Sepsis-3 In Patients With Trauma continued**Table 1. Trauma patient characteristics with and without infection**

	Infected N = 239		Not Infected N = 712		Total N = 951		p
Age, median (IQR)	53	32-73	44	27-62	46	28-64	0.00
Male, n (%)	147	61.5%	517	72.6%	664	69.8%	0.00
Elixhauser, median (IQR)	10	2-16	0	0-8	2	1-11	0.00
Comorbidities, n (%)							
Diabetes	31	13.0%	88	12.4%	119	12.5%	0.81
Hypertension	96	40.2%	230	32.3%	326	34.3%	0.03
Alcohol abuse	43	18.0%	147	20.6%	190	20.0%	0.49
Obese	19	7.9%	42	5.9%	61	6.4%	0.26
Cirrhosis	11	4.6%	32	4.5%	43	4.5%	0.94
CHF	16	6.7%	15	2.1%	31	3.3%	0.00
Race/Ethnicity, n (%)							
White	135	56.5%	369	51.8%	504	53.0%	0.65
Black	52	21.8%	173	24.3%	225	23.7%	
Hispanic	42	17.6%	140	19.7%	182	19.1%	
Other	10	4.2%	30	4.2%	40	4.2%	
Injury Severity Score ≥ 15, n (%)	107	44.8%	172	24.2%	279	29.3%	0.00
SOFA at Admission, median (IQR)	6	2-8	2	0-5	2	1-6	0.00
SOFA at time of infection, median (IQR)	5	2-7	Na	Na	Na	Na	
Days to infection, median (IQR)	5	2-8	Na	Na	Na	Na	
Meet Sepsis 3 Criteria, n(%)	27	11.3%	Na	Na	Na	Na	
Type of Injury, n (%) s							
Blunt	195	81.6%	582	81.7%	777	81.7%	0.96
Penetrating	41	17.2%	128	18.0%	169	17.8%	0.77
Burn	4	1.7%	5	0.7%	9	0.9%	0.18
Other	1	0.4%	7	1.0%	8	0.8%	0.40
In Hospital Mortality, n (%)	22	9.2%	46	6.5%	68	7.2%	0.15

*Elixhauser risk score for mortality. Sepsis 3 criteria adapted from Seymour et. al. Abbreviations: congestive heart failure (CHF).

The Fatigue Index Test (FIT): Clinical Manifestations of a Novel Measure of Inspiratory Muscle Fatigue in COPD

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Rationale: Multiple measures have been employed to quantify respiratory muscle fatigue in COPD. Whereas current guidelines suggest that serial assessment of respiratory muscle pressures in response to electrical or magnetic stimulation is the best technique to identify fatigue, such testing is difficult and time-consuming. The Fatigue Index Test (FIT) is a novel method of quantifying propensity to inspiratory muscle fatigue in clinical practice. The FIT has not been previously studied in COPD. This study examined the relationships between the FIT score and COPD-related outcomes.

Methods: 66 male veterans with mild-to-very severe COPD (aged 70.06 ± 5.68 years) underwent measures of BMI, spirometry, the modified Medical Research Council (mMRC) dyspnea scale and 6-minute walk test (6MWT) from which the BODE index was calculated. Participants also completed the St. George's Respiratory Questionnaire (SGRQ) and the COPD Assessment Test (CAT). The FIT score is calculated from parameters obtained via the Test of Incremental Respiratory Endurance, in which subjects are instructed to inhale with as much force as possible during a maximal and sustained inspiratory effort from residual volume to total lung capacity using the PrO₂ device (Smithfield, RI, USA). Higher FIT scores indicate less susceptibility to inspiratory muscle fatigue.

Results: The mean \pm SD FIT score was 14.58 ± 6.39 . FIT scores positively correlated with pre- and post-bronchodilator FEV₁ %, FVC % and IC/TLC with r-values from 0.29 to 0.45 (all $p \leq 0.02$), as well as with the 6MWT distance ($r = 0.47$, $p = 0.00$). FIT scores were negatively associated with dyspnea ($r = -0.37$, $p = 0.00$), the BODE index ($r = -0.36$, $p = 0.01$), the CAT score ($r = -0.31$, $p = 0.02$), and with the SGRQ total, impacts, activity and symptoms scores with r-values from -0.31 to -0.45 (all ≤ 0.01). No significant relationship between the FIT and BMI was found. Additionally, a significant difference in FIT scores was found between subjects with IC/TLC $> 25\%$ (15.5 ± 6.17) versus IC/TLC $\leq 25\%$ (11.9 ± 6.6), $p = 0.04$.

Conclusion: A higher propensity to inspiratory muscle fatigue as reflected by lower FIT scores was linked to a more severe airflow obstruction, reduced functional exercise capacity, impaired quality of life, increased COPD symptomatology and greater mortality risk in the study sample. The FIT offers promise as a valuable and quick indicator of overall health and fitness status of individuals with COPD.

Assessing the Clinical Utility of Serum ACE, Soluble IL-2 Receptor, and 1-25 Vitamin D in the Diagnosis of Extra-Pulmonary Sarcoidosis

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Introduction: The diagnosis of extra-pulmonary sarcoidosis is challenging and often requires a combination of clinicoradiographic findings and histologic evidence. However, extra-thoracic biopsy, particularly in the case of neuro, cardiac and ocular sarcoidosis, is often not feasible. Angiotensin-converting enzyme (ACE), soluble interleukin-2 receptor (sIL-2R), and 1-25 dihydroxyvitamin D (1,25 vitamin D) levels have been implicated as disease markers in pulmonary sarcoidosis. These markers are routinely tested, yet their utility in the diagnosis of extra-pulmonary sarcoidosis is not well-defined. Our objective was to determine if ACE, sIL-2R, and 1,25 vitamin D serum levels are predictive of neurological, cardiac, and ocular involvement in sarcoidosis.

Methods: We performed a retrospective study of 141 patients with suspected neuro, cardiac or ocular sarcoidosis evaluated by the neurology, cardiology, or ophthalmology departments at the Cleveland Clinic between January 2013 and December 2017. The electronic medical record was queried for patients with available serum ACE, sIL-2R, or 1-25 vitamin D levels tested as part of the initial workup. These levels were compared in patients with and without a final diagnosis of sarcoidosis and their diagnostic accuracy was determined by calculating the area under receiver operating characteristic curves.

Results: ACE, sIL-2R, and 1-25 vitamin D levels were tested in 116, 36, and 24 patients respectively. ACE was elevated in 7 (29%) patients with sarcoidosis and 18 (20%) patients without sarcoidosis, $p=0.308$. No patient had an elevated sIL-2R level. 1-25 vitamin D level was elevated in 1 (8%) patient with sarcoidosis and 2 (18%) patients without sarcoidosis, $p=0.576$. The sensitivity and specificity for ACE testing was 29% and 80% with an AUC of 0.560 [95% CI 0.430-0.690, $p=0.368$]. The AUC for sIL-2R was 0.672 [95% CI 0.491-0.852, $p=.079$]. The sensitivity and specificity for 1,25 vitamin D was 8% and 82% with an AUC of 0.399 [95% CI 0.164-0.633, $p=0.401$].

In a secondary analysis excluding patients with pulmonary involvement, the AUC for sIL-2R increased to 0.796 [95% CI 0.559-1.000, $p=.033$].

Conclusion: Serum ACE and 1-25 vitamin D levels are not useful in predicting neurological, cardiac, or ocular involvement in sarcoidosis. sIL-2R levels may aid in diagnosing isolated extra-pulmonary disease, but larger studies are needed. In the era of rising healthcare costs and the Choosing Wisely campaign, these tests should not be performed routinely for diagnostic workup.

Dysregulation of microRNA-25 Modulates Airway Smooth Muscle Cell Proliferation via the Mammalian Target of Rapamycin Pathway by Targeting Tuberous Sclerosis Complex 1

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Rationale: Our laboratory identified a panel of microRNAs (miRs) that are dysregulated in human airway smooth muscle (ASM) cells upon pro-inflammatory stimulation, including members of the miR-106b~25 family. Of these, miR-25 targets the 3' untranslated region (UTR) of tuberous sclerosis complex 1 (TSC1) gene, an upstream negative regulator of mammalian target of rapamycin complex 1 (mTORC1). A regulator of proliferation and survival, mTORC1 is associated with aberrant inflammation of the airways and is further implicated in asthmatic ASM remodeling. We further corroborated that this subset of miRs is differentially regulated in asthmatic ASM cells, and more specifically that, inhibition of miR-25 promotes ASM cell proliferation and correlates with enhanced Akt phosphorylation in vitro. Here, we continue these studies by further investigating the hypothesis that miR-25 targets TSC1 to attenuate ASM cell proliferation via mTORC1 signaling.

Methods. ASM cells were transduced with lentiviral construct to knock-down miR-25. QPCR analysis in proliferating and growth arrested cells was used to assess the role of miR-25 expression on TSC1 gene-silencing. Western blot analysis assessed Hamartin (TSC1) expression and phosphorylation of mTORC1 at activator (Ser-2481) and repressor (Ser-2448) sites, and several downstream effectors, including S6 kinase and proliferating cell nuclear antigen (PCNA).

Results. miR-25 knock-down decreased phosphorylation of pmTOR (Ser-2448) by 30% in proliferating cells, indicating enhanced mTORC1 activity and activation of its downstream effectors. Furthermore, pmTOR (Ser-2481) expression in proliferating cells was increased by about 700%, confirming differential regulation of mTORC1 phosphorylation sites in an active protein complex. Additionally, PCNA protein expression was enhanced in miR-25 knockdown ASM cells, suggesting a hyperproliferative phenotype.

Conclusions. These data support the involvement of miR-25 in asthmatic ASM cell proliferation. miR-25 silences TSC1, resulting in a reduction of its protein product, Hamartin. Deregulation of mTORC1 by hamartin subsequently resulted in differential regulation of activator and repressor phosphorylation events of mTORC1. Further downstream effector enhancements, such as that of PCNA, strengthen the hypothesis that gene-regulation by miR-25 plays an important role in modulating ASM phenotype. Further experiments enhancing miR-25 expression will clarify the role of miR-25 in proliferating asthmatic ASM cells and its effect on the mTORC1 proliferative pathway. These findings will provide more insight on the pathogenesis of asthma disease and potentially provide a novel mechanism by which asthma is targeted to reduce hyperproliferation.

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ICU-Based Palliative Care for Older Adults with Traumatic Head Injuries: A Feasibility Study

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Rationale: Geriatric head trauma patients in the Intensive Care Unit (ICU) present a large unmet need for palliative care and advanced care planning. Over half of older adults with head trauma require ICU care and overall have in-hospital mortality ranging from 30-80%. Despite best practice recommendations emphasizing early goal-related communication, few to no evidence-based approaches have been tested in this population. Evidence from 18 clinical trials across diverse care settings supports the efficacy of a video-based decision support tool developed by ACP Decisions. The 5- minute video outlines treatment options including full care (full code), limited care (do not resuscitate/do not intubate), and comfort care. The video's feasibility, tolerability, and efficacy has not yet been demonstrated in an ICU-based, geriatric head trauma population.

Methods: After identification by the research team and oral consent, participants (patients and/or family) watch the ACP Decisions video and then complete a 9-question survey. Goal enrollment is 35 participants. Inclusion criteria are: (1) Patients must be >age 65, presenting with head trauma, ability to consent, admitted/being considered for admission to the ICU, and able to speak one of the 17 languages in which the ACP Decisions video is offered; and (2) Family member participants must be >age 18, able to consent, and able to speak one of the 17 languages in which the video is offered. Results: Data collection is ongoing with 11 participants already enrolled in the study (7 patients, 4 family and/or friends) and 87% enrollment to date of patients approached. Of the 7 patients enrolled, 57% were over the age of 80. The population studied had all attended at least some college. The majority of patients felt comfortable (54.5%) or very comfortable (9.1%) watching the video. 36.4% of the population studied found the video contents helpful, with an equal number stating "no opinion". Over half of participants (54.5%) would recommend the video to another person.

Conclusions: Preliminary results of our feasibility study for implementation of a video decision support tool among older adults admitted to the Stanford ICU with traumatic head injury have been encouraging. Participants report comfort watching the video and would recommend its contents to another person. We anticipate that enrollment will be completed by December 2018. This study is the first phase of a broader goal to implement an evidence-based advance care planning and palliative care intervention among older adults with traumatic head injuries.

Cleaved RAGE Modifies the Association between Matrix Metalloproteinase-3 and Mortality in Sepsis

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Rationale: Sepsis is a leading cause of death during critical illness. However, the interplay between the numerous molecular pathways implicated in sepsis is poorly understood. Plasma levels of matrix metalloproteinase (MMP)-3 and MMP-9, and the receptor for advanced glycation endproducts (RAGE) are associated with sepsis mortality and may act in concert to propagate a dysfunctional inflammatory response. In preclinical models, MMP-3 and MMP-9 shed RAGE from vascular endothelium, thereby generating circulating cleaved RAGE (cRAGE). We aimed to quantify the association of plasma MMPs with cRAGE levels and to determine whether cRAGE acts as an effect modifier for the relationship between MMPs and shock or mortality.

Methods: We enrolled 200 critically ill patients with sepsis and measured cRAGE, MMP-3, and MMP-9 plasma concentrations (ELISA) at ICU admission. Shock was assessed by vasopressor use or mean arterial pressure <65mmHg despite 30cc/kg fluid resuscitation within the first 24 hours. We defined mortality at 30 days. We used logistic regression to test the association between metalloproteinases, cRAGE, MMP-cRAGE interaction, and sepsis outcomes of shock and mortality adjusting for age, sex, and race. We used adjusted linear regression to test the association between cRAGE and metalloproteinases. When a statistically significant interaction was detected ($p < 0.05$), we dichotomized continuous variables at the median to test strata-specific odds ratios.

Results: Shock and mortality incidences were 44% and 45%, respectively. Plasma cRAGE was associated with mortality (OR 1.45 per log increase [95%CI 1.25-1.70], $p < 0.01$), shock (OR 1.32 [95%CI 1.10, 1.58], $p < 0.01$) and MMP-3 levels (b 0.10 [95%CI 0.01, 0.21], $p = 0.04$) but not MMP-9 levels. Higher MMP-3 concentrations were associated with shock (OR 1.55 per log increase [95%CI 1.01, 2.42]; $p = 0.05$) and mortality (OR 1.65 [95%CI 1.14, 2.40], $p < 0.01$) whereas MMP-9 levels were not associated with outcomes. We detected a statistically significant interaction between cRAGE and MMP-3 on sepsis mortality ($p = 0.02$) whereby in the presence of high plasma cRAGE, MMP-3 levels were associated with lower mortality (Table). There was no significant interaction between cRAGE and MMP-3 on shock, and there was no interaction between MMP-9 and cRAGE for either outcome.

Conclusion: Higher plasma concentrations of cRAGE and MMP-3 are associated with shock and mortality in sepsis. Plasma cRAGE acts as an effect modifier for the relationship between MMP-3 and mortality; we hypothesize that this may occur via MMP-3-related cleavage of RAGE. Our findings highlight the complexity of the RAGE-MMP-3 interplay in sepsis and suggest there may be context specific effects.

Table. Plasma cRAGE Acts an Effect Modifier of the Association between Plasma MMP-3 and Mortality in Sepsis

Plasma cRAGE	Adjusted Odds Ratio for Plasma MMP-3 on Mortality (95%CI)	<i>p</i>
Low < 934 pg/mL (<i>n</i> =100)	3.06 (1.52, 6.17)	<0.01
High ≥ 934 pg/mL (<i>n</i> =100)	0.82 (0.47, 1.43)	0.44

Subjects with low plasma cRAGE had increasing odds of mortality with increasing plasma MMP-3 levels whereas subjects with high plasma cRAGE did not. Baseline plasma cRAGE was dichotomized at the median.

High-flow nasal oxygen versus standard oxygen therapy in immunocompromised patients with acute respiratory failure: A meta-analysis of randomized controlled trials

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Rationale: Acute hypoxemic respiratory failure (AHRF) in immunocompromised patients represents the commonest reason for intensive care unit (ICU) admission. Although AHRF in critically ill immunocompromised patients is associated with high mortality rates, the best strategy for oxygen therapy remains controversial. Therefore, we performed a meta-analysis of all randomized controlled trials (RCTs) to evaluate the safety and efficacy of high-flow nasal oxygen vs standard oxygen therapy in this population.

Methods: We performed a systematic database search (PubMed, Embase, and Cochrane Library) for all RCTs that compared high-flow nasal oxygen with standard oxygen therapy (nasal prongs or mask with or without a reservoir bag and with or without a Venturi system) from inception to October 2018. Primary outcome was endotracheal intubation rate. Secondary outcomes were short-term mortality, acquired ICU infection, ICU length of stay (LOS), and hospital LOS. We used a random-effects model to calculate risk ratios (RRs) and weighted mean differences (MDs) with their 95% confidence intervals (CIs) for dichotomous and continuous data, respectively. Heterogeneity was explored by Cochran Q statistic ($p < 0.05$) and I-squared (I^2) statistic. P-values < 0.05 were considered statistically significant. Additionally, meta-regression analysis was performed for baseline study covariates.

Results: We identified 4 RCTs with a total of 1,112 patients (mean age 63 ± 12 ; 65.9% males). The leading cause of AHRF was pneumonia (43.4%). High-flow nasal oxygen was delivered via a heated humidifier and initiated at a gas flow of 40-50 L/min and fraction of inspired oxygen (FiO_2) of 100%, with a subsequent flow rate increase to achieve peripheral capillary oxygen saturation (SpO_2) of $\geq 92\%$. Compared with standard oxygen therapy, high-flow nasal oxygen was not associated with significant reduction in the rate of intubation (RR 0.87; 95% CI: 0.75-1.00; $P=0.05$), short-term mortality (RR 0.96; 95% CI 0.83;1.11; $P=0.57$), acquired ICU infections (RR 0.86; 95% CI 0.63-1.18; $P=0.35$), ICU LOS (MD -0.86; 95% CI -4.63-2.92; $P=0.66$), or hospital LOS (MD -2.77; 95% -7.14-1.60; $P=0.21$). Meta-regression analyses based on study-level covariates (mean age, respiratory rate, and Sequential Organ Failure Assessment [SOFA] score) did not suggest significant covariates effect for the intubation rates.

Conclusions: Among critically ill immunocompromised patients with AHRF, high-flow nasal oxygen compared with standard oxygen therapy did not significantly reduce the rates of intubation, short-term mortality, acquired ICU infection, or ICU/hospital LOS. Further long-term adequately powered trials are needed to establish the best strategy for oxygen therapy in this high-risk population.

Using Community Misconceptions about Asthma as a Vessel for Asthma Education: The Development and results of a Hispanic Community Asthma Leader Training Program

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Rationale: Despite substantial investments in research, asthma continues to pose a significant health burden for Hispanics in the US. In NYC, Hispanics have higher asthma prevalence than non-Hispanics (10.9% vs. 7.4%), and more than double the rate of ER visits (33.8% vs.12.8%). Community Based Participatory Research (CBPR) works with community members to define research questions and collaborate on the conduct of the research in order to derive more pertinent research questions with results that may be more readily adopted. We describe the results of collaboration with a community outreach organization to understand Hispanic asthma research needs and misconceptions, and the resulting intervention to improve asthma knowledge.

Methods: Partnering with the community outreach organization, El Poder de Decidir, we conducted 12 Hispanic community asthma meetings to identify research priorities. This was augmented by asthma education literature, and discussions with organizations experienced with Hispanic asthma outreach. Six Hispanic Asthma Community Leaders (ACLs) were trained using educational modules highlighting misconceptions about asthma. Modules were summarized in a brief asthma education card. At the completion of training, ACLs were also asked to react to 4 hypothetical asthma patient scenarios to test their knowledge. ACLs then assembled members from their community for teaching using the brief asthma education card, and observed by members from El Poder de Decidir and a bilingual pulmonologist. Participants completed pre- and post- asthma knowledge surveys, and results were analyzed using a two-tailed paired t-test.

Results: The development of the intervention including the community workshops, education modules and brief asthma education card for ACLs was collaboratively developed over two years. The 6 ACLs were trained over the course of 3 months and were then observed as they taught a total of 104 participants. Among all participants, the mean knowledge score increased from 0.56 (sd 0.18) to 0.85 (sd 0.13) pre- and post- teaching by ACLs (0.282 (95%CI 0.25-0.31) $t(103)=17.62$; $p=0.0001$). At baseline the most commonly missed questions were about asthma symptoms and triggers, and that daily need to use asthma indicated the need for controller medication.



Conclusions: We have successfully developed and tested an asthma education intervention for the Hispanic community which includes tailored education materials to build on identified misconceptions, and Hispanic ACLs to teach to their own community members. Further studies are warranted to determine whether these results persist among a larger group, what the comparative effectiveness is versus other education-based interventions and the longer-term health benefits.

Lymphocyte Landscape of Heterotypic Immune Murine Lungs

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Rationale: Previous non-lethal exposure to *Streptococcus pneumoniae*, the leading cause of bacterial pneumonia, confers subsequent protection against lethal serotype-mismatched pneumococci. This heterotypic protection is driven by lung T-cells, including CD4⁺ tissue-resident memory T-cells (TRM) that secrete cytokines orchestrating a rapid immune recall and bacterial clearance. This study aims to elucidate the dynamics and landscape of other lung lymphocytes that may contribute to heterotypic immunity in experienced lungs.

Methods: Intratracheal instillation of *S.pneumoniae* serotype-19F strain EF3030 (Sp19F) was used to incite non-lethal experience. Influx and establishment of extravascular lung lymphocytes was determined by flow cytometry. Heterotypic immunity was established with two Sp19F exposures a week apart. Four weeks later, lungs were collected for histology, fluorescence in-situ hybridization (FISH) or flow cytometry.

Results: To elucidate the kinetics of lung adaptive immune cell recruitment post initial Sp19F exposure, intrapulmonary B-cell and T-cell populations were temporally enumerated. Flow cytometric analyses after the initial infection demonstrated a robust influx of B-cells and T-cells 5 days post infection, which expanded thereafter. After resolution of the second Sp19F exposure, lungs exhibited perivascular lymphocytic aggregates but lacked bronchoalveolar associated lymphoid tissue (BALT). FISH for IgG1 transcripts exhibited presence of class-switched antibody-secreting cells within the lymphoid aggregates. Since heterotypic immunity could involve multiple T-cell populations, we sought to delineate the T-cell subsets enriched within experienced lungs. Flow cytometric analyses indicated increased numbers of extravascular CD4⁺ and CD8⁺ T-cells, a subset of which were CD4⁺ TRMs (identified by CD4⁺CD11a^{bright}CD69⁺), as well as gd T-cells. Numbers of Natural killer (NK) T-cells did not change and no follicular helper T-cells (TFH) were detectable. Further analysis of the enriched CD4⁺ T-cell subsets revealed enlargement of a previously uncharacterized population of CD4⁺CD11a^{bright}CD69⁻ cells which were distinct from TRMs. These cells were CD62L^{lo}CD44^{hi} which suggests an effector memory cell phenotype.

Conclusions: In summary, our preliminary studies suggest that the first pneumococcal exposures act as important recruitment stimuli for establishment of an effective adaptive immune landscape responsible for heterotypic immunity. This includes B-cells and gd T-cells in addition to CD4⁺ and CD8⁺ T-cells. We observed an unappreciated CD4⁺CD11a^{bright}CD69⁻ memory T-cell subset in experienced lungs. The biological functions of these non-TRM lymphocytes elicited by experience, and their contributions to heterotypic immune protection are the focus of ongoing studies.

Impairment of Endothelial Caveolin-1-TRPV4 Channel Signaling in Pulmonary Hypertension

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Rationale: Endothelial dysfunction is a hallmark of pulmonary hypertension (PH) resulting in impaired arterial vasodilation and elevated pulmonary arterial pressure (PAP). However, the underlying mechanisms for the loss of endothelium-dependent vasodilation in PAH remain unknown. We recently identified endothelial TRPV4 (transient receptor potential vanilloid 4; TRPV4_{EC}) channels as a key Ca²⁺ influx pathway that promotes vasodilation via endothelial nitric oxide synthase (eNOS) activation in small, resistance pulmonary arteries (PAs). The goals of this study were to determine the role of TRPV4_{EC} channels in maintaining resting PAP, and the pathological mechanisms that impair TRPV4_{EC} channel regulation in PH, thus contributing to the loss of endothelium-dependent vasodilation. Caveolin-1 (Cav-1_{EC}) is an important structural protein in the pulmonary circulation, and is known to associate with TRPV4 channels in cultured endothelial cells. We hypothesized that Cav-1_{EC} enhances TRPV4_{EC} channel activity, and impaired Cav-1_{EC}-TRPV4_{EC} vasodilatory signaling contributes to the loss of endothelium-dependent vasodilation in PH.

Methods: Three-week chronic hypoxia (CH) model was used to induce PH in mice. TRPV4_{EC} channel activity was determined using high-speed confocal recording of Ca²⁺ influx through single TRPV4_{EC} channels (TRPV4_{EC} sparklets) in small PAs from C57BL6, inducible endothelium-specific TRPV4 or Cav-1 knockout (TRPV4_{EC}^{-/-} or Cav-1_{EC}^{-/-}), or wild-type mice. Pressure myography in small PAs was used to investigate the functional consequence of TRPV4_{EC} channel activation. Right ventricular systolic pressure (RVSP) was measured as an indicator of PAP.

Results: TRPV4_{EC}^{-/-} mice showed impaired endothelium-dependent vasodilation of small PAs and elevated RVSPs, highlighting the crucial role of TRPV4_{EC} channels in lowering resting PAP. Moreover, Cav-1_{EC} enabled protein kinase C (PKC) activation of TRPV4_{EC} sparklets, thus enhancing TRPV4_{EC} channel activity. Consistent with our hypothesis, PKC-TRPV4_{EC} channel signaling was significantly impaired in PAs from CH mice, which was accompanied by attenuated Cav-1_{EC}-PKC localization. PH has been commonly associated with elevated levels of the deleterious reactive nitrogen species peroxynitrite (PN). We found that PN inhibitors rescued PKC-TRPV4_{EC} channel signaling and vasodilation, and lowered RVSP in CH mice. Furthermore, exogenous PN also disrupted Cav-1_{EC}-PKC-TRPV4_{EC} channel vasodilator signaling.

Conclusion: Cav-1_{EC}-PKC-TRPV4_{EC} channel signaling regulates endothelium-dependent vasodilation in small PAs. Disruption of Cav-1_{EC}-PKC-TRPV4_{EC} channel signaling by elevated PN levels contributes to the loss of endothelium-dependent vasodilation and elevation of PAP in PH.

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Maternal Outcomes in Pregnant Women with Cystic Fibrosis

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Rationale: Cystic fibrosis (CF) is the most common lethal inherited disorder in Caucasians. With recent advances in therapies, median survival now exceeds 47 years. Given increased survival, pregnancy in CF women is becoming more common. Prior studies have demonstrated increased morbidity and mortality in pregnant CF individuals. However, there is a paucity of updated data describing maternal outcomes in this population. On average, adults with CF have a 1-2% decline in FEV₁/year. Thus, the objective of our study was to examine change in FEV₁ after pregnancy in CF.

Methods: This retrospective cohort study was conducted in CF patients ≥ 18 years of age who were seen in the Johns Hopkins Adult CF Center from June 2006 to June 2017. Baseline demographic and clinical variables were extracted from electronic record databases. Lung function measured as FEV₁% predicted was identified for three specific time periods: 12 months prior to pregnancy (baseline), during pregnancy, and 12 months after pregnancy. The highest FEV₁ (% predicted) for each time period was captured for analysis. A one-way analysis of variance (ANOVA) with post hoc pairwise comparisons (Tukey test) was conducted to evaluate for differences in FEV₁ over time.

Results: There were 36 pregnant women identified in our center during the timeframe selected. The median age was 29.2 years (SD 5.72) with 38% of women being homozygous for F508del. Twenty-five (69.4%) women had a pregnancy that resulted in a live birth, with 7 (19%) spontaneous abortions, and 4 (11%) therapeutic abortions. 22% of women had CF-related diabetes, and 72% were pancreatic insufficient. Results yielded significant variation among differences in mean FEV₁% at baseline (73.5% (SD 27.9%)), during pregnancy (70.0%, (SD 29.3%)), and after pregnancy (69.8% (SD 29.7%)) (p=0.02). There was a significant decline in FEV₁% of 3.6% from baseline to after pregnancy (p=0.04). However, there was no significant difference in FEV₁% before and during pregnancy (p=0.053) or during pregnancy and after pregnancy (p=0.991). When looking at the subgroup of patients starting with an FEV₁ less than 60%, there was a larger decrease in FEV₁% from baseline (48.4% (SD 8.1%)) to after pregnancy (43.3% (SD 7.1%)) (-5.5%, p=0.038).

Conclusions: We found a significant decrease in FEV₁% after pregnancy. In the cohort with moderate to severe lung function at baseline (FEV₁ $\leq 60\%$), this difference was more pronounced. Overall, we found a decline in FEV₁ over time that is larger than expected compared to the general adult CF population.

Perforin inhibition blocks NK-mediated in vitro killing of human lung epithelial cells in COPD

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Rationale: We have previously shown that human lung natural killer (NK) cells are able to kill autologous lung epithelial cells and that this natural cytotoxicity is increased in COPD. NK cell killing can be mediated by various molecules, including perforin/granzyme, Fas ligand, and TNF-related apoptosis inducing ligand (TRAIL). It is not known which of these mechanisms is necessary for the killing of lung epithelial cells by NK cells in COPD.

Methods: We collected lung tissue from consented patients undergoing clinically-indicated lung resections ($n = 4$ subjects without COPD; $n = 7$ subjects with COPD). We isolated NK cells from dispersed lung tissue using CD56+ immunomagnetic beads. RNA was isolated from the purified NK cell population and we used real-time PCR to measure transcripts for perforin, granzyme A, granzyme B, Fas ligand, TRAIL, and TNF-alpha. In separate COPD subjects ($n = 3$), we isolated both CD56+ cells and CD326+ epithelial cells using immunomagnetic beads to measure epithelial cell apoptosis under various conditions. Epithelial cells were cultured in one of four conditions: 1) in media alone to measure baseline apoptosis; 2) with the perforin inhibitor, concanamycin A (10nM); 3) with autologous lung NK cells at a ratio of 10 NKs to 1 epithelial cell; 4) with concanamycin A plus NK cells. After four hours, cells were collected and viability of epithelial cells was measured by flow cytometry using Annexin-V and 7-AAD staining. Results were normalized to the baseline viability of epithelial cells cultured alone.

Results: Human lung NK cells expressed transcripts for all genes that we measured: perforin, granzyme A, granzyme B, Fas ligand, TRAIL, and TNF-alpha. However, only transcripts for perforin, granzyme A, and granzyme B were significantly increased in COPD (perforin: 6.5-fold increase, $p = 0.02$; granzyme A: 8-fold increase, $p = 0.006$; granzyme B: 12-fold increase, $p = 0.009$). As previously shown co-culture of lung epithelial cells from COPD subjects with autologous lung NK cells led to increased apoptosis of epithelial cells. However, including the perforin inhibitor, concanamycin A, blocked this effect. Concanamycin A had no effect on epithelial cells by themselves.

Conclusions: These preliminary results suggest that human lung NK cells are using the perforin/granzyme pathway to kill autologous lung epithelial cells. The RNA transcript data also suggests that lung NK cells from COPD patients may have greater reserves of pre-formed cytotoxic molecules although protein analyses would be necessary for confirmation.

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Predictors and Barriers to Uptake of Influenza Vaccination among African American Patients with Chronic Obstructive Pulmonary Disease in a Large Academic Hospital

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Background: Influenza infection in patients with chronic obstructive pulmonary disease (COPD) is associated with more frequent exacerbations and increased mortality risk. Therefore, the Center for Disease Control and Prevention (CDC) recommends annual influenza vaccination for patients with COPD to improve outcomes. African-Americans (AAs) with COPD tend to have higher disease burden and experience worse quality of life compared to other ethnic groups. Influenza vaccination rate among African American patients with COPD remains suboptimal. We sought to identify factors that influence influenza vaccine uptake among African American patients with COPD.

Methods: We selected 298 (simple random sampling) African American patients with COPD on their list of diagnosis, who visited one of the out-patient departments of Grady Memorial Hospital, Atlanta between October 1, 2017 and February 28, 2018. Patients were asked to participate in the study by completing a questionnaire. All participants had at least one pulmonary outpatient visit during the study period. Co-morbidities self-reported by the patients and included in the analysis were asthma, coronary artery disease, heart failure, kidney diseases, liver diseases, diabetes mellitus, cerebrovascular accident, HIV or AIDS and sickle cell disease. MS Excel 2011 and STATA for Mac (Stata Corp, College Station, TX, USA) were used for data management and analyses respectively. A two-sided P-value < 0.05 was considered statistically significant.

Results: A total of 268 (55% female) patients completed the questionnaire. Mean (\pm SD) age was 48.5 years (\pm 8.5 years). The influenza vaccination rate among the patients was 59% (n=158/268). Number of co-morbidities and female gender were significantly associated with a positive influenza vaccination status. 63% received flu vaccine information and recommendation from their Pulmonologist and 76% of these patients received influenza vaccine. The probability of being vaccinated against influenza was about 12 times higher among patients who received flu vaccine recommendation and information from a pulmonologist (P<0.05) compared to other providers (Table 1). Identified reasons for declining vaccination included fear of getting sick from flu vaccine (58%), fear that the vaccine will make their COPD worse (50%), belief that the vaccine is not effective (41%) and distrust of the pharmaceutical companies that produce vaccines (48%).

Conclusion: Patients with COPD are most likely to receive influenza vaccination if the information and recommendation are provided by their pulmonologists. Education of patients during pulmonology clinic visits focused on addressing influenza vaccine concerns identified in this study may improve vaccination rate and chances of meeting CDC Healthy people 2020 target of 90% influenza vaccine coverage in high-risk conditions including COPD.

Predictors and Barriers to Uptake of Influenza Vaccination among African American Patients with Chronic Obstructive Pulmonary Disease in a Large Academic Hospital
continued

Table: Predictors of flu vaccination among African American Patients with COPD based on physician recommendation (OR adjusted for age, gender, educational status and presence of co-morbidities)

Information and Recommendations on Flu vaccine provided	Vaccinated [n (%)]	Not Vaccinated [n (%)]	Crude OR	Adjusted OR
Pulmonologist				
No (n=69)	22 (31)	47 (69)	Reference	Reference
Yes (n=169)	128 (76)	41 (24)	14.4 (4.3 – 20.2)	12.1 (3.8 – 19.2)
Can't remember (n=30)	8 (28)	22 (72)		
Internist(Primary Care Provider)				
No (n=76)	43 (56)	33 (44)	Reference	Reference
Yes (n=143)	92 (64)	51 (36)	6.9 (3.7 – 14.2)	5.2 (2.9 – 9.2)
Can't remember (n=49)	23 (47)	26 (53)		
Other physicians*				
No (n=100)	68 (68)	32 (32)	Reference	Reference
Yes (n=142)	80 (56)	62 (44)	2.8 (0.5 – 3.6)	1.6 (0.6 – 2.8)
Can't remember (n= 26)	10 (38)	16 (62)		

*Family physicians, other internal medicine and non-internal medicine specialists

Impact of Prolonged Mechanical Ventilation on Ability to Perform Everyday Activities

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Rationale: Patients who require prolonged mechanical ventilation are at risk for respiratory muscle dysfunction, which could adversely affect their functional recovery and limit their physical activities in daily life. The aim of this study was to determine the long-term impact of prolonged ventilation on physical, mental and social functioning, using a disease-specific outcome measure (pulmonary functional status scale [PFSS]), in patients managed at an long-term acute care hospital (LTACH.)

Methods: In 20 patients (age 62 ± 12 (SD) years; 11 woman) transferred for weaning from prolonged ventilation (duration of ventilation on enrollment, 34 ± 16 days), the PFSS questionnaire was administered on admission and discharge from the LTACH and six months later. (On LTACH admission, when answering the questionnaire patients/surrogates were asked to estimate patient functional status two weeks before hospitalization for critical illness; this was considered pre-illness function). PFSS is a 35-item questionnaire that measures the difficulty in performing daily activities in patients with chronic lung disease. PFSS measures functional status in 3 domains: physical activity/social functioning, emotional functioning and sexual functioning; scores in each domain range from 0 to 5 (higher score indicates greater functional independence). Mean scores from each of the domains were summed to generate a summary PFSS score (range, 3-15); score of 15 indicated that a task could be performed without difficulty.

Results: Before critical illness, PFSS-summary score was 13.08 ± 2.9 . At LTACH discharge, the score was 7.8 ± 2 . At six months, the score increased to 10.4 ± 2.8 ($p < 0.05$), yet it remained less than pre-illness value ($p < 0.05$). Of the three domains included in the PFSS-summary score, scores at six months returned to pre-illness value for emotional and sexual functioning domains ($p = ns$) but not for physical activity/social functioning domain ($p < 0.05$). At six months, the major limitation to physical activity that patients reported was dyspnea; patients were able to walk no more than half a block at six months, compared with four blocks or more before the critical illness.

Conclusion: Patients weaned from prolonged mechanical ventilation reported an improvement in their ability to perform everyday activities six months after discharge from an LTACH. Despite the improvement, patients reported that their physical activity was not back to pre-illness level, with dyspnea being a major contributor to their limitation in performing everyday activities.

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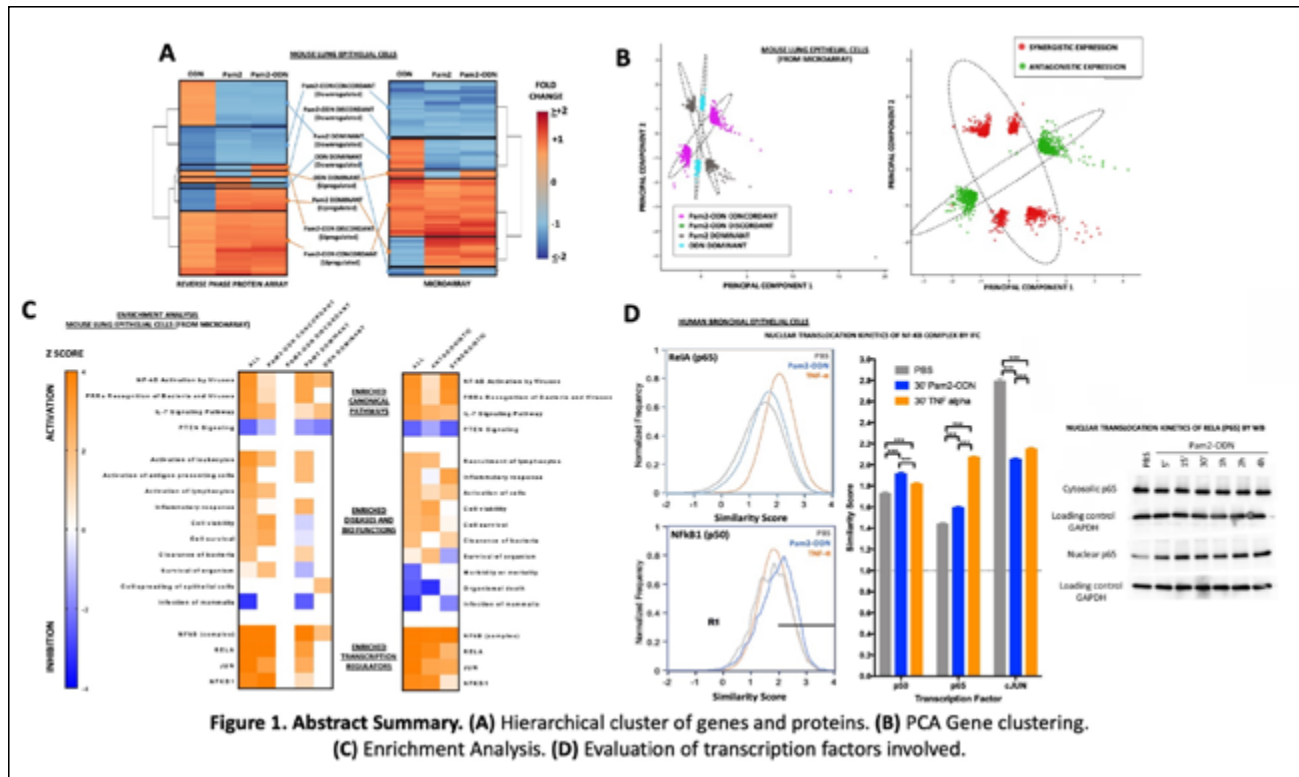
Synergistic Gene and Protein Expression Analyses Reveal Roles of NFkB and JUN in Inducible Epithelial Resistance Against Infection

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Rationale: Lower respiratory tract infections are a major cause of worldwide morbidity. Our group has previously reported that therapeutic stimulation of innate immune responses from the lung epithelium, referred to as inducible epithelial resistance, results in generation of a microbicidal intrapulmonary environment that promotes enhanced survival of infectious challenges. This protective response is achieved through concurrent aerosolized delivery to mice of Pam2CSK4, a Toll-like receptor (TLR)-2/6 agonist, and ODN M362, a TLR-9 agonist. Delivery of the individual ligands alone confers little *in vivo* protection against pneumonia, and results in only modest gene expression changes from the lungs of treated animals or from isolated mouse or human lung cells. Conversely, delivery of the two ligands together results in robust enrichment of antimicrobial genes and yields a substantial survival benefit, despite the fact that both ligands are believed to signal through MyD88-dependent pathways. This study investigates the mechanisms underlying this surprising coordinate detection of two ligands resulting in greater than additive protection.

Methods: We stimulated human bronchial epithelial cells, mouse lung epithelial cells and mouse lung homogenates with single (Pam2 or ODN) or dual (Pam2-ODN) ligand treatment, and performed microarray and reverse phase protein array analyses to identify signal-specific effector molecule enrichment. Analysis with IPA software was done after principal component analysis (PCA) clustering considering treatment responses, synergistic and antagonistic expression. Nuclear translocation and activity of transcription factors behind these patterns were studied by Western blotting (WB), Imaging Flow Cytometry (IFC) and DNA-binding activity ELISAs.



Synergistic Gene and Protein Expression Analyses Reveal Roles of NFkB and JUN in Inducible Epithelial Resistance Against Infection *continued*

Results: 1906 genes and 163 proteins differentially expressed were clustered into 4 subgroups: Pam2 dominant, ODN dominant, Pam2-ODN concordant or discordant responses. After PCA, Pam2 and ODN dominant genes and proteins were mainly synergistic, while concordant and discordant molecules were mostly antagonistic. In all sample types, enrichment analysis revealed activation of NFkB complex and pattern recognition receptor pathways against bacteria and viruses, including cell and organismal viability and survival. NFKB1, RelA and JUN-c were predicted as key regulators, and their increased expression, nuclear translocation, and activity was confirmed by WB, IFC or DNA-binding ELISA at 30' after Pam2-ODN treatment.

Conclusions: Altogether, differential synergistic and antagonistic expressions of effector genes and proteins led to function-specific activation of pathways and responses that better describe the phenomenon of inducible resistance as a novel epithelial function against infection. More so, our data suggest that this occurs through a novel interaction between NFKB and JUN transcription factor families that requires further studies.

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Mucus Matters: Ferrets Demonstrate Restrictive Lung Physiology, Sustained Fibrosis, Mucociliary Decrement in Airways, and Aberrant Repair Following Bleomycin-Induced Pulmonary Fibrosis

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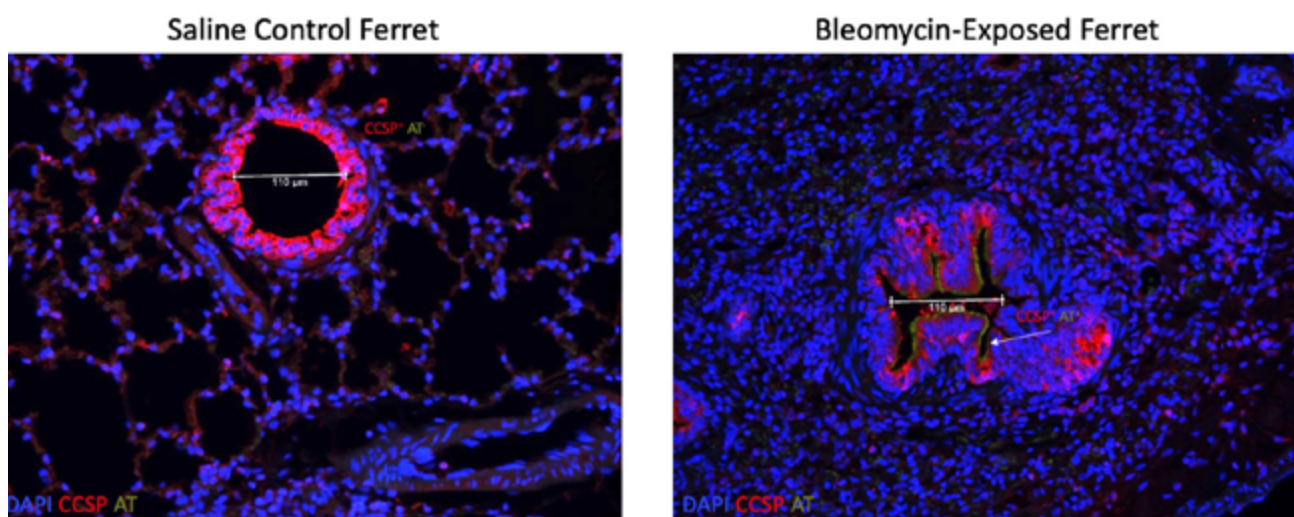
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Rationale: A gain-of-function promoter variant for MUC5B is a strong risk-factor for the development of idiopathic pulmonary fibrosis (IPF); yet, the role of MUC5B mucin in IPF pathogenesis is unknown. Ferrets, unlike mice, have submucosal glands and human-like distribution of MUC5B in the lung. We hypothesize the mucus microenvironment matters for the initiation and propagation of fibrosis in IPF, and developed a novel bleomycin(BL)-exposed ferret model to test whether it exhibits injury-repair patterns more akin to human IPF pathophysiology.

Methods: BL (5U/kg) or saline-control(SCT) was administered via intratracheal microspray to wild-type (WT) ferrets. Fibrosis was assessed with μ CT, hydroxyproline (Hyp), and histology. Respiratory system mechanics (inspiratory capacity(IC), compliance(Crs), and elastance(Ers)) were measured by forced-oscillation-technique using the FlexiventFX6. Muc5B, alpha-smooth-muscle-actin(α SMA), acetylated tubulin(AT) and club-cell-secretory-protein(CCSP) expression were assessed by immunohistochemistry (IHC) and immunofluorescence (IF). Mucociliary transport (MCT) rate and ciliary beat frequency (CBF) were assessed *ex-vivo* using micro- optical coherence tomography (μ OCT).

Results: Apparent at 2wks, ground-glass opacities persisted through 6wks on μ CT. Volumetric, threshold-based μ CT

Aberrant ciliation in CCSP+ small airways of bleomycin-exposed ferrets at 3-weeks



Mucus Matters: Ferrets Demonstrate Restrictive Lung Physiology, Sustained Fibrosis, Mucociliary Decrement in Airways, and Aberrant Repair Following Bleomycin-Induced Pulmonary Fibrosis continued

analysis revealed increased fibrosis in BL lungs (increased $18.1 \pm 2.2\%$ vs $-0.8 \pm 0.8\%$ in SCt, $P < 0.001$). At 6wks, Hyp was elevated ($8.1 \pm 1.4 \mu\text{g}/\text{mg}$ BL vs $4.3 \pm 0.7 \mu\text{g}/\text{mg}$ SCt, $P < 0.04$), indicating buildup of collagenous extracellular matrix and fibrotic injury. At 3wks, BL-ferrets had significantly reduced IC (mean decrement -10.5 mL vs SCt, $N=7/\text{group}$, $P < 0.0001$), decreased Crs ($3.8 \pm 0.58 \text{ mL}/\text{cmH}_2\text{O}$ BL vs $4.3 \pm 0.35 \text{ mL}/\text{cmH}_2\text{O}$ SCt, $N=7/\text{group}$, $P=0.026$), and increased Ers ($0.27 \pm 0.05 \text{ cmH}_2\text{O}/\text{mL}$ BL vs $0.23 \pm 0.02 \text{ cmH}_2\text{O}/\text{mL}$ SCt, $N=7/\text{group}$, $P=0.026$), demonstrating restrictive lung physiology. IF revealed collagen-rich matrices, scattered myofibroblasts, αSMA^+ fibroblastic foci (FF), and diffuse expression of Muc5b in areas of severe interstitial fibrosis. BL-ferrets had abnormal expression of mucin-rich proximal airway markers in cystic distal airspaces and aberrant AT⁺ ciliation of CCSP⁺ epithelium, akin to MUC5B positive honeycomb change and bronchiolization of the distal lung. μOCT demonstrated reduced CBF in the bronchi at 3wks and 6wks post-BL exposure (mean decrement -2.4 and -1.8 Hz BL vs. SCt, $P < 0.01$) and decreased MCT at 6wks (mean decrement $-3.2 \pm 0.19 \text{ mm}/\text{min}$ BL, $P < 0.01$).

Conclusion: BL-exposed ferrets exhibit features of IPF not found in rodent models and may be related to human-like Muc5b expression: FF, mucin-rich honeycomb cysts, bronchiolized distal airspaces, and sustained fibrosis associated with aberrant lung and mucociliary physiology. Our **ongoing studies** are investigating fibrosis development and mucociliary physiology contemporaneously in BL-exposed ferrets with genetic and pharmacologic modulation of Muc5b-expression to elucidate the role of Muc5b microenvironments in pathogenesis of fibrosis and dysregulated repair. <!--EndFragment-->

Differences in characteristics and outcomes of high-risk ward patients evaluated by compared to those not evaluated by rapid response teams

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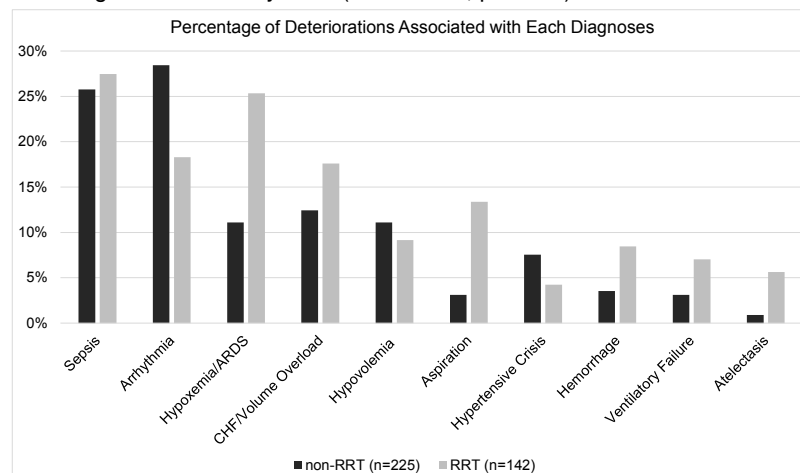
Rationale: Rapid response teams (RRTs) have been implemented in hospitals to improve the care of deteriorating patients. However, there is a critical gap in understanding how high-risk deteriorating patients evaluated by RRTs differ from other high-risk patients on the wards. Therefore, we compared the demographics, diagnoses, and outcomes of high-risk patients seen by an RRT to high-risk patients not seen by an RRT.

Methods: Among adult inpatients at an urban academic medical center between 2011 to 2016, 6,020 admissions were identified as high-risk for deterioration as denoted by an eCART score above the 95th percentile, a previously validated early warning score. Of these admissions, 780 were randomly selected for manual chart review by one of three trained individuals to determine if the patient experienced clinical deterioration at the time of the high eCART score, the causative diagnoses, and outcomes. Comparisons were made between high-risk patients evaluated by the RRT and high-risk patients not evaluated by the RRT.

Results: Of the 780 reviewed admissions, 367 (47%) were determined to have true deterioration events. Of these, 142 (39%) were evaluated by the RRT at the time of deterioration. Patients seen by the RRT did not differ significantly in terms of age, sex, race, or eCART score as compared to those not seen by the RRT. RRTs most frequently responded to sepsis (27%, n=39), hypoxemia (25%, n=36), volume overload (18%, n = 25), arrhythmia (18%, n = 26), and aspiration (13%, n =19). Compared to patients not seen by the RRT, those seen were over two times as likely to be diagnosed with hypoxemia (25% vs. 11%, $p < 0.001$), four times as likely to be diagnosed with aspiration (13% vs. 3%, $p < 0.001$), and significantly less likely to be diagnosed with arrhythmia (18% vs. 28%, $p = .028$). Patients seen by the RRT were more likely to be transferred to the ICU, (72% vs. 38%, $p < .001$), receive goals of care discussions (22% vs. 10%, $p = .018$), and die during their hospital stay (26% vs. 10%, $p < 0.001$).

Conclusions: Among high-risk ward patients, RRTs are more likely to be called for hypoxemia and aspiration, and less likely to be called for arrhythmias. Understanding which patients RRTs are triggered for is critical for informing which providers should be a part of the RRT and to help tailor training for RRT members, which could improve the care delivered to deteriorating patients.

Figure 1: This figure portrays the conditions responsible for the deteriorations in high-risk patients treated by the rapid response team (RRT) in comparison to high-risk patients not treated by the RRT (limited to the ten most frequent diagnoses). Patients treated by RRT were over two times as likely to be diagnosed with hypoxemia (25% vs. 11%, $p < 0.001$) and four times as likely to be diagnosed with aspiration (13% v 3%, $p < 0.001$), and less likely to be diagnosed with arrhythmia (18% v 28%, $p = .028$).



Hyperoxia-induced Soluble Guanylyl Cyclase (sGC) Dysfunction in Developing Airway Involves the Calcium Sensing Receptor (CaSR)

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Background: Hyperoxia with/without respiratory support is a vital intervention following premature birth. However, early oxygen exposure leads to subsequent airway hyperreactivity, remodeling (proliferation, fibrosis) and asthma; necessitating understanding of mechanisms that promote contractility/remodeling, vs. bronchodilation/anti-remodeling. Here, airway smooth muscle (ASM) is a key cell type. In adult ASM, we previously found the extracellular calcium sensing receptor (CaSR) is important. Given higher Ca²⁺ in developing lung, studies suggest CaSR in fetal ASM (fASM) is important, and enhanced by hyperoxia. Conversely, we find that the bronchodilatory NO-sGC-cGMP axis in adults is dysfunctional in prematurity and with oxygen, and thus direct sGC activation may be beneficial (e.g. cinaciguat, BAY58). In this study, we tested the hypothesis that CaSR and sGC dysfunction are linked in enhanced contractility and remodeling.

Methods: Human fASM cells were isolated from canalicular stage (18–22 week gestation) lung tissue following fetal demise (StemCell Express; Mayo IRB exempt). Cells were exposed to 21% O₂ vs. 50% O₂ (hyperoxia) for 48h with/without heme-independent sGC activator BAY58 or with/without CaSR antagonist NPS2143 (10uM). Expression of CaSR and sGC isoforms, and changes in cGMP, p-VASP-Ser 239 (PKG target) and extracellular matrix deposition were assessed. Extracellular Ca²⁺ was altered (0, 0.5, 1 or 2 mM) with/without CaSR agonist R568 (10 uM) vs. antagonist NPS2143 (10uM), and concurrent presence of BAY58. [Ca²⁺]_i responses to 10uM histamine were recorded in fura-2AM loaded cells.

Results: Hyperoxia increased fASM expression of CaSR but not sGC isoforms. Both NPS2143 and BAY58 individually suppressed hyperoxia effects on collagen deposition and [Ca²⁺]_i responses to histamine. [Ca²⁺]_i responses to agonist increased with extracellular Ca²⁺: exacerbated with hyperoxia or R568, but suppressed by NPS2143. In the presence of R568, BAY58 was without effect but in the presence of NPS2143, BAY58 more potently reduced [Ca²⁺]_i than by itself. Neither NPS nor R568 altered expression of sGC isoforms.

Conclusion: In developing human ASM, CaSR regulates [Ca²⁺]_i, with enhanced effects in hyperoxia. sGC effects on fASM are blunted by CaSR, with greater impact in hyperoxia. These novel data point to the potential for concurrent application of CaSR antagonists with sGC activators to overcome effects of hyperoxia in developing airways.

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Chitinase 3 Like 1 Protein Promotes Host Tolerance During Lung Infection with Influenza Viral Infection

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Rationale: Influenza is the most common seasonal viral infection that killed more than 50,000 people in the United States in 2017. The financial burden of influenza is estimated to be between \$ 3-14 billions. The current therapeutic options for influenza are limited to antiviral drugs that are effective only when administered early during the infection. There is an urgent need for new therapeutics. Chitinase and chitinase like proteins have been shown to play an instrumental role in host response to bacterial infections. Chitinase 3 like 1 (Chi3l1) known as Breast Regression Protein (BRP39) and YKL40 in humans is abundantly present in the lungs and have been shown to play important roles in host response against bacterial pathogens. The role of Chi3l1 is not known in influenza viral infection. Here we sought to explore the role of Chi3l1 in influenza infection using mice that are deficient in this protein (BRP39 KO) and transgenic mice overexpressing human version (YKL40) of the protein in the lung under the CC10 promoter.

Method: Mice were inoculated with H1N1/PR8 strain of influenza (10 PFU) intranasally and observed for weight loss. Mice were euthanized at different time points to measure viral load, inflammatory response, lung pathology and protein content in the broncho-alveolar lavage (BAL) fluid.

Results: Our results show that Chi3l1 ^{-/-} mice have high susceptibility to infection as evident by significant increased weight loss, and failure to recover their weight to baseline even after the virus is cleared. Similarly, these mice had significantly elevated WBC counts in their BAL at day 16 post infection, suggesting that the failed recovery of body weight may be due to persistent inflammation. This persistent inflammation lead to increased lung damage and vascular leak as measured by total protein content in the BAL samples of Chi3l1 ^{-/-} mice. This persistent injury also led to increased fibrotic changes that were observed in Chi3l1 ^{-/-} mice at day 16 post influenza infection. In contrast, mice that overexpress the protein recovered faster from influenza induced weight loss. These protective effects were independent of viral clearance as both KO and overexpressing mice showed similar viral load kinetics.

Discussion: Our data suggest that Chi3l1 plays a key role during influenza infection. The important roles of Chi3l1 may be through regulating the host tolerance rather than host resistance. Our laboratory is investigating the molecular mechanisms by which Chi3l1 affects the host tolerance during influenza infection.

Exhaled Nitric Oxide as a Predictor of Exercise Induced Bronchoconstriction in Children with Well Controlled Asthma

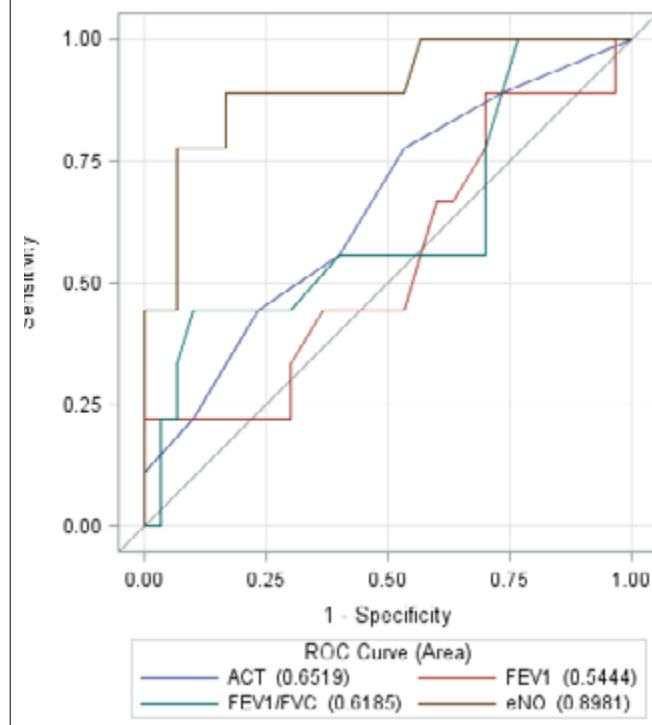
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Rationale: Exercise induced bronchoconstriction (EIB) recently has been identified as an independent risk factor for asthma morbidity and health care utilization. While many tools exist to quantify asthma severity and control, few studies have examined the strength of these tools as predictors of EIB specifically. The Asthma Control Test (ACT) is a standardized, yet highly subjective self-administered method of monitoring asthma symptoms that is readily available in the clinical setting. More objective measures of asthma control include lung function and exhaled nitric oxide (eNO), a biomarker of airway inflammation. In a cohort of well controlled asthmatics defined by normal ACT and thus, low symptom burden, our objective was to determine if spirometry or airway inflammation predicted EIB.

Methods: We recruited 40 African-American and Latino children ages 11-13 in New York City (NYC) with well-controlled asthma, defined as an ACT score ≥ 20 . Children performed spirometry (Vmax CareFusion) and eNO measurement (NIOX Vero) prior to progressive cardio-pulmonary exercise test (CPET) (Vmax CareFusion). Spirometry was repeated after CPET. Children were defined as having EIB if they demonstrated a $\geq 10\%$ fall in FEV₁ following CPET according to ATS criteria. T-tests and logistic regression models were used to determine the association between respiratory measures and EIB.

Figure 1: Receiver operator curves (ROC) comparing asthma control test (ACT), spirometry measures (FEV₁ and FEV₁/FVC), and exhaled nitric oxide (eNO).



Results: Twenty-three percent of children (N=9) experienced EIB. There was no difference in spirometry between children who experienced EIB and those who did not ($P > 0.05$). However, baseline eNO was significantly higher among children who experienced EIB ($P < 0.01$). A receiver-operator curve demonstrated a statically significant greater area under the curve for eNO (AUC=0.90; 95% CI: 0.77, 1.00) than for ACT or spirometry ($P < 0.01$) (Figure 1). In a multivariable logistic regression model adjusted for race/ethnicity, sex, and second hand smoke exposure, eNO remained a significant predictor of EIB (OR=13.3; 95% CI: 2.0, 88.3; $P < 0.01$).

Conclusion: In our cohort of 11-13 year olds in NYC, despite having normal ACT and thus well controlled asthma, 23% demonstrated EIB. Airway inflammation, as indicated by high eNO, was more significantly associated with EIB compared to spirometry. Thus, even seemingly well controlled asthmatics based on ACT can have EIB and EIB is predicted by eNO, not spirometry. Our findings offer rationale for the use eNO in the clinical setting to determine children at risk for EIB. More specifically, in children that appear to have well controlled asthma, eNO may be used to determine appropriate counseling, such as stressing the importance of pre-exercise warm-up.

CELL RESISTANCE MEASUREMENTS REVEALS DEFECTIVE PERMEABILITY IN CYSTIC FIBROSIS BRONCHIAL EPITHELIAL CELLS

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Objectives: Integrity of epithelial cell barrier is maintained by cell-cell junction proteins. Emerging data show that these proteins are compromised in Cystic Fibrosis (CF) human bronchial epithelial cells (HBECS). Cystic Fibrosis transmembrane conductance regulator (CFTR) regulates cell to cell junctions function. Loss of CFTR function may impair cell permeability. Increased permeability of CF airway epithelia increase susceptibility to bacterial infection and exacerbating inflammation.

Methods: We used CRISPR/Cas9-gene edited 16HBE14o- (16HBEge) bronchial epithelial cell lines carrying CFTR nonsense mutations (W1282X or G542X) in homozygosity, and the parental 16HBE14o- cell line as isogenic WT control (kindly provided by the Cystic Fibrosis Foundation Therapeutics Lab, CFFT). Ussing chamber assay was used for validating absence of CFTR function in 16HBEge cell lines.

Electric Cell-substrate Impedance Sensing (ECIS[®]) instrument was used to measure cell permeability in real time. Cell resistance was monitored either in the absence of inflammatory stimuli, and in response to 10, 1 or 0.1 µg/ml lipopolysaccharide (LPS) (acute and chronic exposure). Cells were grown in ESIS 96-wells arrays. Resistance was measured for 10 days to ensure formation of cell junctions and measured continuously after LPS exposure for 96 hours. Tight-junction (TJ) (Claudin 4, Occludin, ZO-1) and adherens-junction (AJ) (E-cadherin and B-catenin) protein levels were assessed by western blot.

Results: Ussing chamber analysis shows that CF W1282X and G542X cells displayed no response to forskolin/IBMX compared to parental WT cells, confirming absence of CFTR function. ECIS analysis revealed that all cell lines gained resistance overtime. At 14 days of culture, WT cells showed statistically significant higher resistance compared to CF cells (WT=2027.9±123.3 Ω; CF W1282X 1494.9±35.2 Ω; CF G542X 1604.8±57.7 Ω). Short exposure to LPS (24 hours) minimally affected resistance in WT cells (drop of 43± 11.2 Ω). However, pronounced drop in resistance was seen in CF cells: W1282X =242.6± 89.3 Ω, G542X=126.2± 74.5 Ω. After chronic LPS exposure (96 hours), WT cells regained resistance, which did not differ from untreated WT cells (2154.9± 112 Ω). Both CF cells, failed to regain resistance compared to WT treated cells (W1282X= 1485.8±22; G542X= 1644.4±35, P<0.001). Preliminary data suggest that, at steady-state and LPS exposure, CF W1282X cells grown in monolayers and at ALI have decreased expression of adherens junction proteins (E-cadherin and B-catenin) when compared to WT cells. No differences were observed in tight junction proteins (Claudin 4 and Occludin).

Conclusions: Our preliminary data suggest that loss of CFTR function impairs cell barrier permeability at steady state. Loss of CFTR function causes a pronounced loss of resistance in CF cells after short exposure to LPS. CF cells fail to regain resistance after chronic exposure. CF cells display decreased expression of AJ proteins compared to WT cells, which may explain the barrier permeability dysfunction. TJ proteins did not show any difference in expression between WT and CF cells.

Microbiome Protects against Pulmonary Fibrosis through TLR5 Activation

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Rationale: Idiopathic pulmonary fibrosis (IPF) is a devastating lung disease resulting from maladaptive responses to lung epithelial injury. Epithelial homeostasis can be promoted via activation of innate immunity by the microbiome. TLR5 is an innate immune receptor which recognizes bacterial flagellin and has been linked to maintenance of epithelial integrity in the gut.

Methods: TLR5 deficient and wildtype (C57BL/6) mice were dosed with bleomycin (3U/kg) via oropharyngeal exposure and lung tissue was collected at day 21. To determine the relationship between the microbiome and TLR5 activation, TLR5 deficient and wildtype mice received an antibiotic cocktail (ampicillin, vancomycin, neomycin and metronidazole), for 4 weeks before being dosed with bleomycin (3U/kg) and throughout the post- bleomycin period. Some mice were reconstituted via oral gavage of diluted stool contents after the antibiotic period and exposed to bleomycin 2 weeks later. A recombinant flagellin analogue was used to activate TLR5 to determine protection against fibrosis in the bleomycin model. Patients with IPF and at-risk control subject were genotyped for TLR5 rs5744168 and the association of the dominant-negative minor allele with IPF evaluated.

Lung-healthy smokers and non-smokers were genotyped for rs5744168 and the association of the dominant-negative minor allele with serum markers of epithelial injury was evaluated.

Results: Genetic TLR5 deficiency was associated with IPF in humans, and increased susceptibility to experimental fibrosis in mice. Elimination of the microbiome through antibiotics abolished the protective effect of TLR5. Activation of TLR5 through a flagellin analogue protected from experimental fibrosis. TLR5 activation ameliorated epithelial injury after bleomycin exposure. TLR5 deficiency in human smokers without lung disease was associated with increased levels of serum epithelial injury markers.

Conclusion: Our data suggest that the microbiome activates TLR5 and thereby protects from epithelial injury and pulmonary fibrosis. Furthermore, pharmacological TLR5 activation is a possible treatment in IPF.

Genetic and Infectious Regulators of Baseline and Acute Immunity in Human Populations

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Rationale: Influenza virus is a respiratory pathogen that causes annual epidemics and periodic pandemics, leading to a heavy financial, morbidity, and mortality disease burden. Previous studies have identified genetic, environmental, and immunological associations with the quality of anti-influenza immunity and/or influenza disease severity, however, there has not been an integrative analysis of the collective, unique, and interactive effects of host genetics and chronic viral infections on baseline and acute immune responses.

Methods: We utilized samples from healthy or influenza infected subjects from 7 distinct populations comprised of 5 independent human cohorts from 5 countries. Nasal wash and plasma cytokine levels were measured using Milliplex HCYTOMAG-60K kits, and herpesvirus serostatus was determined with ELISA kits and plasma samples. For influenza infected cohorts, influenza viral load was determined using quantitative RT-PCR (qRT-PCR) following RNA extraction from nasal swabs and functional antibody titers were determined using microneutralization assays.

Results: We have found that herpesviruses have unique and interactive effects on cytokine levels that are specific to anatomical location, both at baseline and during acute influenza virus infection. Additionally, herpesvirus infection is associated with decreased influenza symptom severity and virus shedding, and increased antibody titers. Associations between cytokine levels and influenza severity were consistent in cohorts of similar ancestral and environmental backgrounds, but were not observed in populations from distinct backgrounds. In further support of the potential effects of host genetics in immune variation across populations, we identified approximately 100 variants in immune related genes that are either enriched in or absent from a given population of ancestral background, a portion of which are expression quantitative trait loci (eQTLs) at baseline.

Conclusions: Variation in herpesvirus seroprevalence, and expression of immune transcription factors and effector molecules across distinct environmental and ancestral populations may result in unique immune phenotypes. Understanding these interactions allow for the identification of therapeutic targets, more representative animal models, improved clinical study design by inclusion of populations with distinct immune profiles, and personalized medicine through the identification of optimum treatments.

Outcomes of Positive Pressure Ventilation in Acute Respiratory Distress Syndrome and Their Predictors: A National Cohort

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Rationale: Although noninvasive positive pressure ventilation (NIPPV) is commonly used in acute respiratory distress syndrome (ARDS) to avoid invasive mechanical ventilation (IMV), the data supporting its benefit for this indication is lacking. To address this evidence gap, we analyzed the outcomes of NIPPV and IMV in ARDS and studied the predictors of NIPPV failure.

Methods: We used the latest version of the largest publicly available inpatient database in the United States (U.S.). The 2016 National Inpatient Sample (NIS) database covers 97% of the U.S. population and encompasses data for about 7,000,000 hospitalizations. We queried the database using International Classification of Diseases 10th codes and identified 26,911 adult records with a primary diagnosis of ARDS. We found 4,627 discharges that required positive pressure ventilation during hospitalization and were not diagnosed with acute cardiogenic pulmonary edema. Then, we divided the cohort into initial treatment with IMV (IMV group), NIPPV only and NIPPV failure groups. We defined NIPPV failure as use of NIPPV and IMV either on the same day or IMV at a later date. The studied outcomes were in-hospital mortality, length of stay (LOS) and NIPPV failure rate. Patient- and hospital-level characteristics, ARDS etiologies, comorbidities and complications were also analyzed. The data were analyzed using Stata 13.0. Linear regression of log-transformed LOS and logistic regression of binary outcomes were used to test for associations.

Results: Patients were 49.9% females, 61.9% Caucasian, and the median age was 59 years, interquartile range: 47-71. NIPPV success had the lowest mortality rate (4.85%, 95% CI: 3.7-6.0) and shorter LOS (7 days, 95% CI: 6.6-7.4) in the cohort. NIPPV failure rate was 20%, 95%CI: 18.1-22.2. Patients diagnosed with sepsis (OR: 4.63, 95%CI: 3.42-6.28), pneumonia (OR: 2.43, 95%CI: 1.8-3.29) or chronic liver disease (OR: 2.38, 95%CI: 1.45-3.89) had the highest adjusted odds for NIPPV failure. When we compared NIPPV failure vs. IMV groups (Figure 1), there was no significant difference in mortality (OR: 1.04, 95% CI: 0.78-1.38) or LOS (5.7%, 95% CI: -6.6, 19.6).

Conclusions: If NIPPV is successful in ARDS, it carries significantly lower mortality rates and shorter LOS compared to IMV. If NIPPV fails, our study showed no significant difference in mortality or LOS compared to if the patient was initially intubated. Patients with sepsis, pneumonia and chronic liver disease have the highest odds of NIPPV failure.

Funding sources: Self

How the Antiviral Signature in Neutrophils from Patients with Pulmonary Arterial Hypertension Affects Function

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Rationale: Patients with idiopathic pulmonary arterial hypertension (IPAH) have high circulating levels of neutrophil elastase (NE) and low levels of the endogenous NE inhibitor elafin. Furthermore, IPAH patients have increased neutrophil to lymphocyte ratio. Our previous studies showed functional abnormalities in PAH vs. control neutrophils including increased adhesion, impaired migration, elevated NE, and PMA--induced neutrophil extracellular trap (NET) formation. Additionally, the transcriptomic analyses indicated an innate immune response and an antiviral signature. We hypothesized that there is a direct link between the antiviral signature and the dysfunction observed in PAH neutrophils related to increased adhesion, reduced migration and heightened production of NE and release of NETs.

Methods: We compared proteomic analyses by mass spectrometry with transcriptomic analyses on neutrophils from IPAH patients (n=12 and 9) and age and gender matched healthy controls (n=6). The results of these analyses were further investigated using confocal microscopy, western blot and FACS analyses.

Results: Proteomic analyses revealed a central role for increased vinculin and reduced beta 2 integrin in PAH neutrophils that can explain the heightened adhesion and decreased migration. Both these features have also been implicated in suppressing the response to a retrovirus. The top pathway implicated in the transcriptomic analyses demonstrated heightened expression of interferon--signaling genes in the PAH vs. control neutrophils, with increased gene expression of the type 1 interferon receptor consisting of 2 subunits IFNAR1 and IFNAR2. Furthermore, cytoplasmic RNA viral sensors RIG--I and PKR were notably heightened in the IPAH vs. control neutrophils. We then determined whether an increase in endogenous retroviral expression was present and altered in IPAH vs. control neutrophils. Indeed, an increase in protein expression of human endogenous retrovirus K (Herv--K) envelope protein was observed by confocal microscopy in the PAH vs. control neutrophils (n=3). Moreover, stimulation of a neutrophil like cell line (differentiated HL--60) with the Herv--K dUTPase protein resulted in an elevation in NE linked to NET formation. As HL--60 promyelocytes express higher levels of Herv--K than differentiated HL--60 cells, we determined whether PAH neutrophils were immature. Consistent with this, PAH neutrophils expressed low levels of CD10 and CD14, but CD11b levels were also reduced.

Conclusion: Our data suggest that in association with persistent elevation in Herv--K, PAH neutrophils produced elevated levels of NE, a propensity to NET formation, decreased migration, increased adhesion, and evoked an anti--viral signature. These features can potentiate the negative impact of NE mediated NET formation on vascular function in PAH.

Investigating susceptibility to ozone-induced lung inflammation and injury using the Collaborative Cross mouse genetic reference panel

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Rationale: Exposure to ambient ozone, a common air pollutant, causes lung injury and inflammation and decrements in pulmonary function; thus, it is a public health priority to identify susceptibility factors. Previous studies in both humans and rodents have established that differential responsiveness to ozone exposure is under some genetic control, even when controlling for other known susceptibility factors (e.g., age, sex, pre-existing lung disease). We seek to further identify and characterize the genes and molecular mechanisms that confer susceptibility to ozone responses by using the Collaborative Cross (CC) genetic reference population.

Methods: The CC is a panel of recombinant inbred mouse strains generated from 8 existing founder strains (A/J, C57BL/6J, 129S1/SvImJ, NOD/LtJ, NZO/HILtJ, CAST/EiJ, PWK/PhJ, WSB/EiJ). Female and male mice, aged 10-12 weeks, from 55 CC strains were exposed to 2 ppm O₃ or filtered air (FA) for 3 hours. Mice were phenotyped for airway inflammation by measuring cell counts in lung lavage fluid collected 21 hours after cessation of ozone exposure. A subset of strains were phenotyped for lung injury by measuring total protein in lung lavage fluid.

Results: We observed a wide range of the magnitude of neutrophilic recruitment to the airways after ozone exposure (from 0% to 82% of total airway cells). In a 13-strain subset, we measured total protein concentration in lavage fluid, which ranged from 1 mg/ml to 4 mg/ml. Interestingly, we noted that neutrophilic recruitment and total protein in lavage (i.e., lung injury) are not necessarily correlated, with some strains having high neutrophilic recruitment with low protein in lavage and vice versa.

Conclusions: Consistent with previous studies, the present work demonstrates that genetic background influences hallmark responses to ozone exposure and highlights the utility of the CC as a resource for uncovering genetic determinants of ozone responses. Current efforts are underway to perform quantitative trait locus (QTL) mapping and related statistical analyses to identify specific genetic drivers of susceptibility to ozone exposure.

Incidence of Thromboembolic Events in a Pediatric Cardiac Intensive Care Unit and Proposed Protocol for Early Detection and Management

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Introduction: Thromboembolic events are known complications associated to central venous catheters and arterial catheterization. The exact mechanism of thrombus formation is not completely understood, but it has been suggested that intimal flaps and dissection can occur after direct vessel puncture, leading to occlusion of the true lumen and predisposition to thrombosis and limb ischemia. Thromboembolic complications are particularly important in the pediatric population due to its association with other morbidities. Prior studies identified risk factors associated with thromboembolic events in pediatric population, but there is limited information about the incidence of thromboembolic events in children with congenital heart disease. Our aim is to identify cases of thromboembolic events, risk factors, management and outcomes in pediatric patients who were admitted to the Cardiac Intensive Care Unit as part of a quality improvement initiative to develop a detection and management protocol.

Methods: A cross – sectional, descriptive database review was performed on patients admitted to the cardiac intensive care unit at the Centro Cardiovascular de Puerto Rico y del Caribe that developed thromboembolic complications and had positive Doppler/duplex ultrasound for thrombus formation. Study period from January 2016 – March 2018. Data included patient demographics, weight, diagnosis, procedure performed, type and location of thrombus, management, and other complications.

Results: A total of 35 patients were identified with a thromboembolic event in the study period. Incidence overall was 10.9%, 5.7% and 9.2% in surgery and catheterism group, respectively. They were most common of arterial origin (89.7%). Cases were most frequent in male patients, a mean age of 1.7 ± 4.8 months and with mean weight of 8.3 ± 12 kg. The events occurred mostly after congenital heart disease surgery and associated with femoral artery placement (60%). Management included heparin infusion (37%) and there were no major sequelae or complication related to thrombotic event nor therapy.

Conclusion: In review of the findings we conclude that thromboembolic events were mostly seen in patients of low weight and were most likely of arterial origin, with a higher incidence when compared to literature elsewhere. These findings guided us to design a protocol, tailored to our institution and patient population, to decrease the likelihood of these events and risk of major complications. The proposed protocol includes guidelines for prevention, monitoring, evaluation of suspected events and management to be followed once an event is diagnosed. Protocol will be implemented in the Unit with follow up evaluation after 6 months.

μ-Optical Coherence Tomography to Characterize Pharmacologic Induction of Muc5B in WT and Muc5b KO Primary Ferret Bronchial Epithelial ALI Cultures

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a progressive fibrotic lung disease with median-survival ranging from 3-5 years after diagnosis. The greatest risk factor for developing IPF is a gain-of-function promoter variant in the mucin MUC5B; which accounts for ~30% of the risk for developing IPF. Our lab has generated a novel bleomycin-induced ferret model of pulmonary fibrosis which better recapitulates human IPF. Our lab's long-term objective is to understand fibrotic mechanisms in altered mucin microenvironments in a ferret model. My short-term goal was to identify pharmacologic agents that are capable of inducing preferential Muc5b overexpression in an *in vitro* ferret system.

Methods: Primary wild type (WT) and Muc5B knockout (KO) ferret bronchial epithelial cells (FBEs) were cultured at air liquid interface (ALI) in Pneumacult media. FBE filters were induced basolaterally with either pyocyanin (PCN), phorbol myristate (PMA), or prostaglandin D2 (PGD2). Micro-optical coherence tomography (μOCT) was used to assess functional microanatomy of the FBEs including the following parameters: air surface liquid (ASL) depth, periciliary liquid (PCL) depth, ciliary beat frequency (CBF), and mucociliary transport (MCT). Ongoing studies include Muc5b and Muc5ac mucin protein expression and gene expression by Western blot and q-PCR.

Results: *In vitro* evaluation of the mucociliary transport apparatus in FBEs prior to drug induction by μOCT demonstrated that Muc5b KO ferret ALI filters had significantly reduced ASL depth (mean difference -1.3 μm, P=0.0026, N=15-17 filters/group), reflecting diminished mucus. A trend in reduced PCL depth in Muc5b KO compared to WT (mean decrement -0.35 μm, P=0.0588) FBEs suggested airway dehydration. 24-hours post drug induction, μOCT demonstrated that only PCN significantly increased mucus depth (i.e. ASL) in WT relative to control, but not in Muc5b KO ferret ALI filters (mean difference 23.6 μm, P=0.015, N=3 filters/group x 3 ROI per filter), indicating that PCN increased mucus in a Muc5b-specific manner. PMA and PGD2 significantly increased CBF in WT relative to control (mean difference for PMA 3.4 Hz, P=0.0018; mean difference for PGD2 4.8 Hz, P<0.0001).

Conclusions: Muc5B KO FBE ALIs demonstrate significantly decreased mucus and a trend towards decreased PCL compared to WT controls. PCN significantly increases mucus in WT, but not in Muc5b KO primary FBE ALI cells, indicating PCN increases ASL in a Muc5B-dependent fashion. *In vivo* studies of PCN-induced Muc5b-overexpression and response to injury are ongoing.

Characteristics Associated with COPD in People Living with HIV in the Current Antiretroviral Therapy (ART) Era

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Background: People living with HIV (PLWH) are at increased risk for chronic obstructive pulmonary disease (COPD) compared to uninfected persons. We determined the characteristics and risk factors associated with COPD in a large, multicenter national cohort of PLWH in care in the current ART era in order to inform subsequent interventions to ameliorate the burden of COPD. We hypothesized that while smoking would be an important risk for COPD, other factors such as substance use would also be key in PLWH.

Methods: We utilized data from five sites in the CFAR Network of Integrated Clinical Systems (CNICS) cohort. COPD was defined based on an algorithm we previously validated against spirometry requiring a COPD diagnosis code and ≥ 90 -day continuous supply of short-acting or long-acting inhalers, or other COPD medications. We compared demographic and clinical characteristics among those with and without COPD. Predictors of incident COPD were examined in Cox proportional hazards models adjusted for age, sex, race/ethnicity, current or former smoking status, drug use (current and former heroin/opioid, cocaine/crack, methamphetamine/crystal, and marijuana use), injection drug use (IDU), alcohol use, HIV viral load, nadir CD4 count and site. We also examined whether ART was associated with COPD in separate models given covariance.

Results: 13,462 PLWH were included from 2010-2017, of whom 1031 met our COPD definition. PLWH with COPD were older at their most recent visit than those without COPD (mean 56 vs. 48 years), more likely to be current smokers (59% vs. 34%), and to have ever used illicit opioids/heroin (26% vs. 17%), cocaine/crack (55% vs. 39%), marijuana (63% vs. 58%), and ever IDU (58% vs. 43%). In adjusted analyses, factors associated with incident COPD included older age and female sex (Table). Hispanic ethnicity was associated with decreased incident COPD. The hazard ratios (HR) for current and prior smoking with incident COPD were large (HR 6.69 and 2.96, respectively). In addition, current and prior illicit opioids/heroin, and prior cocaine/crack were associated with incident COPD. Baseline viral load and nadir CD4 were not significant in adjusted models, nor was ART use in a separate adjusted model.

Table. Factors associated with incident COPD among people living with HIV in Cox proportional hazards model

Covariate	Hazard Ratio [95% CI]
Age (per 10 years)	1.96 [1.76 – 2.20]
Female	1.80 [1.41 – 2.29]
Race	
White	1.00
Black	0.79 [0.62 – 1.01]
Hispanic	0.58 [0.40 – 0.83]
Other	0.57 [0.26 – 1.07]
Smoking	
Never	1.00
Past	2.96 [2.05 – 4.27]
Current	6.66 [4.75 – 9.32]
Illicit opioids/heroin	
Never	1.00
Past	1.37 [1.02 – 1.83]
Current	1.83 [1.13 – 2.97]
Cocaine/crack	
Never	1.00
Past	1.39 [1.06 – 1.82]
Current	1.38 [0.91 – 2.97]

*Also adjusted for alcohol, marijuana, methamphetamine/crystal and injection drug use, as well as CD4 nadir, baseline viral load, and site, none of which were statistically significant.

Conclusions: COPD is a common comorbidity in PLWH and is strongly associated with older age and current smoking. Women were also at greater risk. Both current and prior illicit opioid/heroin and prior cocaine/crack were predictors of incident COPD in adjusted analyses suggesting future interventions should focus on substance use in addition to smoking cessation.

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