CARDIOLOGY/CARDIOVASCULAR DISEASE

FINAL ID: 2

PROMOTERS OF SPHINGOSINE 1-PHOSPHATE RECEPTORS ARE DIFFERENTIALLY REGULATED BY GROWTH FACTORS AND HYPOXIA


Rationale: Endothelial dysfunction plays critical a role in pulmonary arterial hypertension (PAH). We and others have previously demonstrated that sphingosine 1-phosphate (S1P) and its receptors, S1PR1, S1PR2 and S1PR3, are intimately involved in lung vascular proliferation and endothelial function. Objective: To explore the transcription regulation of S1PR1, S1PR2 and S1PR3 by hypoxia and growth factors. Methods: In silico analysis of three S1PR promoters and identification of cis-regulatory elements by TRANSFAC and JASPAR was performed. S1PR1, S1PR2 and S1PR3 promoters were constructed into a luciferase reporter vector in transfected human pulmonary artery endothelial cells (PAECs), stimulated by growth factors VEGF, PDGF, HGF or hypoxia (2% oxygen) for 24 hours. The promoter activities were assessed for functionality by luciferase assay. Results: Results: In silico analysis of three S1PR promoters identified several putative cis-regulatory elements, including binding sites for growth factor induced transcription factors and hypoxia inducible factor. In PAECs, basic promoter activity of S1PR1 was higher than S1PR2 and S1PR2. Promoter activity of S1PR1 was significantly increased by VEGF, PDEF and HGF (p<0.05). Promoter activity of S1PR2 was significantly increased by PDGF, HGF and hypoxia (p<0.05). Promoter activity of S1PR3 was significantly increased by VEGF and PDGF only (p<0.05). Conclusion: These data suggest that S1P receptors are differentially regulated by growth factors and hypoxia. We speculate that S1PR1, S1PR2 and S1PR3 may play distinct roles in pulmonary vascular remodeling.

FINAL ID: 6

OVER-EXPRESSION OF TNNI3K, A CARDIAC SPECIFIC KINASE, EXACERBATES INFLAMMATION IN COXSACKIEVIRUS B3 (CVB3) INFECTED TISSUE

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Background: Patients with dilated cardiomyopathies (DCM) account for the majority of heart transplants performed. Studies reveal a 30% detection of viral myocarditis in the majority of heart transplant recipients. We hypothesized that TNNI3K plays a role in the etiology of heart failure (1). Treatment of VM is currently supportive at best (2). Methods: To assess the role of TNNI3K in the acute inflammatory phase of VM, 8–10 week old male mice on an FVB/N background were inoculated with 10^3 PFU of heart passaged CVB3 containing infectious virus in PBS. Experimental mice include cardiac specific Tnni3k transgenic (Tg) mice that over-express human TNNI3K or kinase-inactive (KI) mutant via a αMHC promoter versus wild-type (WT) control litter-mates. Myocarditis was scored at 10 days post infection through microscopic analysis of percentage of heart with inflammation compared to overall heart size. Results reveal up to 15% increase in inflammation in Tg mice versus KI and WT controls. Conclusions: This is the first to demonstrate a deleterious role of TNNI3K in a model of VM and presents a novel cardiac-specific therapeutic target for combating VM. Future directions aim to assess the role of TNNI3K in the progression to DCM, investigate stress kinase activated signal transduction, and survey the benefit of pharmacological inhibitors of TNNI3K for therapeutic development.


FINAL ID: 8

MICRONRONA-130A REGULATION OF ATRIOVENTRICULAR NODAL FUNCTION

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Purpose: Conduction system disease and arrhythmia represent a major source of cardiovascular morbidity and mortality, yet the transcriptional networks required to maintain function of the adult conduction system are largely undefined. The cardiac conduction system is composed of multiple components, including the sinoatrial (SA) and atrioventricular (AV) nodes, and the ventricular conduction system. Interestingly, the AV node maintains a unique expression profile of connexin isoforms with no expression of connexin43 (Cx43) in the compact portion of the AV node and re-expression within the distal portion of the AV node. We have previously described the development of atrial and ventricular tachyarrhythmias with microRNA-130a overexpression in the adult heart via downregulation of Cx43. We hypothesized that miR-130a overexpression affects AV nodal conduction due to its effects on connexin43.

Methods: Utilizing a cardiac-restricted inducible overexpression transgenic mouse model, we induced microRNA-130a overexpression in the mouse heart under the control of an inducible α-MHC promoter.

Results: Using fluorescent situ hybridization, we found high expression of miR-130a at the junction of the atria and the interventricular septum coinciding with the location of the AV node. Using immunofluorescence, we demonstrated reduced expression of Cx43 within the AV node. Overexpression of miR-130a resulted in a progressive increase in the PR interval (±35.2ms vs ±48.5ms) indicative of delayed conduction from the SA node through the AV node. To define this further, we performed intracardiac measurements in control vs transgenic hearts and found a prolonged HV interval indicative of conduction delay in the His-Purkinje system (9ms vs 12ms). H&E, Masson Trichrome, and picosirius red staining demonstrated no structural changes in the atria in transgenic hearts compared to controls. We also did not detect significant changes in the other connexin isoforms (Cx40 and Cx45) within and beyond the AV node in the transgenic mice compared to controls.

Conclusions: Taken together, miR-130a is an important regulator of normal cardiac conduction via regulation of genes such as connexin43. One possible role for miR-130a may be for regulation of Cx43 expression within the AV node.

FINAL ID: 12
TRANSCATHETER EMBOLIZATION OF A PULMONARY ARTERIOVENOUS MALFORMATION IN A PATIENT WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA
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Pulmonary arteriovenous malformations (PAVM) are rare pulmonary vascular anomalies. More than 80% of cases are congenital with more than 50% associated with the autosomal dominant syndrome hereditary hemorrhagic telangiectasia (HHT) or Osler-Weber-Rendu Syndrome. Untreated PAVMs progressively enlarge over time and can cause significant right-to-left shunting. There is no consensus on the management of PAVM. Surgical and catheter based approaches have been used. We report a case of a 74 year-old man who presented with decompensated congestive heart failure (NYHA Class III-IV). He also reported occasional nose bleeds and was noted to have numerous small telangiectasias over his lips and tongue. Chest computed tomography angiogram revealed a large right middle lobe and small left lower lobe PAVM. Angiography of the pulmonary arteries confirmed these findings. The patient underwent percutaneous closure of the right PAVM with a size 10 vascular plug type 2 (St. Jude Medical, St. Paul, Minnesota, U.S.A.) with complete cessation of blood flow through the malformation. During the immediate post intervention period, the patient's oxygen saturation continued to improve. On a post-discharge follow-up two months later, the patient reported significant symptomatic improvement from his baseline condition (NYHA II). Based on the clinical history, the patient met the diagnostic criteria for HHT. The Curacao criteria which is used to diagnose HHT includes the presence of spontaneous and recurrent epistaxis; multiple telangiectasias present on lips, oral cavity, fingers, and nose; presence of visceral AVMs; and one first degree relative with HHT. Genetic testing confirmed the diagnosis of HHT as a result of heterozygous deletion of the ENG gene. This case illustrates an integrated approach to the management of a complex patient with an uncommon genetic disorder whose symptomatic manifestations had a high impact on his quality of life.

FINAL ID: 14
WHICH CAME FIRST, THE CHICKEN OR THE EGG?
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Takotsubo's cardiomyopathy is a transient left ventricular dysfunction that usually follows an acute stressful event like an acute medical illness or other forms of emotional or physical stress. There are few case reports of this cardiomyopathy associated with high degree AV block reported in the literature. Left ventricular dysfunction usually normalizes within 2 to 12 weeks in most cases. But it is not well known if AV block in the setting of Takotsubo's cardiomyopathy recovers.

Case Report: A 65 Yo female presented to the hospital for an elective wrist ganglion cyst removal and pre op vital check showed bradycardia in the low 40’s. Patient was complaining of dizziness and indigestion and sent to the Emergency Department. EKG showed 2:1 AV block with a LBBB. Her lab work was unremarkable except for small elevation of troponins. Her prior baseline EKG showed a normal sinus rhythm with RBBB.

Decision Making: Echocardiography showed a wall motion abnormality consistent with LAD territory ischemia with an LV EF of 35%. Coronary angiography showed no significant occlusive CAD and LV gram was consistent with Takotsubo's Cardiomyopathy.

EP study showed spontaneous infra-Hisian block with prolonged HV interval, at which time the patient was implanted with a BIV pacemaker. The decision to implant the BIV is made due to the fact that BIV pacing may worsen the underlying left ventricular dysfunction. Patient was discharged home on standard heart failure regimen. At three months follow up her LV ejection fraction had normalized and she was 100% BIV paced and the AV conduction had not normalized.

Conclusion and Discussion: This case demonstrates the possible link between AV block and Takotsubo's cardiomyopathy and the management dilemma it poses. In the literature, there are very few case reports of AV induced cardiomyopathy associated with a high degree AV block, including a complete heart block. The QRS duration in all these case reports was a narrow complex and this may raise a question that our patient might have had a long standing intermittent AV block and the stress of undergoing a surgery might have precipitated the Takotsubo's cardiomyopathy. It is also equally possible that the stress induced cardiomyopathy may be the cause of the high degree AV block. Although the left ventricular dysfunction normalizes in almost all patients with this cardiomyopathy, it is not well known if the AV block does. All the case reports described in the literature are treated with implantation of a permanent pacemaker and the longest wait time before implantation was 18 days. In one case report from Quebec, Canada, long term follow up showed that patient had resumed 1:1 AV conduction two years after she presented with 2:1 AV block and Takotsubo's cardiomyopathy. This emphasizes the importance of long term follow up to avoid unnecessary pacing.

FINAL ID: 18
A NOVEL REGULATOR OF ENDOTHELIAL CELL MIGRATION: SEROTONIN 5-HYDROXYTRYPTAMINE 7 RECEPTOR
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The 5-hydroxytryptamine type 7 receptor (5-HT7R) regulates many physiological processes, including learning and memory, circadian rhythm, and behavior. Its role is also implicated in psychiatric disorders. Little is known about the 5-HT7R function outside of CNS. Here, we report that 5-HT7R, endogenously expressed in endothelial cells (EC), may promote cell migration and adhesion. Using Boyden chamber migration assay and wound healing “scratch” assay we demonstrated that stimulation of the receptor with 5-HT7R agonists 5-CT and AS 19 significantly increased EC migration. In addition, 5-CT and AS 19 treatment increased EC adhesion to extracellular matrix. Downregulation of 5-HT7R using specific siRNA significantly inhibited baseline and 5-HT-induced EC migration. Additionally, pretreatment of ECs with PKA inhibitor 14-22 amide significantly reduced 5-CT-or AS 19-induced EC migration, suggesting that PKA is involved in the regulation of EC migration mediated by 5-HT7R. Our results suggest a prominent role of 5-HT7R in promoting cell migration and adhesion and identify 5-HT7R as a potential regulator of physiological and pathophysiological processes involving cell migration and adhesion.

FINAL ID: 20
SKELETAL MYOSIN BINDING PROTEIN-C ARE UNIQUE REGULATORS OF CARDIAC AND SKELETAL MUSCLE FUNCTION
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Myosin Binding Protein-C (MyBP-C) is a family of thick filament proteins that interact with both myosin and actin within the sarcosome of striated muscle. Three isoforms of MyBP-C are currently known: slow skeletal, fast skeletal, and cardiac (sMyBP-C, fMyBP-C, and cMyBP-C). While the function of cMyBP-C in the heart has been relatively well-studied, the function of the skeletal isoforms in muscle remains enigmatic. Contrary to nomenclature, slow and fast skeletal MyBP-C are both expressed in slow-twitch and fast-twitch skeletal muscles, as well as co-expressed within the same sarcomere. All three isoforms are also co-expressed in the heart during fetal development. However, skeletal MyBP-C are dysregulated in both heart failure and muscular.
dystrophy. Healthy, adult mammalian hearts only express cardiac MyBP-C, but cMyBP-C is re-expressed during heart failure. In addition, expression of slow and fast MyBP-C is dramatically altered in skeletal muscle during the onset of muscular dystrophies. The role of skeletal MyBP-C has yet to be fully elucidated in health and disease. We demonstrate that differences in the N-terminal structure may be responsible for subtle differences in how each of the three isoforms regulate cardiac contraction in heart. Skeletal MyBP-C lacks an N-terminal domain, designated “C0 domain,” that is specific to the cardiac isoform. Without this domain, the cardiac cell exhibits enhanced relaxation kinetics. In skeletal muscle, we demonstrate how altered expression of skeletal MyBP-C can regulate function in skeletal muscle, and contribute to functional deficits observed in muscular dystrophy. The contractile kinetics of a muscle cell are reduced with increasing levels of skeletal MyBP-C, and our results demonstrate that both skeletal MyBP-C isoforms can act to limit force generation and increase tension cost at the myofilament level. Together, our experiments demonstrate that skeletal MyBP-C proteins are unique molecular regulators of contraction in both cardiac and skeletal muscles. Future studies may elucidate whether modulation of skeletal MyBP-C can be useful for therapies in muscle disease.

**FINAL ID: 22**
**BENEFITS AND HARMs OF ACUTE ENDOVASCULAR REPERFUSION THERAPy IN ISCHEMIC STROKE: A META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS**

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**Background and Purpose:** Endovascular (intra-arterial, IA) therapy for acute ischemic stroke has become part of acute therapy, but a few randomized controlled trials (RCTs) have had inconsistent results. We evaluated the efficacy and safety of endovascular therapy in published RCTs.

**Methods:** We performed a systematic review of RCTs of endovascular therapy with thrombolytic or mechanical reperfusion compared with interventions without IA therapy. Use of systemic thrombolysis was not excluded. Primary outcome was the frequency of good functional outcome (modified Rankin scale (mRS) of 0–2 at 90 days) and secondary outcomes were mortality at 90 days and symptomatic intracranial hemorrhage (sICH). Two groups of independent reviewers searched and identified studies and extracted data. Random-effects meta-analysis was performed and GRADE was used to evaluate quality of evidence and provide recommendations for use.

**Results:** Ten studies were included (n=1,612), of which 9 studies reported the primary outcome. IA therapy was not significantly associated with good functional outcome (Relative Risk [RR] =1.17; 95% CI, 0.97 to 1.42; p=0.10 and Absolute Risk Difference [ARD]=7%; 95% CI, 0.1% to 14%; p=0.05). Heterogeneity was moderate among studies (I2=50%). Mortality was unchanged with IA therapy (RR=0.92; 95% CI, 0.75 to 1.13; p=0.45) and there was no difference in sICH (RR=1.20; 95 % CI, 0.79 to 1.82; p=0.39). The quality of evidence was low for all outcomes and the recommendation is weak for the use of IA therapy as per GRADE methodology.

**Conclusions:** IA therapy has no significant increase in good outcomes, and no changes in either mortality or sICH in patients with acute ischemic stroke.

**ENDOCRINOLOGY/METABOLISM**

**FINAL ID: 25**
**HETEROGENEITY OF SECRETORY GRANULE STRUCTURE IN HUMAN PANCREATIC ISLETS**

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Recent studies on human islets have demonstrated considerable species differences from morphogenesis to gene expression. We studied the ultrastructure of the human pancreas throughout the lifespan (n=45, 2–mo to 85-yr old) including pathological conditions such as obesity, substance abuse, type 1 diabetes (T1D) and T2D. Characteristic species differences were examined in comparison to the mouse pancreas. Mouse insulin granules were largely homogeneous in structure with a dense core and a halo. In contrast, marked variability in individual human beta-cells was observed related to development, aging and disease conditions. Our analysis shows at least six types of granules based on size, density of the core and various forms of crystallization (light core, moderately dense core, dense core, square crystals, fragmented crystals and needle crystals; Fig 1). A large percentage of light core granules was observed in children, obese subjects and T2D patients. The formation of lipid droplets in beta-cells was prominent beginning in adolescence and increasing in severity in adults. However, no direct correlation was noted with age, obesity or T2D. Finally, no bi-hormonal endocrine cells or acinar-insulin cells were observed in humans, which should mark transitional cells undergoing dedifferentiation. These studies provide a foundation for understanding the role of changes in beta-cell ultrastructure in disease.

**FINAL ID: 26**
**IDIOPATHIC SUDDEN SENSORINEURAL HEARING LOSS: ASSOCIATION WITH PREVIOUSLY UNDIAGNOSED THROMBOPHILIA AND SUBSEQUENT THROMBOTIC EVENTS**

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**Introduction:** Many cases of sudden sensorineural hearing loss (SSHL) remain idiopathic. Idiopathic sudden sensorineural hearing loss (ISSHL) may be secondary to inner ear thrombosis, and the development of ISSHL should trigger evaluation for previously undiagnosed thrombophilia to facilitate primary prevention of subsequent thrombotic events.

**Case descriptions:** 1. A 62 y.o Caucasian male, being evaluated for hyperlipidemia and insulin resistance, developed SSHL of the left ear. He had no precedent viral symptoms, trauma or ototoxic medication exposure. He was evaluated with audiometry and a sub-total severe left SSHL was confirmed. MRI head and X-ray of the auditory canal were normal. Laboratory evaluation revealed the following major gene thrombophilas: MTHFR gene mutation (C677T homozygous) elevated homocysteine levels (19.6 umol/l); high factor VIII (175%); high Factor XI (179%); high anti-cardiolipin IgG antibody (27) 2. A 67 y.o male developed left sided ISSHL coupled with loss of word recognition. MRI did not reveal any abnormality and he was treated with prednisone 40 mg/day. He developed knee pain, and was diagnosed with Ficat Stage 1 osteonecrosis both knees six months later. He was found to have high ACA (75 MPL, medium positive range 20–80, repeated 82 MPL), hyperfibrinolitic 4G4G homozgyosity of the plasmogen activator inhibitor-1 gene (PAI-1). He was also found to be homozygous for the 6A6A-stromelysin mutation and heterozygous for the EnoS T786C mutation, both associated with development of osteonecrosis.

**Conclusion:** After the development of ISSHL, it is important to evaluate for pathoetiologic familial and acquired thrombophilia in order to prospectively protect against future venous and arterial thrombosis which may result in significant thrombotic morbidity. High dose prednison therapy, often used after diagnosis of ISSHL, has the potential to cause development of osteonecrosis, particularly in the concurrent presence of thrombophilia-hypofibrinolysis. In patients with ISSHL, PCR evaluation for the Factor V Leiden, G20210A-PAI-1 gene mutations, along with Factors VIII, XI, homocysteine, and the antiphospholipid antibody syndrome may help to detect patients at potential risk of thrombosis and osteonecrosis.

**FINAL ID: 27**
**ENDOPLASMATIC RETICULUM STRESS SELECTIVELY UP-REGULATES TRIP-BR2 IN VISCERAL FAT, A NOVEL TRANSCRIPTIONAL CO-REGULATOR FOR ADIPOSY AND ENERGY METABOLISM, DURING OBESITY**

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Obesity results from storage of excess nutritional calories as fat in adipose tissue. Excess nutritional influx has been shown to promote endoplasmonic reticulum (ER) stress leading to insulin resistance, inflammation and metabolic disorders. We have recently shown that a novel transcriptional co-regulator, TRIP-Br2, is selectively up-regulated in visceral fat of obese humans and mice and TRIP-Br2 ablation in mice exerts a protective effect on obesity as well as associated metabolic dysfunctions. Accumulation of excess fat in visceral but not subcutaneous fat depot has been associated with increase metabolic risks during obesity. However, the regulation of functional heterogeneity among white fat depot is poorly understood. In this study, we observed that TRIP-Br2 in adipocytes is induced by high-fat diet and inflammatory cytokines (IL-6 and TNF alpha) in visceral fat is mediated by a previously unknown ER stress inducible-transcription regulation factors including XBP1, ATF4, ATF6, C/EBPb, CREB3 and CREB3L3. Hence, our data suggest that ER stress-induced TRIP-Br2 expression in visceral fat is mediated by a previously unknown ER stress inducible-transcription regulator, which is absent in subcutaneous fat and other metabolic tissues including liver and heart, despite elevated levels of ER stress markers. Thus far, our expression analyses ruled out the involvement of known ER stress activated transcription factors including XBP1, ATF4, ATF6, C/EBPb, CREB3 and CREB3L3. Hence, our data suggest that ER stress-induced TRIP-Br2 expression in visceral fat is mediated by a previously unknown ER stress inducible-transcription regulator, which is absent in subcutaneous fat and other metabolic tissues. Taken together, our study suggests that identification of the visceral fat-specific TRIP-Br2 regulator could potentially reveal novel mechanism regulating differential metabolic risks in different white fat depots during obesity.
Global metabolic health has deteriorated dramatically with the burgeoning epidemic of obesity and diabetes. While consumption of a calorically-dense diet coupled with increased physical inactivity are central drivers of metabolic diseases, these factors alone fail to fully account for the magnitude and rapidity with which metabolic health has deteriorated. As such, increasing attention has turned to contributing factors that may potentiate the effects of lifestyle changes; this includes exposure to endocrine disrupting chemicals (EDCs), exogenous chemicals with the capacity to modulate endogenous hormonal and metabolic signaling pathways.

Importantly, exposure to EDCs during critical developmental periods has been implicated in aggravating global energy homeostasis later in life, potentially through epigenetic modulation or a reprogramming of metabolic axes. Tolylfluoride (TF) is a phenylsulfamidine fungicide used on fruit crops in agricultural regions outside of the United States as well as a booster biocide in marine paints to improve hydrodynamics. In previous work, this novel EDC was shown to promote preadipoctye-to-adipocyte differentiation in the 3T3-L1 cell line. TF also was shown to induce a state of insulin resistance in primary murine and human fat through a specific down regulation of the key insulin signaling intermediate, insulin receptor substrate-1 (IRS1). These effects resulted from activation of glucocorticoid signaling, suggesting that TF is a novel environmental glucocorticoid.

While these effects suggest a potential role for TF in inducing insulin resistance and diabetes, whether cellular effects are recapitulated after in vivo exposure is not known. Furthermore, despite the fact that adipose tissue plays a central role in metabolism, the ultimate metabolic phenotype of an exposed individual is likely dictated by the balance of effects mediated through a network of metabolic tissues, including liver, muscle, pancreas, and brain in addition to adipose tissue.

In the present study, the metabolic effects of chronic exposure to C57BL/6 male mice via TF incorporated into the diet were determined. Compared to control-fed animals, those mice on a TF-supplemented diet experienced a 23% increase in adiposity that was most pronounced in the perigonadal fat pads. Interestingly, the effects of TF on adipocyte physiology recapitulated findings obtained ex vivo. Specifically, dietary supplementation with TF resulted in a 30% reduction in adipocyte IRS-1 levels as well as potential effects on other key intermediates in the signal transduction cascade. In addition, TF exposure led to a 37% increase in fasting blood sugars and reduced adiponectin RNA levels in perigonadal adipocytes by 31%, suggesting a potential role in the induction of insulin resistance through modulation of adipocyte function. In a related study, C57BL/6 dams were exposed to dietary TF throughout pregnancy and lactation, and the perinatally exposed pups were then followed to adulthood. Both male and female pups exhibited reduced body weight at weaning and impaired glucose tolerance in adulthood. These findings suggest that exposure throughout the life span to the novel EDC TF has the capacity to globally disrupt energy homeostasis in a manner that could contribute to the development of metabolic diseases. Further work is required to characterize the specific mechanisms of metabolic disruption in all tissues contributing to energy homeostasis. Moreover, the currently assembled data supports investigations into human exposure and its potential metabolic consequences in order to understand whether TF may be a pathogenic contributor to the development of obesity and diabetes in exposed populations.

**FINAL ID: 33**

**SOLUBLE RECEPTOR OF ADVANCED GLYCAZATION ENDOPRODUCTS (sRAGE) IS DECREASED ACROSS THE GLUCOSE TOLERANCE CONTINUUM AND CORRELATES WITH SEVERITY OF INSULIN RESISTANCE**

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The receptor for advanced glycation endproducts (RAGE) binds AGES and other ligands to induce a potent and cyclical cascade of pro-inflammatory events. Apart from full length RAGE, which is expressed in the cell membrane, soluble isoforms of RAGE (sRAGE) are formed by proteolytic cleavage of the RAGE ectodomain and through alternative splicing mechanisms. Upon cleavage or exocytosis, sRAGE is released into the interstitial space where it acts as a decay receptor for RAGE ligands and thus attenuates full length RAGE-mediated events. Low circulating sRAGE levels are associated with conditions such as obesity, T2DM, and arthritis. However, the relationship between decreased sRAGE levels and its contribution to the etiology of disease is controversial and requires further population-specific inquiry. Currently, no data exist that examine circulating sRAGE concentrations across the natural history of type II diabetes. The purpose of this study was to examine circulating sRAGE levels across three groups of adults stratified by glucose tolerance status (GTS): normal glucose tolerant (NGT: n=31, BMI=27.3±1.2 kg/m², 45±3 yrs), impaired glucose tolerant (IGT: n=15, BMI=36.4±1.3 kg/m², 66±1 yrs), and type II diabetes mellitus (T2DM: n=11, BMI=38.3±2.1 kg/m², 56±2 yrs). Fast- ing plasma samples were assayed for sRAGE by commercial ELISA. Mean plasma circulating sRAGE differed significantly across the three groups (NGT: 1322±108; IGT: 735±58; T2DM: 728±62 pg/mL, p<0.001). Bonferroni correction showed NGT to be significantly different from IGT (p=0.002) and T2DM (p=0.001), with no significant difference between IGT and T2DM. Sub-analysis of the NGT group, when stratified by obesity, revealed additional sRAGE disparity (p=0.014) between lean-NGT (n=18, BMI=22±1.6 kg/m², 159±136 pg/ml) and obese-NGT (n=13, BMI=34±2.9 kg/m², 1049±150 pg/ml). Similarly, when groups were stratified by fasting glucose status (FGS), significant differences to GTS stratification were seen (NEG: 1411±116, IFG: 756±49, T2DM: 749±79 pg/mL, p<0.001). Bivariate correlations showed plasma sRAGE was negatively correlated with BMI (kg/m²: r = -0.529, p<0.001), impaired glucose tolerant (IGT: r = -0.503, p<0.001). These data suggest that insulin sensitivity, glucose metab- olism, and also prevent the unnecessary performance of pancreatectomy as a treatment option.

FINAL ID: 34 POSTPRANDIAL RESPONSE TO ALTERED FEEDING ROUTES AND REVERSAL SURGERY IN ROUX EN Y GASTRIC BYPASS-RELATED HYPOGLYCEMIA

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Background: Roux-en-Y (R-YGB) surgery provides dramatic reductions in weight loss and improvement in metabolic control. However, post-prandial hypoglycemia is a late complication of RYG surgery. The pathophysiology of this disorder remains unclear. A key debate is whether the post prandial hyperinsulinemia observed is due to inherent changes in pancreatic beta cells, such as overgrowth, or to alterations in food delivery and absorption leading to altered gut hormone profiles.

Study Design: We studied four patients with symptomatic postprandial hypoglycemia in the absence of other endocrine conditions. All patients had finished medical therapy and continued to have multiple daily episodes of hypoglycemia. We performed mixed meal testing orally and then through a gastrostomy tube into the excluded stomach. In this way, we intended to determine if restoring nutrient delivery through the stomach and duodenum would ameliorate postprandial glucose and insulin excursions. We measured glucose, insulin, and a number of gut peptides including glucagon-like peptide 1 (GLP-1), gastric inhibitory peptide (GIP), and Peptide YY (PYY) with frequent sampling over 3 hours after meal ingestion. We then performed a reversal of the gastric bypass, restoring a more normal transit pathway of nutrients through the stomach and duodenum. In this way, we intended to determine if restoring nutrient delivery through the excluded stomach gastrostomy tube and after reversal. Oral feeding led to an increased rise in glucose between 0 and 15 minutes (paired t test, p = 0.01 and 0.003, respectively) and a greater decline in glucose between 15 and 90 minutes (p = 0.01 and 0.003, respectively). Insulin secretion was also diminished, with area under the curve (AUC) between 90-90 minutes significantly decreased between oral and G-tube feeding (p = 0.037). We also found that postprandial GLP-1 levels were dramatically reduced with feeding through the excluded stomach and after reversal (AUC, p = 0.002 and 0.003, respectively). PYY secretion also trended higher with oral feeding (AUC, p = 0.07). Glucagon levels were not significantly different across the groups.

Conclusions: Overall, we find that reversal of gastric bypass is an effective treatment option for severe postprandial hypoglycemia. The pathophysiology of this disorder seems to be primarily due to altered gut anatomy resulting in altered nutrient absorption and gut peptide hormone release, rather than inherent beta cell hyperplasia or hyperfunction. We specifically found that postprandial GLP-1 levels were higher in patients after oral nutrient intake through the RYG anatomy. Increased GLP-1, which is a known insulin secretagogue, in combination with the larger rise in serum glucose levels likely lead to enhanced postprandial insulin secretion in RYG patients. In some insulin sensitive patients, this results in a rapid drop in serum glucose levels approximately 1 hour after the meal, ultimately leading to postprandial hypo- glycemia. If nutrients are delivered through the “normal” anatomy, these large increases in glucose and GLP-1 are not seen and insulin production is diminished, resulting in euglycemia. We hope that these insights will help develop new strategies for the treatment of this devastating complication and also prevent the unnecessary performance of pancreatectomy as a treatment option.

FINAL ID: 35 INHIBITION OF THE NLRP3 INFLAMMASOME REDUCES THE SEVERITY OF EXPERIMENTALLY-INDUCED ACUTE PANCREATITIS IN OBESSE MICE

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Obesity affects more than one third of American adults, and is a risk factor in the progression from mild (MAP) to severe acute pancreatitis (SAP). However, the mechanism of this association has yet to be elucidated. Concur- rent with inflammation, hyperactivation of the NLRP3 inflammasome occurs in obesity. Lean knockout mice for components of the NLRP3 inflammasome are protected from cerulein-induced MAP, indicative of a direct involvement of this pathway. We hypothesized that inhibition of the NLRP3 inflammasome with the sulfonlyurea drug glyburide would reduce disease severity in obese mice with cerulein-induced SAP. Pretreatment with glyburide significantly reduced serum IL-6, amylose and lipase, pancreatic mass/body mass, pancreatic histology score, acinar cell death and peritonal cell production of LPS-induced IL-1, in obese ob/ob mice with SAP compared to vehicle-treated ob/ob mice. Similarly, glyburide-pretreatment in wild-type diet-induced obese (DIO; fed a high-fat diet for 16 weeks) mice with SAP exhibited lower serum IL-6 and lipase, pancreatic histology score and acinar cell death, compared to vehicle-pretreated DIO mice. These data suggest an important role for the NLRP3 inflammasome in obesity-associated SAP pathophysiology, and expose the potential therapeutic usefulness of its inhibition in the prevention or treatment of SAP in obese individuals.

FINAL ID: 36 HYPERGLYCEMIA IMPAIRS FIRST TRIMESTER CYTOTROPHOBLAST FUNCTION AND INDUCES ANTI-ANGIOGENIC MILIEU VIA STRESS SIGNALING

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Objective: Preeclampsia (preE) is a pregnancy disorder characterized by the de novo development of hypertension and proteinuria and is the leading cause of fetal-maternal morbidity and mortality. The specific etiologies of this syndrome remain undefined. It seems clear that preE is a syndrome with vari- ous pathophysiologic triggers and mechanisms. Approximately 20% of diab- etic pregnant women develop preE. The mechanisms contributing to this
effect are not well understood. It has been evident that dysfunction of cytotrophoblast (CTB) cells, which are critical for formation of the fetal-maternal interface, may play a central role in the pathogenesis of preE. Excessive circulating glucose, caused by diabetes or other conditions, is one well-characterized precursor of preE. The CTBs invade the decidua using plasmin. The inactive plasminogen is converted into plasmin by urokinase plasminogen activator (uPA), which is regulated by plasminogen activator inhibitor 1 (PAI-1). It has also been suggested that peroxisome proliferator-activated receptor gamma (PPARγ) is involved in the dysfunction of CTBs in hyperglycemia. This study assesses the signaling mechanisms of excess glucose-induced CTBs dysfunction.

Study Design: Human CTBs (Sw. 71) were treated with 45, 135, 225, 495 or 945 mg/dL glucose for 48h. Some cells were pretreated with a p38 inhibitor (SB203580) or a PPARγ ligand (rosiglitazone). Thereafter, cell lysates were utilized to measure uPA, PAI-1 and PPARγ expression and p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation by western blot (WB). The mRNA expression of uPA and PAI-1 in CTBs lysates was measured by qPCR. Levels of angiogenic and anti-angiogenic factors (sFlt-1, sEng, VEGF165, PIGF) and IL-6 were measured in the media by ELISA kits. To evaluate the apoptotic signaling, pro-apoptotic Bel-2-associated X protein (Bax), pro-apoptotic Bel-2 protein (Bad) and pro-inflammatory protein cyclooxygenase-2 (Cox-2) expressions were assessed by both WB and immunohistochemistry. The p38 MAPK phosphorylation was evaluated by WB. Statistical comparisons were performed using analysis of variance with Duncan’s post hoc test.

Results: Both uPA (0.6 fold, 0.5 fold) and PAI-1 (0.5 fold, 0.5 fold) protein and mRNA expression, respectively, were downregulated (p<0.05) in CTBs treated with >135 mg/dL glucose compared to basal (45 mg/dL). The anti-angiogenic factors (sEng (2.8 fold) and sFlt-1 (2.1 fold)) and IL-6 (1.9 fold) were upregulated, while the angiogenic factors (VEGF (0.4 fold) and PIGF (0.5 fold)) were downregulated in the presence of >135 mg/dL glucose. The p38 MAPK phosphorylation and PPARγ expression were upregulated (p<0.05) in hyperglycemic CTBs. The expression of Bax (2.1 fold), Bad (2.6 fold), Cox-2 (1.9) were upregulated (p>0.05) in CTBs treated with >135 mg/dL glucose compared to basal (45 mg/dL). The SB203580 or rosiglitazone pretreatment showed an attenuation of glucose-induced downregulation of factors involved in CTBs invasion. The hyperglycemia-induced apoptotic and stress signaling proteins were attenuated by the SB203580 or rosiglitazone pretreatment.

Conclusions: 1) Exposure of CTBs to excess glucose inhibits the invasive profile of CTBs by decreasing the expression of uPA and PAI-1, by downregulation of VEGF and PIGF; and upregulation of sEng, sFlt-1 and IL-6. 2) Hyperglycemia induced the apoptotic signaling in CTBs by upregulating Bax, Bad and Cox-2 protein expression. The attenuation of hyperglycemia-induced downregulation of proteins in CTBs invasion and upregulation of apoptotic and stress signaling proteins by SB203580 or rosiglitazone pretreatment suggests the involvement of stress signaling mechanisms in CTBs dysfunction.

**FINAL ID: 37**

**FAMILIAR THROMBOPHILIA (FACTOR V LEIDEN HETEROZYGOSITY, HIGH FACTOR VIII) IN WOMEN WITH “IDIOPATHIC” OSTEONECROSIS**

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Common causes of osteonecrosis include long-term high doses of corticosteroids, alcoholism, and trauma or dislocation of the affected joint. In some cases, a clear etiology cannot be found and the case may be labeled “idiopathic”. Our specific aim was to assess associations of thrombophilia and hyperglycemia with “idiopathic” osteonecrosis (ON, 8 hips, 5 knees, 3 multifocal, 1 metastatic) in 18 women whose ON was not secondary to long-term high dose corticosteroids, alcoholism, or trauma-dislocation. We studied the women in the consecutive order of their referral to the Cholesterol Center. PCR and serologic measures of T-H in the 18 cases were compared to those in 152 and 61 healthy female controls. Factor V Leiden heterozygosity was found in 3 of 18 cases (16.7%) vs. 5 of 151 controls (3.4%), Fisher’s p<0.05. High Factor VIII was present in 12 of 26 (46.2%) of cases vs. 6 of 56 controls (10.7%), p<0.009. Homozygote/homozygote for the endothelial nitric oxide synthase (eNOS) T-786C mutation, which reduces nitric oxide production required for healthy bone, was present in 7 of 8 (87.5%) of cases vs 12 of 26 (46.2%) of controls, p=0.053.

Our data demonstrate a clear association between Factor V Leiden heterozygosity, high factor VIII levels, eNOS hetero-homozygosity and increased incidence of “idiopathic” osteonecrosis. Prior studies have also shown that familial thrombophilia is associated with increased risk of osteonecrosis.

**FINAL ID: 38**

**TESTOSTERONE THERAPY, THROMBOPHILIA, AND HOSPITALIZATION FOR DEEP VEIN THROMBOSIS-PULMONARY EMBOLUS, AN EXPLORATORY STUDY**

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In our study of 141 men hospitalized (Mercy Hospitals, Cincinnati OH) in the last year for deep vein thrombosis-pulmonary embolism (DVT-PE), we determined the prevalence of use of exogenous testosterone (T) with subsequent development of DVT-PE. We speculated that exogenous T was aromatized to estradiol (E2) and that high E2 interacted with previously undiagnosed thrombophilia-hypofibrinolysis to produce thrombosis. Of the 141 men, 34 (24%) were deceased or in hospice care, 6 (4%) had cancer thought to be a cause of DVT-PE, 63 (45%) could not be contacted, leaving 44, 2% of whom had taken T before and at the time of their admissions, 1.4 % of the total cohort. One patient (case #1) had 2 PE, 6 and 24 months after starting T; DVT in the second patient (case #2) occurred 24 months after starting T. Case #1 was found to have high homocysteine (18 xx) and low antigenic protein S (39%, lower normal limit [LNL] 70%), and his antigenic protein S/antigenic factor VII ratio was 0.5, LNL – 0.5. Case #2 had high Factor VIII activity (190%), LNL 150. In case #2, while taking 100 mg T/day as a gel, serum estradiol was high, 51 pg/ml, upper normal limit 42.6 pg/ml. Of 141 men hospitalized for DVT-PE, a conservative estimate of the percentage who had taken T before and at the time of their DVT-PE was 1.4% (2/141). These 2 men who sustained DVT-PE after starting exogenous T were found to have previously undiagnosed familial thrombophilia, suggesting a thrombotic interaction between exogenous T and thrombophilia-hypofibrinolysis.

**FINAL ID: 39**

**SEXUAL DIMORPHIC IMPACT OF ADULT-ONSET HEPATIC GH RESISTANCE ON GLUCOSE AND LIPID METABOLISM**

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The liver partitions nutrients for appropriate utilization/storage by other tissues. Defects in hepatic nutrient processing can lead to inappropriate fat accumulation (hepatosteatosis), insulin resistance, hyperglycemia, hyperlipidemia.
and associated pathologies. The liver is a major target of growth hormone (GH), where development-related, gender- and disease-related changes in GH secretion/action are associated with alterations in hepatic nutrient processing. However, since GH receptors (GHR) are ubiquitously expressed and are required for normal structural growth, it has been difficult to tease apart the direct vs. indirect effects of experimental or disease-related changes in circulating GH levels on adult hepatic metabolism. In order to better define the primary actions of GH on hepatic metabolism in adults, we have knocked down the GH receptor (GHR) in the liver of mice (aLivGHRkd), by treating 1wk-old GHR-/- mice with adeno-associated vectors expressing liver-specific, Cre recombinase. Hepatic glycogen and triglyceride (TG) content dramatically increased 7d post-GHR knock-down in male, but not female mice. The increase in hepatic nutrient storage cannot be attributed to changes in systemic or hepatic insulin sensitivity or GH-mediated increases in lipolysis. Interestingly, under both basal and fasted conditions, hepatic PPArγ mRNA and protein levels were increased. This was reflected by increased expression of glucokinase and lipogenic genes that are known targets of PPArγ. In addition, expression of PEPCK was decreased. Phosphorylation of Stat5 (pStat5) was reduced in livers of male aLivGHRkd mice. pStat5b has been reported to suppress glucokinase and lipogenic genes that are known targets of PPArγ. In addition, expression of PEPCK was decreased. Phosphorylation of Stat5 (pStat5) was reduced in livers of male aLivGHRkd mice. pStat5b has been reported to suppressed PPArγ transactivation activity, as well as directly increase PEPCK gene transcription. Therefore, we hypothesize that in males, GH directly suppresses hepatic nutrient storage by pStat5b-mediated processes. The lack of an effect of aLivGHRkd in female mice may be in part mediated by estrogen, because livers of ovariecotomized (OVX) aLivGHRkd mice accumulated TG and increased expression of lipogenic genes, which was blocked by estrogen replacement. Interestingly, in females, hepatic lipogenesis was not increased in OVX-females with intact hepatic GHR, as compared to SHAM-operated controls. These results clearly demonstrate that the hepatic GHR, or estrogen alone, are sufficient to suppress lipogenesis. It is possible that estrogen may modify GHR through regulation of pStat5. However, estrogen may also bypass the effects of hepatic GHR resistance and act directly to suppress the activity of PPArγ and/or SREBP1c. Studies are ongoing to differentiate between these possibilities.

**FINAL ID: 40**

**INTEGRATED MULTIVARIABLE ARTIFICIAL PANCREAS CONTROL SYSTEMS WORK AS WELL AS OPERATOR CONTROLLED SYSTEMS**

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Our goal is to develop a closed-loop (CL) artificial pancreas (AP) control system for those with T1DM to achieve normal blood glucose (BG) and prevent hypoglycemia (HG). We used continuous measurements of glucose (CGM) and physiological variables (i.e., energy expenditure [EE] and galvanic skin response [GSR]), integrated with HG early alarm modules (EAM) during real-time. We compared an operator to AP-driven BG control, to assess AP performance against current care (insulin pump [IP] / CGM).

Nine open-loop (OL) and nine CL experiments were performed on T1DM subjects. A multivariable adaptive AP (MAAP) system was used in the first six CL and a MAAP integrated with a HG EAM, was used in the last three CL experiments. BG and physical activity (PA) information were sent to the AP every 10 minutes (mins). All experiments were performed in a clinical research center over 60 hrs (24 OL and 36 CL). Meals and PA occurred without warning the AP (no insulin boluses were given in CL). Subjects wore an IP, two CGMs and a SenseWear Pro3 armband reporting EE and GSR. Subjects had single bouts of exercise (20 mins of treadmill running) before/after lunch daily. Speed/incline of the treadmill increased until subjects completed the bout or reached self-identified endpoints (e.g., fatigue). Subjects exercised at 8 ± 3.8% (Mean ± SD) of age-predicted max heart rate of 220-age (range: 70-97%).

We analyzed time in BG ranges: >55, 55-70, 70-180, 180-250 and >250 mg/dl, OL vs. CL. Mean BG for each was compared by two tailed *t-*test. There were no significant differences between OL vs. CL, except in >250 range where CL > OL. CL had two severe HG episodes vs. none in CL. CL time resulting in BG>250 were partially caused by CGM issues.

CL results were comparable to OL without causing HG despite similar exercise protocols implemented. Subjects responded to AP and no episodes of severe low during CL. Advantages are no warning by users for meals or exercise. This is the first automated multivariable AP to use EE and GSR information to predict insulin needs.

**FINAL ID: 41**

**COMPARATIVE EFFECTIVENESS OF NATIVE AND NANOFORMULATED SUPEROXIDE DISMUTASE IN MODULATING AT INFLAMMATION IN OBESITY**

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Hypertension is a prevalent condition in obesity and obesity-linked hypertension is resistant to anti-hypertensive drugs. Adipose Tissue (AT) inflammation is considered to play an important role in mediating obesity-associated comorbidities. Nanomedicine is an emerging field applying nanotechnology to improve the efficacy of therapeutic drugs. Of note, nanofomualted superoxide dismutase (SOD) has been suggested to ameliorate hyper-tension. Because oxidative stress is often associated with inflammation and hypertension, and is, increased in obese AT, we sought to determine the effectiveness of native and nanofomualted superoxide dismutase (SOD) in modulating AT inflammation in a mouse model of diet-induced obesity. Wild type C57BL/6J mice were fed a high fat (HF) diet (45% fat) for 10 wk followed by 2 wk treatment with native or nanofomulated SOD (nanoSOD). The mRNA expression of monocyte chemoattractant protein (MCP-1), an inflammatory marker, in visceral AT (VAT) and the macrophage-enriched stromal vascular cells collected from AT showed a significant decrease in both native and nanoSOD treated mice, the later being more potent in mediating this effect. In the liver, the expression of CD68, a macrophage marker, was increased in both native and nanoSOD treated mice; however, the inflammatory markers were not altered in any of the groups. The expression of carnitine palmitoyl transferase-1 (CPT-1), an enzyme involved in fatty acid beta-oxidation, was significantly increased in nanoSOD treated mice compared to control and native SOD groups (<0.01 vs control and P<0.05 vs native SOD) indicating increased fatty acid catabolism in liver. Plasma total cholesterol showed a trend towards a decrease in nanoSOD treated mice compared to HF control. Notably, native SOD treatment resulted in a significant increase in blood glucose levels (P<0.01 vs control) compared to control and nanoSOD groups, indicating impaired metabolic homeostasis. In Together, our data show that both native and nanoSOD were effective in reducing AT inflammation but native SOD treatment led to an increase in fasting blood glucose. Our data suggest that nanofomulation of SOD is beneficial in improving the effectiveness of SOD to ameliorate obesity-linked AT inflammation without undesirable changes in metabolic profile.

**FINAL ID: 42**

**THE ROLE OF VITAMIN D IN PREVENTING LEAKY GUT-INDUCED ENDOXEMIA IN SUBJECTS AT RISK FOR DIABETES**


The role of gut microbiota in obesity and insulin resistance has been recently under scrutiny. A diet high fat and low fiber may lead to changes of microbiota and damage of the intestinal epithelium, leading to a leaky gut and release of bacterial products (i.e., lipopolysaccharide, LPS) in the circulation. Inflammatory effects of LPS are mediated cellulary by a complex of molecules such as LPS binding protein (LBP) and cell bound CD14. It has been suggested that shedding of membrane CD14 in circulation as soluble CD14 (sCD14), as well as an increase in anti-LPS core antibodies (Endocab) may have anti-inflammatory effects. Zonulin has emerged as a marker of inflammatory effects. Zonulin has emerged as a marker of inflammatory process triggered in the gut of the metabolically impaired individuals. Methods: Twenty subjects participating in a vitamin D supplementation study at Jesse Brown VA center in Chicago were selected if they achieved a plasma vitamin D level of 50-90 ng/dl after 12 mo of weekly 50,000 U vitamin D. Patients were African-American males, with baseline vitamin D insufficiency, pre-diabetes, and obesity. Serum samples were collected at baseline and 12 mo to measure sCD14, LBP, Endocab, and zonulin. Dietary information were collected using 24-h dietary recalls.

Results: Data were analyzed as differences from baseline (Final-Initial). The effects of vitamin D on HbA1c, BMI, and inflammatory markers were
analyzed using a paired t-test. Correlations between various parameters were performed using Pearson coefficient. Mean baseline characteristics were: age 60 yo, BMI 32.7, HbA1c 6.2%, and vitamin D 11.7ng/dl. Average 24h caloric intake was 2070kcal, fat 80g, saturated fat 25g, carbohydrate 26g, protein 73g, and fiber 15g. Baseline sCD14 was 1428 ng/ml, LBP 18519 ng/ml, Endocap 237 GMU/ml, and zonulin 6 ng/ml. At 12 mo, BMI was 32.6 (p=0.92), HbA1c 6.4% (p=0.18), and vitamin D 56 ng/dl (p=0.000). After 12 mo of supplementation, vitamin D significantly increased sCD14 (P< 0.003) and Endocap (p= 0.037), decreased zonulin (p= 0.01), but did not significantly change LBP. Changes in all four markers had strong inverse correlations to changes in HbA1c and BMI (r=-0.4 to -0.6). Changes in all four markers had strong positive intercorrelations (r= 0.6 to 0.9). The strongest correlation was between zonulin and sCD14 (r= 0.9) and Endocap and sCD14 (r= 0.85). There were moderate to strong correlations between changes in all four markers and total caloric intake (kcal, r=0.3-0.4), as well as carbohydrate and fiber intake (g, r=0.37-0.45). The change in LBP had a moderate correlation to fat intake (g, r=36), and a weak correlation to saturated fat intake (g, r=21).

Conclusion: Vitamin supplementation with 50000 U vitamin D resulted in changes of inflammatory markers associated with gut derived endoxemia. Vitamin D decreased zonulin suggesting a protective effect on the intestinal barrier. Also an anti-inflammatory effect is suggested by the fact that vitamin D increased sCD14 and antibodies to core LPS, and hence preventing LPS triggered inflammatory cascade. Also data suggest that BMI and HbA1c may attenuate these effects, as they inversely correlated with changes in all four markers. Lastly, our data suggest that caloric intake and specifically carbohydrate and fiber intake are important players in gut permeability and endoxemia. This study sheds light on vitamin D as a potential agent that modulates gut permeability and prevents low grade inflammation associated with obesity, insulin resistance, and unhealthy diets.

**FINAL ID: 43**

**BREATHE MEASUREMENT OF ALTERED TISSUE GLUCOSE OXIDATION IN OBESE AND DIABETES MELLITUS**

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**Objective:** To evaluate the determinants of the carbohydrate oxidation expressed as the exhaled 13CO2 following ingestion of [13C] glucose, in lean, obese, and T2DM in humans.

**Research Design and Methods:** Men and women, age 18–65 from across the spectrum of obesity and glycaemia, were studied on 2 consecutive days. On the first day, a 75g oral glucose load spiked with 13C-glucose was administered with subsequent breath sampling over 3 hours. On day 2, a hyperinsulinaemic euglycemic clamp was performed.

Body composition was measured by dual energy x-ray absorptiometry (DEXA) at the time of screening evaluation. Full datasets were available in 53 participants.

**Age, sex, and race-adjusted. Correlations were evaluated between the body index of glucose oxidation and clamp-derived glucose disposal rate (GDR), Body Mass Index (BMI), and body compositions. Stepwise multivariate analyses were performed to evaluate the concurrent contribution of age, BMI, GDR, and body component compositions to the body index of glucose oxidation.**

**Results:** Adjusted Correlation of the breath measurement (13CO2 AUC 180) With GDR and BMI were (r=0.39, p=0.003), and (r=-0.474, p=0.002) respectively. Statistically significant correlations were present between 13CO2 AUC 180 and all body composition indices (total tissue mass (g) r=−.540, P<0.0001; fat tissue mass (g) r=−.350,p=0.02; lean tissue mass (g) r=−.560, P=0.0001). In multivariable analysis the concurrent effect of BMI, total tissue mass, and total fat mass (g) on breath measurement did not retain significance whereas the contribution of total lean mass and insulin resistance remained significant.

**Conclusion:** 13CO2 appearance in exhaled breath following a standard oral glucose load with added 13C-glucose is a determinant of body mass rather than fat tissue mass. These observations demonstrate using a novel and simple methodology that skeletal muscle in Obese and T2DM is the main site of impaired carbohydrate oxidation.

**FINAL ID: 44**

**GCK-RELATED HYPERGLYCEMIA: FREQUENTLY MISDIAGNOSED AND INAPPROPRIATELY TREATED IN THE US**

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Heterozygous mutations in GCK have an estimated prevalence of 1 in 1000 individuals and lead to a stable fasting plasma glucose concentration ≥100 and <126 mg/dl. GCK-related hyperglycemia (or MODY2) does not need glucose-lowering therapy, except in some instances during pregnancy. Through the web-based US Monogenic Diabetes Registry (http://monogenicdiabetes.uchicago.edu/registry/) we assessed demographics, time to diagnosis and longitudinal treatment patterns of individuals with GCK-related hyperglycemia (Table). 85% received genetic testing on a research basis due to barriers to clinical genetic testing. 76% were referred to the Registry by physicians, and 79% of referring physicians were endocrinologists. Zip-code data analysis shows that the majority of participants have education levels and household incomes exceeding the US national average. Nearly half were on medical therapy prior to genetic diagnosis.

Our data suggest that GCK-related hyperglycemia is frequently misdiagnosed and inappropriately treated as diabetes, unnecessarily driving up health care costs. An over-representation of individuals from higher socioeconomic status and under subspecialty care in the Registry underscores the importance of efforts to educate all physicians and patients about monogenic forms of diabetes and increase access to genetic testing to inform appropriate clinical care in an equitable fashion.

**FINAL ID: 46**

**IMPROVING DIABETES EDUCATION FOR PEDIATRIC RESIDENTS WITH AN ONLINE MODULE**

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Background: Medical resident lack of confidence and knowledge regarding diabetes management represents a significant barrier to optimal management of patients with diabetes. Online computer modules designed to educate...
residents about diabetes management have previously been shown to be efficacious. However to our knowledge, no previous studies have used a randomized-controlled design to study efficacy while accounting for differences in curriculum and patient exposure inherent to resident medical education and training. We hypothesize that an online computer module will result in sustainable improvements in resident knowledge and confidence regarding diabetes in comparison to standard resident diabetes education.

Methods: All University of Chicago pediatric and internal medicine-pediatric residents during fall 2013 were eligible to participate. Participants were randomized to a control group and an intervention group. Both groups completed a survey assessing their level of training, experience caring for patients with diabetes in comparison to standard resident diabetes education. Both groups also completed a 20-question diabetes knowledge test on 10 key domains of diabetes care. The intervention group was then administered an online diabetes education module and completed an immediate post-test.

Retention of knowledge and differences over time between groups will be assessed via repeat survey and knowledge test 3 months after the initial test. The intervention group will determine if the module results in long-term gains in knowledge and confidence in diabetes management.

Conclusions: Our data show that the majority of participating University of Chicago pediatric and medicine-pediatric residents lack confidence and knowledge in important areas of diabetes care. The online module resulted in immediate gains in knowledge. Follow-up data on resident diabetes knowledge and confidence will be collected in February 2014. These data will determine if the module results in long-term gains in knowledge and improves confidence in comparison to standard resident diabetes education, supporting its incorporation into the resident medical education curriculum.

GASTROENTEROLOGY/CLINICAL NUTRITION

FINAL ID: 50
LIVER FUNCTION TESTS AND OCCURRENCE OF ANEMIA, THROMBOCYTOPENIA AND LEUCOPENIA - RETROSPECTIVE COHORT STUDY IN US ADULTS
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Background: Chronic liver disease is often associated with hematologic abnormalities. However, limited studies exist that have investigated whether abnormalities in specific liver function tests (LFTs) are associated with the occurrence of anemia, leucopenia or thrombocytopenia. Objective: To use prospectively collected data from the National Health and Nutrition Evaluation Survey (NHANES) between 1999-2010 to analyze the relationship between LFTs and abnormalities in hematologic indices.

Methods: Demographic (age, race, gender), socioeconomic (poverty to income ratio) and laboratory data (AST, ALT, Alkaline phosphatase, GGT, total bilirubin, total protein, albumin, hemoglobin, white cell, platelet count, serum B12, folic acid, ferritin) were retrieved from publically available data files for adults 18 years and older on the NHANES website. Multivariate logistic regression was employed to study association between LFTs and hematologic abnormalities. Further studies are needed to investigate the prognostic importance of these patient clusters. Significance: The study suggests a relationship between abnormalities in specific liver tests and blood cell counts. Further studies into the underlying mechanisms could throw more light on occurrence of cytopenias in patients with chronic liver disease.

FINAL ID: 52
THE INCIDENCE OF CLOSTRIDIUM DIFFICILE INFECTION IN HOSPITALIZED PATIENTS WITH CYSTIC FIBROSIS IN THE UNITED STATES
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Background: The occurrence of Clostridium difficile (C. difficile) infection (CDI) in patients with cystic fibrosis (CF) has not been extensively investigated and its study remains limited to case series and reports. C. difficile bacterium or its toxins may be frequently isolated in up to 30% of CF patients, however experts believe this reflects an asymptomatic colonization while actual disease remains rare in the CF population.

Objective: To investigate the occurrence of CDI in hospitalized patients with CF in the United States.

Methods: Data were obtained from the Nationwide Inpatient Sample (NIS), Healthcare Cost and Utilization Project (HCUP), Agency for Healthcare Research and Quality for the years 2002 to 2010. Data were weighted to generate national-level estimates.

Results: For the year 2010, there were a total of 9,706,097 weighted discharges in the age group 18–44 years. In this age cohort, 32,541 patients had a diagnosis of CDI and 19,278 patients had a diagnosis of CF. There were 1,849 patients with CF who also carried a diagnosis of lung transplant. The incidence of CDI in the hospitalized CF population was 1.6% compared to an incidence of 0.3% in the non-CF hospitalized population (P<0.05). In the subset of patients with CF who had undergone lung transplantation, the incidence of CDI was 3.1%. After high-dimensional propensity score matching to control for demographic factors and comorbidities; patients with CF continued to have a higher risk for CDI than their matched counterparts (OR 3.0, 95% CI 2.6, 3.5).

Patients with CF + CDI had an overall worse outcome than patients with CDI only (all differences significant at the level of P<0.05). Utilizing a multiple variable regression model to control for demographic factors and comorbidities, patients in the CF + CDI group continued to demonstrate poor outcomes compared to patients in the CDI only group. This was evident as a higher risk of death (adjusted odds ratio (aOR) 3.1; 95% CI 1.9, 5.1), coelectomy (aOR 2.6; 95% CI 1.3, 5.3) and higher hospital charges (adjusted regression coefficient 524,000; 95% CI $22,000, $62,000). The difference in LOS between the two groups did not achieve statistical significance (adjusted regression coefficient 3.3 days; 95% CI 0.81, 5.8 days).
Finally, we assessed the trend of CDI in the CF and non-CF population. Between the years 2002 – 2010, the incidence of CDI in the hospitalized CF population (ages 18 – 44 years) increased from 0.9% to 1.6% whereas the incidence of CDI in the corresponding non-CF population increased from 0.2% to 0.3%. For both these groups, this represented a significant increasing trend in the incidence of CDI (P<0.05).

**Conclusions:** Clostridium difficile infection (CDI) incidence in patients with cystic fibrosis (CF) was 1.6%. CF had worse outcomes (higher risk of death, colectomy and hospital charges) in the setting of CF. There was an increasing trend in the incidence of CDI complicating CF in the years 2002 - 2010.

**FINAL ID: 54**

**INCREASING SERUM PRE-ADIPOCYTE FACTOR-1 (PREF-1) CORRELATES WITH DECREASED BODY FAT, INCREASED FREE FATTY ACIDS, AND THE LEVELS OF RECENT ALCOHOL CONSUMPTION IN EXCESSIVE ALCOHOL DRINKERS.

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**Background:** Under physiological state, free fatty acids (FFA) enter adipocytes and stored in the adipose tissues in the form of triglycerides (TG). Patients with alcoholic liver disease have been shown to have significantly lower percentage of body fat (%BF). This results in reducing TG store as reflected by increasing serum FFA. In adipose tissue, Pref-1 is specifically expressed in preadipocytes but not in adipocytes. Increasing Pref-1 leads to inhibition of adipogenesis and reduced adipose tissue mass. Our aim is to investigate the association between alcohol consumption, serum Pref-1, and %BF in heavy drinkers compared to controls.

**Methods:** 97 chronic heavy drinkers (mean age 41.3 years/65% men/81% Caucasian) were enrolled from Fairbanks Alcohol Treatment Center. 53 non-heavy drinkers (mean age 31.8 years/88% men/84% Caucasian) were recruited from Roudebush VAMC. Time Line Follow-Back (TLFB) was used to quantify the amount of alcohol consumed in the past 30 days before enrollment. Anthropometric measurement was performed to calculate %BF. Serum Pref-1 and FFA were measured. Alcohol intake was considered as categorical variable (heavy/non-heavy) using NIAAA criteria. It was also modeled as a continuous variable and divided by quartiles by calculating the total amount of alcohol consumed in the past 30 days from TLFB. Linear regression was used in the analyses.

**Results:** Heavy drinkers had higher levels of Pref-1 (0.32±0.13 vs 0.13±0.06, p<0.01), FFA (2.31±0.78 vs 0.42±0.28, p<0.001), and lower %BF (29.7±4.7 vs 31.7±5.7, p<0.03). There were no differences in the BMI and waist circumferences. Serum Pref-1 was significantly associated with the amount of alcohol consumption during the past 30 days. There was the trend on the paradoxical relationship between %BF and the amount of alcohol consumed. In the sub-analyses, %BF was significantly decreased with the increased amount of alcohol consumption, specifically drinking in the 3rd (r=0.11, p<0.03) and 4th quartiles range (r=0.32, p<0.005). Serum Pref-1 is negatively correlated with %BF, but positively associated with serum FFA.

**Summary:** Our data suggest that Pref-1 might play a role in the inhibition of adipogenesis and thus decreasing %BF in alcoholics. Further work is needed to validate these findings and to better understand the role of Pref-1 and its clinical significance in subjects with heavy alcohol use.

**GENETIC & MOLECULAR MEDICINE**

**FINAL ID: 56**

**ACTIVATION OF ALTERNATIVE RECEPTOR TYROSINE KINASE IN GLOBLASTOMA MEDIATES RESISTANCE TO PDGFR INHIBITION.

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Several receptor tyrosine kinase (RTK) pathways including the pathways mediated by PDGFR and EGFFR have been implicated in the pathogenesis of glioblastoma multiforme (GBM) and identified as potential therapeutic targets. The use of RTK inhibitors have been evaluated in the treatment of GBM; however, has not significantly improved patient survival due to tumor resistance to such therapy.

Our laboratory has described a mouse model of glioma that is dependent on PDGFR activation for tumor initiation and progression. This model mimics the features of human proneural GBM, a subtype of GBM that is closely associated with PDGFR activation. From this model, we developed primary cell cultures enriched for stem and progenitor populations (spheroid cultures) in which doxycycline exposure inhibits PDGFR expression, resulting in reduced level of PDGFR activation. We believe that these populations of cells are more relevant to the development of resistance to such therapies in GBM. Utilizing this mouse model and culturing system, we identified tumors that were resistant to the therapeutic inhibition of PDGFR and used them to investigate the molecular mechanisms underlying that resistance. We found that tumor spheroid cultures (TSCs) derived from PDGFR-inhibition resistant glioma required activation of two closely related RTKs, insulin receptor (IR) and insulin growth factor receptor (IGF1R) for proliferation, PI3K and MAPK pathways as critical downstream mediators to maintain growth in these cultures. IR and IGF1R pathways are surprisingly not sufficient for proliferation in PDGFR-inhibition sensitive TSCs, but when PDGFR is inhibited in these sensitive cultures, IR and IGF1R activation sustains survival in select subpopulations, which eventually emerge as the resistant TSC. In an in vitro system mimicking the development of therapeutic resistance, co-targeting IR, IGF1R and PDGFR using small molecules reduced the frequency of emergence of resistance. Published public databases describing the molecular and biological changes in brain tumors, we found that high levels of PDGFR expression are correlated with high levels of signaling molecules in the IGF1R pathway. We also found that patients with proneural GBMs, but not other subtypes of GBM, had a worse prognosis if there was evidence of IR or IGF1R pathway over-expression. This observation suggests a role for IR and IGF1R in PDGFR-driven tumor progression in GBMs, as proneural GBM has a signature of high PDGFR expression and downregulation of IGF1R activation. These findings together suggest a rationale for targeting IR, IGF1R and PDGFR simultaneously in carefully selected GBM patients, whose tumor is highly active for PDGFR.

**FINAL ID: 58**

**ANALYZING AND COMPARING RNA-SEQ DATA FROM LUNG BIOPSY AND PERIPHERAL BLOOD MONONUCLEAR CELLS OF AN IDIOPATHIC PULMONARY FIBROSIS PATIENT.

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**Rationale:** Microarrays profiling of peripheral blood mononuclear cells (PBMCs) gene expression have been applied in the past to recapitulate disease development in idiopathic pulmonary fibrosis (IPF) patients as a proxy for the molecular perturbations occurring in the lung. However, since PBMCs are the first line of defense against infection and adapt to intruders, it is still debatable whether PBMCs gene expression profiling can reflect IPF-specific gene expression profiling in lungs. Objective. To directly compare and contrast differentially expressed genes (DEGs) in IPF lungs and IPF PBMCs by grouping DEGs based on functional similarity to systematically enhance biological interpretation derived from RNA-Seq.

**Methods:** Total RNA from lung biopsy (n=1) and PBMCs (n=1) were isolated from the same IPF patient and subjected to direct sequencing of transcripts by high-throughput sequencing technologies using Illumina HiSeq 2500 following manufacturer’s protocols. RNA-sequencing data was verified using an Illumina HiSeq system. Raw Illumina reads (fastq.gz) were preprocessed using Illumina RNA-Seq pipeline developed by University of Chicago Center for Research Informatics. The Database for Annotation, Visualization and Integrated Discovery1,2 (DAVID) v6.7 tools (http://david.abcc.ncifcrf.gov) were used to conduct functional annotation enrichment analysis on the DEGs identified within each contrast.

**Results:** Quality scores (Qphred) across all bases for the sequence files were >30, suggesting the accuracy of base call >99.9%. From each contrast (i.e.
IPF lungs vs normal lungs and IPF PBMCs vs normal lungs), 586 and 1070 DEGs were identified respectively, at 0.05 false discovery rate (FDR). Among them, only 229 genes overlapped; while the majorities were unique DEGs in these tissues. Notably, lungs- and PBMC-specific DEGs were also enriched in the same GO terms listed, suggesting the complexity of gene interaction networks contributing to the pathogenesis of IPF. Furthermore, genes involved in “cell motility” biological process was significantly enriched in IPF PBMCs but not in IPF lungs.

Conclusions: Analyzing RNA-seq data of IPF lungs and IPF PBMCs from the same patient has provided a list comprehensive DEGs involved in pathological processes, suggesting an incomplete overlap of DEGs in these tissues.

FINAL ID: 60
GENETIC ASSOCIATION OF A MAPK8 EXPRESSION QUANTITATIVE TRAIT LOCUS WITH PRE-CAPILLARY PULMONARY HYPERTENSION IN SICKLE CELL DISEASE

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Sickle cell disease (SCD) is associated with pleiotropic clinical outcomes, the severity of which exhibits remarkable inter-individual variability. Investigations of the pathophysiology of SCD have focused on the adverse effects of vaso-occlusion, chronic inflammation, and hemolysis. SCD is also characterized by up-regulation of the hypoxic response to chronic anemia. We postulated that the up-regulated hypoxic response in SCD contributes to altered gene expression that might impact pulmonary hypertension, a complication associated with early mortality.

To identify genes regulated by the hypoxic response and not other effects of chronic anemia, we compared expression variation in peripheral blood mononuclear cells from 13 sickle cell anemia untreated with hydroxyurea and 11 healthy controls (HC) patients characterized by homozygous VHL R200W-induced constitutive up-regulation of hypoxia inducible factors in the absence of anemia or hypoxia. Gene expression of both cohorts was profiled on identical Affymetrix exon arrays. The degree and direction of differential gene expression were highly correlated between sickle cell anemia and CP (Spearman’s r = 0.73 between regression coefficients of differential gene expression), suggesting that 53% of expression variation in sickle cell anemia is related to hypoxic transcriptional responses. At 5% false discovery rate (FDR), 1040 genes exhibited a >1.15 fold change in both sickle cell anemia and VHL R200W homozygotes, among which 297 were up-regulated and 743 down-regulated. Hypoxia strongly induced inflammatory response pathways but suppressed T-cell activation in sickle cell anemia. MAPK8, encoding a mitogen-activated protein kinase important for stress-induced apoptosis, T-cell differentiation and inflammatory responses, was a hypoxia down-regulated gene and played a central role in hypoxic gene regulation in sickle cell anemia according to gene network analysis.

To assess the genetic contribution to hypoxic transcriptional variation among SCD patients, we mapped expression quantitative trait loci (eQTL) for the 1,040 hypoxia response genes. Associations mapping with a focus on local regulatory polymorphisms in 61 SCD patients identified eQTL for 103 of the hypoxia response genes at 5% FDR. We further tested the hypothesis that these hypoxic eQTL potentially underlie heterogeneity in risk of pulmonary hypertension in an additional SCD cohort (University of Illinois cohort). In this cohort, the A allele of an eQTL of MAPK8, rs10857560, was associated with pre-capillary pulmonary hypertension defined as mean pulmonary artery pressure ≥ 25 and pulmonary capillary wedge pressure ≥ 15 mm Hg at right heart catheterization (allele frequency=0.66; OR=13.8, P=0.00037, n=238). This association was confirmed in another independent cohort (Walk-PHaSST cohort) (allele frequency=0.65; OR=11.3, P=0.00025, n=519). These results are congruent with previous findings. Notably, lungs- and PBMC-specific DEGs were also enriched in the same GO terms listed, suggesting the complexity of gene interaction networks contributing to the pathogenesis of IPF. Furthermore, genes involved in “cell motility” biological process was significantly enriched in IPF PBMCs but not in IPF lungs.

Conclusions: Analyzing RNA-seq data of IPF lungs and IPF PBMCs from the same patient has provided a list comprehensive DEGs involved in pathological processes, suggesting an incomplete overlap of DEGs in these tissues.

FINAL ID: 62
DNA damage response during mitosis induces cancer chromosomal instability


Cancer is an evolutionary disease. This evolutionary capacity largely depends on the marked genetic heterogeneity that characterizes tumor cell populations. As such, many cancer types display both structural and numerical chromosomal aberrations. However, the relationship between DNA damage, which leads to structural rearrangements of chromosomes (s-CIN), and mitotic processes causing whole chromosomal instability (w-CIN) remains poorly understood, and the reason s-CIN and w-CIN frequently co-exist in cancer is unknown. Here we show that induction of DNA damage response during mitosis selectively stabilizes the attachments of a subpopulation of microtubules, termed kinetochore-microtubules, to chromosomes. This elevates the rate of whole-chromosome mis-segregation leading to w-CIN and consequently generates a preponderance of micronuclei that predispose chromosomes to pulverization. These events are mediated by the ATM/Chk2 arm of the DNA damage response, which induces mitotic defects through Aurora A and Plk1 kinases. Inhibiting the DNA damage response during mitosis suppresses w-CIN discriminately in cancer cells with inherently persistent mitotic DNA damage, a hallmark of colorectal cancer cells. Furthermore, selectively destabilizing microtubule attachments to chromosomes substantially increases the viability of irradiated mitotic cells and generates radiation resistance in orthotopically transplanted human glioblastoma tumors. Thus, DNA damage response in mitosis that results from s-CIN collaboratively induces w-CIN and negatively impacts cellular viability. Our work uncovers the synergistic relationship that governs structural and numerical cancer chromosomal instabilities and provides a mechanism for the generation of continuous genetic heterogeneity during tumor evolution.

FINAL ID: 64
STATHMIN PROTEIN EXPRESSION MAY BE MORE RELIABLE THAN P16 PROTEIN EXPRESSION AS A MARKER FOR HIGH-GRADE CERVICAL AND LARYNGEAL DYSPLASIAS.

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Introduction: The dysplasia-carcinoma continuum has been studied extensively for high papilloma virus-driven cervical and laryngeal disease. While morphology is the mainstay of detection and grading of dysplasia, there are many situations where immunohistochemistry for p16 assists in grading of dysplasia and for therapeutic decision making in head and neck cancer. However, there are limitations to the use of p16. We tested a new antibody to stathmin, a 17-kDa cytosolic protein that is involved in cellular proliferation in both normal and malignant cells, as an alternate for p16. Earlier studies have demonstrated that p16 has greater sensitivity for cervical squamous dysplasia but that stathmin has greater specificity for high grade lesions.

Methods: Ten cervical and ten laryngeal biopsies from individuals with morphologically-proven dysplasia were immunostained with antibodies to p16 and stathmin. The areas of dysplasia were scored for expression of the two
antibodies on a scale of 0-3 for intensity. A comparison of positivity for dysplasia with the two antibodies was performed.

**Results:** p16 was found to be strongly positive, in both the nucleus and cytoplasm, in areas of high- and low grade dysplasia. On the other hand, staining was positive in the cytoplasm only in areas of high grade dysplasia and did not stain normal tissue and areas of low grade dysplasia.

**Discussion:** In our study stathmin was able to better distinguish high grade dysplasia from low grade precursor lesions and benign reactive epithelial changes. In comparison to p16, stathmin was more specific for high-grade dysplasia. This technique has the potential to improve and refine the characterization of dysplasia in patients with laryngeal papillomas and cervical dysplasia.

**HEMATOLOGY AND ONCOLOGY**

**FINAL ID: 70**

**LONG TERM ANTICOAGULATION (4 TO 16 YEARS) STOPS THE PROGRESSION OF OSTEONECROSIS IN PATIENTS HETEROZYGOUS FOR THE FACTOR V LEIDEN MUTATION OR WITH RESISTANCE TO ACTIVATED PROTEIN C.**

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In 5 patients, 4 heterozygous for the Factor V Leiden mutation 1 with resistance to activated protein C (RAPC) and Ficat stage 1 or II idiopathic osteonecrosis (ON) of the hips, we assessed whether, and to what degree continuous long term (4-16 year) anticoagulation started before collapse of the head of the femur would prevent progression of ON, ameliorate pain, and facilitate fully functional recovery.

The 5 patients, 1 female African American, 4 Caucasian men, had, at study entry, idiopathic hip ON without collapse (Ficat stage 1 or II), 9 hips. Because each had sustained 2 or more thrombotic events, after 3 months treatment with Lovenox, all 5 were anticoagulated for life with Coumadin, or, subsequently, Xarelto. At follow-up after 4, 9, 13, 16 and 16 years on anticoagulants, there was no progression to hip collapse, their hip Ficat stage II remained unchanged, and there was no progression to severe osteoarthritis. Within 3, 3, 9, and 16 months after initiation of anticoagulation, 4 of the 5 patients were pain-free, and remained asymptomatic throughout follow-up, carrying on their usual daily activities including vigorous exercise, while 1 patient required Percocet for pain. None of the patients sustained clinically significant bleeding during 4 to 16 years of anticoagulation.

The natural history of untreated ON is that 60% to 80% of patients starting at Ficat Stage II (before hip collapse), will progress to collapse, Ficat stage III or IV, requiring total hip replacement within 2 years of initial diagnosis. Thrombophilia mediated by Factor V Leiden heterozygosity or RAPC plays a central etiologic role in the development and progression of ON. Long term anticoagulation can very favorably change the natural history of ON.

**FINAL ID: 72**

**MEDICAL TREATMENT OF IDIOPATHIC OSTEONECROSIS OF THE KNEE: A PRELIMINARY STUDY**

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**Background:** In patients with idiopathic knee osteonecrosis, we determined prospectively whether Enoxaparin or Metanx could prevent collapse and rapid progression to osteoarthritis, ameliorate pain, and facilitate full function. We assessed relationships of thrombophilia-hypo fibrinolysis and the eNOS T786C mutation to osteonecrosis.

**Methods:** Methods: Of 15 patients (3 (3 knees) stage I, 11 (17 knees) II, 1, (2 stage III), 6 with thrombophilia-hypo fibrinolysis were treated with Enoxaparin (40-60 mg/day for ≥ 3 months) and (5 homocysteinemia) with Metanx (L-methylfolate/B6/B12) for ≥ 3 months with reassessment every 4-6 months and repeat X-rays every year. The amount and duration of Enoxaparin were mandated by an FDA-approved protocol.

**Results:** Of 6 Enoxaparin-treated patients, with follow-up for ≥1 year (15.1, 7.5, 3.9, 2.25, 2, 1 years), none progressed to joint collapse or severe osteoarthritis; 4 became and remained asymptomatic and fully functional (2,3,9,7,5,15.1 year follow-up). A 5th patient did not progress to collapse.

is the primary cause of their conjunctival epidermidalization. Potential mechanisms include conjunctival inflammation and dry eye. Since conjunctival epidermidalization constitutes a serious, vision threatening condition, clinicians should be aware of it as a potentially dangerous adverse effect of risedronate sodium.
for pancreatic ductal adenocarcinoma development in normal diet mice. C677T homozygosity, 4G4G homozygosity for the Plasminogen Activator inhibitor, recent sepsis, and severe orongs of bone disease. Serum levels of the Factor V Leiden mutation (FV) is the most common familial thrombophilia, predominantly associated with deep venous thrombosis (DVT) and pulmonary embolus (PE). We hypothesize that ocular thrombosis [central retinal vein occlusion (CRVO)], central retinal artery occlusion (CRAO), amaurosis fugax (AF) is an uncommon but significant presenting manifestation of FV.

Methods: We prospectively performed PCR determinations of FV in 3515 patients seen at The Jewish Hospital of Cincinnati for evaluation of thrombotic events and assessed the nature of thrombotic events in those 286 patients found to be FV heterozygotes. Complete evaluation of coagulation measures was accomplished via PCR assays and serologic measures of thrombophilia and fibrinolysis. Comparisons were made against a group of healthy normal controls (n=105) via chi-squared and Student's unpaired t-test analyses. Personnel and laboratory staff were appropriately blinded to the subjects' diagnosis and severity of illness.

Results: Of the 3515 patients studied, 206 females and 80 males were found to have the FV mutation (8.1% of the cohort; 279 heterozygous and seven homozygous). Of these 286 FV patients, 14 (5%; seven female and seven male; mean age 52±11) had ocular thrombosis as the presenting thrombotic event. Of these 14 patients (all non-smoking), three had CRAO, one had AF, and ten had CRVO. Two women with CRVO were taking Premarin at time of presentation. In 13 of the 14 patients, ocular thrombosis was the initial thrombotic event leading to thrombophilia screening; the other patient had a prior DVT but had not been genotyped. Two of the 14 patients later developed osteonecrosis of the hip and jaw, respectively. Other previously undiagnosed coagulation abnormalities present in the 14 patients included MTHFR C677T homozygosity or MTHFR C677T-A1298C compound heterozygosity in seven of nine (78%) patients compared to 31 of 102 (30%) healthy normal controls (p=0.007), 4G4G homozygosity of the PAI-1 gene in seven of 13 (54%) patients compared to 26 of 100 (26%) normal controls (p=0.05), and low antithrombin III in two of eight (25%) patients compared to two of 92 (2.2%) normal controls (p=0.03).

Conclusions: In a cohort of 3515 subjects having coagulation profiles, with 286 found to have the FV mutation (8.1%), ocular thrombosis was the presenting clinical event leading to testing for procoagulants in 5% of the 286 patients. Although comprising a minority of the clinical thrombotic events and assessed the nature of thrombotic events in those 286 patients found to be FV heterozygotes. Complete evaluation of coagulation measures was accomplished via PCR assays and serologic measures of thrombophilia and fibrinolysis. Comparisons were made against a group of healthy normal controls (n=105) via chi-squared and Student's unpaired t-test analyses. Personnel and laboratory staff were appropriately blinded to the subjects' diagnosis and severity of illness.

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Conclusions: In a cohort of 3515 subjects having coagulation profiles, with 286 found to have the FV mutation (8.1%), ocular thrombosis was the presenting clinical event leading to testing for procoagulants in 5% of the 286 patients. Although comprising a minority of the clinical thrombotic events bringing patients to study of procoagulants, ocular thrombosis should be considered in the broad group of thrombotic events that trigger laboratory assessment of coagulation abnormalities.
in making diagnosis, implicating prognosis and guiding treatment. 2010 and 2013 CAP surveys identified that for HER2 validation most laboratories felt the minimum number of 25 cases was appropriate; however, only 56% of laboratories used this minimum number and only 66% of predictive markers were revalidated after introduction. This highlights the fact that an easily used and cost effective process is needed in order to validate and revalidate HER2. We question whether the incorporation of tissue microarrays (TMAs) with an adequate number and appropriate types of specimens could serve as the gold standard for antibody validation, in the validation and revalidation of HER2.

Methods: Seventy five specimens with mammmary carcinoma were identified and added to two TMAs by using TMA Master (Caliper 3D HISTECH) with 34 and 41 samples (1.5mm each) respectively, and stained using HER2 antibody (Ventana, 4B5 clone). All grades and stages of mammary carcinoma and ranges of ER/PR status were used. All cases were compared using fluorescence in-situ hybridization performed by two other academic centers.

Results: The tissue microarray chips are tested for HER2/neu antibody IHC validation. The immunostaining of each tissue sample is evaluated by three independent pathologists in double-blind manner and compared to the genetic test results of HER2/neu amplification. The recut chips are also sent to other clinical laboratories for IHC validation. The quality, accuracy, and cost of the IHC validation by using tissue microarray is equivalent to the traditional validation and verification process. However, the TMA provide effective platform for further quantitative measure of the level of HER2 expression in a more accurate and reliable pattern. The cost to validate HER2 using TMA significantly decrease the cost 30 times compared to the traditional validation ($104 vs. $3002).

Conclusions: The recent CAP surveys have identified a critical need for easily performed and cost effective processes for the validation and revalidation of HER2. The incorporation of tissue microarrays into routine daily practice is an effective tool for the initial validation of HER2 and will result in even greater cost savings for ongoing validation due to changing analytic variables. Savings are greatly enhanced if onsite staff is capable of creating TMAs.

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**FINAL ID: 82**

**ACUTE MYELOID LEUKEMIA: PRETREATMENT BIOCHEMICAL AND CLINICAL VARIABLES PREDICTIVE FOR RISK STATUS BASED ON VALIDATED CYTOGENETIC AND MOLECULAR ABNORMALITIES**

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**Introduction:** Insurance status has been found to affect treatment outcomes in various solid tumors. However, less data is available in patients with acute myeloid leukemia (AML). We conducted a comprehensive retrospective analysis to further investigate whether insurance status affects treatment outcomes in AML patients. Our analyses evolved to explore the role potential biochemical and clinical pre-treatment variables play in affecting treatment outcomes and predicting known cytogenetic risk groups.

**Methods:** From 2000 to 2011 we identified 269 patients with AML at the University of Oklahoma Health Sciences Center. A total of 217 patients with usable medical records were included in the final analysis. In the study, 28 pretreatment variables were examined to assess their effect on prognosis in terms of treatment outcomes and their ability to predict cytogenetic/molecular risk status. Cytogenetic risk groups were created based on the National Comprehensive Cancer Network (NCCN) guidelines algorithm. Primary outcomes were complete remission (CR), relapse, and overall survival (OS) rates. In order to assess survival, Kaplan-Meier curves and log rank tests of equality were performed on categorical variables. Cox proportional hazard models were used to assess continuous variables. A multivariate Cox proportional hazard model was created to explore associations between overall survival times with all covariates. Pre-treatment variables were then used to predict cytogenetic risk status using a multinomial logistic regression.

**Results:** Of the 217 patients (52.2% males, 47.8% females) included in the study, 81.5% were white, 9.0% African American, and 6.2% Native American. Median age at diagnosis was 51.0 years. 36.3% had private insurance, 45.8% had public insurance, and 17.3% were uninsured. There was no significant association found between insurance status and treatment outcomes (CR, OS, and relapse rates). Cytogenetic risk status was independently associated with insurance status. Cytogenetic risk status was also significantly related to complete remission (p=0.0007), status at last follow up (p = 0.0405), and overall survival (p = 0.0002 for better-risk and p < 0.0001 for intermediate-risk, compared to poor-risk group).

Among 28 tested variables, we found 3 factors significantly predict cytogenetic/molecular risk status. Having the better-risk group as the reference group, diabetes (OR-12.7, 95% CI = 1.44-111.7) and elevated creatinine (OR-0.07, 95% CI = 0.00-0.95) were predictive for intermediate-risk, where as diabetes (OR-6.9, 95% CI = 1.07-44.8) and elevated uric acid (OR-0.60, 95% CI = 0.39-0.91) were predictive for poor-risk group.

**Conclusion:** Based on cytogenetic and molecular classification, our results on survival analysis are consistent with previous reported studies. There was no association between treatment outcomes and insurance status. Among the 28 pretreatment variables studied, we found diabetes, uric acid, and creatinine might be useful markers in predicting cytogenetic risk category. This could aid in risk-adaptive treatment plans before cytogenetic tests become available or tests prove to be unfeasible to be done. Larger studies are needed to corroborate our findings.

**INFECTIOUS DISEASE**

**FINAL ID: 90**

**RISK FACTORS FOR RECURRENT CLOSTRIDIUM DIFFICILE INFECTION: A SYSTEMATIC REVIEW AND META-ANALYSIS**

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**Background and Aim:** The risk of acquiring Clostridium difficile infection (CDI) during a hospital admission is estimated to be 1 in 100 patients with a 10% risk of in-hospital death. In the past 2 decades, the prevalence of hospital-acquired CDI has more than doubled in the United States. CDI therapy is initially effective for most patients, but an estimated 20-30% of patients develop symptomatic recurrence within 2 weeks of successful completion of therapy. While the mechanism of recurrence has not been elucidated, a variety of risk factors have been suggested. Our aim was to evaluate current evidence on the risk factors for recurrent CDI (rCDI).

**Methods:** We searched MEDLINE, Web of Science, Cochrane library, and SCOPUS databases for the following search terms: CDI, risk factor, predictor, marker, relapse, recurrence and recurrent . All studies that investigated the risk factors of rCDI using multivariate methods were considered eligible. Information on assessed risk factors was collected and data were combined by means of a random-effects model. Pooled odds ratios (ORs) and 95% CIs were calculated.

**Results:** Of the 310 citations identified, 31 studies (n= 16,280 patients) met the inclusion criteria. The most frequent risk factors associated with rCDI were advanced age, antimicrobial therapy and use of proton pump inhibitors (PPI). Age (per year increase) and ≥ 65 years were associated with an increased risk of rCDI (OR 1.02, 95% CI 1.01-1.02, P=0.001 and OR 1.63, 95% CI 1.22-2.17, P=0.001 respectively), as was additional non-CDI antibiotic therapy after treatment (OR 2.18, 95% CI 1.63-2.93, P<0.0001). Risk was also greater for patients who were prescribed fluoroquinolones before the initial occurrence of CDI (OR 1.48, 95% CI 1.19-1.83, P=0.0004) and patients taking a PPI during CDI treatment (OR 1.80, 95% CI 1.17-2.77, P=0.007).

**Conclusion:** Advanced age, additional antimicrobial therapy during follow up and use of PPI during CDI treatment were associated with a greater risk of developing recurrent CDI. In older patients with a history of CDI, judicious use of antibiotics and PPIs might help reduce CDI recurrence.

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FINAL ID: 92
SEVERE RHABDOMYOLYSIS AS THE PRESENTING SIGN OF HIV INFECTION
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A 29-year-old African-American homosexual male presented to the hospital with a 4-day history of nausea, vomiting, diarrhea, and abdominal pain. He had similar but less severe symptoms two months prior after taking cephalaxin and TMP-SMX for a subungual infection. He reported taking TMP-SMX 4 days ago (just prior to his current symptoms) for the same subungual infection. He admitted to sexual activity with one monogamous male partner and endorsed using protection. He denied history of sexual transmitted infection, sick contacts, trauma, myalgia, skin rash and illicit drug use. He drinks alcohol infrequently. He denied use of over-the-counter medication.

Pertinent findings on physical exam included tachycardia with heart rate 103 beats per minute, blood pressure 160/86 mm Hg, dry oral mucosa with oral thrush, mild epigastric tenderness to palpation, and a left thumb healing ulcer with partially removed nail without erythema or discharge. Labs included WBC 13 K/uL, hemoglobin 11.4 g/dL, and platelets 94,000 K/uL. Chemistry profile showed blood urea nitrogen 67 mg/dL, creatinine 3.4 mg/dL, total creatine kinase 70,000 U/L, lactate dehydrogenase 9802 U/L, total bilirubin 1.3 mg/dL, AST 606 U/L, ALT 182 U/L, ESR 74 and haptoglobin 304 mg/dL. Urinalysis showed large blood without red blood cells, negative drug screen, and no schistocytes on blood smear. He was diagnosed with acute kidney injury secondary to rhabdomyolysis and was treated with IV fluid. He was evaluated for HIV and hepatitis. Hepatitis panel was negative, but HIV 1.2 antibodies tested positive and this was confirmed by western blot. Patient’s viral load was 420,000 copies/mL and CD4 count was 3 cu mm. During his stay his creatinine, CK, AST and ALT improved with IV fluid treatment.

Common etiologies of rhabdomyolysis in general include crush injury, prolonged immobility, states of myositis, seizures, strenuous exercise or heat stress, hypokalemia or hypophosphatemia, severe volume contraction, and influenza. This patient had no injury, prolonged immobility, strenuous exercise, or history of seizures, no abnormalities of potassium or phosphorus, and he was not hypovolemic. Polymyositis was considered less likely because of the extremely high level of creatine kinase (350 times the upper limit of normal, whereas in polymyositis 5-50 times is expected). Hemolytic uremic syndrome (HUS), G6PD deficiency, and TMP-SMX (trimethoprim-sulfamethoxazole)-induced rhabdomyolysis were also considered. The patient had no schistocytes on peripheral blood smear, anemia was consistent with chronic disease, and renal function improved quickly with IV fluids, thus HUS and G6PD were unlikely. HIV infection itself can cause rhabdomyolysis, rhabdomyolysis may even lead physicians to suspect undiagnosed HIV infection. It is important to recognize this adverse effect in HIV patients. TMP-SMX-associated anemia in 1973. While rhabdomyolysis associated with TMP-SMX is rare, it is important to recognize this adverse effect in HIV patients. TMP-SMX-associated rhabdomyolysis may even lead physicians to suspect undiagnosed HIV infection if this adverse effect occurs.

Background: Solid organ transplant (SOT) patients are at increased risk of infections due to immunosuppressive treatment. Previously, we had reported that pediatric small bowel transplant (SBT) patients have a high incidence of blood stream and systemic fungal infections (70% each). Clostridium difficile infection (CDI) is reported in between 3-31% of adult SOT recipients. The type of transplant and use of corticosteroids are factors associated with significant risk of CDI in adult SOT patients. However, the risk factors for CDI in pediatric SBT patients have not been investigated.

Aim: To investigate the risk factors for CDI and the impact of CDI on survival within one year from the date of transplant in pediatric SBT recipients.

Methods: We identified 12 pediatric SBT patients (cases) who developed CDI within one year of their first transplant during the study period 2000-2009. We matched the cases by their age, by the year of transplant and by the type of transplant with 36 SBT recipients who did not develop CDI during the first post-SBT year (controls). Using univariate regression analysis, we investigated the effect of patient and treatment related factors on occurrence of CDI.

Results: The mean age at the time of transplant was 2.7±2.3 years for cases and 2.5±2.9 years in controls (p=0.81). Cases and controls were comparable in terms of ethnicity, gender, induction therapy for transplant, and maintenance immunosuppression drugs. However, compared to controls, cases had a significantly higher percentage of CMV sero positivity among transplant recipients (66.7% vs. 24.3%, p=0.007). In univariate analysis, CMV sero-positivity of the recipient (OR=6.22, 95% CI=1.51-25.64, p=0.011), presence of jejunostomy (OR=6.0, 95% CI 1.48-24.27, p=0.012) and use of a proton pump inhibitor (PPI) in the preceding 4 weeks (OR=4.2, 95% CI=1.06-16.58, p=0.04) were significantly associated with CDI. Due to the small sample size, multivariate analysis could not be performed. Overall survival (measured from the date of transplant) was not significantly different between cases and controls (7.9±0.7 vs 8.1±1.3 years, p=0.81).

Conclusion: CMV positive sero-status of the recipient, use of a PPI within four weeks preceding CDI and presence of a jejunostomy were found to be significant risk factors for CDI in this cohort. CDI did not seem to influence overall survival in our pediatric SBT patients.

Significance: Our study suggests greater vigilance for occurrence of CDI in SBT patients with jejunostomies and/or on PPIs.

FINAL ID: 96
VITAMIN D, BACTERIAL VAGINOSIS, AND THE VAGINAL MICROBIOME
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Objective: Several studies suggest that low serum vitamin D levels are associated with increased prevalence of bacterial vaginos (BV), a highly prevalent vaginal infection that is causally linked to adverse reproductive outcomes. BV develops when the concentration of healthy Lactobacillus species in the vagina declines and is replaced by other bacterial species. We recently conducted a randomized, double-blinded, placebo-controlled, 24-week trial of high-dose vitamin D supplementation in 118 women with BV, to examine the effect of vitamin D plus standard metronidazole therapy on subsequent BV recurrence. The purpose of the substudy described here was to characterize the composition of the vaginal microbiota of 30 participants (15 in the vitamin D arm and 15 in the control arm) at enrollment and after 4 weeks and 24 weeks in the trial. We assessed whether vitamin D supplementation led to meaningful shifts in the composition and diversity of the vaginal microbiome over time.

Methods: Non-pregnant, reproductive-age women with BV were recruited from a public sexually transmitted disease clinic in Columbus, OH. Using self-reported adherence data (taking vitamin D or placebo pills on time, completing the full metronidazole course at enrollment, and returning for all scheduled study visits), 30 “perfect compliers” were selected from the full study population for inclusion in this substudy. Bacterial species and genera from vaginal samples collected at baseline and after 4 and 24 weeks were identified by sequencing the V1 to V3 region of the 16S rRNA gene using a Roche 454 GS FLX instrument. Sequence reads were used as queries for a blast search of
an extended version of the CORE database that included non-redundant sequences from the vaginal reference package. Changes in community diversity over time were analyzed using the Shannon diversity index.

**Results:** Women randomized to vitamin D and placebo had similar vaginal microbiota at enrollment, considering both species composition and diversity. Lactobacillus iners was the most prevalent bacterial species detected in both groups at enrollment. Median Shannon diversity index was 1.8 among women randomized to vitamin D (range: 0.96 to 2.1) and 1.9 (range: 0.96 to 2.6) among women randomized to placebo. Decreases in microbial community diversity—suggesting a shift toward vaginal health—were observed in both the vitamin D and placebo arms 4 weeks after enrollment, with a less pronounced shift in women taking vitamin D (median Shannon diversity index: 1.0, range: 0.04 to 2.5) than among women randomized to placebo (median Shannon diversity index: 0.3, range: 0.0 to 2.1). By the 24-week visit, community diversity in both groups had increased—suggesting a shift toward BV near enrollment levels (median Shannon diversity index in vitamin D arm: 1.6, range: 0.0 to 2.3; in placebo arm: 1.3; range: 0.01 to 2.33).

**Conclusions:** Although we detected changes in the composition and diversity of participants’ vaginal microbial communities over time, we observed no significant differences between randomization groups after 24 weeks. Vitamin D supplementation, in addition to standard metronidazole therapy, does not alter vaginal microbiota. These findings agree with those of the parent trial, which found no significant difference in BV recurrence between randomization arms.

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**Nephrology**

**FINAL ID: 100**

**THE VALUE OF REPEAT KIDNEY BIOPSY OF QUIESCENT LUPUS NEPHRITIS**

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Currently the effectiveness of LN therapy is determined mainly by improvements in the proteinuria and renal function. The histologic response to therapy is generally not evaluated because serial kidney biopsies are not usually obtained in patients who have improved. Furthermore, discontinuation of therapy in responders is a clinical decision, and kidney pathology is not taken into account. Serial renal biopsies were done in a cohort of Hispanic LN patients (n=25) as part of the local standard of care. These biopsies were correlated with clinical response to determine the concordance of clinical and histologic findings. Biopsies were done at the time of diagnosis (BX1), after 6 months of induction therapy with either MMF (2.4g/d) or cyclophosphamide (1g/mo X6) (BX2), and during maintenance therapy with either MMF or azathioprine, after at least 42 months of total treatment, and at least 24 months of clinical inactivity (BX3). Biopsies were read by a single renal pathologist (VA) and NIH activity (AI) and chronicity (CI) indices were calculated. Changes in serum creatinine (Scr) and proteinuria (Pr) were calculated over time. Between Bx1 and Bx3 Scr and Pr improved significantly, (1.0±0.4 vs 0.80±0.3 mg/dl and 3.3±2.1 vs 0.3±0.2 g/dl respectively, P<0.001). The AI between Bx1 and Bx3 declined (9±4 vs 1.9±1.7, P<0.001), and the CI increased between Bx1 and Bx3 (2.8±1.4 vs 4±2.1, P<0.05) but did not increase between Bx2 and Bx3. However looking at individual patients who were complete responders (n=16, SCR normal; PR 0-0.5g/d) only 11 had an AI of 0-1 at Bx3, and 3 patients had an AI>2 at Bx3 (range 3-5). A multivariate analysis was done to identify predictors of AI at Bx3. The independent variables associated with improvement in AI at Bx3 were initial therapy with cyclophosphamide, improvement in Scr between Bx1 and Bx2, and increase in complement component C4 between Bx1 and Bx3.

In conclusion, these data demonstrate discordance between clinical and histologic responses in LN patients on immunosuppressive therapy for over 3 years, with 30% of complete responders still having active histologic lesions. The implications of this continued low-level activity for discontinuation of therapy remains to be determined.

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**Final ID: 102**

**PLACENTAL EXPRESSION OF RENIN-ANGIOTENSIN SYSTEM COMPONENTS AND (PRO)REIN RECEPTOR IN NORMAL AND PREECLAMPTIC PREGNANCY**

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**Objective:** Preeclampsia (preE), a syndrome of hypertension, proteinuria, and each patient compared to the normal pregnant (NP) patient. In this study, the placentals expressions of (P)RR, angiotensin type 1 (AT1), angiotensin type 2 (AT2) and placental concentration of angiotensin II (AngII) were evaluated in preE patients and in a rat model of preE as well as in nonhuman primates.

**Study Design:** (1) Placentas were collected from 10 NP and 10 preE consenting patients in an IRB approved prospective study at Scott & White Healthcare, Temple, Texas. Inclusion criteria for determination of preE patients included blood pressure ≥140/90 and presence of proteinuria ≥100 mg of protein/24h in urine. Samples of placenta and umbilical cord were collected from those subjects after deliveries. (2) An established rat model of preE and NP rats (n=10 each) was used to evaluate the expression of (P)RR, AT1 and AT2. Placental homogenates were analyzed to determine the concentration of (pro)renin and AngII by commercially available kits. (3) The placentals samples from squirrel monkey (NP; n=10) and owl monkey (both early and term, NP; n=2 each) were collected. (P)RR, AT1 and AT2 expressions were measured by gel electrophoresis of the placental and umbilical cord homogenates followed by detection with western blotting (WB). Immunohistochemistry (IHC) was also utilized to visualize (P)RR, AT1 and AT2 expression.

**Results:** The expressions of (P)RR (2.1 fold, 2.5 fold), AT1 (1.6 fold, 1.9 fold) and AT2 (1.8 fold, 2.1 fold) were significantly higher (p<0.05) in the placenta and umbilical cords, respectively, of preE patients compared to normal pregnancies as evaluated by WB and IHC. The placental expressions of (P)RR (2.8 fold), AT1 (1.8 fold) and AT2 (2.2 fold) were also significantly higher (p<0.05) in preE rats compared to NP. The placental concentrations of (pro)renin (preE: 352±41; NP: 172±28 pg/mL) and AngII (preE: 57±6; NP: 41±3 fmol/mL) were significantly elevated (p<0.05) in the preE patients compared to NP patients. The early placentas of owl monkey expressed higher (p<0.05) levels of (P)RR compared to term placentas. The (P)RR was expressed in the placentals samples from squirrel monkey as evaluated both by WB and IHC.

**Conclusions:** These data suggest that increased expression of (P)RR, AT1, AT2, AngII, and (pro)renin in the placenta are related to the occurrence of preE in both human patients and animal models. The higher expression of (P)RR in early owl monkey in comparison to term placentas suggests that the (P)RR is important for normal placental development. The expression of (P)RR in nonhuman primates revives the approach of future studies on owl monkey and squirrel monkey preE models.

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**Final ID: 104**

**ACTH A NOVEL TREATMENT FOR IGA NEPHROPATHY**

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IgA nephropathy is the most prevalent primary chronic glomerular disease worldwide. Despite this, treatment options remain limited as current data on immunosuppressive therapy including MMF and cyclosporine have been inconclusive. This leaves supportive therapy and corticosteroids to be the cornerstone of current therapy for aggressive disease. We report a case of
IgA nephropathy treated with ACTH with marked improvement in proteinuria and stabilization of renal function.

Our patient was treated conservatively with fish oil and ACE-1 prior to presenting to our clinic. He had marked elevation in both proteinuria in addition to rising creatinine. A renal biopsy was performed showing IgA nephropathy and patient was started on course of prednisone. His proteinuria persist and was started on Cellcept. However, his renal function continues to decline and his serum creatinine is now 3 ml/min with a proteinuria of 3.3 g/g.

Given the progression of his disease, a repeat renal biopsy was performed showing significant mesangial staining for IgA, IgM and C3. At this point, patient had failed prednisone and Cellcept; a decision was made to start an ACTH gel therapy. His serum creatinine stabilizes at 2.5-3.0 ml/dL and proteinuria decreases to 800 mg/g.

ACTH analog has been used in Europe with success in treating idiopathic membranous nephropathy dating to the 1990’s. In the United States a published retrospective case series reporting treatment with ACTH gel therapy in idiopathic nephrotic syndrome reported complete or partial remission in 11 out of 21 patients. As of now, one patient had IgA nephropathy and achieved complete remission with ACTH gel therapy. The studied population had failed on an average of 2.3 immunosuppressive regimen prior to ACTH gel therapy. Likewise, our patient has failed both prednisone and MMF, therefore we believe that ACTH gel therapy is a viable option for patients with progressive IgA nephropathy who has failed prednisone and MMF therapy.

Case Report: A 54yo man with strangulated inguinal hernia had emergent bowel resection. He stayed intubated for more surgery on day 2 & 3, all under sevoflurane. On d1, urine specific gravity was 1.005. He had since been polyuric up to 4-9 L/day.Serum (S)Na,139 mEq/L at day 1, rose to 165 by day 6. By deleting IV sodium & giving free water, S Na fell to 154 by day 7. Polyuria (10-11 L/d) remained & urine osm fell from 823 Osm/kg on day 6 to 190 by day 8. Despite serum osm of 331, ADH was repeatedly <0.8 pg/ml, confirming DI. Sevoflurane via FiO2 can cause nephrogenic diabetes insipidus (NDI). To differentiate CDI from NDI, he got 80ug IV DDAVP & raised Uosm to 563 & 578 respectively by 3rd & 5th day. Peak Uosm of 756 post-DDAVP showed intact concentration ability.

On MRI, posterior pituitary had no T1 hyperintensity (i.e. no ADH) confirming CDI. With delirium from increased S Na & no PO intake due to intubation, he could not drink to prevent dehydration. Thus he got 11 days of DDAVP to keep S Na 139-149 & allow extubation. When conscious & talking, he denied history of DI but admitted to 12L of water intake per day for 30 years. For 7days before discharge, S Na was normal by ad lib fluids.

Conclusions: This man shows classical elusive nature of DI due to usually effective polyuria. He became dehydrated when prolonged intubation precluded drinking and perpetuated his inactivity to voice & quench thirst & aggravated S Na, which in turn caused more encephalopathy. Once lucid, drinking kept S Na normal better than DDAVP&CDI was diagnosed by >500 mosm/kg rise in Uosm with DDAVP, but peak Uosm of only 756 belied a partial NDI. Loss of MRI T1 hyperintensity in posterior pituitary confirms its utility to diagnose CDI.

FINAL ID: 106

ACTIVATED OMENTUM SLOWS PROGRESSION OF CHRONIC KIDNEY DISEASE


Introduction: Two groups of rats were studied, an experimental group which underwent 5/6 nephrectomy (removing left kidney and 2/3 of the remaining kidney) and a control group underwent 5/6 nephrectomy as well as complete omentectomy. Polydextran particles were added intraperitoneally only in the experimental group in order to activate the omentum and facilitate its attachment to the injured kidney. Control omentomized rats did not receive polydextran particles.

Methods: After 12 weeks the experimental rats having omentum attached to the remnant kidney had 30% lower plasma creatinine and 50% lower urea nitrogen levels, 30% less glomerulosclerosis, 30% less tubulointerstitial injury, reduced extracellular matrix and reduced thickening of basement membranes. A fusion zone formed between the injured kidney and the omentum abounded in sc-1, Wt-1 transcripts and proteins, increase of HGF and IGF-1 levels, increased number of proliferating cell nuclear antigen (PCNA), Ki-67 (an index of proliferation activity), and CD 34 positive cells suggestive of an active and healing tissue; and kidney function tests improved in the experimental group in order to activate the omentum and facilitate its attachment to the injured kidney.

Results: After 12 weeks the experimental rats having omentum attached to the remnant kidney had 30% lower plasma creatinine and 50% lower urea nitrogen levels, 30% less glomerulosclerosis, 30% less tubulointerstitial injury, reduced extracellular matrix and reduced thickening of basement membranes. A fusion zone formed between the injured kidney and the omentum abounded in sc-1, Wt-1 transcripts and proteins, increased number of proliferating cell nuclear antigen (PCNA), Ki-67 (an index of proliferation activity), and CD 34 positive cells suggestive of an active and healing tissue; and kidney function tests improved in the experimental group in order to activate the omentum and facilitate its attachment to the injured kidney.

Conclusions: These results suggest that the activated omentum attached to the injured kidney and slowed the progression of chronic kidney disease. The effect appears to be brought about by the presence of osteomal cell stem cells and their secretary products in the vicinity of the injured kidney.

FINAL ID: 108

CENTRAL DIABETES INSIPIDUS (CDI) : UNDIAGNOSED FOR 3 DECADES TILL UNMASKED BY DEHYDRATION DUE TO ENDOTRACHEAL INTUBATION AND STUPOR

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DI is marked by excessive dilute urine usually controlled by polydipsia. We here report a man with chronic CDI undiagnosed till revealed by severe hypernatremia from inavertant water deprivation due to prolonged intubation & stupor.

FINAL ID: 110

FIBRINOGEN AMYLOIDOSIS WITH CNS MANIFESTATION

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Fibrinogen amyloid is the most common form of familial amyloidosis. Organ involvement is mainly renal with sporadic reports of cardiac involvement. Most patients progress to end stage renal disease. Combined liver and kidney transplantation is curative. In this case report we present a case of fibrinogen-A alpha amyloidosis which was diagnosed after a kidney biopsy. Although the patient presented with low grade proteinuria and chronic kidney disease, the presence of unusual CNS findings prompted kidney biopsy. The biopsy showed a Congo red positive stain and amyloid fibrils on electron microscopic examinations. Mass spectroscopy analysis of the renal biopsy revealed a fibrinogen-A alpha amyloid and genomic sequence analysis revealed the common mutation E526V.

Brain biopsy was done to evaluate the brain lesions. The brain biopsy stained with Congo red showed only florescence and immunohistochemistry using fibrinogen specific antibodies showed staining of the perivascular spaces as well as within astrocytes. In systemic amyloidosis, more evidence is accumulating that significant pathology and tissue damage occur as a result of amyloid monomer deposition long before the full scale amyloid plaque accumulation. Previous experimental data showed that serum amyloid protein cross the blood brain barrier and stabilize amyloid formation only after disruption of the barrier. This might explain the perivascular fluoresence.

This is first time fibrinogen amyloid is shown to produce CNS pathology. Genetic predisposition might have precipitated the development of CNS manifestation in our patient. Although his family history of significant for unexplained kidney failure and heart disease, none of this family members were diagnosis with amyloidosis despite some receiving kidney transplant. This highlights the importance of kidney biopsy in patients with poorly explained renal disease.

PEDIATRICS

FINAL ID: 116

THE HIGHER EXPRESSION OF APOPTOTIC AND STRESS SIGNALING MARKERS IN PREECLAMPTIC UMBILICAL CORDS AND PLACENTAS INDICATE AN ADVERSE IMPACT ON OFFSPRING

Objective: Preeclampsia (preE), a syndrome of hypertension and proteinuria in pregnancy, is thought to be initiated with alterations of placental function. Hypoxia and oxidative stress can lead to placental apoptosis. preE is the most frequent complication of pregnancy. It is associated with endothelial dysfunction in the mother, which is related to the release of circulating vasculotoxic factors and the induction of augmented oxidative stress by the diseased placenta. These circulating factors may pass the placental barrier and leave persistent defects in the circulation of the offspring that may predispose to a pathological response later in life. We assessed apoptotic signaling in umbilical cord from patients with or without preE. We also evaluated the status of these stress signaling markers in a rat model of preE.

Methods: In this study, we recruited 10 normal pregnant (NP) and 10 preE consenting patients in an IRB approved prospective study from Scott & White hospital, Temple, Texas. Inclusion criteria for determination of preE: patients include blood pressure >140/90 and presence of proteinuria >300 mg of protein/24h urine. Samples of placenta and umbilical cord were collected from those subjects after deliveries. Two groups of rats were also used in this study to assess the stress signaling markers. These rats were: normal pregnant (n=10) and preE rats (n=10) which were given weekly injections of deoxycorticosterone acetate and 0.9% saline to drink. Apoptotic and stress signaling proteins; Bcl-2-associated X protein (Bax), pro-apoptotic Bcl-2 protein (Bad) and pro-inflammatory protein cyclooxygenase-2 (Cox-2) expression were assessed both by western blot and immunohistochemistry. The p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation was evaluated by western blot. Comparisons were performed using ANOVA with Duncan’s post-hoc test.

Results: Expression of Bax (Placenta: 1.2 fold, Cord: 1.5 fold), Bad (Placenta: 1.7 fold, Cord: 1.7 fold), Cox-2 (Placenta: 0.8 fold, Cord: 2.5 fold) and p38 MAPK phosphorylation (Placenta: 1.5 fold, Cord: 1.7 fold) were up-regulated (p<0.05) in placental tissues and umbilical cords of preE compared to NP patients. Apoptotic signaling was upregulated in preE rats placenta (Bax: 1.4 fold, Bad: 2.3 fold, Cox-2: 2.5 fold, p38: 3.0 fold) compared to NP. We did follow up examination of the babies for both groups of patients to assess the pregnancy outcome. Average hospital stay for preE babies were significantly longer than those of NP babies (3.4 vs 6.1). There were no complications has been reported for the NP babies. However, Out of 10 babies; 1 had hypoglycemia; 4 had hyperbilirubinemia; 3 had respiratory distress syndrome; 1 had bilateral polydactyly with bilateral syndactyly; 1 had large for gestational age; 1 had fetal ascites; 1 had intrauterine growth restriction.

Conclusions: Apoptotic signaling is augmented in preE which may lead to reduced nutrient transport capacity triggering placenta release of vascular factors that modulate maternal and fetal vascular responses characteristic of this syndrome. PreE alters the intracellular environment by modulating the pattern of hormonal signals and activating the detrimental cellular signaling that has been transported to the fetus. The fetus has to adapt to this impending intracellular environment and detrimental signaling and this adaptation increases the risk of disease to the offspring.

PULMONARY/CRITICAL CARE

FINAL ID: 118
EPIGENETIC REGULATION OF P. AERUGINOSA MEDIATED ACUTE LUNG INJURY BY SINGPOSINE KINASE 2
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Rationale: Sphingolipids have emerged as important signaling molecules that can regulate a vast range of cellular processes, including cell survival, proliferation, migration, differentiation and inflammation. Although many enzymes are involved in regulating the levels of the sphingolipids, sphingosine kinase 1 (SphK1) and sphingosine kinase 2 (SphK2) are of particular interest. The role of SphKs in inflammation and immune cell function has been widely investigated with most studies focusing on SphK1, while limited evidence of SphK2 in inflammation is controversy.

Methods: In vivo, wild type, SphK1 knockout and SphK2 knockout mice were challenged with P. aeruginosa strain PA03 (1x105 cfu/mouse) intratracheally for 24 h. Bronchial alveolar lavage (BAL) fluid was performed. Pulmonary permeability was assessed by measurement of BAL protein and cell count. Lung inflammation and injury was assessed by measurement of BAL TNF-α, IL-1β, IL-6 and MIP-2. Lung tissue was collected for histology analysis and TUNEL staining for apoptosis. Acetylation of histone protein H3 and H4 in the lung was analyzed by Western blot. In vitro, mouse lung epithelial cell line (MLE12) and human lung microvascular endothelial cell (HLMVECs) were pretreated with vehicle, SphK1 inhibitor (5µM), or SphK2 inhibitor (5µM) followed by PA03 treatment (heat killed 1x108 cfu/ml). Cell culture medium was collected for TNF-α, IL-1β, IL-6, MIP-2/IL-8 measurements. Cell lysate was collected for detection of acetylation of H3/H4. SphK2 phosphorylation and localization was assessed by immunofluorescent staining. MLE12 and HLMVECs monolayer permeability were assessed by measurement of transmonolayer electrical resistance (TER).

Results: P. aeruginosa induced significant lung inflammation and injury in wild type and SphK1 knockout mice, but in SphK2 knockout mice. There is significant acetylation of H3 and H4 in the lung of wild type and SphK1 knockout mice after P. aeruginosa challenge, which is attenuated in SphK2 knockout mice. P. aeruginosa induced obvious alveolar endothelial and endothelial cells apoptosis, which is attenuated in SphK2 knockout mice. P. aeruginosa induced significant acetylation of H3 and H4 in both MLE12 cells and HLMVECs, which is inhibited by SphK2 inhibitor, but not by SphK1 inhibitor. Phosphorylation and nuclear translocation of SphK2 was observed in both MLE12 and HLMVECs upon P. aeruginosa treatment. TNF-α, IL-6 and IL-1β were increased in the cell culture medium of MLE12 and HLMVECs after P. aeruginosa treatment. SphK2 inhibitor significantly attenuated the inflammatory response to P. aeruginosa.

Conclusion: Epigenetic regulation of inflammatory genes expression is a critical mechanism underlying P. aeruginosa mediated lung inflammation and injury. P. aeruginosa activates SphK2, resulting in histone protein acetylation and initiation of inflammatory genes expression. Therefore, targeting SphK2 may serve as highly effective therapeutic targets against P. aeruginosa mediated lung inflammation and injury.

FINAL ID: 120
ARG INHIBITION EXACERBATES ENDOTHELIAL DYSFUNCTION INDUCED BY PATHOLOGIC STRETCH
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Introduction: Ventilator-Induced Lung Injury (VILI) is a pathophysiologic syndrome that can occur in critically ill patients supported by mechanical ventilation (MV). VILI results from lung overdistention and is characterized by increased alveolar-capillary permeability, endothelial dysfunction and infiltration of inflammatory cells into the lungs. Imatinib is a pharmacologic inhibitor of tyrosine kinases with clinical efficacy against some malignancies that recently has been identified as a possible endothelial barrier protective agent. In contrast, preliminary studies from our laboratory indicate that imatinib exacerbates VILI-induced endothelial dysfunction and inflammation in vivo. In the present study, we further explore the mechanisms by which imatinib exerts its effects on cultured lung endothelium exposed to mechanical stretch in vitro.

Methods: Human pulmonary artery endothelial cells (ECs) were pre-treated with imatinib (0-40 µM) and then exposed to pathologic cyclic stretch (18% CS, 24h). VCAM and VE-cadherin protein expression levels were determined by western blotting. Cytokine levels (IL-8 and IL-6) were measured in cell supernatants by ELISA. For permeability studies, ECs were exposed to 18% CS for 24h, then replated onto microelectrode plates, treated with imatinib, and transendothelial electrical resistance (TER) determined. Small interfering RNA knock-down of imatinib target kinases, c-abl and Arg, was used to characterize the differential roles of these kinases in regulating human lung EC function.

Results: In lung ECs exposed to pathologic stretch in vitro, imatinib (40µM) induced significant upregulation of VCAM, a cell adhesion protein that is NADH-dependent, and loss of VE-cadherin, an important cell-cell junctional protein. In the presence of imatinib, IL-8 levels were significantly increased after CS compared to vehicle-treated CS cultures, whereas IL-6 levels were decreased. In addition, imatinib increased permeability in preconditioned stretched ECs. Selective downregulation of individual tyrosine kinases with siRNA revealed that inhibition of Arg expression induces loss of VE-cadherin after exposure to CS, while inhibition of c-Ab1 expression had no effect. Conclusion: These studies demonstrate that imatinib exacerbates endothelial barrier dysfunction induced by pathologic mechanical stretch, and that this response is mediated in part through inhibition of the Arg tyrosine kinase. Future investigation into the Arg-related mechanisms responsible for these...
effects may provide useful insights into the pathogenesis of VILI in critically ill patients.

FINAL ID: 122
A MYLK VARIANT REGULATES ASTHMATIC INFLAMMATION VIA ALTERATIONS IN MESSENGER RNA SECONDARY STRUCTURE
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The C721T polymorphism within the gene encoding myosin light chain kinase (MYLK) is significantly associated with severe asthma in African Americans. Here we further examine the molecular function of this single nucleotide polymorphism (SNP) located in the non-muscle isomform (nmMLCK) in asthma susceptibility and pathology. We identified nmMLCK variant (C721T) with a distinct mRNA secondary structure from other MYLK coding variants. The secondary structure of this nmMLCK variant (721C) is more stable with an elongated half-life in human lung endothelial cells, and this SNP conferred increased efficiency in protein translation initiation due to an increased accessibility to translation start site. Finally, nmMLCK expression of C721- and T721-containing MYLK transgenes was compared in nmMLCK--mice and confirmed deleterious effects of nmMLCK expression on asthmatic inflammatory indices and implicated the augmented influence of MYLK C721T SNP on asthma severity. These studies indicate that non-synonymous SNPs may lead to phenotypic influences via alterations in protein expression as well as by changes in protein structure and function. The confirmation of this novel mechanism of the regulation of asthmatic inflammation by MYLK advances knowledge of the genetic basis for asthma disparities and further underscores the therapeutic potential of targeting nmMLCK.

FINAL ID: 124
GENE EXPRESSION PROFILING OF NON-INJURIOUS MECHANICAL VENTILATION IN AN ANIMAL MODEL OF SEPSES-INDUCED ARDS
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Rationale: Caspase-12, an inflammation caspase, regulates cytokine levels in in vitro system can be used as a tool to study hypoxia-induced endothelial dysfunction. This in vitro system can be used as a tool to study hypoxia-induced endothelial dysfunction. This in vitro system can be used as a tool to study hypoxia-induced endothelial dysfunction. This in vitro system can be used as a tool to study hypoxia-induced endothelial dysfunction. This in vitro system can be used as a tool to study hypoxia-induced endothelial dysfunction.
higher survival rate in a sepsis model. Because caspase-12 regulates cytokine production, we sought to determine whether caspase 12 deficiency protects mice against bleomycin-mediated acute lung injury and fibrosis.

**Methods:** Caspase-12 deficient (caspase-12/-/-) mice and their WT siblings (7-wk old) were used in this study. Bleomycin sulfate (0.05 U/mouse, dissolved in phosphate-buffered saline [PBS] solution) was delivered into lungs via intratracheal instillation after anesthesia, with PBS as a control. Mice were harvested in day 7 (D7) and day 21 (D21) after bleomycin instillation. Lungs were lavaged with 1 ml cold PBS solution. Total cell number, protein concentration and cytokines (IL1, IL6, TNFx, interferon-gammar [IFNG]) in bronchoalveolar lavage fluid (BALF) were measured. In separated experiments, lungs were harvested for mRNA isolation and collagen measurement. The isolated mRNA was used to measure collagen and fibronectin levels normalized to the expression of a housekeeping gene, hexose-6-phosphate dehydrogenase by Taqman gene expression assays. Collagen protein levels were measured and normalized the total protein amount using the Sircol Collagen Assay kit.

**Results:** Our data show that caspase-12/-/- mice have significantly lower BALF total cell number and protein concentration than their WT siblings in both D7 and D21 bleomycin-treated groups (p < 0.05), while no significant difference for BALF total cell number and protein concentration was found between caspase-12/-/- and WT mice without bleomycin treatment. In terms of cytokine levels in BALF at the D7 group, IFNG and IL6 were significantly lower in caspase-12/-/- mice than in WT mice (IFNG: [22.79 +/- 4.28] vs [51.54 +/- 5.23] μg/ml, p = 0.0046; IL6: [16.11 +/- 1.78] vs [33.02 +/- 4.16] μg/ml, p = 0.011). IL1 and TNFx levels were lower in caspase-12/-/- mice, but not statistically significant. At the D21 group, IL1 and TNFx levels were significant lower in caspase-12/-/- mice than in WT mice (IL1: [18.66 +/- 4.52] vs [9.44 +/- 3.15] μg/ml, p = 0.032; TNFx: [122.9 +/- 56.24] vs [502.50 +/- 150.60] μg/ml, p = 0.017), but no significant difference in IL6 and IFNG levels occurred between caspase-12/-/- and WT mice. Real-time PCR and the Sircol Collagen assays did not show significant difference in collagen and fibronectin mRNA and collagen protein levels in lungs between caspase-12/-/- and WT mice, respectively.

**Conclusion:** Caspase-12 deficiency protects against bleomycin-mediated acute lung injury but not fibrosis in mice.

**Funding:** Northwestern Faculty Foundation Dixon Innovation Research Award and Pfizer Investigator-initiated Grant (JC)

**RHEUMATOLOGY/IMMUNOLOGY/ALLERGY**

**FINAL ID: 134**

**PITYRIASIS RUBRA PILARIS TREATED WITH USTEKINUMAB**

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**Introduction:** We report a 43-year-old man who presented with a severe pityriasis rubra pilaris (PRP) successfully treated with ustekinumab. Therapeutic options described for PRP comprise topical vitamin D analogues, keratolytics, systemic acitretin, methotrexate, cyclosporine, azathioprine, fumaric acid esters, phototherapy and anti-TNF. Three PRP cases successfully treated with ustekinumab have been reported yet.

**Observations:** The patient presented at our clinic with a generalized puritic skin rash of recent onset. Physical examination revealed palmoplantar hyperkeratosis, erythema of the face and the scalp, as well as keratosis pilaris on the trunk and limbs with erythematous plaques on the elbows. Our differential diagnosis comprised PRP or guttate psoriasis, and the diagnosis of type 1 PRP was confirmed by histological examination. A topical therapy comprising vitamin D analogues and steroids was insufficient, with a worsening of the skin status and deformation of the nails. A treatment with acitretin or methotrexate being contraindicated in this patient because of a status post drug-induced hepatitis with elevated transaminases, we introduced a treatment with anti-interleukin (IL)-12/23 (ustekinumab). The patient noted a rapid improvement, with a regression of the lesions after two weeks already, and a complete resolution after one month. Six months (4 injections) after ustekinumab introduction, the patient is still in remission with a treatment suppression. Three months after the last injection, the skin status stays in remission.

**Discussion:** Ustekinumab, a monoclonal antibody approved for the treatment of psoriasis, acts by inhibiting the p40 subunit shared by IL-12 and -23. In psoriasis Th17 cells, activated by IL-23, produce IL-17 and -22. These cytokines are key mediators linking the adaptive immune response to keratinocyte dysregulation. IL-22 induces keratinocyte hyperproliferation, inducing acanthosis, which is also a PRP characteristic. This let us hope for a response to ustekinumab in this dermatosis, as recently confirmed in the three recently published cases.

**Conclusion:** Ustekinumab represents an interesting therapeutic alternative for PRP resistant to retinoids or in patients having contraindications to retinoids. Supplementary studies are now required to determine the safety and efficacy of this treatment in PRP.

**FINAL ID: 136**

**INFLAMMATORY PROTEASE, CASPASE-1, IS RELEASED FROM MONOCYTES IN A STABLY ACTIVE FORM IN RESPONSE TO ENDOTOXIN**

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**Introduction:** Caspase-1 is an inflammatory protease, which is part of the cysteine-aspartate protease (caspase) family, and is required for cleavage of the acute pro-inflammatory cytokines, interleukin-1β (IL-1β) and IL-18, as well as for a form of cell death termed pyroptosis. Caspase-1 is synthesized as a zymogen which requires the assembly of a multi-protein complex, termed the inflammasome, for its activation. This assembly forms in response to pathogen or danger associated molecular patterns, triggering an acute inflammatory response, but where and how this assembly happens in the cell is poorly understood. To address this question we compared the activity of caspase-1 in the released fraction as compared to the cytosolic form in the human monocyte cell line, THP-1.

**Results:** In response to endotoxin, pro-caspase-1 is rapidly processed and released from monocytes in its mature, p20/p10, form. We found that the released form has activity (4.2 ±1.3 AU/min) by WEHD-afc cleavage which is stable for over 12h and this activity is inhibited by the tetra-peptide caspase-1 specific inhibitor (YVAD-cmk). Released activity was enhanced in...
the presence of serum in the media. Interestingly, however we were unable to completely immunodeplete the released caspase-1 activity or show its ability to cleave exogenously added proIL-1β. This contrasts to the in-vitro cell-extract model, where concentrating monocytic lysates to 10μg/μl spontaneously activates caspase-1. This cytosolic caspase-1 cleaves endogenous proIL-1β but rapidly loses function, with a t(1/2)=12 min.

Conclusions: These results suggest that caspase-1 activation requires a multi-level regulation that links assembly of the inflammasome complex and enzymatic activity to its interaction with its target substrates during release. Understanding these differences between the released and cytosolic inflammasomes provides a novel opportunity to gain fundamental insight into the details that regulate the acute inflammatory response.

FINAL ID: 138
A NOVEL MICROBIOME-ASSOCIATED LIPID PLAYS A PUTATIVE IMMUNOREGULATORY ROLE IN MULTIPLE SCLEROSIS
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Multiple sclerosis (MS) is a widespread and devastating disease. The microbiome has been implicated in the pathogenesis of many autoimmune diseases; however, its role in MS is inconclusive. We have characterized a novel pathogen-associated molecular pattern and toll-like receptor 2 agonist, called Lipid 654, which is produced by many gastrointestinal commensal bacteroidetes species. We found that Lipid 654 is not only present in human serum but that its levels are also significantly lower in the serum of MS patients. The purpose of this study was to translate our human findings into murine models to more precisely study the role of Lipid 654 in MS. To do this, we first measured endogenous levels of Lipid 654 by MRM-mass spectrometry in serum, spinal cord, and brain of mice with and without experimental autoimmune encephalomyelitis (EAE). Additionally, we determined the effect of treatment with exogenous Lipid 654, purified from Porphyromonas gingivalis, on EAE disease severity. We found that endogenous levels of Lipid 654 in serum, brain and spinal cord were reduced in mice with EAE. Additionally, Lipid 654 treatment significantly reduced EAE disease severity. Together, these results suggest an immunoregulatory role for Lipid 654. Paralleling our human studies, Lipid 654 is higher in the serum of healthy mice compared to mice with EAE. Additionally, our findings suggest that Lipid 654 or its downstream effects may be utilized in the treatment of MS. Finally, by moving to a murine model, we plan to further dissect the molecular mechanisms underlying Lipid 654's role in EAE and MS.

FINAL ID: 140
THE IMPACT OF GOT ON PATIENT’S LIVES AND DIFFERENCE BY GENDER AND RACE: A PATIENT PERSPECTIVE
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Background: No qualitative research has been done in African-Americans with gout, who bear a major burden of gout. Past research offers little or no knowledge in women with gout.

Objective: To examine the impact of gout on QOL and study differences by gender and race.

Methods: Eight race- and sex-stratified nominal groups were conducted, oversampling for African-Americans and women with gout. Patients presented, discussed, combined and ranked their concerns.

Results: 52 patients with mean age 66.3 years, 52% men, 77% African-American, participated in eight nominal groups: African-American men (n=23; 3 groups); African-American women (n=18; 3 groups); Caucasian men (n=5; 1 group); and Caucasian women (n=6; 1 group). The most frequently cited highly-ranked concerns among the eight nominal groups were: (1) effect of gout flare on daily activities (n=8); (2) work disability (n=7); (3) severe pain (n=6); (4) joint swelling and tenderness (n=6); (5) food restrictions (n=6); (6) medication related issues (n=4); (7) difficulty with shoes (n=4); (8) sleep disruption (n=3); (9) emotional Impact (n=3); (10) dependency on family and others (n=3); and (11) interference with sexual function (n=3). Compared to men, women ranked the following concerns high: problems with shoes (n=4 vs. 0); dependency (n=4 vs. 1); and joint or limb deformity (n=2 vs. 1). Compared to Caucasians, African-Americans more often ranked the following concerns high: dietary restrictions (n=6 vs. 0); severe pain (n=6 vs. 0); gout bringing the day to a “halt” (n=2 vs. 0); effect on emotional health (n=2 vs. 0); and the need for canes/brutches during flares (n=2 vs. 0).

Conclusions: Gout has significant impact on patient’s QOL. Important differences in the impact of gout by gender and race were noted.

CARDIOLOGY/CARDIOVASCULAR DISEASE

FINAL ID: 3
AMELIORATION OF Atherosclerosis Enabled by RECOGNITION of THROMBOPHILIA AND ANTICOAGULATION
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Premise: In patients with CVD with a progressive downhill course despite maximal lipid lowering, commonly have previously undiagnosed thrombophilia interacting with atherosclerosis, producing rapidly progressing ischemia.

Introduction: Most familial and acquired thrombopathias increase venous thrombosis but, in the presence of atherosclerosis, can accelerate arterial occlusion with rapidly progressing ischemic despite optimal lipid lowering interventions with diet medications.

Case Description: We describe 3 men, one woman, non-smokers free of type 2 diabetes mellitus, all with angiographically documented occlusive coronary disease. Their CVD was clinically and symptomatically refractory to...
Patient #1: 76 year old male with a history of myocardial infarction (MI) at the age of 49 had treatment refractory chest pain. He developed atrial fibrillation, was successfully cardioverted and started on Coumadin therapy with immediate resolution of angina. After cessation of Coumadin following resumption of sinus rhythm, he complained of progressively severe angina unresponsive to nitrates. After documentation of the Lupus anticoagulant he was restarted on Coumadin and has remained asymptomatic for 37 years.

Patient #2: 52 year old male with history of MI at age 42 underwent PCTA and later repeat PCTA. Chest pain persisted and progressive occlusion led to a 5 vessel CABG 4 years later. 1 year later, and again secondary to chest pain, a 3rd PCI was done with 5 stents to diseased grafts. He was found to have High Factor 8 and Factor 9, On Coumadin therapy, symptoms improved, and he was stable for 4 years. After being taken off Coumadin because of bruising, he had resurgence of angina, underwent PCI for 4th time, had new native vessel lesion. He was restarted on Coumadin and symptoms have begun to resolve.

Patient #3: 41 year old male had MI at 25 with refractory chest pain found to have anti-phospholipid antibody syndrome. He was started on Coumadin therapy, rapidly became asymptomatic and now, 16 years later, has not suffered any cardiac complications and has remained chest pain free.

Patient #4: 57 year old female with history of CABG at 48, presented with chronic angina. She was found to have high factor 8 and be homozygous endothelial nitric oxide synthase mutation and Plasminogen activating inhibitor mutation 4G:5G heterozygote. Patient was started on Coumadin and demonstrated significant symptomatic improvement. Coumadin was stopped secondary to mild bleeding but patient’s symptoms returned and eventually wished to be restarted on Coumadin. She has been asymptomatic and in stable cardiac health since.

**Discussion:** These cases illustrate the morbidity benefit realized from discovery of underlying thrombophilia and treatment with Coumadin. The interaction between existing and progressive atherosclerotic disease with underlying thrombophilia can be qualitatively assessed but quantitative benefit is evident from these cases. While, the underlying thrombophilia was addressed much later in life for these individuals it certainly brings into question the possibility of decreased CVD clinical and symptomatic burden had diagnosis been made and treatment started earlier—an argument for screening of thrombophilia in individuals with treatment refractory CVD and even possibly early CVD.

**FINAL ID: 5**

**INCREASED OXIDATIVE STRESS AND INFLAMMATION IN A MOUSE MODEL OF DILATED CARDIOMYOPATHY**

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Mutations within contractile proteins cause dilated cardiomyopathy (DCM), a leading cause of heart failure. However, it is unclear whether the resulting contractile dysfunction is associated with increased oxidative stress and inflammation. In this study, we tested whether inflammation and oxidative stress are associated with the development of DCM and contractile dysfunction mediated by mutation in the cardiac myosin binding protein-C (MYBPC3) gene. The present study utilized a mouse model of DCM, expressing a previously characterized homozygous C’ mutation in the MYBPC3 gene (cMyBP-C<sup>C’</sup>). DCM and contractile dysfunction were confirmed in the hearts of cMyBP-C<sup>C’</sup> mice with echocardiographic analysis revealing increased left ventricular internal diameter and reduced ejection fraction and fractional shortening. Histopathological analysis indicated increased chamber size, heart area, and fibrosis while immunohistochemical staining showed elevated CD68 positive cells in DCM hearts, verifying the presence of inflammation. Oxidative stress was observed in DCM hearts by the increased and decreased levels of GSSG and GSH/GSSG ratio, respectively. Electron paramagnetic resonance (EPR) spectroscopic study demonstrates the reduced signals of mitochondrial semiquinone radical and Fe-S centers in the hearts of DCM animals. In addition, the increased EPR signals of CM<sub>6</sub> formed from the oxidation of spin probe CMH by the reactive oxygen species validates the increased oxidative stress in the hearts of DCM animals. These results suggest that oxidative stress and inflammation

**FINAL ID: 4**

**VASCUULAR STEM CELL THERAPY OF THE DIABETIC RETINA WITH COMP-ANG1 AND ENDO THELIAL PROGENITOR CELLS**

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**Background:** Diabetic retinopathy (DR) is the leading cause of blindness in the working-age population. Hyperglycemia-induced loss of retinal vascular cells and vascular dysfunction are primary pathophysiologic mechanisms of DR, which are unaddressed by current therapies. Restoring vascular homeostasis and replacing lost endothelial cells (reparative angiogenesis) could reduce the neurovascular damage that occurs in DR. An important factor lost in diabetic retinopathy is Angiopoietin-1 (Ang1), which promotes endothelial survival and vascular stability. Outgrowth endothelial cells (OECs) are a specific subtype of endothelial progenitor cell that have the potential to reintegrate into damaged vascular beds and promote reparative angiogenesis. The purpose of this study was to determine whether a novel Ang1 analog, COMP-Ang1, could prevent the structural and functional hallmarks of DR if given alone before the onset of retinopathy and reverse diabetic damage if given after the onset of retinopathy in conjunction with OECs by increasing OEC integration into the diabetic retina.

**Methods:** Diabetic Ins2Akita mice were treated at 2 months (before the onset of DR) with a single intravitreal dose of adeno-associated virus (AAV2) encoding COMP-Ang1 (AAV2.COMP-Ang1). These mice were compared to those treated with a control virus (AAV2.AcGFP) or PBS injection. Four months later, vascular structure (retinal flatmount staining and treypsin digest) and function (Evans blue dye and microbroad permeability assays) were compared between groups. Inflammation was assessed by acridine orange and leucocyte fluorography. Neuronal structure and function were measured using spectral domain optical coherence tomography and optokinetic tracking response, respectively. A second cohort of 7 month-old mice (after the onset of DR) were treated with AAV2.COMP-Ang1 (or control) as well as labeled OECs (harvested from the mononuclear layer of donated cord blood). Three days later retinas were harvested and analyzed with confocal microscopy to assess OEC integration into the retinal vasculature.

**Results:** AAV2.COMP-Ang1 preserved vascular structure in 6-month-old diabetic mice and decreased acellular capillary formation compared to controls in the first cohort of mice. This was accompanied by preservation of vascular function, demonstrated by decreased vascular leakage. Additionally, AAV2.COMP-Ang1 decreased retinal inflammation shown as a decreased number of leucocytes adherent to the vascular wall. These results correlated with increased VE-cadherin and decreased VEgf-A expression in the retina of AAV2.COMP-Ang1 treated mice. Furthermore, retinal neuronal structure and function were preserved with AAV2.COMP-Ang1 therapy. Retinal thinning and ganglion cell layer dropout were prevented and treated mice retained near-normal visual acuity and electoretinographic response compared to controls.

OEC migration speed, tube formation, and Akt phosphorylation were increased by COMP-Ang1 in a dose-dependent manner in vitro. In the second cohort of mice, preliminary results suggest that COMP-Ang1 increases OEC integration into the retina and stabilizes vasculature compared to control. These results demonstrate a reparative role for OECs in the diabetic retina.

**Conclusions:** AAV2.COMP-Ang1 may be useful in preventing diabetic neurovascular dysfunction. Vascular and neuronal health were preserved in diabetic mice despite persistent hyperglycemia. COMP-Ang1 can enhance the intrinsic angiogenic properties of endothelial progenitor cells. OECs and COMP-Ang1 may play a functional reparative role in diabetic retinopathy and other diabetic microvascular diseases. Future studies will determine whether newly integrated OECs reduce functional deficits in DR.

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are associated with the development of DCM mediated by contractile protein dysfunction.

**FINAL ID: 7**

**RELATIONSHIP OF PULMONARY ARTERIAL COMPLIANCE AND PULMONARY VASCULAR RESISTANCE IN CRITICALLY ILL MECHANICALLY VENTILATED SURGICAL PATIENTS**

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**Background:** Pulmonary arterial capacitance (PAC), a recently proposed determinant of right ventricular afterload, has been shown to be a better predictor of mortality than pulmonary vascular resistance (PVR) in pulmonary arterial hypertension. In healthy individuals and in patients with pulmonary artery hypertension, PVR and PAC are inversely related. The understanding of the hemodynamic response of the pulmonary circulation in mechanically ventilated critically ill surgical patients remains limited. We prospectively determined the relationship between serial PAC and PVR and compared mean values of PAC and PVR among survivors and non-survivors in the surgical intensive care unit.

**Methods:** Thirty-two critically ill and/or injured mechanically ventilated adult surgical patients admitted to a Level I Trauma Center were enrolled in this 48-hour study. Pulmonary artery catheter derived hemodynamics were obtained every 12 hrs (total = 5 points/patient). Pulmonary arterial capacitance (mL/mmHg) was defined as the ratio of stroke volume over pulmonary pulse pressure. Pulmonary vascular resistance (Wood units) was calculated as the transpulmonary pressure gradient divided by cardiac output. Spearman’s rank correlation assessed relationship. The Independent Samples Mann-Whitney Test compared means of PAC and PVR amongst survivors and non-survivors.

**Results:** For the 32 patients, the mean age was 49±20 years, 69% were male, and 84% were trauma patients with a mean Injury Severity Score of 24±10. Serial PAC showed a strong inverse association with PVR (r = -0.74, -0.62, -0.78, -0.72, and -0.68, p < 0.001). Pulmonary capacitance and right ventricular stroke work index showed a weak inverse relationship (r = non-significant). At 48 hrs non-survivors had lower mean PAC (non-survivors, 3.1±1.5 versus survivors, 5.4±1.7, p = 0.007) and higher mean PVR (non-survivors, 4.1±1.8 versus survivors, 1.8±0.9, p = 0.001).

**Conclusion:** The strong inverse relationship of PAC and PVR is maintained in mechanically ventilated critically ill surgical patients. The prognostic role of PAC in this population should be addressed by larger studies.

**FINAL ID: 9**

**HAPLOINSUFFICIENCY OF CMYBP-C EXACERBATES PRESSURE-OVERLOAD INDUCED HYPERTROPHY**

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**Rationale:** Mutations in MYBPC3, encoding for cardiac myosin binding protein-C (cMyBP-C), accounts for nearly 40% of identified hypertrophic cardiomyopathy (HCM) causing mutations and often results in an absence of protein (haploinsufficiency). Haploinsufficient human carriers and mouse models have variable disease penetrance and late onset HCM.

**Objective:** To determine if symptomatic heterozygous MYBPC3 mutant carriers are at a greater risk for HCM after cardiac stress.

**Methods and Results:** Transverse aortic constriction or sham surgery was performed on 10-12 week old wild-type (WT) and MYBPC3 heterozygous (Het) mice, followed by 12 weeks to allow progression through hypertrophy. Heart weight/ body weight ratios were significantly different post-TAC compared to WT and Het sham (23.0 ± 2.5, 24.8 ± 2.3 %MHC) compared to WT and Het sham (8.5 ± 0.4 %, 12.0 ± 1.0 %MHC). Levels of cMyBP-C assessed by Western blot do not show a significant change in cMyBP-C levels between WT and Het TAC or Sham groups.

**Conclusions:** These observations suggest that haploinsufficiency of MYBPC3 does hasten the pathogenesis of HCM after cardiac insult; however the mechanism causing this increase in hypertrophy is unclear. Further exploration of how heterozygous mutations in MYBPC3 could provide novel therapeutic targets for the large number of human carriers of similar mutations.

**FINAL ID: 11**

**MECHANICAL CIRCULATORY SUPPORT IMPROVES DIABETIC CONTROL IN PATIENTS WITH ADVANCED HEART FAILURE**

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**Purpose:** Heart failure (HF) can contribute to insulin resistance. We evaluated the effect of continuous-flow low ventricular assist device (LVAD) implantation on glycemic control in diabetic patients.

**Methods:** Charts were retrospectively reviewed for 244 patients receiving LVAD support between February 2006 and April 2013. We excluded patients who did not have diabetes or had incomplete data regarding diabetic control. Pre-LVAD variables were compared to data obtained approximately six months post-LVAD.

**Results:** 103 (42%) patients had Type II diabetes mellitus, of whom 66 had complete data for analysis (mean age 59 ± 12 years, 83% men, 83% destination therapy, 62% ischemic). Glycosylated hemoglobin (HbA1c) decreased from 7.5 ± 1.6% to 6.1 ± 1.3% after LVAD support (p<0.0001). Concurrently, fasting blood glucose and insulin requirements decreased. Of the 22 patients taking oral hypoglycemic agents pre-LVAD, six discontinued them successfully post-LVAD. There were no significant changes in weight and body mass index. High-density lipoprotein, creatinine, albumin, and B-type natriuretic peptide also improved significantly (Table). Echocardiographic imaging confirmed improvement of left ventricular ejection fraction and size.

**Conclusion:** Significant improvement in glycemic control is noted after LVAD implantation. Left ventricular unloading with LVAD appears to significantly improve metabolic dysfunction including glycemic control in advanced HF. Prospective studies evaluating diet and level of activity are required to further assess the impact of LVAD therapy on diabetes and other metabolic parameters.

**FINAL ID: 13**

**IMATINIB ATTENUATES VASCULAR DYSFUNCTION IN LPS-INDUCED ACUTE LUNG INJURY**

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**Rationale:** Acute lung injury (ALI) is characterized by inflammation-induced dysfunction of the endothelial cell (EC) barrier lining the pulmonary vasculature, which causes leakage of fluid, protein, and inflammatory cells into the airspaces. The mechanisms underlying ALI are critically dependent on EC cytoskeletal regulation of vascular permeability. Recently published work indicates that the tyrosine kinase Abi regulates the cytokinesis to maintain vascular integrity. Conversely, the closely related kinase abl related gene (arg) regulates actomyosin contraction, lamellipodia dynamics, and focal adhesion (FA) formation to increase endothelial permeability. The tyrosine kinase inhibitor imatinib inhibits both Abi and Arg and is used to treat patients with chronic myelogenous leukemia and other malignancies. However, numerous case reports have established that imatinib can cause significant vascular leak and edema.
Given these effects on permeability and current clinical usage, we sought to determine the effects of imatinib in LPS-induced ALI in vitro and in vivo.

**Methods:** Transendothelial electrical resistance (TER) was measured in LPS-challenged human pulmonary artery ECs treated with imatinib or vehicle. Complementary western blots (WB) for EC functional proteins (VE-Cadherin) and inflammatory markers (VCAM) were conducted. Abl or Arg expression was selectively decreased via siRNA, and the LPS responses were assessed. C57BL/6 mice received intraperitoneal imatinib (75 mg/kg) or saline either immediately prior to, or 4 hours after, intratracheal LPS (1 mg/kg). After 18 hours, bronchoalveolar lavage (BAL) and lungs were harvested. Lung injury was assessed by BAL cell counts, protein content and cytokine levels, as well as Evans blue dye extravasation and histologic lung injury scoring.

**Results:** Imatinib inhibits the LPS induced transendothelial electrical resistance (Figure 1). Imatinib dose-dependently decreased production of inflammatory cytokines (IL6 and IL8) and expression of VCAM in LPS-challenged ECs. Additionally, imatinib restored VE-Cad expression in LPS-challenged ECs. Silencing Abl or Arg attenuated LPS-induced VCAM expression in ECs. Additionally, imatinib treatment, both pre- and post-LPS challenge, significantly decreased LPS-induced vascular leak and inflammatory response in mice. LPS-challenged mice that received imatinib pre-treatment had a decrease in total cell counts of 59% (p=0.025) and a decrease in BAL protein of 53% (p=0.0015).

**Conclusions:** Imatinib dose-dependently inhibits LPS-induced inflammation and vascular leak in vitro and in vivo. These effects may be mediated in part via the tyrosine kinases Abl and Arg, which play a critical role in the regulation of the EC cytoskeleton. Thus, imatinib may be a potential therapeutic option for patients with ALI.

**FINAL ID: 15**

**NOVEL BALLOON CATHETER DEVICE WITH PACING, ABLATING, ELECTROPORATING, AND DRUG ELUTING CAPABILITIES FOR ATRIAL FIBRILLATION TREATMENT - ABLATION INSIDE PULMONARY VEINS AND PREVENTION OF PULMONARY VEIN STENOSIS IN CANINES**

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**Introduction:** Pulmonary veins harbor the triggers for atrial fibrillation (AF) from atrial muscle sleeve extensions. Electrical isolation of this muscle tissue is a common means of treating AF by lesion application encircling the pulmonary vein ostia. However, this method is fraught with AF recurrence because of incomplete ablation lesions and/or reconnections to the atrial myocardial tissue. One method to enhance efficacy of ablation and electrical isolation is to ablate inside the pulmonary veins. This strategy is intentionally avoided because of the complication of pulmonary vein stenosis post-ablation.

**Hypothesis:** Ablation inside the pulmonary vein without the adverse effect of stenosis would be an ideal and efficacious strategy to treat and possibly cure patients with AF.

**Study Rationale and Methods:** Direct ablation of pulmonary vein myocardium provides a more permanent tissue lesion, but can lead to a life-threatening complication of pulmonary vein stenosis. We propose a novel strategy that offers the ability to 1) pace inside the pulmonary veins to detect the desired site for ablation, 2) ablate inside the pulmonary vein with D/C energy to ensure electrical isolation without thermal energy to the tissue, 3) ablation of a pro-fibrotic agent at the site of ablation to prevent tissue hyper-proliferation and or stenosis, thus enabling a safe and permanent tissue ablation. The use of electroporation, along with drug elution may prevent pulmonary vein stenosis, thus enabling a safe and permanent tissue ablation.

**Future Research and Clinical Implications:** If efficacy and safety is displayed in chronic canine studies, we will move towards applying for experimental use of our prototype and novel method to conduct the first-in-human trials for refractory atrial fibrillation patients. Even more groundbreaking will be the understanding of how we can utilize D/C energy to ablate myoccardial tissue without causing thermal injury and therefore provides a very attractive approach for future electrophysiologic ablation.

**FINAL ID: 16**

**AUTOANTIBODIES AGAINST CARDIAC MYOSIN BINDING PROTEIN-C IN PATIENTS WITH DILATED AND HYPERTROPHIC CARDIOMYOPATHY**

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This study sought to determine the presence of autoantibodies (AAbs) targeted to the N-terminal domains of cardiac myosin binding protein-C (eMyBP-C) and to other contractile proteins in patients with dilated (DCM), hypertrophic (HCM) and hypertrophic obstructive (HOCM) cardiomyopathy. The presence of AAbs to cardiac proteins, including eMyBP-C, has been demonstrated to be involved in the progression of myocarditis into DCM. However, the existence of such AAbs in patients with HCM and the antigenic potential of the N-terminal region of eMyBP-C have not been fully elucidated. The presence of AAbs to the N-terminal CO3 domains of eMyBP-C and to cardiac troponin-I (CtnI) and α-tropomyosin (α-TM) were evaluated in the sera of healthy volunteers (n = 14) and in patients with DCM (n = 23), HCM (n = 81) and HOCM before and after surgical septal myectomy (n = 18) by enzyme linked immunosorbant assay (ELISA) and Western blot analysis. AAbs to the N-terminal domains of eMyBP-C, and to CtnI and α-TM, were detected in the sera of a subset of patients with DCM, HCM and HOCM. A significant correlation existed between eMyBP-C and CtnI AAb levels in DCM, HCM and HOCM patients and between eMyBP-C and α-TM AAbs in HCM patients. This study provides evidence for the presence of AAbs targeting the N-terminal region of eMyBP-C in patients with cardiomyopathies. Isolating the antigenic and immunogenic residues within this region will provide further insight into the pathomechanisms by which eMyBP-C-AAbs induce myocarditis and cardiomyopathy.

**FINAL ID: 17**

**B-TYPE NATRIURETIC PEPTIDE IN THE SURGICAL INTENSIVE CARE UNIT: ASSOCIATION WITH WEDGE PRESSURE AND SIGNIFICANCE OF CHANGE**

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**Background:** B-type natriuretic peptide (BNP), a biomarker of hemodynamic stress, may be elevated in critically injured trauma patients without congestive heart failure. However, the clinical usefulness of BNP in critically ill surgical patients is not well established. We prospectively determined the association between serial BNP and pulmonary capillary wedge pressure...
is characterized by abnormal placentation. Marinobufagenin (MBG), a Preeclampsia (preE), a hypertensive disorder of pregnancy, was administered to critically ill mechanically ventilated surgical patients was low to moderate. The change in pulmonary capillary wedge pressure and BNP levels were obtained within 6 hours of admission and then every 12 hours for 48 hours. Delta BNP was calculated as BNP at 48 hours minus initial BNP. Spearman’s rank correlation assessed relationship. The Independent Samples Mann-Whitney Test compared delta BNP amongst survivors and non-survivors. Results: For the 32 patients, the mean age was 49±20 years, 69% were male, and 84% were trauma patients with a mean Injury Severity Score of 24±10. Serial BNP showed low to moderate association with PCWP (r = 0.32, 0.43*, 0.38, 0.20, 0.25*, non-significant except *). Delta BNP at 48 hours for survivors was significantly lower than non-survivors (-101±346 versus 141±263 respectively, p = 0.048).

Conclusion: The association of serial BNP and PCWP in critically ill mechanically ventilated surgical patients was low to moderate. The change in BNP at 48 hours may have a prognostic role.

FINAL ID: 19
STUDYING THE INTERACTION BETWEEN CMYBP-C AND MYOSIN S2
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There are over 5 million patients suffering from heart failure in the United States resulting in over 56,000 deaths each year. In hearts from patients or animal models with heart failure, cardiac myosin binding protein-C (cMyBP-C) is known to be dephosphorylated. Phosphorylation is proposed to modulate the regulatory role of cMyBP-C by controlling cMyBP-C’s N terminal interaction with the Subfragment 2 (S2) region of myosin heavy chain. Dephosphorylation of cMyBP-C binds strongly to S2, while phosphorylation of phosphorylation sites in the M-domain leads to less strong binding. The exact site of interaction has been an intense source of debate. To further investigate this, recombinant COC2 N-terminal domains of cMyBP-C is cross-linked with cMyBP-C binding fragment from S2 (1-126 amino acids) using cross-linkers DST, DTSSP, and BS3. Multiple cross-linking reactions were run in order to optimize cross-linking by changing peptide equilibration time, cross-linking time, peptide concentration, cross-linker type, and cross-linker concentration. Peptides were tested at 5μM and 10μM, with a cross-linker concentration ranging from 0μM, 10μM, 50μM, 100μM, and 500μM. We determined the optimal reaction conditions to be 30 minutes of equilibration at room temperature followed by 30 minutes of cross-linking on ice with the cross-linker BS3. Next, we tested the effects of phosphorylation on binding. Two recombinant CO-C2 peptides that replace phosphorylatable Serines were used. One phosphoablated (AAA), the other phosphomimetic (DDD). Unexpectedly, cross-linking occurred in both the AAA and DDD, when we expected only the AAA to cross-link. Further investigation is needed to establish the exact sites of interaction.

FINAL ID: 20
CARDIOTONIC STEROIDS INDUCE APOPTOTIC AND STRESS SIGNALING IN FIRST TRIMESTER CYTOTROPHOBLAST CELLS
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Objective: Preeclampsia (preE), a hypertensive disorder of pregnancy, is characterized by abnormal placentation. Marinobufagenin (MBG), a cardiotonic steroid (CTS), inhibits cytotrophoblast (CTB) cell functions that are critical for normal placental development. In a previous study, we have demonstrated that CTSs induce anti-angiogenic and anti-proliferative effects in CTB cells. This study tests the hypothesis that CTSs induce apoptotic and stress signaling in CTB cells.

Methods: Human extravillous CTB cells of the line Sw.71, derived from first trimester chorionic villus tissue, were incubated with 0, 0.1, 1, and 100 nM of each of three CTSs (MBG, cinobufatulin (CINO) and ouabain (OUB)) for 48 hours. Some cells were pretreated with a p38 inhibitor (SB203580). Thereafter, cell lysates were utilized to measure the p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation by western blot (WB). Levels of angiogenic and anti-angiogenic factors (sFLT-1, sENG, VEGF165, PIGF) and IL-6 were measured in the media by ELISA kits. To evaluate the apoptotic signaling, pro-apoptotic Bel-2-associated X protein (Bax), pro-apoptotic Bel-2 protein (Bad) and pro-inflammatory protein cyclooxygenase-2 (COX-2) expressions were assayed both by WB. Statistical comparisons were performed using analysis of variance with Duncan’s post hoc test.

Results: The p38 MAPK phosphorylation was upregulated (p<0.05) in CTBs in the presence of ≥1 nM CTSs (CINO, MBG, OUB). The anti-angiogenic factors (sENG and sFLT-1) and IL-6 were upregulated, while the angiogenic factors (VEGF and PIGF) were downregulated in the presence of ≥1 nM CTSs. The expression of Bax, Bad, Cox-2 were upregulated (p<0.05) in CTBs treated with ≥1 nM CTSs compared to basal. The SB203580 (a p38 inhibitor) pretreatment showed an attenuation of CTSs-induced apoptotic and stress signaling proteins in CTB cells.

Conclusions: Exposure of CTBs to ≥1 nM CTSs (MBG, CINO, OUB) induced the apoptotic and stress signaling by downregulation of VEGF and PIGF and upregulation of sENG, sFLT-1, and IL-6. CTSs induced the apoptotic and stress signaling in CTBs by upregulating of Bax, Bad and Cox-2 protein expression. The attenuation of CTSs-induced upregulation of apoptotic and stress signaling proteins by SB203580 pretreatment suggests the involvement of stress signaling mechanisms in CTBs dysfunction.

FINAL ID: 21
INTERLEUKIN 22 DOWN REGULATES ABCG1 AND IMPAIRS CHOLESTEROL EFFLUX IN MACROPHAGES
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Background: IL-22 belongs to the IL-10 cytokine family and is expressed by T helper cells. IL-22 functions on epithelial cells and has been shown to improve epithelial barrier functions. IL-22 is induced locally in inflammatory bowel disease, asthma, and psoriasis. Patients with psoriasis have increased coronary artery disease and it was previously shown that macrophages from patients with psoriasis have impaired cholesterol efflux. The function of IL-22 on macrophage cholesterol metabolism is not known.

Methods: ABCA1, ABCG1 and CD36 mRNA and protein expression, cholesterol uptake and efflux were studied in murine macrophages and human THP-1 macrophages. C57BL6/J mice with transgenic expression of S100A12 and S100A8/9 in myeloid cells were generated by using a bacterial artificial chromosome (hBAC/S100 mice). hBAC/S100 and WT littermates were bred into mice lacking receptor for advanced glycation end products, RAGE.

Results: Peritoneal macrophages from hBAC/S100 mice had reduced ABCG1 mRNA and protein expression, increased cholesterol uptake and reduced cholesterol efflux compared to WT. This was abolished in hBAC/S100 mice lacking RAGE, the receptor for S100/calgranulin. Recombinant S100A12 or S100A8 protein (2.5ug/ml) had no effects on ABCG1 expression in WT peritoneal macrophages or human THP-1 cells suggesting other systemic intermediary products in hBAC/S100 mice. Plasma IL-22 and mRNA in bone marrow derived macrophages were increased in hBAC/S100 mice and this was abolished in mice lacking RAGE. Moreover, IL-22 mRNA increased by 2 fold in cultured human THP-1 cells in response to rS100A12. Importantly, THP-1 treated with rIL-22 (100ng/ml) had reduced expression of ABCG1 and impaired cholesterol efflux compared to mouse se rum (mostly HDL), but not ApoA1. Up regulation of ABCG1 and ABCA1 in response to LXR agonist TO901317 abolished the detrimental effects of IL-22 on cholesterol efflux.
Conclusion: S100/calgranulin promotes secretion of IL-22 in a RAGE dependent manner. IL-22 down regulates ABCG1 and impairs cholesterol efflux in macrophages. This raises the hypothesis that elevated IL-22 associated with autoimmune diseases may improve epithelial barrier function via down regulation of cellular cholesterol efflux but thereby could possibly augment atherosclerosis.

For example, repetitive bilateral or unilateral arm training or shifting body weight to the affected lower extremity is utilized in rehabilitation interventions for people with hemiplegia. Pushing an object with one hand while standing with one foot in front (described as a combined asymmetry) is often used during occupational and leisure activities. Individuals with unilateral impairments commonly perform daily tasks implementing such combined asymmetries. However, the effects of both asymmetries on postural control are not well investigated. The aim of the present study was to investigate effects of symmetric and asymmetric stance and pushing movement on anticipatory and compensatory postural adjustments. Ten healthy volunteers stood symmetrically (feet parallel) or asymmetrically (one foot forward and the other backward) and pushed a handle with both hands or right or left hand. Bilateral electromyography (EMG) activities of the trunk and leg muscles were recorded and the EMG data were integrated during the two epochs: 1) from -150 ms to +50 ms (anticipatory postural adjustment, APA) and 2) from +50 ms to +250 ms (compensatory postural adjustment, CPA) in relation to the initiation of pushing movement. Center of pressure (COP) displacements in the anterior-posterior (AP) and medial-lateral (ML) directions were recorded and analyzed during the APAs and CPAs. Isolated asymmetry of stance was associated with larger muscle activity of the backward leg while isolated asymmetry of pushing movement induced larger trunk muscle activity on the contra-lateral side. A combined asymmetry of stance and pushing movement resulted in the increase or decrease of the thigh muscle activity and COP displacement in the ML direction depending on whether both asymmetries were induced on the same side of the body or on the opposite sides. Both isolated and combined asymmetries affect APAs and CPAs in pushing. The findings highlight the importance of considering the isolated and combined effects of asymmetry-related changes in postural control when optimizing the work and leisure environment. Given the fact that rehabilitation interventions involving asymmetric and symmetric arm movements are not always effective in resolving impairment and disability in individuals with stroke, future studies of the effect of combined asymmetries in individuals with unilateral impairment are needed.

**FINAL ID: 47**

**IMPROVING PATIENT SATISFACTION: RESIDENT LED INITIATIVE TO PROVIDE REAL TIME FEEDBACK**

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Background: In the continuously evolving realm of medical education and healthcare reimbursement, patient satisfaction is an integral part of evaluating physician and hospital performance. HCAHPS scores account for 30% of the Value-based purchasing (VBP) score with three of the questions focusing specifically on physician-patient communication. Results are only available long after patient discharge and residents are rarely involved in improving these scores.

Purpose: Our aim was to integrate real-time feedback about patient satisfaction in resident training in order to impact performance and meet Milestones for practice-based learning.

Methods: We devised a twelve-item questionnaire to assess various aspects of the physician-patient relationship. The questionnaire was administered by a resident to patients cared for by 4 Internal Medicine resident teams. Each patient was surveyed once during their hospitalization. Each team was made aware of their patient’s pooled responses at weeks 1 and 3 of their 4 week rotation. After giving the teams real-time results, we continued to measure patients’ responses and evaluated the impact on their satisfaction.

Results: A total of 55 patients completed the questionnaire. Patient responses in five of the seven key question areas improved significantly over the course of the study. 97% of patients perceived that their physicians spent enough time with them on rounds at week 3, improving from 83% during week 1. At week 1, only 75% of patients believed their physicians communicated their daily plan of care with them. By week 3, this improved to 84%. When asked if they understood their laboratory/test results, patients who responded “well” or “perfectly” improved from 58% to 84% from week 1 to week 3. Additionally, when asked if their physician adequately answered their questions, responses of “usually” or “always” improved from 67% to 97%. Finally, perception of good communication with family improved from 78% to 96%.

Discussion or Conclusion: Negative patient satisfaction scores have potentially serious consequences on hospital rankings and reimbursement. For
residents, we found that providing real-time feedback about their performance led to a remarkable improvement in their patients’ perception.

**FINAL ID: 48**

**A PEER MENTORING PILOT PROJECT TO IMPROVE DIABETES OUTCOMES**

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The prevalence of diabetes has increased at an astounding rate over the last three decades, placing an enormous strain on health care systems across the globe. Furthermore, despite intensive efforts, many individuals with diabetes do not achieve their glycemic targets and are at heightened risk of diabetes complications. The development of low-cost interventions to improve diabetes care is critical to limit the individual and societal burden of this disease. The University of Chicago Kovler Diabetes Center’s Peer Mentoring Program is a health behavior change intervention to help patients improve their diabetes outcomes by increasing patient empowerment and augmenting patients’ sense of social connectedness. This intervention was piloted by recruiting six patients with diabetes mellitus from the Kovler Diabetes Center.

The goal was to determine whether a peer mentor modeling was feasible and whether the intervention was seen as a positive experience by study participants, and survey data were collected to assess changes in attitudes regarding diabetes after the intervention. The study used a primarily qualitative design that consisted of diabetes education, role playing, and discussion sessions. In addition, the study participants were asked to contact their peers by phone once weekly outside of the group meetings. Approximately 10 weeks after the initial implementation of the Program, participants were invited to attend a focus group facilitated by Kovler Diabetes Center staff. Of the six Program participants, four shared their views regarding the intervention, duration, and focus of the Program.

Program participants also completed surveys that assessed their attitudes regarding diabetes before and after the intervention. All participants found the Program to be beneficial in helping them self-manage their diabetes as well as giving them tools to assist others in doing the same. The role playing, mutual support, diabetes education, and physician access components were all thought to be successful components of the Program.

Participants found the peer support to be an integral aspect of the Program. The role playing, mutual support, diabetes education, and physician access components were all thought to be successful components of the Program. The participants’ main critique was a lack of information on how to inform others about the Program. In addition, although participants conveyed an interest in continuing in the Program, they hoped to be given more advanced educational material about diabetes management. The survey results suggested that participants had decreased feelings of anger regarding living with diabetes and reduced worry about hypoglycemia. Participants also reported feeling less overwhelmed by diabetes. Based on data gathered from the pilot study, an expanded 12-week Peer Mentoring Program has been developed with over 40 participants recruited for the study. Participants found the Program to be beneficial in helping them self-manage their diabetes as well as giving them tools to assist others in doing the same.

**FINAL ID: 51**

**TRENDS IN ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY IN CHILDREN WITHIN THE UNITED STATES FROM 2000 TO 2009**

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**Background:** Endoscopic retrograde cholangiopancreatography (ERCP) is an effective and commonly performed procedure in children. However, data pertaining to the number of diagnostic and therapeutic ERCP performed in children are very limited with the absence of large-scale studies.

**Objective:** To investigate the volume of ERCP performed in hospitalized children in the United States.

**Methods:** Data were obtained from the Kids Inpatient Database (KID), Healthcare Cost and Utilization Project (HCUP), Agency for Healthcare Research and Quality for the years 2000 to 2009. Data were weighted to generate national-level estimates.

**Results:** A total of 22,153 pediatric cases of ERCP were identified; 6,372 diagnostic and 17,314 therapeutic ERCPs were performed (1,533 cases were recorded as undergoing both during the single hospitalization). Children in the KID without an ERCP totaled 11,000,639. Children who had undergone ERCP were more likely to be female (82.6% vs. 66.8%; odds ratio [OR] OR 3.06; CI 2.95, 3.16), older (children 16-20 years; 81.0% vs. 48.2%; OR 4.58; CI 4.43, 4.74) and Hispanic (28.3% vs. 17.2%; OR 1.89; CI 1.84-1.95). The number of diagnostic ERCPs decreased from 2,047 in 2000 to 1,161 in 2009 representing an overall decline of 43%. Therapeutic ERCPs increased from 3,290 in 2000 to 5,572 in 2009 (69% increase). There was a significant decreasing trend for diagnostic and increasing trend for therapeutic ERCPs (P<0.001 for each analysis). During the period of this study, significant increasing trends were observed for gallbladder/biliary disease and pancreatitis in hospitalized children.

**Conclusions:** Our results indicate a significant increasing trend for therapeutic ERCPs in hospitalized children in the United States from 2000-2009.
This trend is coincident with increases in gallbladder/biliary disease and pancreatitis.

**FINAL ID: 53**
**DIAGNOSTIC DILEMMA: EBV AND CMV CO-INFECTION IN A PATIENT WITH CROHN’S DISEASE**

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**Purpose:** Combination therapy with immunomodulators and anti-tumor necrosis factor (anti-TNF) drugs has been proposed for moderate to severe Crohn’s disease, especially in high risk individuals. However, the risk of infections especially opportunistic infections, becomes a major clinical concern due to profound immunosuppression. Cytomegalovirus (CMV) and Epstein Bar virus (EBV) infections often present with colonic involvement that mimics a disease relapse and can pose a diagnostic and management dilemma. Co-infection with CMV and EBV has not been previously reported. Here, we present a novel case of patient with Crohn’s disease maintained on combination therapy, found to have an EBV and CMV co-infection.

**Case Report:** A 66 year old male with Crohn’s disease of the J pouch was being treated with certolizumab pegol (CZP) 100mg q 4 weeks subcutaneously and oral 6-mercaptopurine 150mg daily for the recurrence of strictureing disease in the pouch for about 6 months. He presented with progressively worsening bloody diarrhea and weight loss. Initial endoscopic evaluation showed severe active inflammation with mucosal ulceration and anastomotic strictures. Clostridium difficile PCR was negative. A repeat psochocystitis at a tertiary care center showed chronic active ileitis with ulcerations. Histopathological analysis showed pyloric gland metaplasia and multiple CMV viral inclusions. Azitha and multiple CMV viral inclusions. Azitha and multiple CMV viral inclusions. Initial tests showed positive CMV immunostain and positive EBER in situ hybridization for EBV with appropriate controls. Blood test for CMV and EBV PCR was negative. Cetorlizumab pegol and 6-mercaptopurine were discontinued and treatment was initiated with valganciclovir 900 mg daily. Repeat psochocystis 3 months later showed eradication of both CMV and EBV with improvement of symptoms.

**Conclusion:** Immunosuppressive agents are the mainstay of therapy for moderate to severe Inflammatory Bowel Disease. Combination therapy with immunomodulators and anti TNF agents has been advocated in advanced disease and certain high risk patient for better therapeutic outcomes. CMV and EBV infections have been independently reported as opportunistic infections effecting immunosuppressed patients. This is the first case report of EBV and CMV co-infection in Crohn’s disease patient receiving combination therapy. This case emphasizes the need of clinical vigilance in patients receiving such therapy and presenting with worsening symptoms.

**FINAL ID: 55**
**CLOSTRIDIUM DIFFICILE INFECTION RELATED EMERGENCY DEPARTMENT VISITS IN THE UNITED STATES 2006-2009**

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**Background:** Cl22106115 C. difficile is a gram positive spore-forming anaerobic bacterium that is believed to cause 15% to 25% of antibiotic-associated diarrhea in the United States. In the last decade, there has been an alarming up-trend in the number of cases of CDI; both hospital- and community-acquired cases have increased.

**Objective:** To interrogate a nationwide emergency department (ED) database to determine the trend of ED visits related to CDI for the years 2006–2010. Data were weighted to generate national-level estimates.

**Methods:** Data were obtained from the Nationwide Emergency Department Sample (NEDS), Healthcare Cost and Utilization Project (HCUP), Agency for Healthcare Research and Quality for the years 2006–2010. Data were weighted to generate national-level estimates.

**Results:** For the calendar years 2006 – 2010, a weighted total of 462,160 patients were discharged from the ED with a primary diagnosis of CDI. The rate (cases/100,000 population) of ED visits with CDI as a primary diagnosis increased from 34.08 in 2006 to 42.37 in 2010; this represented an increase of 24.32% (P<0.01). There was also a significant overall increased trend in the number of ED visits with CDI as a primary diagnosis from 2006 – 2010 (P<0.01). The highest incidence rate (cases/100,000 population) of CDI related ED visits was observed patients >= 65 years (163.35), while the lowest incidence was in patients ages 18 – 24 years.

**Factors associated with an increased risk of hospital admission included**
- female sex, a comorbid burden of >=3 (aOR 1.21, 95% CI 1.18 – 1.25), Medicaid or Medicare insurance (aOR 1.21, 95% CI 1.18 – 1.25), and presentation to a facility in the Southern region of the United States (aOR 1.06, 95% CI 1.02 – 1.09).

**Conclusions:** CDI related ED visits represent a considerable burden on the healthcare system in the United States. Additionally, an increasing trend in the incidence of these cases was observed for the years 2006 – 2010.

**GENETIC & MOLECULAR MEDICINE**

**FINAL ID: 57**
**IDENTIFYING AND VALIDATING A COMBINED MRNA AND MICRORNA SIGNATURE IN RESPONSE TO IMATINIB TREATMENT IN A CHRONIC MYELOID LEUKEMIA CELL LINE**

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Imatinib, a targeted tyrosine kinase inhibitor, is the gold standard for managing chronic myeloid leukemia (CML). Despite its wide application imatinib resistance occurs in 20-30% of individuals with CML. As so clinicians and researchers have searched for putative biomarkers to predict imatinib response; however, the majority of these studies remain externally uncorroborated potentially because many of these investigations rely on only one type of marker (DNA or miRNA) in complex traits prediction. In order to improve imatinib sensitivity biomarker discovery, we evaluated whole genome mRNA and microRNA (miRNA) expression changes post imatinib treatment together. Through a Gene Expression Omnibus (GEO) search, we identified two genome-wide expression datasets that contain expression changes in response to imatinib treatment in a CML cell line (K562): one for mRNA and the other for miRNA. Comparing treated versus untreated, significantly differentially expressed transcripts/miRNA were selected from both datasets. Three additional filtering criteria were applied 1) miRbase miRNAa predictive association; 2) negative expression correlation between gene-miRNA; and 3) literature support. These criteria narrowed our candidate gene-miRNA to a single pair: IL8 and miR-493*. Using PCR we confirmed the significant up-regulation and down-regulation of miR-493* and IL8, respectively after imatinib exposure (1 µM for 24 and 48 hours) in K562 cells (p<0.05). In addition, IL8 expression was significantly down-regulated 24 hours after transfecting K562 cells with 15 nM miR-493* mimic (p<0.05). This further support the relationship between miR-493* and IL8. More importantly, we observed significant cellular growth inhibition after inhibiting IL8 or over-express miR-493* in this CML line. The inhibition of IL8 through siRNA also sensitized K562 cells to imatinib treatment when compared to that of scramble control. The study combined expression changes in the transcriptome and microRNA after imatinib exposure to identify a potential gene-miRNA network that is critical in imatinib response. Experimental validation further supports the relationship between IL8 and miR-493*, as well as between this gene-miRNA
pair and imatinib sensitivity in a CML cell line. Our data suggest integrative analysis of multiple omic level data may improve biomarker discovery.

**FINAL ID: 59**

**EPIGENETICS AND HEALTH DISPARITIES OF ACUTE RESPIRATORY DISTRESS SYNDROME: IDENTIFYING CYTOSINE MODIFICATIONS IN MYLK IN A NESTED CASE–CONTROL STUDY**

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**Background:** Acute respiratory distress syndrome (ARDS) is a destructive clinical syndrome with a substantial mortality rate of ~40%, affecting an estimated 190,600 people and attributing to 3.6 million hospital days annually in the United States. Survivors of ARDS also have considerable morbidity, including interstitial lung disease and neurological outcomes. African-American (AA) patients display a higher mortality than Caucasian (Cau) patients after adjusting for demographic and clinical variables. However, after controlling for severity of illness this association disappears in some studies. Aside from genetic variation, differentially modified cytosines (primarily 5-mC), an epigenetic gene regulatory mechanism, have recently been identified between individuals of AA and Cau race. Given that a wide investigation of cytosine modification in ARDS-candidate genes may offer insights into how these genes play a role in health disparities, we performed a search for ARDS-specific CpGs (cytosine-phosphate-guanine) in MYLK (encoding myosin light chain kinase, MLCK), a recognized ARDS gene with genetic variants and expression related to ARDS severity.

**Methods:** isolation of genomic dna was performed on whole blood collected from 39 ards cases (18 cau and 21 aa) and 75 norm-ards icu controls (39 cau and 36 aa). Real-time pcr analysis and immunoblotting were performed according to illumina's annotations. bivariate and stratified analysis as well as multinomial logistic regression and linear regression modeling were performed utilizing sas 9.3.

**Results:** of the 52 cpq probes located within mylk, five cpq probes were differentially modified (p<0.05) between ards patients and icu controls, while controlling for age, sex, race and diabetes. For example, cg17787502 and cg23344121, located in the gene body in exon 11 and 19 respectively, showed a 2.6±0.6 protein and 8.6±2.6 fold increase in AA patients compared to CAU patients (p = 0.0267 respectively).

**Conclusions:** these findings are preliminary and require confirmation by independent replication. Forthcoming studies will also incorporate genetic variation to illuminate the genetic architecture of these cytosine modification differences as well as their interactions with environment to determine causality.

**Rationale:** increased vascular permeability and alveolar edema are cardinal features of inflammatory conditions such as acute lung injury (ALI), acute respiratory distress syndrome (ARDS) and ventilator–induced lung injury (VILI). We previously demonstrated that PBEF/NAMPT, the pro-inflammatory cytokine known as pre-B cell colony enhancing factor (PBEF) is a viable candidate gene in ALI and ventilator-induced lung injury (VILI) syndromes. PBEF/NAMPT is highly expressed in LPS- and cyclic stretch (CS)-stimulated endothelial cells (EC), is up-regulated in murine, canine and human ALI, and is spatially localized to lung endothelium. MicroRNAs (miRNAs) regulate gene expression post-transcriptionally through binding to 3’ UTR of mRNA and are linked to a variety of inflammatory conditions, cancer, and cardiovascular diseases. Preliminary in-silico analysis identified two miRNA candidates, hasa-mir-374a and hasa-mir-568 as potentially binding to the 3’UTR of PBEF/NAMPT. We investigated whether these miRNAs participate in regulation of LPS- and 18% CS-induced nmMLCK expression in vitro.

**Methods:** the functions of hasa-mir-374a and hasa-mir-568 were studied in cultured human pulmonary artery ec (hpaec). rna extraction, quantitative real-time pcr analysis and immunoblotting were performed according to manufacturer's recommendations for each assay. reporter constructs (switchgear genomics, menko path, ca) containing the luciferase gene fused to the pefn568 3’utr were used in dual luciferase assays to determine the effects of individual mirnas on pbefnampt expression in 18% cs- and lps-stimulated ec.

**Results:** increased pbefnampt transcription (rt-pcr) and expression (western blotting) induced by 18% cs (2 hrs-3.4±0.6 protein fold increase, 10 hrs-1.5±0.6 protein fold) and by lps (4 hrs-3.8±0.2 protein fold, 18 hrs-2.6±0.2 protein fold) were significantly attenuated by transfection with mimics of hasa-mir-374a (40-60% reductions each). Lps and 18% cs each increased the luciferase activity of the human 3’utr luciferase reporter (2-3.2 fold) with induction reduced by mimics of each mirna (44-60% reduction). Specific mirna inhibitors (antagomirs) for each pbefnampt mirna significantly increased the endogenous pbefnampt mRNA (1.4-3.4±0.1 fold) and protein levels (1.2-1.4±0.1 fold) and 3’utr luciferase activity (1.4-1.7±0.1 fold) compared with negative antagomir controls.

**Conclusion:** collectively, these data demonstrate that increased pbefnampt expression induced by pro-inflammatory cytokines (cytosine-phosphate-guanine) is dependent on the regulation of pbefnampt expression by cs and lps.

**FINAL ID: 63**

**DEVELOPMENT OF MACROLACTAMIZATION METHODS FOR SYNTHESIS OF CYCLOTETRAPEPTIDES – AN UNDEREXPLOITED CLASS**

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Cyclo-tetrapeptides are a rich source of key precursors in synthesis of drug molecules. This is mostly attributed to their low molecular weight, favorable pharmacokinetic characteristics, and structural support for a wide range of functional groups. Although cyclic tetrapeptides have these great potentials, their current applications in the pharmaceutical industries are highly limited, primarily due to ineffective cyclization of a linear tetrapeptide. Bringing the two termini sufficiently close to induce cyclization in such small molecules is challenging, and virtually impossible with the currently used synthetic methods. Linear peptides prefer more extended conformations, while peptide bonds adopt E-conformations, resulting in the discussed conformational issues. Utilizing a Pd assisted tandem-deprotection-cyclization reaction, we successfully developed a synthetic strategy to convert open-chain N-Chz-dipeptidobenzotriazoles to form both symmetric and asymmetric cyclic tetrapeptides. This methodology was successfully demonstrated by ring-closure of a series of dipeptidobenzotriazoles yielding cyclo-tetrapeptides, which cannot be prepared efficiently using previously reported methods. The approach described here should provide a convenient entry for the design of a variety of cyclo-tetrapeptides with potential utility in medicine, catalysis, and ma.

**FINAL ID: 65**

**HSA-MIR-374A AND HSA-MIR-568 EPIGENETICALLY REGULATE PBEF/NAMPT GENE EXPRESSION IN HUMAN LUNG ENDOTHELIUM**

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**Background:** Acute respiratory distress syndrome (ARDS) is a destructive clinical syndrome with a substantial mortality rate of ~40%, affecting an estimated 190,600 people and attributing to 3.6 million hospital days annually in the United States. Survivors of ARDS also have considerable morbidity, including interstitial lung disease and neurological outcomes. African-American (AA) patients display a higher mortality than Caucasian (Cau) patients after adjusting for demographic and clinical variables. However, after controlling for severity of illness this association disappears in some studies. Aside from genetic variation, differentially modified cytosines (primarily 5-mC), an epigenetic gene regulatory mechanism, have recently been identified between individuals of AA and Cau race. Given that a wide investigation of cytosine modification in ARDS-candidate genes may offer insights into how these genes play a role in health disparities, we performed a search for ARDS-specific CpGs (cytosine-phosphate-guanine) in MYLK (encoding myosin light chain kinase, MLCK), a recognized ARDS gene with genetic variants and expression related to ARDS severity.

**Methods:** isolation of genomic dna was performed on whole blood collected from 39 ards cases (18 cau and 21 aa) and 75 norm-ards icu controls (39 cau and 36 aa). Real-time pcr analysis and immunoblotting were performed according to illumina's annotations. bivariate and stratified analysis as well as multinomial logistic regression and linear regression modeling were performed utilizing sas 9.3.

**Results:** of the 52 cpq probes located within mylk, five cpq probes were differentially modified (p<0.05) between ards patients and icu controls, while controlling for age, sex, race and diabetes. For example, cg17787502 and cg23344121, located in the gene body in exon 11 and 19 respectively, showed significantly lower modification in ards patients (p = 0.0305 and 0.0061 respectively). these same cytosines were also found to have lower modification in AA versus cau patients (p = 0.0029 and 0.0267 respectively).

**Conclusions:** these findings are preliminary and require confirmation by independent replication. Forthcoming studies will also incorporate genetic variation to illuminate the genetic architecture of these cytosine modification differences as well as their interactions with environment to determine causality.
Cell-free DNA (cfDNA) can be collected from human plasma after spinning at high-speeds to ensure removal of all white cell material. Isolation and sequencing of cfDNA provides a way to sample the individual from several diverse tissue types, as DNA is released into the blood from all tissues during apoptosis, necrosis, or as a result of cell damage. Work has been done to identify the size distribution of DNA within plasma, but little has been done using next-generation sequencing to identify the exact sequence of these fragments. In this study we have isolated and sequenced cfDNA from healthy human volunteers. We will present the results of the sequencing including the size distribution of the fragments we recovered and the optimal library preparation procedures.

**FINAL ID: 66**

**HYPERGLYCEMIA DOWN-REGULATES THE CGMP-DEPENDENT PROTEIN KINASE I EXPRESSION IN FIRST TRIMESTER CYTOTROPHOBLAST CELLS**

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**Objective:** Diabetes is associated with an increased risk of microvascular complications including nephropathy and hypertension. The signaling pathway of nitric oxide (NO), cGMP, and cGMP-dependent protein kinase (PKG) has been shown to be down-regulated under diabetic conditions and contributes to the development of diabetic vascular complications. It has been shown that high glucose concentrations significantly reduce PKG-I production as well as PKG-I activity in cultured vascular smooth muscle cells. However, there has been no data so far on the effect of hyperglycemia on PKC expression in cytotrophoblast cells (CTBs). It has also been suggested that the pexorosine proliferator-activated receptor gamma (PPAR γ) is involved in the dysfunction of CTBs in high glucose conditions. The present study was undertaken to investigate how high glucose concentrations regulate PKG-I expression in CTBs.

**Study Design:** Human CTBs (Sw. 71) were treated with 45, 135, 225, 495 or 945 mg/dL glucose for 48h. Some cells were pretreated with a p38 inhibitor (10 μM SB203580) or 10 μM rilsiglotitin. After the treatment, the media was removed from cells and a lysis buffer containing 50 mM Tris at pH 7.4, 50 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.3 mM Na-orthovanadate, 50 mM NaF, 1 mM EDT, 10 μg/ml leupeptin, and 5 μg/ml aprotonin was added to the cells. Cells were scraped and put into tubes. Protein concentrations were determined and 10 μg protein from each sample was separated using NuPAGE Novex 4-12% Bis-Tris gels (Invitrogen) and transferred to nitrocellulose membranes. Membranes were blocked in 5% milk and probed with anti-PKG1α, anti-PKG1β, anti-sGC1α, or anti-sGC1β antibodies. After incubation with the corresponding secondary antibody, proteins were visualized with chemiluminescence detection system (Pierce). The intensity of the bands were determined using ImageQuant LAS 4000 (GE Healthcare, Life Sciences). Protein kinase G1α (PKG1α), protein kinase G1β (PKG1β), soluble guanylate cyclase 1α (sGC1α), and soluble guanylate cyclase 1β (sGC1β) expression was measured by ImageJ software.

The p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation and PPARy expression were evaluated by western blot. Statistical comparisons were performed using analysis of variance with Duncan’s post hoc test.

**Results:** Both The expression of PKG1α, PKG1β, and sGC1α were significantly down-regulated (p<0.05) in CTBs treated with >135 mg/dL glucose compared to basal (45 mg/d). The expression of sGC1α was significantly down-regulated (p<0.05) in CTBs treated with >135 mg/dL-glucose compared to basal (45 mg/dL). The p38 MAPK phosphorylation and PPARy expression were upregulated (p<0.05) in excess glucose-treated CTBs. The hyperglycemia-induced down-regulation of cGMP, and cGMP-dependent protein kinase (PKG) were attenuated by the SB203580 or rilsiglotitin pretreatment.

**Conclusions:** Exposure to excess glucose down-regulates the cGMP, and cGMP-dependent protein kinase (PKG), and thus contributes to the development of vascular complications in diabetic during pregnancy. The attenuation of hyperglycemia-induced down-regulation of PKG proteins by SB203580 or rilsiglotitin pretreatment suggests the involvement of stress signaling mechanisms in this process.

**FINAL ID: 67**

**A NOVEL SNP IN TNFRSF1B IS ASSOCIATED WITH RESPONSE TO ANTI-TNF THERAPY IN INFANTILARY BOWEL DISEASE PATIENTS**

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**Background:** Infantile inflammatory bowel disease (IBD) is a chronic and debilitating gastrointestinal disease estimated to affect 1.4 million individuals in the US. Although the recent advent of anti-TNF antibodies has led to a dramatic improvement in IBD treatment, more than 20% of patients fail to respond to these therapies. Identifying predictors of response to anti-TNF drugs is essential to determine which patients could be spared the expense and side effects and which will benefit from this class of therapy. In a retrospective candidate gene study of 167 IBD patients, we identified a SNP in the TNF receptor TNFRSF1B (rs1061628) that was associated with response to anti-TNF agents (OR=1.8; p=0.048). In this study, we aimed to validate our findings in an independent cohort of IBD patients.

**Methods:** Our validation cohort was recruited from the University of Chicago Medical Center and was composed of 84 patients diagnosed with IBD and treated with an anti-TNF agent. DNA was isolated from blood samples and genotyped for rs1061628. Using electronic medical records, patients were classified as responders or primary non-responders to anti-TNF therapy. An allelic association was performed using this response data.

**Results:** We found the presence of the minor allele (T) to be associated with an increased risk of being a primary non-responder to anti-TNF agents (OR=4.3, p=0.03). Furthermore, in vitro studies revealed an increase in gene expression with the T allele by qPCR analysis (p=0.002) and by luciferase assay (p<0.05) in colon cells.

**Conclusion:** We have shown that the TNFRSF1B SNP rs1061628 is predictive of response to anti-TNF therapy. This SNP is located in the 3' UTR of TNFRSF1B and may modulate gene expression, potentially as a miRNA binding site. Increased receptor expression may result in decreased sensitivitiy to anti-TNF drugs. This can be seen in the association with anti-TNF drug response and is consistent with previous studies showing TNFRSF1B to be overexpressed in areas of active inflammation in IBD patients.

**GERIATRICS**

**FINAL ID: 69**

**RAPI RELOCALIZATION CONTRIBUTES TO THE CHROMATIN-MEDIATED GENE EXPRESSION PROFILE AND PACE OF CELL SENESCENCE**


Cellular senescence is accompanied by characteristic changes in chromatin structure and gene expression. To investigate the mechanisms underlying these changes we used S. cerevisiae lacking telomerase (tcl1Δ). A candidate transcription factor for regulating senescence-specific gene expression is Rap1 (Repressor Activator Protein 1), which normally localizes to telomeres and binds as a transcriptional activator or repressor at other genomic loci. We hypothesized that Rap1 might relocalize from shortened telomeres to new genomic sites and thus regulate gene expression at senescence. Rap1 ChIP in WT and senescent cells revealed Rap1 relocalization from subtelomeres to hundreds of new Rap1 targets at senescence (NRTS). NRTS promoters contain low affinity Rap1 binding sites and weak nucleosome excluding sequences. Most NRTS are upregulated at senescence, and Rap1 appears to activate them because the expression is similarly upregulated by a two-fold increase in Rap1 levels in wild type cells and 2) their upregulation at senescence is suppressed by a two-fold decrease in Rap1 levels (via the RAP1 DAmP allele). Remarkably, the RAP1 DAmP allele also slows the rate of senescence, indicating...
Introduction: Rap1 relocalization is not passive but rather requires the Mec1/ATR checkpoint kinase, and is associated with Mec1-dependent post-translational modifications of Rap1 itself. In addition, we have observed an important interplay between Rap1 and histones at senescence as follows. First, we find diminished levels of the core histone proteins at senescence, similar to what others have observed in senescent human fibroblasts and in replicatively aged yeast mother cells. Second, Rap1 targets the promoters of all core histone loci at senescence, and Rap1 over/under-expression experiments demonstrated that Rap1 represses histone gene expression at senescence. Third, Rap1 and histone occupancies have a reciprocal relationship at NRTS as demonstrated by preferential loss of histone H3 at these targets at senescence, and by selective loss of Rap1 and restoration of histone H3 at these targets when histones H2A/H2B/H3/H4 are all overexpressed. Thus in senescent cells Rap1 plays a general role in downregulation of global histone levels along with a site specific role associated with histone loss at upregulated genes. The importance of histone antagonism by Rap1 is underscored by our finding that overexpression of H2A/H2B/H3/H4 delays senescence, similar to under-expression of Rap1. Rap1 relocalization is a novel mechanism that connects DNA-damage responses at telomeres to global changes in chromatin and gene expression that drives the pace of senescence.

HEMATOLOGY AND ONCOLOGY

FINAL ID: 71
HIGH-GRADE B-CELL NON-HODGKIN LYMPHOMA DIAGNOSED BY SIGMAID POLYP
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Introduction: diffuse large cell lymphomas are a heterogenous group of lymphoid neoplasms making up 25-31% of non-hodgkin lymphomas. among these is diffuse large b-cell lymphoma (dlblc). dlblc most commonly arises from a mature b cell but can also develop via the transformation of low grade b-cell lymphomas. extranodal extramedullary disease occurs in up to 40% of cases of dlblc. among gi lymphomas, b-cell lymphomas are uncommon and high-grade dlblc is rare.

Case Presentation: The patient is a 74 year-old male with newly diagnosed diabetes mellitus type 2 who presented with complaints of a mass in his neck and increased fatigue. On exam, there was bilateral cervical lymphadenopathy. MRI showed multiple enlarged enhancing lymph nodes predominantly involving the right submandibular, jugulodigastric, bilateral internal jugular and bilateral suprACLavicular regions. One lymph node enhanced homogeneously, suggesting central necrosis, suspicious for neoplasm, lymph node biopsy was obtained. A few days later and before the results of the biopsy, the patient presented to the ED with several episodes of bleeding with dark maroon stools. Patient complained of fatigue and decreased oral intake. His hemoglobin had dropped from 10 to 8.5. Patient underwent colonoscopy, at which time a number of polyps was removed from the sigmoid colon. Patient also underwent endoscopy which was unremarkable. The pathology report of the colon polyps and lymph node, found dysplastic cells consistent high grade b-cell non-hodgkin lymphoma. He is currently receiving R-CHOP therapy.

Conclusion: high-grade diffuse b-cell lymphoma involving a colon polyp is rare. this type of polyp tends to undergo necrosis and bleed after chemotherapy, therefore, they may require removal before starting chemotherapy to avoid perforation or worsening bleeding.

FINAL ID: 75
ERLOTINIB IS NOT EFFECTIVE IN PATIENTS (PTS) WITH JAK-2 V617F POSITIVE POLYCYTHEMIA VERA
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Introduction: Erlotinib is an epidermal growth factor receptor small-molecule inhibitor and is FDA approved for the treatment of lung and pancreatic cancers. In preclinical study, in vitro colony culture assays revealed that erlotinib at micro-molar concentrations effectively suppressed the growth and expansion of Polycythemia Vera (PV) hematopoietic progenitor cells while having little effect on normal cells. Several JAK inhibitors are being studied for the management of PV, one of which has been approved for the treatment of myelofibrosis (ruxolitinib).

Aim: To study the clinical effect of erlotinib in pts diagnosed with JAK2V617F + PV.

Methods: We conducted a single arm, prospective phase II study at the University of Oklahoma and the Oklahoma City VA hospitals in pts with WHO defined JAK-2 V617F positive PV from June 2010 to August 2012. Appropriate IRB approval was obtained in accordance with Hilsinki declaration. Pts had to be failing phlebotomy. Toxicity was assessed by treating physicians using NCI version 4. Dose modification for erlotinib was done using label recommendations.

Results: Five Caucasian pts were enrolled (3 (60%) males, with median age at enrollment of 63 years, range 26-79). Pts had pretreatment median hemoglobin14.4 g/dL (10.4-19.2 g/dL), median platelet count 511 x109 (424-681 x109), median white blood cell (WBC) 14.4 x109 (7.8- 18.3 x109). Three pts had JAK-2 V617F+PV. Two patients (40%) pts had to discontinue treatment due to toxicity (grade 3 toxicity in 1 case and grade 4 facial rash in 1 case). No therapy continued beyond 16 weeks (due to toxicity or lack of response). All pts in the study developed rash (grade 1 – 3) and diarrhea (grade 1 – 2). Three pts developed mucositis. No
death was observed during the study and follow up period (median follow up was 23 months, range 12-37 months). Study was closed due to lack of efficacy.

Conclusions: Despite in vitro efficacy of erlotinib as potent inhibitor of JAK-2 activity, erlotinib is not effective in pts with JAK-2 V617F positive PV with poor toxicity profile. Poor accrual was related to potential toxicity of erlotinib compared to alternative treatments in view of lack of clinical efficacy.

**FINAL ID: 77**

**CLINICAL DRUG RESPONSE CAN BE PREDICTED USING BASELINE GENE EXPRESSION LEVELS AND IN VITRO DRUG SENSITIVITY IN CELL LINES**

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Chemothapeutic responders were typically identified based on patients’ gross histological cancer diagnosis. More recently, molecular markers have also been discovered which predict response to certain drugs, for example, ERBB2 amplification in breast cancer. However, the overwhelming majority of biomarkers reported in the scientific literature do not reproducibly predict drug sensitivity in vivo, or do not have an effect size large enough to be of practical use. We demonstrate for the first time that, from primary tumor gene expression data, drug response in patients can be predicted using models generated from only in vitro drug sensitivity and baseline gene expression in a very large panel of cell lines. Our method uses a type of model that allows every gene to influence the prediction by the prediction by a small amount, thus accounting for the cumulative effect of many genes. First, the models are fit for whole genome gene expression against drug sensitivity in the panel of cell lines. Then, following (crucial) data homogenization and filtering steps, these models are applied to baseline expression levels from primary tumor biopsies, yielding an in vivo drug sensitivity prediction. This enriches for drug responders in three clinical trials, even in the absence of any known drug sensitivity biomarker. In all publicly available datasets in which an approach is testable, it is shown to perform equally well, or better than, gene signatures that were derived directly from the clinical data itself. Such prediction can potentially transform personalized medicine. It could also be applied to drug development, particularly in developing companion diagnostic tests.

**FINAL ID: 79**

**CENTRAL RETINAL VEIN OCCLUSION AFTER HORMONE THERAPY IN A PATIENT WITH UNKNOWN HYPERHOMOCYSTEINEMIA**

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**Introduction:** Retinal vascular occlusion (RVO), including central retinal vein occlusion (CRVO), central retinal artery occlusion (CRAO), and amaurosis fugax (AF), can be caused by inherited and acquired thrombophilia, particularly hyperhomocysteinemia. Thrombophilia associated with exogenous estrogens, estrogen-progestin oral contraceptives, clomiphene citrate, or selective estrogen receptor modulators may promote CRVO, AF, and CRAO. Most reports of CRVO or CRAO correlated with estrogens or estrogen agonists have not assessed the interactions between pharmacologic thrombophilia and acquired thrombophilia conferred by estrogens or estrogen agonists and inherited or acquired thrombophilia known to be causally linked to the development of RVO.

**Case Description:** A 53-year-old Caucasian female, who was two years post-menopausal and a non-smoker, with no significant past medical history developed painless complete vision loss in her left eye sixteen days after being started on an implantable estrogen/testosterone pellet (estradiol 75 mg, testosterone 75 mg). Ophthalmologic evaluation with Fluorescein angiography and fundus photography confirmed central retinal vein occlusion (CRVO) of the left eye, based on characteristic retinal hemorrhages in all four quadrants with a dilated, tortuous, retinal venous system. Patient was started on Bevacizumab intra-ocular injections.

Family history ultimately revealed a niece and brother with history significant for deep venous thrombosis and a brother with a fatal myocardial infarction at age 48. Laboratory studies found that the patient had compound heterozygosity of the MTHFR gene with C677T and A1298C mutations, high serum homocysteine (22.1 umol/L, upper normal limit 15), and 4G4G homozygosity for the plasminogen activator inhibitor gene. Other analyses for thrombophilia were normal. Patient was subsequently started on L-methylfolate, vitamin B6, and vitamin B12 therapy. After six weeks, serum homocysteine normalized, and patient has since been event-free.

**Discussion:** Our current report along with prior studies has established thrombophilia as a common pathogenic cause of RVO. This case illustrates how acquired thrombophilia (estrogen-testosterone pellet in the current case) superimposed upon familial thrombophilia facilitates the development of CRVO. In particular, this demonstrates increased serum homocysteine levels, which normalized after treatment with folate acid, vitamin B6, and vitamin B12, as an imperative mechanism that significantly reduces the threshold for thromboembolic phenomena. The data collected from this report and other studies reveal the importance of screening for hyperhomocysteinemia, the MTHFR genotype, Factor V Leiden, and Factors VIII and IX levels prior to the initiation of exogenous estrogen (particularly estrogen-testosterone) therapy to prevent thrombus formation in females with previously undiagnosed thrombophilia.

**FINAL ID: 81**

**AXITINIB IN TREATMENT OF METASTATIC CHROMOPHORE RENAL CELL CARCINOMA (MET CH RCC)-ACASE REPORT**

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**Introduction:** Renal cell carcinoma (RCC) constitutes about 4% of all the cancers in the United States. The predominant and the most common histologic variant is the clear cell accounting to about 80% of the RCC’s. Chromophobe RCC is a less common variant making up for about 5% of all the RCC’s. Hence, there is limited amount of literature on the treatment strategies of Ch RCC. Here we present a case of metastatic Ch RCC that has positively responded to Axitinib, a VEGF selective inhibitor. To our knowledge, this is the first reported case of successful response of chromophoric renal cell carcinoma to Axitinib therapy.

**Case:** Our patient is a 59 Y old female who presented initially in July of 2006 with back pain. CT scan showed a 7 × 9 cm mass at the upper pole of the left kidney and a thrombus at the renal vein extending to the inferior vena cava. The patient underwent a left radical nephrectomy and IVU tumor thrombectomy on 9/18/2006. Pathology revealed Ch RCC, Fuhrman nuclear grade III. No initial metastatic disease was identified in the sampled lymph nodes. Treatment: at initial evaluation in October 2006 an adjuvant therapy was not recommended. She was observed until March 2009 when an isolated lung nodule was noted and resected. Pathology revealed met Ch RCC. As the disease progressed with mediastinal adenopathy Temsirolimus therapy was started in May 2010 but was discontinued shortly due to progressive disease and progression of adenopathy. In March 2012 Sorafenib was initiated at 200mg BID and later increased to 400 mg BID. CT of chest in October 2012 showed two new lung nodules not meeting the criteria for progression (sum of diameters > 25%). Along with moderate toxicities she had an upper GI bleed and Sorafenib had to be held for 2 months. An EGĐ revealed a gastric mass obstructing the pylorus and its biopsy was consistent with a metastatic Ch RCC. Sorafenib was discontinued at this time and Axitinib was started in March 2013. The initial dose was 5 mg BID and it was slowly escalated to 7mg BID and finally to 10 mg BID. In July 2013, she presented with another episode of GI bleed. EGĐ and capsule endoscopy were unrevealing for any ulcers or new growths. Axitinib is being continued and at the time of writing this report, nearly 10 months since initiation of therapy, she has been tolerating it well. There are no major toxicities reported so far and dose reduction was not needed. The latest follow up imaging with CT scan showed stable lung nodules, no new lung lesions and stable low density pre carinal and right hilar lymphadenopathy indicating stable disease in response to the therapy.

**Discussion:** Treatment of Renal cell carcinoma has made remarkable progress with the development of new targeted therapies like selective and nonselective VEGF inhibitors, m-TOR pathway inhibitors, c-Kit inhibitors and second generation TKI’s. Due to lack of adequate Ch RCC patients in major treatment guidelines of met Ch RCC are lacking. Available data from retrospective studies, sub group analyses of major trials and case reports suggest Temsirolimus as an effective agent in all forms of met RCC’s. However, role of Axitinib and other targeted agents have not been studied in prospective
Acute megakaryoblastic leukemia (AMKL), otherwise known as M7 leukemia is a rare form of leukemia occurring mostly in pediatric population especially with Down syndrome. AMKL in adults is extremely rare with reported incidence ranging between 1-12% of all the leukemias. AMKL in adults is seen commonly as secondary leukemia and with underlying myeloproliferative disorders and Myelodysplastic syndromes. We report a case of AMKL transforming from an underlying CMMML with t(2;8) which has not been described in prior literature. Case: A 76 yo male with a known history of CMMML diagnosed in 2008 presented with symptoms of shortness of breath and fatigue worsening over the previous few weeks. The patient has not received any prior treatment for CMMML and has been on routine monitoring of blood counts. Lab data at admission revealed Hb 7.7 gm/dL, Platelets 488 K/m2, WBC 50,000 with 59% peripheral blasts. Bun: 23, Cr 1.3 (baseline). Fibrinogen: 144. WBC: 50,000, Hemoglobin: 7.7 gm/dL, Platelets: 488 K/m2. Bone marrow biopsy showed a hypercellular bone marrow with comorbid conditions is not well described in literature. Our patient’s labs showed worsening thrombocytopenia, with a drop in her platelets from 130,000 to 29,000 in the span of two days, a bilirubin of 21, LDH of 1165, reticulocyte count of 6.2 and schistocytes noted on peripheral smear. Her LFTs were also noted to be elevated at the time of admission. The patient did not have any signs of fever, neurological changes or renal insufficiency. An abdominal ultrasound did not show any biliary problems and nor did it reveal any splenomegaly, which is one of the commonest causes of thrombocytopenia in liver failure patients. The patient’s platelets continued to drop to 21,000 at which point she developed spontaneous menstrual bleeding as well as a hemoglobin drop from 8.8 to as low as 6.4. The patient then had her ADAM TS 13 drawn and she was started on emergent plasmapheresis. After five days of plasmapheresis, the patient’s platelet count improved to 99,000, and her LDH, bilirubin and reticulocyte count all decreased to 605, 118 and 2, respectively. The patient’s ADAM TS 13 activity was confirmed to be low at 44%. The patient tolerated the plasmapheresis well and was subsequently discharged.

Discussion: Thrombocytopenia is a common finding in patients with severe alcoholic hepatitis, especially in patients with splenomegaly who have splenic sequestration of platelets, or those with bone marrow toxicity and decreased megakaryocyte production from alcohol. However, the effects of alcohol will normally suppress all cell lines and in this case the patient had an increased reticulocyte count, which was atypical of bone marrow suppression. The common findings in severe hepatitits are also seen in patients with TTP, which makes it difficult to differentiate these patients and to start emergent plasmapheresis when needed. It is especially difficult in patients where there is an absence of the classic pentad for TTP including fever, renal insufficiency, MAHA, neurological symptoms, and thrombocytopenia. It is therefore, imperative that one should maintain a low threshold in these patients to consider the possibility of TTP, in order to quickly treat them as necessary, even in the presence of severe alcoholic hepatitis and liver failure.

Final ID: 85

Signaling Kinase PKD-1 regulates Endothelial Cell CD36 Transcription and Stimulates Angiogenic Responses

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CD36 is a scavenger receptor that plays an important role in ischemic diseases, diabetes and cancer. Lysophosphatidic acid (LPA), a bioactive signaling phospholipid and angiogenic factor, down-regulates CD36 expression in microvascular endothelial cells (MVECs) via protein kinase PKD-1 signaling, thereby abolishing endothelial cell responses to its antiangiogenic ligand thrombospondin-1 (TSP-1). However, little is known regarding how angiogenic signaling is integrated to establish and regulate endothelial specific CD36 transcription. We here describe that in MVECs LPA represses transcription of CD36 by activating a PKD-1 signaling pathway that induces the formation of a HDAC7/NCOR1-FoxO1 complex in the nucleus. We then consider the use of PKD-1 gene transduction with co-immunoprecipitation assay, we showed an increased interaction of HDAC7/NCOR1 with FoxO1 in response to LPA treatment. However, the interaction between HDAC7 and FoxO1 was attenuated with PKD-1 silencing. More intriguingly, by using angiogenic profiling with real time quantitative PCR and mammosphere and angiogenesis assay, we showed that transcription reprogrammed MVECs to express ephrin B2 and to activate MAPK/ERK1/2 signaling, which are two critical “molecular signatures” involved in arteriogenesis. Moreover, three dimensional spheroid assay, a modified Boyden Chamber assay and in vivo Matrigel assay revealed that turning...
off CD36 transcription promoted angiogenesis in vitro and in vivo in a PKD-I-dependent manner. We also demonstrated the presence of this signaling pathway in the vasculature of Lewis lung carcinoma of CD36 deficient mice by using immunofluorescence microscopy, demonstrating its importance in angiogenesis, specifically arteriogenic responses. In summary our data suggest that a LIM-PKD-I-BetaC7/Ncor1-FOXO1 signaling axis is critical for epigenetic regulation of CD36 and mediates silencing of this antiangiogenic switch, subsequently resulting in reprogramming of MVECs for proangiogenic and arteriogenic responses. Therefore, targeting this signaling cascade could be a novel approach for cancer, cardiovascular ischemia and other thrombotic diseases.

**Final ID: 86**

**Analysis of Impact of Distance from Residence to Treatment Center on the Outcome of Patients with Acute Myeloid Leukemia**

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**Introduction:** Acute myeloid leukemia (AML) is the most common form of acute leukemia among adults and accounts for the largest number of annual deaths from leukemia in the United States. Limited data is available comparing epidemiology and treatment according to the distance from patient residence to treatment center. Oklahoma University Health Sciences Center (OUHSC) is the major tertiary center for Oklahoma residents to receive treatment for AML. We utilize a retrospective analysis of adults with AML treated at our institution evaluating the impact on distance from center with treatment outcomes of survival, remission, and relapse.

**Methods:** From January 2000 until June 2011 we identified a total of 269 patients with 216 meeting inclusion criteria for the study with the diagnosis of AML. To evaluate the relationship between overall survival and distance a Kaplan-Meier (with a log rank test) and a Cox proportional hazard model evaluating covariates of interest were performed. A logistical regression was performed to assess association of all covariates with relapse and complete remission.

**Results:** Distance of residence to OUHSC of 50-75 miles and 75-100 miles (compared to >100 miles) indicates an increased hazard of death with hazard ratio of 2.61 (p = 0.0014) and 1.83 (p=0.0481) after adjusting for age. Patients living closer to OUHSC (0-25 miles & 25-50 miles) as compared to areas further from OUHSC (>100 miles) do not have a significantly greater hazard of death. There are differences in the survival curves for the five different distance groups (Figure 2; p=0.0040). The predicted probability of complete remission is lower in patients 50-75 miles from OUHSC (OR =0.317; p = 0.0386) as compared to patients living >100 miles away adjusting for age and risk type. Other distance groups did not demonstrate statistical significance for complete remission.

**Conclusions:** Patients living 0-50 and over 100 miles have a similar hazard of death, which could be explained by proximity to OUHSC or outlying tertiary care centers, with most being located over 100 miles from OUHSC. The increase in hazard of death in patients living 50-100 miles from OUHSC may be attributed to increased distance to a tertiary care center that provide variable treatment for AML. We utilize a retrospective analysis of adults with AML treated at our institution evaluating the impact on distance from center with treatment outcomes of survival, remission, and relapse.

**Final ID: 87**

**High Levels of CC-Chemokine Expression and Downregulated Levels of CCR5 During HIV-1/HTLV-2 Coinfection**

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**Background:** HTLV-1 and HTLV-2 are common copathogens among HIV-infected individuals. Cohort studies have shown that HTLV-2 may confer a survival benefit among patients with HIV-1/HTLV-2 coinfections, with lower plasma HIV-1 levels and delayed rates of CD4+ T cell decline. These effects have been attributed to the ability of the HTLV-2 viral transactivating protein, known as Tax2, to induce the production of high levels of the antiviral CC-chemokines CCL3/MIP-1α, CCL4/MIP-1β, and CCL5/RANTES by PBMCs and monocyte derived macrophages (MDMs); and to downregulate expression of the HIV-1 CCR5 receptor on CD4+ T lymphocytes. Additionally, Tax2 protein was shown to mediate inhibition of HIV-1 replication in peripheral blood mononuclear cell (PBMC) cultures in vitro. In this study, we investigated the innate immunity of coinfected HIV-1/HTLV-2 individuals by testing the ability of patient PBMCs to induce CC-chemokines in association with the expression of CCR5 receptor.

**Methods:** Cryopreserved PBMC aliquots from HIV-1/HTLV-2, HIV-1/HTLV-1 coinfected, HIV-1 monoinfected, and uninfected donors (HTLV and HIV-1 seronegative) were cultured in vitro for 24 and 72 hours. Levels of CC-chemokines (MIP-1α, MIP-1β, and RANTES) were measured in cell-free supernatants by ELISA (DuoSet ELISA development kits, R&D Systems). In parallel, cells were collected to determine CCR5 expression by flow cytometry analysis using PE-labeled anti-CCR5 monoclonal antibody (clone 2D7, BD Bioscience). ANOVA with Bonferroni’s multiple post-test comparison were used to analyze the data using GraphPad Prism software.

**Results:** After 24 hours of culture, higher levels of MIP-1α, MIP-1β, and RANTES were found in HIV-1/HTLV-2 coinfected (462.3±296.2, 1512.1±1133.7, 8044.0±1081.3 respectively) compared to HIV-1 mono-infected (14.5±7.6, 12±6.8, 2345±1197.2 respectively) populations (p<0.05). Higher levels (p<0.05) of RANTES were found in HIV-1/HTLV-1 after 24 and 72 hours of culture. Lymphocytes from HIV-1/HTLV-2 coinfected individuals showed a significant downregulation in the expression of CCR5 after 24 and 72 hours of culture compared to lymphocytes from HIV-1 and uninfected groups (p<0.05). Lower percentages of CCR5-positive cells were found in HIV-1/HTLV-1 coinfected after 72 hours of in vitro culture (p<0.05).

**Conclusion:** HTLV-2 infections may result in activation of innate immunity against HIV-1 via stimulation of CC-chemokines and receptors and therefore might modify CCR5/HIV-1 binding and HIV-1 progression in coinfected individuals.

**Final ID: 89**

**An Unusual Cause of Chest Pain: A Case of Left Pectoral Pyomyositis Secondary to Community Acquired MRSA**

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**Objectives:** 1. Enhance clinical awareness of the increasing incidence of pyomyositis in temperate climates. 2. Consider the presence of pyomyositis in patients who present with localized musculoskeletal complaints in the setting of bacteremia.

**Case:** A 28-year-old African American female with a past medical history of hypertension presented with two days of persistent, left-sided, “burning,” chest pain exacerbated by palpation, deep breathing, and lying supine. While in the emergency department the patient was found to be afebrile and tachycardic with a mild leukocytosis of 14,700/μL. A chest X-ray was performed, and no abnormalities were noted. Computed tomography (CT) of the chest with contrast was negative for pulmonary emboli or other...
pathology. The patient was subsequently discharged home with a presumed muscle strain, only to return to the emergency department two days later with complaints of worsening left-sided chest pain. The patient had a temperature of 100.1 F and a worsening leukocytosis. An electrocardiogram showed sinus tachycardia, and a chest x-ray was unremarkable. The patient was admitted for further evaluation and management. Two days after admission, blood cultures returned positive for methicillin-resistant Staphylococcus aureus (MRSA). The patient was subsequently started on intravenous vancomycin. Transthoracic and transesophageal echocardiograms were performed and showed no valvular or other abnormalities. An indium-tagged white blood cell scan showed an area of intense uptake in left mid-anterior hemithorax, consistent with the distribution of the chest pain. A repeat CT scan revealed an ill-defined mass-like inflammatory lesion in the left anterior chest wall extending into the intercostal fat, mediastinal fat, and pleural spaces, with some compression of the right ventricular outflow tract, as well as a moderate left-sided pleural effusion. A thoracentesis produced 250mL of exudative and culture-negative fluid. Surgical drainage of the affected muscle was considered, but not performed, as a focal fluid collection or abscess was not identified. The patient was continued on IV vancomycin for a total 4 weeks and her symptoms gradually improved. A repeat chest CT was performed at the end of therapy, which showed resolution of the inflammatory mass and pleural effusion.

Discussion: Pyomyositis is a purulent infection involving skeletal muscle associated with bacteremia and abscess formation. Classically seen in the tropics, this disease has been recognized with increasing frequency in temperate climates. Pyomyositis is often not an early diagnostic consideration due to its rarity in temperate climates and non-specific presenting signs and symptoms (fever, muscle pain and leukocytosis), often delaying initiation of appropriate therapy. If not recognized and treated in a timely manner, pyomyositis can progress to septicemia and septic shock. Predisposing factors include immunodeficiency, trauma, intravenous drug use and malnutrition. The most common associated pathogen is Staphylococcus aureus, responsible for approximately 95% of tropical and 70% of temperate cases. Treatment includes drainage of abscesses and empiric intravenous antibiotics initially directed against MRSA and adjusted according to gram stain and culture results. It is generally recommended to continue IV antibiotics for four weeks and to repeat imaging at the end of therapy. This case illustrates the importance of considering the diagnosis of pyomyositis in patients who present with persistent fever, leukocytosis and focal muscle cramping and tenderness.

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Kaposi’s sarcoma-associated herpes virus (KSHV) is etiologically associated with Kaposi’s sarcoma (KS) and primary effusion lymphoma (PEL). KS lesions are characterized by endothelial cells with multiple copies of latent KSHV episomal genome, lytic replication in a low percentage of infected monocytes, inflammatory cytokines plus growth factors. Our earlier studies demonstrated that KSHV utilizes inflammatory COX-2/ PGE2 to establish and maintain its latency (Sharma-Walia, N., A. G. Paul, V. Bottero, S. Sadagopan, M. V. Veettil, N. Kerur, and B. Chandran. 2010. PLoS Pathog 6: ). KSHV latency, monocyte recruitment and lipogenesis

KSHV INDUCED 5-LIPOXYGENASE-LEUKOTRIENE B4 (5LO/LTB4) CASCADE PLAYS KEY ROLES IN KSHV LATENCY, MONOCYTE RECRUITMENT AND LIPOGENESIS
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Kaposi’s sarcoma-associated herpesvirus (KSHV) is etiologically associated with Kaposi’s sarcoma (KS) and primary effusion lymphoma (PEL). KS lesions are characterized by endothelial cells with multiple copies of latent KSHV episomal genome, lytic replication in a low percentage of infected monocytes, inflammatory cytokines plus growth factors. Our earlier studies demonstrated that KSHV utilizes inflammatory COX-2/PGE2 to establish and maintain its latency (Sharma-Walia, N., A. G. Paul, V. Bottero, S. Sadagopan, M. V. Veettil, N. Kerur, and B. Chandran. 2010. PLoS Pathog 6: e1000777). Here, we evaluated the role of 5-lipoxygenase (5LO) and its potent chemoattractant metabolite leukotriene B4 (LTB4) in KSHV biology. Abundant staining of 5LO was detected in human KS tissue sections. We observed elevated levels of 5LO and high secretion of LTB4 during primary KSHV infection of endothelial cells and in PEL–B cells (BCBL-1 and BC-3). Blocking the 5LO/LTB4 cascade inhibited viral latent ORF73, immunomodulatory K5, vMIP1, and vMIP2 gene expression, without much effect on lytic switch ORF50, immediate early lytic K8 and vMIP2 gene expression. 5LO/LTB4 inhibition downregulated TH2 and elevated TH1 related cytokine secretion, and decreased human monocyte recruitment, adhesion and transendothelial migration. 5LO/LTB4 inhibition reduced fatty acid synthase (FASN) promoter activity and its expression. Since FASN, a key enzyme required in lipogenesis, is important in KSHV latency, collectively, these findings suggest that the 5LO/LTB4 play important role in KSHV biology, and that effective inhibition of the 5LO pathway via clinically approved SLO inhibitors could potentially be used in treatment to control KS and PEL.

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Nanovesicles (NVs) production, transport and their significance in tumor microenvironment has been shown in many types of cancers but the prevalence and role(s) of NVs in Kaposi’s sarcoma herpes virus (KSHV) associated human malignancies has never been studied. Here, we characterized and assessed the biological role(s) of KSHV-infected cell milieu derived NVs. We worked with latently infected (KSHV+) primary effusion lymphoma (PEL) cell lines, which included PEL (KSHV+/EBV--; BCBL-1, BC-3) and non-infected (KSHV+/EBV-; BJAB) human Burkitt’s lymphoma (BL) cell lines. Here, we characterized KSHV+ cell line derived NVs and compared them with NVs obtained from the KSHV+/EBV- cell line. We found that KSHV+/EBV- PEL cells secrete a significantly high level of NVs. Fluorescently labeled NVs derived from either KSHV+ or KSHV- cell lines were efficiently/rapidly internalized by primary endothelial cells (ECs). We further showed that the KSHV+ cell line derived NVs were enriched in inflammatory protein leukotriene A4 hydrolase (LTA4H) when compared to BJAB derived NVs. Most interesting was the induction of endothelial mesenchymal transformation (EndMT), migration, vascular permeability and invasion potential of the recipient EC’s exposed to KSHV+/PEL derived NVs. Together, our study for the first time reported that NVs secreted from KSHV+/EBV- cells have a unique inflammatory signature and these NVs induce EndMT via binding to endothelial adhesion junctions and TGF-β1. This study is the first time reported that NVs secreted from KSHV+/EBV- cells have a unique inflammatory signature and these NVs induce EndMT via binding to endothelial adhesion junctions and TGF-β1.

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Objectives: To determine factors that influence outcomes of diabetic foot ulcers (DFUs) treated with skin substitutes (SSs).

Methods: A retrospective review of all patients with DFUs treated with a SS (Apigraf or Dermagraft) between 2011 and 2012 in a tertiary care regional wound center was conducted. Demographic information, ulcer size and location, comorbidities, other treatments received (debridement, hyperbaric oxygen, offloading, compression and antibiotics when needed) and outcomes were determined. Primary outcome studied was complete wound healing. Statistical analysis was done using chi-square test for categorical variables and t-test for continuous variables.

Results: Mean age of the patients studied was 67.6 years (range: 46 – 92 years) and 57% were male. Complete healing occurred in 26 (70%). Factors associated with successful wound healing included smaller ulcer size (p=0.009) and shorter lead time defined as the time from DFU identification to referral to a wound center for treatment. The mean lead time was 1.87 months for healers versus 7.28 months for non-healers (p=0.003). Patients with healed DFUs had fewer debridements (mean: 13.5) compared to non-healers (mean: 20.5).

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 факторы, влияющие на заживление язвенной язвы (ДУУ) при дерматите (ДУУ) и сахарном диабете (СД)

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26.7), (p=0.017). More wound infections were seen in the ulcers that failed to heal compared to the ulcers that healed; however this was not statistically significant (54% versus 28%, p= 0.126). Location of the wound, associated comorbidities, and adequacy of blood sugar control did not significantly impact healing.

Conclusions: Education leading to early identification of a smaller DFU becomes paramount to healing when using SSs. Additionally, repeated debridement deemed detrimental to DFUs being treated with SSs. Wound infections did not significantly affect wound healing when treated adequately. Given the cost of SSs, our findings emphasize appropriate selection of patients for these novel methods to maximize their healing potential.

NEPHROLOGY

FINAL ID: 99
UREMIC FROST

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A 31 year-old Hispanic male was admitted to the hospital for shortness of breath, weakness, and a pruritic rash. He was found to have microcytic anemia (hemoglobin 4.7; reference range 13.0 - 17.0 g/dL), elevated blood urea nitrogen (213 mg/dL; reference range 10-25 mg/dL) and creatinine (265 mg/dL; reference range 0.70 - 1.40 mg/dL), unmasking uremia and acute kidney failure of unknown etiology. Our dermatology team was consulted due to concern for erythema papulatum uremicum.

Uremic frost. Both uremic frost and erythema papulatum uremicum are now clinical rarities in developed countries due to the wide availability of dialysis. Given different manifestations are associated with specific risks of morbidity and mortality, and respond to different treatment regimens, it is important for dermatologists to be aware of the full breadth of dermatologic conditions associated with renal disease.

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FINAL ID: 101
URINARY EXOSOMAL CERULOPLASMIN IS A POTENTIAL BIOMARKER OF EARLY DIABETIC NEPHROPATHY

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Background: Urine exosomal proteins are fingerprints of the metabolic state of the kidney and offer new biomarkers for diagnosing early diabetic nephropathy. Previously we found that urinary exosomal ceruloplasmin, a redox enzyme sensitive to oxidative stress, was up-regulated several fold in nephrotic patients. We also observed that high exosomal ceruloplasmin in these patients did not correlate with the ceruloplasmin levels in the urine suggesting that urinary exosomal ceruloplasmin was derived from the kidney and not from filtered ceruloplasmin. In this study we investigated whether urinary exosomal ceruloplasmin could also serve as a biomarker for diagnosing early diabetic nephropathy prior to onset of microalbuminuria.

Methods: Urine samples from control subjects (n=15) and diabetic patients (n=54) were collected from renal and diabetic clinics at JSH. Exosomal extracts were prepared by differential ultracentrifugation. Ceruloplasmin levels were measured in the exosomal extracts by ELISA. For analysis of results the diabetic patients were subdivided as: non-albuminuric (<0.03 g albumin/g of creatinine, n=20), microalbuminuria (0.03-0.3 g albumin/g of creatinine, n=17), sub-nephrotic (0.31-2.0 g albumin/g of creatinine, n=12), and nephrotic (>2.1 g albumin/g of creatinine, n=5).

Results: Compared to controls (1.3 ± 0.4 (mean ± SEM) ng ceruloplasmin/ mg exosomal protein), ceruloplasmin levels increased 5 fold in diabetics without albuminuria (p<0.05; n=20), and continued to be high (5-6 fold, p<0.05) in microalbuminuria (n=17), sub-nephrotic (n=12) and nephrotic groups (n=5). Importantly, ceruloplasmin levels in the non-albuminuric diabetic patients were 5 fold higher (p<0.05) than controls, suggesting that urinary exosomal ceruloplasmin levels increased before the onset of microalbuminuria.

Conclusions: We conclude that early increase of urinary exosomal ceruloplasmin levels could be potentially predictive of onset of diabetic nephropathy.

FINAL ID: 103
REDUCED URINARY ANGIOTENSINOGEN EXCRETION IN PREECLAMPSIA

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Objective: Preeclampsia (preE) is a hypertensive disorder of pregnancy. We reported the suppression of circulating renin-angiotensin system (RAS) in a rat model of preE. Urinary angiotensinogen has been considered as an indicator of intrarenal angiotensin status in hypertension. Little is known about the relationship of urinary angiotensinogen and intrarenal RAS in preE. The aim of this study was to evaluate the level of urinary excretion of angiotensinogen in preE to assess the RAS status.

Methods: Normal pregnant (n=57) and preE (n=32) patients were recruited from Scott & White Hospital and had their blood drawn between 21 to 739

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40 weeks of pregnancy. Inclusion criteria for determination of preE patients include blood pressure >140/90 mm Hg and presence of proteinuria >300 mg of protein/24h in urine. Two groups of rats were used in this study: normal pregnant (n=10) and preE rats (n=10) which were given weekly injections of desoxycorticosterone acetate and 0.9% saline to drink. The preE rats developed hypertension and proteinuria. Urinary angiotensinogen levels were assayed by commercially available ELISA kits. The plasma AngII levels were measured by AngII ELISA kit. The kidney expression of (pro)renin receptor, AT1, and AT2 receptors and renin for two groups of rats were measured both by western blot and immunohistochemistry.

**Results:** In preE patients, the mean urinary excretion of angiotensinogen (2.9 ± 1.1 ng/mg creatinine) level was suppressed (p<0.05) compared to that in normal pregnancy (2.7 ± 1.5 mg/mg creatinine). The urinary excretion of angiotensinogen (NP: 2 ± 0.4, PDS: 1.5 ± 0.3, nmol/day) was lower (p<0.05) in PDS rats compared to NP. The plasma concentration of AngII for preE rats (25 ± 3 fmol/mL) was lower (p<0.05) compared to NP rats (39 ± 5 fmol/mL). The kidney expression of (pro)renin receptor was downregulated (0.8 fold), while, the AT1 receptor was upregulated (1.5 fold) in preE rats compared to NP. However, the kidney expression of AT2 and renin were similar in NP and PDS rats.

**Conclusions:** We have demonstrated that urinary excretion of angiotensinogen is reduced in both pregnant women with preE and in a rat model of preE. The kidney expression of RAS components was either downregulated or remained unchanged in preE rats. This finding indicates that preE is an AngII dependent form of hypertension.

**FINAL ID: 105**

**PROINFLAMMATORY CYTOKINES REGULATE OXALATE TRANSPORT BY INTESTINAL EPITHELIAL CELLS**


80% of kidney stones are composed of calcium oxalate, and minor changes in urine oxalate affect the stone risk. The mammalian intestine plays a crucial role in oxalate homeostasis. Intestinal oxalate absorption is largely passive and paracellular, while anion exchanger SLC26A6 (A6) plays a critical role in active transcellular intestinal oxalate secretion. Proinflammatory cytokines disrupt intestinal barrier function and increase passive paracellular flux, as well as inhibit several intestinal transporters. We therefore initiated studies to test the hypothesis that proinflammatory cytokines could enhance passive paracellular intestinal oxalate absorption and/or reduce active transcellular intestinal oxalate secretion, thereby modifying urinary oxalate excretion and the stone risk.

The proinflammatory cytokines IFN-γ and TNF-α caused ~3-fold increase in mucosal to serosal 14C-oxalate & 18-h mannitol (a paracellular marker) absorptive fluxes in human intestinal Caco-2-BBE (C2) cells grown on snapwell inserts and mounted in Ussing chambers. The cytokine-induced increased absorptive fluxes in C2 cells were completely blocked by pretreatment with gastrokine-1/AMP-18 and GLP-2. IFN-γ and TNF-α also significantly inhibited (by 30-40%) apical 14C-oxalate uptake, measured as C1-oxalate exchange, by C2 cells through mechanisms involving reduced A6 mRNA and total protein expression. In addition, IL-4 and IL-6 also significantly inhibited apical oxalate uptake by C2 cells, while IL-1β, 2, & 8 had no effect. The physiological relevance of these findings is underscored by the observation that TNF-α (2 μg IP x 48 hours) caused significant inhibition (~55%) of mouse jejunal oxalate secretion (Control: Jnet = 88.35 ± 15.71, J500 = 99.96 ± 13.95, J50 = −11.61 ± 1.76 pmol/cm²/h, TNF-α: Jnet = 63.85 ± 12.1, J500 = 43.98 ± 7.1, J50 = 19.88±4.99 pmol/cm²/h), converting oxalate transport from net secretion to control tissues to net absorption in TNF-α-treated tissues. These findings are of potential relevance to pathophysiologic of inflammatory bowel disease (IBD) - and obesity-associated hyperoxaluria, in which significantly higher intestinal and systemic proinflammatory cytokines levels respectively have been seen. We conclude that proinflammatory cytokines significantly enhance passive paracellular intestinal oxalate absorption, and that AMP-18 and GLP-2 have therapeutic potential in this process. Proinflammatory cytokines also significantly reduce apical oxalate uptake by C2 cells by reducing A6 mRNA and total protein expression, as well as significantly decreased mouse jejunal oxalate secretion, converting jejunal oxalate transport from net secretion to net absorption, which might lead to hyperoxaluria and increased risk for related kidney stone disease. Future studies will be directed at evaluating the therapeutic potential of AMP-18, GLP-2, anti-TNF-α, anti-IFN-γ, anti-IL-4, and anti-IL-6 neutralizing antibodies in the IBD- and obesity-associated hyperoxaluria.

**FINAL ID: 107**

**ENCEPHALOPATHY AND PROFOUND HYponATREMIA DUE TO SIADH INDUCED BY BORTEZOmb (VELCADE)**

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**Background:** Velcade-induced hyponatremia is not rare, at times symptomatic though under-recognized & underdiagnosed. We report a case to illustrate its insidious onset & slow spontaneous recovery needing brief tolvaptan therapy.

**Method:** We consulted on hyponatremia in a 72-year-old woman getting outpatient velcade for symptomatic multiple myeloma. She was admitted for confusion, ataxia and a fall. A month before, the 1st of 7 doses of velcade was given. Serum (S) Na (in mM) was 140 after the 4th but fell to 125 after the 6th & to 121 after the 7th dose. Though vital signs, volume status & neurologic exam were normal, urine (U) Na (<10) & BNP (15 pg/mL) were low, prompting normal saline for presumed salt depletion. Despite gaining 5.5 L of fluid & accruing edema, SNa peaked only at 127 & then quickly fell to 122. On day 3, U osmolality (osm) (in mOsm/kg) remained high at 567 vs. S osm of 258, despite high UNa of 102. On day 4, when S osm was low at 251-254, plasma ADH was inappropriately normal at 1.1 pg/mL SIADH was diagnosed. Other known etiologies of SIADH were systematically excluded. To prevent worsening hyponatremia, she got 15 mg oral tolvaptan, which promptly dropped S osm to 50, led to 3 L diuresis in 48 h, & briefly raised SNa to 132-139. By restricting fluids to <1.2 L/day, SNa peaked ~130 from days 7-12, at which point, U osm spontaneously fell to 228 & 119. Days 12 after the 7th & last dose of velcade, SNa was 130, slowly climbed to 135 & remained normal for the next month on ad lib fluids.

**Conclusions:**

1. Our case illustrates the potential of symptomatic severe hyponatremia due to velcade-induced SIADH.
2. Frequent & continued monitoring of SNa is key as hyponatremia may emerge insidiously, late after multiple doses, & even in outpatients.
3. Ample hydration during velcade may be unwise.
4. She took > 2 weeks after onset to recover spontaneously, suggesting a brief course of tolvaptan is needed if hyponatremia is severe or fluid restriction ineffective.
5. We need more studies to define the mechanism & address the feasibility of resumption after recovery, with or without concurrent tolvaptan prophylaxis.

**FINAL ID: 109**

**THREE DIMENSIONAL CELL CULTURES DEMONSTRATE ALTERATIONS IN CELL ASSEMBLY WHEN EXPOSED TO HIGH GLUCOSE STATES**

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**Introduction:** Tubular epithelial cells in culture form structures which resemble in vivo tubules. These three dimensional cultures (3d) serve as a model to study the effects of altered glycemic states and the impact of curcumin on cell proliferation.

**Methods:** Human kidney tubular epithelial cells (HK-2) were cultured in 5 and 25 mM glucose with mannitol serving as an osmotic control. After at least three passages in the three states, the cells were transferred to matrigel plated in 24 well plates and cultured with the respective media for an additional twenty days. Additional studies were performed with and without the addition of curcumin (20 mM).

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Results: Individual cells, observed on day one of 3d culture grew into small aggregates and later into tubules and wreath-like structures with lumen formation. Cells within the aggregates showed polarization with primitive microvillous formation observed by light and electron microscopy. Cells cultured in high glucose (25 mM) formed smaller aggregates than in 5 mM glucose. Cell death was observed with the addition of curcumin.

Discussion: High glucose levels alter growth in 3d. The addition of curcumin also affects HK-2 cell growth in matrigel. We present microarray data comparing RNA expression in these three conditions and the impact of curcumin treatment.

FINAL ID: 111
MOLECULAR CHARACTERIZATION OF RENAL RESPONSES IN LUPUS NEPHRITIS USING SERIAL KIDNEY BIOPSY SAMPLES
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Introduction: The molecular events occurring within the kidney during treatment of lupus nephritis (LN) are unknown, but could be used to target and improve therapy. Current therapies are associated with considerable morbidity and provide low complete response rates, around 50% at 12 months. This is in part due to the clinical and pathogenic heterogeneity of LN. We postulate that characterizing the molecular differences between responders and non-responders will yield a set of genes that are being missed by current therapies and may be targets for novel therapeutics.

Methods: We analyzed the molecular expression of 11 pairs of proliferative LN biopsies and 4 normal control biopsies (pre-implantation donor transplant biopsies) using Nanostring RNA extraction kit (Nanostring Technologies, Seattle, WA). The kidney biopsies were formalin-fixed paraffin embedded and supplied by our collaborators in Argentina. All patients had at least 2 biopsies with the first at flare and the second after 6 months of treatment. Patients were treated with standard immunosuppression therapy for LN. Based on clinical parameters for disease activity, 5 patients achieved complete remission (CR), 3 partial remission (PR) and 3 no response (NR). The expression of 511 immune-response genes was analyzed at LN flare diagnosis and after treatment. A linear model was used to compare the 4 groups (control/CR/PR/NR) at baseline and paired t-test was used to compare baseline data to data at follow up for each patient. Linear (mixed) models were used to detect differentially expressed genes for flare samples between responders (CR/PRs) and controls, non-responders (NRs) and controls, and between responders and non-responders. Top transcripts were considered differentially expressed only if they met both criteria of at least a 2-fold change and p-value < 0.01.

Results: Thirteen transcripts were differentially expressed in all 3 groups compared to control. IL-6 and NFATC1 expression were elevated in all groups but statistically significant only in CR group (p = 0.006 for IL-6 and p = 0.005 for NFATC1). Transcript expression varied across the groups. For example, C1QB was differentially expressed in all three groups however it was most highly expressed in the NR group with a 6-fold increase in expression compared to CR (p = 0.001). Overall, 22 transcripts were uniquely expressed in the PR group and 49 in the NR group. At repeat biopsy, three transcripts (FCAR, FKBP5, IL1RL1) showed significant decrease in expression in the CR group. There were no significant changes in expression in either the PR or NR groups. Between groups comparison revealed 7 transcripts differentially expressed in the PR vs. CR and one transcript (TRAF1) in the NR vs. PR.

Pathways showed differences in expression vs. control. There were no significant changes in expression in either the PR or NR groups. Between groups comparison revealed 7 transcripts differentially expressed in the PR vs. CR and one transcript (TRAF1) in the NR vs. PR.

Conclusion: These findings suggest that the molecular make up of LN flares are different. Flares that result in an NR have pathways activated that are unique to LN and that may contribute to treatment failure. Pathways involved in inflammation and autoimmunity remain active despite improvement in clinical parameters suggesting that current clinical markers do not adequately assess disease control. Establishing a molecular model for each flare type may elucidate biomarkers specific to flare type and help guide treatment decisions. Therapeutically targeting the pathways specific to NR may convert non-responders to responders.

FINAL ID: 112
CYSTIC NEPHROMA: A BENIGN TUMOR OR HAMARTOMATOUS PROLIFERATION?
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Background: Cystic Nephroma (CN) has been described as a rare, benign, renal neoplasm. Since 1892, only about 200 cases have been reported in the international literature. Recently, it has been proposed that a unifying term Renal Epithelial and Stromal Tumors (REST) be applied to the spectrum of tumors encompassing Cystic Nephroma and Mixed Epithelial and Stromal Tumors (MEST). The non-specific clinical presentation and similarities in imaging studies with other cystic renal tumors, especially tubulocystic carcinoma and cystic renal cell carcinoma, make preoperative diagnosis of CN difficult.

Methods and Results: We report a 32-year-old Hispanic female, with a history of a right, complex cystic, renal mass treated by robotic decortication 2 years ago, who present with flank pain, hematuria and recurrent urinary tract infection. She denied fever, dysuria and weight loss. Laboratory tests including renal function tests were within normal limits. A CT study showed a 3.4 cm multicystic lesion with thickened septa and enhancement, present at the lower pole of the right kidney. The patient elected to proceed with a right partial nephrectomy. The nephrectomy specimen revealed a well circumscribed, multicystic tumor abutting the renal pelvis, with thick tan-white septa and smooth walls, filled with clear fluid. Microscopic examination showed variably-sized cysts scattered throughout a variable cellular stroma. At higher power, the cysts were lined by cuboidal epithelium with focal hobnailing, without significant cytologic atypia and a low mitotic rate within the epithelium and stroma. The ovarian-like stroma was condensed around the cysts with hyalinization and focal hypercellularity. Scattered areas had thick-walled vessels along with primitive vascular and muscular areas which were positive for desmin. The epithelial lining was positive for CK19, high molecular weight cytokeratin and AMACR suggesting a primitive tubular epithelial phenotype. The stroma was positive for estrogen receptor, progesterone receptor and CD10, an immunoprofile previously described in both CN and MEST. Primitive glomeruli-like structures were also present.

Conclusion: Cystic nephroma is a relatively rare, benign, renal tumor which is composed of epithelial and stromal elements. The epithelium in the current case had features of primitive tubules and areas reminiscent of glomeruli. The stroma is variably cellular with areas of muscle differentiation and vascular structures. These features are reminiscent of renal dysplasia. We suggest that cystic nephroma represents a hamartomatous proliferation akin to cystic dysplasia and that the variable mixture of epithelial and stromal elements and immature glomerular, tubular, muscle and vascular elements may be present in variable proportions creating a spectrum of lesions previously described as CN and MEST. Additionally, these lesions need to be differentiated from cystic renal neoplasms due to similarities in clinic presentation and by imaging with cystic renal cell carcinoma and tubulocystic carcinoma which necessitate histopathological examination and immunohistochemical studies for definitive diagnosis.

FINAL ID: 113
EPOXIECOSATRIENIOIC ACID ANALOG MITIGATES RADIATION NEPHROPATHY
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Radiation nephropathy (rad np) occurs in humans after hematopoietic stem cell transplantation, radionuclide therapies, and could occur after accidental or belligerent radiation exposures. Endothelial injury is an early feature of rad np, and we have found decreased expression of CYP2C epoxidegenase in this model. We therefore tested an epoxiecosatrienioic acid analog as a...
mitigator. Rats underwent single-fraction 11 Gy total body irradiation (TBI), with one leg shielded to enable hematopoietic reconstitution. Epoxyeicosatrienoic acid analog A (EET-A) was given at 10 mg/kg/day, and captopril (cap) was given at 30 mg/kg/day, in the drinking water starting at 2 days after TBI. At 12 weeks after TBI, arterial reactivity to acetylcholine was assessed, along with systolic blood pressure (BP), urine protein and albumin, urine nephrin, and azotemia. BP is systolic blood pressure in mmHg; UP is urine protein, UC is urine creatinine, and BUN is blood urea nitrogen, in mg/dl. U alb and nephrin is urine albumin and nephrin, both in mcg. Data are geometric means.

Afferent arteriolar dilation to acetylcholine was significantly (p<0.05) attenuated 12 weeks post-radiation. Captopril or EET-A mitigation treatment significantly (p<0.05) improved the afferent arteriolar dilation to acetylcholine. Semi-quantitative histology on a subset confirmed the benefit of EET-A and captopril. Both drugs were well-tolerated.

The mitigation benefit of EET-A is less than that of captopril, despite equal lowering of blood pressure. The benefit of both agents on kidney function is parallel with their effect on lowering urinary albumin and nephrin, which indicates mitigation of glomerular injury. Future studies will escalate the dose of EET-A, will test EET-A as a treatment for established rad, and will test that agent in combination with captopril.

**FINAL ID: 114**

**ACUTE KIDNEY INJURY (AKI) DUE TO MULTIPLE MYELOMA (MM) SUCCESSFULLY REVERSED BY BORTEZOMIB: A MEDICAL EMERGENCY REQUIRING A HIGH INDEX OF SUSPICION AND PROMPT DIAGNOSIS BY PLASMA FREE LIGHT CHAIN (PFLC)**

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**Background:** MM is caused by plasma cell dyscrasia & excess M protein. It can cause renal failure due to cast nephropathy or light chain (LC) deposition. We report a case with AKI where MM was promptly diagnosed & renal failure rapidly reversed by bortezomib, a proteasome inhibitor selective against plasma cells.

**Case Report:** We cared for a 56-year-old man with AKI noted in evaluating his back pain. Serum creatinine (Cr) rose from 0.8 to 4.6 mg/dl in 2 months. Serum protein electrophoresis (SPEP) had a small M spike. Urine PEP showed 2 large M spikes (3.1 & 78.9% of 5.1 g protein/d), but dipstick only 1+ positive. PFLC κ was up 2 fold (from renal failure), but λ pathologically up 70-fold to 1890 mg/L (vs. 26 in normal); yielding a low κ:λ ratio of 0.02, diagnostic for MM. Other causes for AKI were excluded. 50% λ-restricted plasma cells in the marrow confirmed MM. He got plasma exchange, steroids & 4 doses of bortezomib in 2 weeks. Cr peaked at 9.9 but needed no dialysis. Bortezomib dropped his PFLC λ to 15 mg/L in 10 days & controlled it over next 4 months. Cr also fell in 10 days & hit 2.4, 1.5 & 1.2-1.4 mg/dL after 0.5, 2, & 3.5-5 months respectively of the 1st dose.

**Conclusions:**
1. Our case illustrates the need for a high index of suspicion for MM in adult AKI.
2. PFLCs are sensitive & specific, useful in monitoring therapy & progression. Myeloma proteins are missed by urine dipstick but detected & quantifiable by UPEP.
3. Contrary to other causes of AKI where therapy is largely supportive, preventing complications & averting nephrotoxins, bortezomib is effective in halting synthesis of LC, the specific culprit, thus reversing AKI in MM. His gratifying response confirms & extends the published short list of similar patients diagnosed & effectively treated by bortezomib without dialysis.

4. Recent experience suggests a much improved outcome in MM despite severe renal failure vs. the era before bortezomib. Myeloma kidneys should be managed as a treatable medical emergency.

**FINAL ID: 115**

**POSTPARTUM ATYPICAL HEMOLYTIC UREMIC SYNDROME (AHUS) SUCCESSFULLY TREATED WITH ECULIZUMAB: A CASE REPORT**

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**Introduction:** Atypical hemolytic uremic syndrome (aHUS) is a rare, life threatening illness characterized by widespread thrombotic microangiopathy (TMA). We report a case of a patient with acquired aHUS treated with eculizumab resulting in resolution of her renal failure without the need for dialysis.

**Case Report:** We were consulted for oliguric acute renal failure in a 36-year-old Hispanic female who had been admitted to the high-risk OB/GYN service with severe pre-eclampsia in her 31st week of pregnancy; had undergone emergent C-section, complicated by post op bleed from her incision site due to which she was taken back to the operating room for re-exploration. No active source of bleed was found; however the patient did require transfusion with blood and fresh frozen plasma.

Physical exam was significant for elevated blood pressure, tending around the incision site & lower extremity edema. Pertinent lab abnormalities included an increase in creatinine from baseline of 0.6 to 6.8 mg/dl, anemia, thrombocytopenia, elevated LFTs, schistocytes on peripheral blood smear, elevated LDH level, low fibrinogen level & high fibrin degradation products (FDPs). She was initially thought to have HELLP syndrome & conservatively managed in the hope that her condition would improve after delivery of the baby, but her condition continued to worsen. Her ADAMTS13 activity was 61% of normal. She was diagnosed to have aHUS & started on eculizumab for treatment. Her renal function, platelet count started improving the very next day, and all other lab abnormalities trended back to normal. She did well over the next few days and was discharged home with renal function slightly above baseline.

**Discussion:** aHUS refers to the constellation of acute renal failure, thrombocytopenia, & microangiopathic hemolysis without antecedent diarrhea (as seen in STEC-HUS), & is caused by chronic uncontrolled activation of complement system. Because of its overlap with TTP, it has been considered a variant of TTP and treated with plasma exchange (PE) in the past. Often PE does not reverse the renal process or stop the TMA and patients end up on dialysis. A major achievement in treatment of aHUS is the recent availability of eculizumab, a humanized monoclonal anti-C5 antibody that targets the underlying cause of TMA in aHUS. This case highlights the importance of distinguishing aHUS from TTP and starting definitive treatment to prevent irreversible loss of kidney function.
Methods: A combination of case-control association study, protein-promoter mobility shift assay, and promoter activity assay was utilized. We performed association studies in case-control samples of unrelated individuals from both African and European American populations in Chicago (218 cases and 378 controls). S1PR1 promoter was cloned from human genomic DNA, constructed into a luciferase reporter vector and assessed for functionality by luciferase assay in transfected human lung endothelial cells (EC). Promoter with SNP was generated by site-directed mutagenesis.

Results: In European Americans, a promoter SNP rs79060434 (-616C/T) was significantly associated with susceptibility of severe sepsis and sepsis-induced ALI/ARDS (p<0.05). Protein-DNA EMSA analysis indicated that -616C significantly increased expression of the transcription factor NFκB to the S1PR1 promoter. TNF-alpha significantly increased promoter activity of S1PR1 gene in time- and dose-dependent patterns (p<0.05). Promoter activity of S1PR1 with -616T in response to TNF-alpha was significantly decreased, compared to S1PR1 with -616C (p<0.05).

Conclusion: These data suggest that a functional S1PR1 promoter variant significantly influences the risk of sepsis and sepsis-associated ALI/ARDS.

PULMONARY/CRITICAL CARE

FINAL ID: 119

JUNCTIONAL COMPLEX AND FOCAL ADHESION REARRANGEMENT MEDIATES PULMONARY ENDOTHELIAL BARRIER ENHANCEMENT BY FTY720 S-PHOSPHONATE

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Rationale: Modulation of pulmonary vascular barrier function is an important clinical goal given the devastating effects of vascular leak in acute lung injury (ALI). We previously demonstrated that FTY720 S-phosphonate (Tysiponate/Tys), an analog of sphingosine 1-phosphate (SIP) and FTY720, has more potent pulmonary barrier protective effects than these agents in vitro and in the LPS- and bleomycin-induced models of mouse ALI. Moreover, Tys preserves expression of the barrier promoting S1P1 receptor (SIP1), whereas SIP and FTY720 induce its ubiquitination and degradation. In this report, we further characterize the novel barrier promoting effects of Tys on intracellular signaling and junctional assembly formation in cultured human pulmonary endothelial cells (EC).

Methods/Results: Mechanistic experiments were performed in vitro using cultured human pulmonary EC. Tys significantly increased peripheral redistribution of adherens junction proteins VE-cadherin and beta-catenin and tight junction protein-ZO-1. Inhibition of VE-cadherin with blocking antibody BV9 significantly attenuated Tys-induced TER elevation, while ZO-1 siRNA partially inhibited Tys-induced TER elevation. Tys significantly increased focal adhesion formations and phosphorylation of focal adhesion kinase (FAK). Pharmacologic inhibition of FAK significantly attenuated Tys-induced TER elevation. Moreover, inhibition of cytoskeleton rearrangement by depolymerizing actin with cytochalasin significantly attenuated Tys-induced TER elevation. Tys significantly increased phosphorylation and peripheral redistribution of the actin-binding protein, cortactin, while cortactin siRNA partially attenuated Tys-induced TER elevation. Tys significantly increased Rac1 activity, while inhibition of Rac1 activity by pharmacological inhibition significantly attenuated Tys-induced VE-cadherin redistribution and TER elevation.

Conclusion: Junctional complex and focal adhesion rearrangement play critical roles in Tys-mediated barrier protection in pulmonary EC. These results provide mechanistic insights into the effects of this potential ALI therapy.

Case Report: A 30 year old man was referred to our pulmonary clinic for evaluation of a dry cough for 2 years. His past medical history was significant for multiple episodes of sinusitis and otitis media starting in his early 20s. He was treated for ITP at age 17 and herpes zoster at age 18. Current physical exam was normal except for a patch of eczema on the chest. Serology was significant for a negative HIV Ab test and markedly reduced IgG, IgA and IgM levels of 465, <6 and <25 mg/dl respectively. Pulmonary func-
tion testing revealed mild restriction. His CT was significant for bilateral, in-
numerable ill-defined nodules with associated ground glass opacities and diffuse mediastinal and hilar lymphadenopathy. These findings were progressive when compared to a CT two years prior. The patient underwent bron-
choscopy with transbronchial biopsy and a CT guided transbronchial needle biopsy both of which showed interstitial fibrosis, chronic inflammation and emphysematous changes but no granulomas. A BAL was negative for infec-
tion. A VATS biopsy was obtained demonstrating extensive granulomatous inflammation and fibrosis in the background of a severe lymphoproliferative interstitial pneumonitis. The patient was diagnosed with CVID and granulo-
matous-lymphoproliferative interstitial lung disease (GLILD). He was started on prednisone 40 mg daily in addition to IVIG 500 mg/kg every 3 weeks. He is doing well with plans to repeat his imaging and pulmonary functions after 6 months of therapy.

Discussion: Patients with CVID are prone to the development of a multisystem granulomatous disease as well as malignancy, particularly non-hodgkins lym-
phoma, making the investigation of lung nodules and lymphadenopathy impor-
tant. Diffuse interstitial lung disease can occur and pathologic examination may reveal a combined granulomatous and lymphoproliferative pattern. The term, granulomatous-lymphoproliferative interstitial lung disease (GLILD), has been coined to characterize this disorder. Open lung biopsy is usually required for the diagnosis, as in this case. The presence of GLILD predicts a poor prognosis with a high prevalence of conversion to lymphoproliferative disorders. There is little guidance available on the treatment of GLILD but corticosteroids have been used with improvement in lung function and radiographs. Alternative immu-
nosuppressive agents such as cyclosporine, infliximab and rituximab have been tried in some cases.

FINAL ID: 123

CONDITIONAL DELETION OF Beta-CATENIN IN SMOOTH MUSCLE CELLS FAIL TO AFFECT THE DEVELOPMENT OF PULMONARY HYPERTENSION

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Rationale: Pulmonary arterial hypertension (PAH) is a rare, progressive and fatal disease in which pulmonary arterial pressure (PAP) is elevated by per-
sistent increases in pulmonary vascular resistance (PVR). Pulmonary vascu-
lar remodeling due to enhanced pulmonary arterial smooth muscle cell (PASMC) proliferation and inhibited apoptosis is one of the major causes for elevated PVR in patients with PAH. Canonical Wnt signaling, through β-catenin, has been shown to regulate vascular smooth muscle cell proliferation and apoptosis. This study aims to investigate whether β-catenin activity in PASMC contribute to pulmonary vascular remodeling in the development and progression of pulmonary hypertension.

Methods: Inducible and smooth muscle cell conditional gene knockout mice of β-catenin were created by crossbreeding floxed mice with SMMHC-CreERT2 mice, in which a tamoxifen-inducible Cre recombinase is under the control of the smooth muscle myosin heavy chain promoter. 8 week old male knockout (KO) mice and wild type (WT) control mice were exposed to 10% oxygen or normoxia for up to three weeks. Pulmonary vascular remodeling was evaluated using tissue morphometrics. Right ventricle systolic pressures (RVSP) and right ventricular hypertrophy, assessed as RV/(LV+S) weight ratios, were measured to evaluate pulmonary hypertensive changes. Iso-
lated PASMC were obtained from WT and KO mice in which cell proliferation assays and histochemistry staining was performed.

Results: The smooth muscle specific deletion of β-catenin in mice after 3-week treatment with tamoxifen significantly decreased mRNA and protein expression levels of β-catenin in whole lung tissues, pulmonary arterial tissues

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and aortic tissues. In the smooth muscle specific β-catenin KO mice, however, chronic hypoxia mediated pulmonary hypertension was comparable to the WT mice. Baseline hemodynamics under normoxia were similar among the two groups. The hypoxia-induced increases in RVSF right ventricular hypertrophy, and small pulmonary arterial wall thickness in smooth muscle specific β-catenin KO mice were all similar to the values obtained in the WT littermates.

Conclusion: In this study we identify no significant difference in the development of hypoxia-induced pulmonary hypertension between smooth muscle specific β-catenin KO mice and their WT littermates. These data indicate that the canonical Wnt pathway via β-catenin in PASMC is not a predominant signaling pathway for the development and progression of pulmonary hypertension.

**FINAL ID: 125**

**REGULATION OF GROUP V PHOSPHOLIPASE A2 GENE EXPRESSION IN PULMONARY INFLAMMATION**

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**Rationale:** Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) is characterized by inflammatory disruption of the alveolar-vascular barrier, resulting in severe respiratory compromise. We previously have demonstrated that Group V phospholipase A2 (PLA2G5) expression is increased in cultured lung endothelium and mouse lungs by LPS and augments pulmonary vascular permeability in vitro and in vivo.

**Objective:** To explore the regulation of PLA2G5 gene expression in lung inflammation by disease-associated genetic variants, microRNA and transcription factors.

**Methods:** A combination of genomic wide association study (GWAS), in silico analysis, transient transfection of PLA2G5 3' UTR luciferase reporter and protein-DNA mobility shift assay of PLA2G5 promoter was utilized. We investigated samples from 250 sickle cell disease patients with and without acute chest syndrome in Chicago, and analyzed PLA2G5 with Genomatix and Haploview for gene regulation and allele linkage disequilibrium, respectively. The interactions of PLA2G5 promoter with lung inflammatory early response transcription factor NFkB and NFAT, and PLA2G5 3' UTR with microRNA hsa-miR-501-5p were studied for PLA2G5 epigenetic regulation.

**Results:** In silico, several promoter regions of PLA2G5 associated with mechanical stretch, fluid shear stress, hypoxia and anti-oxidant response were located. Furthermore, several transcriptional factors of TGF-beta signaling pathway and inflammation are identified. GWAS identified several intronic genetic variants of PLA2G5 that were significantly associated with acute chest syndrome, and these were in high linkage disequilibrium with promoter variants rs1573184 and rs1573188. In silico analysis of promoter variants rs1573185 and rs1573188 predicted that these significantly influence binding of several transcription factors to PLA2G5. Protein-DNA mobility shift assays indicated that lung inflammatory early response transcription factor NFkB and NFAT bind to the PLA2G5 promoter with high affinity. The activity of the PLA2G5 3' UTR luciferase reporter was significantly increased by LPS and 18% cyclic stretch. This induction was enhanced by transfection with mimics of hsa-miR-501-5p.

**Conclusion:** In vitro and in vivo data identify PLA2G5 as a promising candidate gene in inflammatory lung disease. The current study characterizes the regulation of PLA2G5 gene expression by inflammatory disease-associated polymorphisms, microRNA, and acute inflammatory stimuli and advances our understanding of how PLA2G5 levels are regulated during lung injury pathogenesis.

**FINAL ID: 129**

**SPHINGOSINE 1 PHOSPHATE LYASE FUNCTIONS AS AN ENDOGENOUS SUPPRESSOR OF PULMONARY FIBROSIS IN HUMAN AND IN A MOUSE MODEL**

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**Rationale:** Idiopathic pulmonary fibrosis (IPF) is characterized by alveolar epithelial cell injury, accumulation of fibroblasts and myofibroblasts and abnormal deposition of extracellular matrix proteins. Transforming growth factor beta (TGF-β) and sphingosine 1 phosphate (S1P) have been shown to play critical roles in the pathobiology of pulmonary fibrosis.

**Objectives:** To define roles of S1P yase (S1PL) in murine models of pulmonary fibrosis and in humans.

**Methods:** The expression of S1PL was analyzed in peripheral blood mononuclear cells (PBMCs) from control and idiopathic pulmonary fibrosis patients correlated with pulmonary function and overall survival. These results were further verified in bleomycin model of lung fibrosis. Also, modulation of S1PL expression on the regulation of TGF-β1 and S1P-induced fibroblast differentiation was investigated in vitro.

**Results:** S1PL expression was up-regulated in the fibrotic lung tissues and primary lung fibroblasts isolated from IPF patients and bleomycin challenged mice. In vitro, TGF-β1 enhanced the expression of S1PL in human lung fibroblasts through activation and translocation of Smad to the S1PL promoter sequence. Over-expression of S1PL inhibited TGF-β1 and S1P-induced differentiation of human lung fibroblasts through regulating the expression levels of LC3 and Beclin1. S1PL deficiency (Sgpl1−/−) in mice caused exacerbated bleomycin-induced pulmonary fibrosis, and patients with lower expression of S1PL in PBMCs exhibited higher fibrosis and lower survival rate.

**Conclusion:** These studies suggest that S1PL functions as an endogenous suppressor of pulmonary fibrosis, and may serve as a potential therapeutic target human lung fibrosis. This work was supported by P01 HL098050 (VN) and R03HL096933 (SPR).

**FINAL ID: 131**

**INFLAMMATION WITH ALTITUDE AND CIGARETTE SMOKE EXPOSURE**

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**Rationale:** Occupational exposure to passive smoke presents a significant health risk to workers. Never smoking, healthy flight attendants exposed to cigarette smoke (CS) on commercial aircrafts in the United States prior to
the ban on smoking had significant declines in spirometry and diffusion capacity. Thirteen percent of those examined had declines would be classified as mild chronic obstructive pulmonary disease (COPD) by American Thoracic Society (ATS) criteria. COPD is associated with, and likely caused by, chronic inflammation due to environmental irritants including CS. We have previously shown that exposure at cabin altitude causes systemic inflammation. We propose that the source of that inflammation is the lung, and that inflammation is enhanced by cigarette smoke exposure.

Methods: We recruited two groups of individuals, non-smoking and light-smoking individuals (<1 pack per day) both with normal lung function. Subjects are matched against age, gender, ethnicity, and smoking status. Each subject undergoes 2 separate sessions at least 30 days apart in a hypobaric altitude chamber. Subjects are exposed to either ground level pressure or a simulated standard cabin altitude of 8,000 feet for 5.5 hours in random order. Vital signs, pulse oximetry, alveolar gases, and blood samples are obtained before, during, and after the session. Within 1 hour following their session, subjects undergo bronchoscopy for bronchoalveolar lavage (BAL) and brushing.

Results: 227 subjects were initially screened from an original pool of recruitment consisting of patients undergoing pulmonary function testing at the Oklahoma City VA. Only 21 were enrolled, of whom 6 had smoking and non-smoking matches and only 1 consented. Modification of the research protocol allowed a second source of recruitment from the local campus. 57 subjects have been screened, with 43 recruited and 7 subjects ineligible. Of the 43 recruited subjects, 12 are smokers and 24 are non-smokers. 9 subjects have completed at least one phase of testing including BAL. Preliminary results show that both smokers and non-smokers desaturate to an average of 87% with an ascent to 8,000 feet. Although saturations improved during exposure, they remained 5% below baseline.

Conclusions: Exposure to cabin altitude causes hypoxemia in normal subjects. Measuring inflammation with bronchscopy with BAL in subjects with altitude exposure can be practically performed with the use of a hypobaric chamber. Further testing will determine if altitude exposure alone or in combination with cigarette smoke exposure causes lung inflammation.

FINAL ID: 132

INFLUENZA INDUCES IFN THROUGH RIG-I AND TLR3 SIGNALING IN HUMAN LUNG EPITHELIUM
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Rationale: Influenza virus is a major cause of infectious morbidity and mortality. Mortality is primarily related to pulmonary involvement, either through primary viral pneumonia, mixed viral/bacterial pneumonia, or secondary bacterial pneumonia. Unfortunately, it is unknown how the innate immune system responds to influenza virus infection in human lung. Induction of interferon (IFN) is a critical component of the host response to influenza infection. IFNs are further divided into type I (mainly IFN-α and β), II (IFN-γ) and III (IFN-λ) subtypes, based in part on the differential use of unique receptors through which they mediate signal transduction to induce antiviral activity. Previous studies have demonstrated that Retinoic acid-inducible protein 1 (RIG-I), Toll-like receptors 3 and 7 (TLR3 and 7), nucleotide-binding oligomerization domain containing protein 2 (NOD2) all play important roles in the recognition of influenza virus, but their role in IFN induction in unclear, particularly in human lung.

Methods: We investigated IFN induction by influenza virus in the A549 lung epithelial cell line as well as in primary human alveolar epithelial cells purified from normal human donor lungs rejected for transplantation. These cells were infected with A/Puerto Rico/8/1934 H1N1 influenza virus at an MOI of 1. After 24 h of infection, cells and supernatants were collected, and pattern recognition receptor and IFN protein expression level were measured by qRT-PCR and ELISA, respectively. We then used siRNA knockdown to determine the role of TLR3/7, NOD2, and RIG-I in induction of IFN by influenza.

Results: We found that TLR7 and NOD2 were not involved in IFN induction by influenza virus in human lung alveolar epithelial cells as induction was not prevented by the corresponding siRNAs. Neither RIG-I nor TLR3 siRNA alone completely blocked IFN induction. However, double knockdown of RIG-I and TLR3 completely inhibited IFN induction by influenza.

Conclusions: The data shows that signaling through both RIG-I and TLR3 is important for IFN induction by influenza virus in human lung alveolar epithelial cells.

FINAL ID: 133

REGULATION OF EZRIN/RADIXIN/MOESIN PROTEINS GENES EXPRESSION IN PULMONARY INFLAMMATION
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Rationale: The pulmonary vascular endothelium serves as a semi-selective barrier between circulating blood and surrounding tissues with endothelial cell (EC) integrity critical to tissue and organ function. Increased lung vascular permeability, the consequence of endothelial cell (EC) barrier dysfunction, is a cardinal feature of inflammatory conditions such as acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) and sepsis and leads to lethal physiological dysfunction characterized by alveolar flooding, hypoxemia and pulmonary edema. The ezrin, radixin, and moesin (ERM) family of actin-binding proteins link the actin cytoskeleton to the extracellular matrix (ECM). We previously have shown that the ERMs, despite their structural similarities and reported functional redundancy, differentially participate in SIP- and thrombin-induced EC barrier enhancement and hyperpermeability, with radixin promoting barrier function and moesin opposing it. In the present study we explore the regulation of ERM genes expression in lung inflammation by disease-associated genetic variants in promoter regions of genes.

Methods: The functions of ERM proteins were studied in cultured human pulmonary artery EC (HPAEC) and human lung microvascular endothelial cells (HMVEC). Immunoblotting was performed according manufacturer’s recommendation. A combination of genotype-phenotype linkage analysis, in silico analysis, and transient transfection of radixin promoter wild type (wt) and genetic variant with rs1792789 (~9006C>T) SNP were utilized. We analyzed samples from 270 ALI African American patients in Chicago, and analyzed radixin gene expression with Genomatix and Haploviz for gene regulation and allele linkage disequilibrium, respectively.

Results: LPS and 18% CS each increased time-dependently both the protein expression (by western blotting) of individual ERMs and phosphorylation on critical threonine residues (Ezrin-567, Radixin-564, Moesin-558). In silico analysis revealed several promoter regions of radixin associated with mechanical stretch and anti-oxidant responses. GWAS African American study identified several intronic genetic variants of radixin that were significantly associated with ALI, and these were in high linkage disequilibrium with promoter variant rs1792789, significantly associated with ALI. In silico analysis of promoter variant rs1792789 predicted that this SNP significantly influences binding of several transcription factors to radixin. LPS increased the activity of a 2.1 kb radixin promoter wt luciferase reporter (1.5 fold) with induction reduced in cells transfected with 2.1 kb radixin promoter with rs1792789 (33% reduction).

Conclusion: In vitro and in vivo data identify ERM proteins as promising candidate genes in inflammatory lung disease. The current study characterizes the regulation of ERM genes expression by inflammatory disease-associated polymorphisms and acute inflammatory stimuli and advances our understanding of how ERM levels are regulated during lung injury pathogenesis.

RHEUMATOLOGY/IMMUNOLOGY/ALLERGY

FINAL ID: 135

LONG TERM IMPAIRMENT OF CD4 T CELL RESPONSES AFTER SEPSIS
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Sepsis strikes 750,000 Americans every year; an estimated 210,000 of these patients die, making sepsis the leading cause of death in most intensive care units. Patients who survive the acute stages of sepsis often display severely compromised immune function; as a consequence, death in septic patients is oftentimes related to subsequent nosocomial infections. CD4 T cells, which are essential for the coordination of successful immune responses to opportunistic pathogens, are severely depleted during the acute stage of sepsis and gradually recovered afterwards. However, little is known regarding the mechanism(s) behind this recovery or the extent to which sepsis impairs CD4 T cell function in surviving patients.

Using a cecal-ligation and puncture (CLP) model to induce intra-abdominal peritonitis, we tracked endogenous, antigen-specific CD4 T cell populations throughout loss and recovery, and tested their responsiveness to a secondary infectious challenge. Our results demonstrate that a massive loss of CD4 T cells occurs acutely after sepsis induction, and that CD4 T cell recovery is quantitatively apparent 30d post CLP. After recovery, we observed an increased frequency of CD44hiCD11ahiCD49dhi CD4 T cells—a surface phenotype consistent with CD4 T cells that have encountered Ag (e.g. ‘Ag-experienced’). However, the acquisition of this Ag-experienced phenotype was independent of cognate Ag recognition, given that adoptively-transferred, TCR-transgenic CD4 T cell specificity for S. typhymurium flagellin (SM1 cells) or LCMV glycoprotein (SMARTA cells) displayed increased frequencies of Ag-experienced CD4 T cells as well. IL-7 expression was also higher in CD4 T cells from septic hosts when compared to surgical controls, and the frequency of Ag-experienced CD4 T cells was unchanged in IL-7−/− hosts 30d after CLP. Finally, these phenotypic changes were independent of IL15 availability or thymic output. Despite numerically-apparent T cell recovery, we found a sustained impairment of Ag-specific CD4 T cell responses to a heterologous bacterial infection, persisting for as long as 30 days after sepsis induction and resolution. Using a pMHCII tetramer enrichment approach, we found that the recovery of certain Ag-specific CD4 T cell precursor populations was incomplete in septic mice. Our results suggest that CD4 T cells undergo peripheral recovery by homeostatic proliferation after sepsis-induced lymphopenia, and that this is a phenomenon driven by IL-7 and self-Ag:MHCII availability in the system. Furthermore, an asymmetric or skewed recovery of the epitope-specific CD4 T cell repertoire contributes to persistently impaired primary CD4 T cell responses.

Our findings demonstrate that sepsis can result in substantial changes to the available CD4 T cell repertoire, affecting the capacity of the host to respond to newly introduced Ag (infections) long after the septic event has resolved.

**Introduction:** Sickle cell disease (SCD) is associated with a constellation of disturbances using an HDM-induced murine model of asthma in transgenic mice. The purpose of this study was to explore these immunologic mechanisms underlying asthma severity in SCD. Treatment of asthma in SCD could improve patient outcomes and quality of life.


**Final ID: 139**

**MEASUREMENT PROPERTIES OF MULTIDIMENSIONAL HEALTH ASSESSMENT QUESTIONNAIRE IN SYSTEMIC LUPUS ERYTHEMATOSUS**

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**Background:** Patient reported outcome (PRO) tools are uniquely informative for the management of patients with rheumatic diseases. A single, simple PRO measure, irrespective of an individual patient’s specific diagnosis, can easily be integrated into the clinic check-in processes, in the infrastructure of care, with minimal costs or interference with patient flow. A Multi-Dimensional Health Assessment Questionnaire (MDHAQ) is a PRO tool developed for patients with musculoskeletal diseases; RAPID 3 (Routine Assessment of Patient Index Data) index can be calculated in 5 seconds from templates on the MDHAQ and is validated for use in rheumatoid arthritis. We performed a psychometric analysis of MDHAQ/RAPID3 in SLE to study its measurement properties in routine care of patient with systemic lupus erythematosus (SLE).

**Methods:** 161 patients meeting ACR criteria for SLE completed MDHAQ during a routine clinic visit. All patients completed an MDHAQ and LupusPRO (a disease specific PRO in SLE). Disease activity assessments were made according to SELENA-SLEDAI (physician global assessment (SLEDAI- PGA)), Total SLEDAI and the SELENA-Flare Index (SFI) and damage by SLICC damage index. MDHAQ/ RAPID3 was analyzed for: (a) Internal consistency using the Cronbach’s alpha (b) Content validity of 10 physical function (FN) items on principal component analysis, (c) Criterion validity against disease activity and damage, (d) convergent validity against LupusPRO (a disease specific PRO in SLE). We controlled for underlying diagnosis of fibromyalgia. Spearman rho correlation coefficients were used for non-parametric analyses. A p value of 0.05 was considered significant on 2-tailed tests.

**Results:** Mean (SD) age, SLEDAI-Physician global assessment and total SELENA-SLEDAI scores were 44.8 (13.5) yrs, 0.5 (0.6) and 3.8 (4.5). Mean (SD) RAPID3 score was 9.6 (7.4). Internal consistency as measured by Cronbach’s alpha was 0.88 for the FN component of RAPID3. Factor analysis of the 10 FN items, showed 6 items (1a, 1c, 1g, 1h, 1i, 1j) loading onto 2 factors (physical function and Vitality); 4/10 items (1b, 1d, 1e, 1f) showed cross-loading. RAPID3 correlated moderately with disease activity measures (PGA and SFI, rho=0.30 and 0.32, p =0.02 and 0.01 respectively), but not significantly with Total SLEDAI or SLICC. RAPID3 had strong negative correlation with LupusPRO on the composite Health related quality of life domains score (rho -0.73 (p =0.001)), showing convergent validity.
Conclusions: RAPID3 in SLE has good reliability. Some items of physical function may be redundant for SLE patients. RAPID3 has good convergent validity in SLE.

FINAL ID: 141
ALVEOLAR MACROPHAGES TRANSITION TO A REGULATORY PHENOTYPE WITH CHRONIC HOUSE DUST MITE EXPOSURE

Background: Allergic asthma is a leading cause of morbidity in industrialized nations and results from lack of immunological tolerance to innocuous, environmental antigens. While the emphasis of most studies pertaining to the attenuation of asthma after chronic antigen exposure has focused on the local generation of regulatory T cells (Tregs), little attention has been paid to the potentially crucial role of alveolar macrophages (AMs) in disease resolution. AMs are among the first cells to encounter inhaled allergens and have been shown to be active players in the pathogenesis of asthma using mouse models of allergic airway disease (AAD). However, the regulatory actions of AMs have not been well characterized. The purpose of this study was to investigate the role of AMs in tolerance development to house dust mite (HDM), the most common allergen worldwide.

Methods: To investigate the role of AMs in asthma, C57BL/6 mice were challenged intranasally (i.n.) with 25 µg HDM extract 5 days/week for two weeks (acute) or eleven weeks (chronic). Control animals received equal volumes of PBS. Upon sacrifice, cells from bronchoalveolar lavage fluid (BAL) and lung tissue were isolated and stained for cellular differentials (using May-Grünwald Giemsa) and flow cytometric analysis. Airway hyper-reactivity (AHR) was assessed via response to methacholine challenge. Total serum IgE levels were assessed via ELISA. To examine whether AMs converted to regulatory (IL-10+TGF-β+) macrophages, IL-10gfp (Vert-X) mice were also injected with HDM extract and AMs were examined via flow cytometry. TGF-β production was determined via %AMs positive for latency associated peptide.

Results: Mice that were acutely exposed to HDM developed hallmark features of AAD, including a significant increase in frequency of airway eosinophils (p < 0.001), increased airway hyper-reactivity (AHR) upon methacholine challenge (p < 0.05), and an increase in total serum IgE levels (p < 0.01) compared to control mice. Conversely, chronic HDM-exposed mice developed tolerance to HDM, as noted by resolution of AHR and BAL eosinophilia and a reduction in total serum IgE levels. Chronic HDM exposure was associated with a 9-fold increase in AMs compared to control mice (p < 0.001) and a 3-fold increase in AMs compared to acute HDM-exposed mice (p < 0.001). Phenotypic analysis of these cells demonstrated a 3-fold reduction in frequency of CD206+ alternatively-activated AMs, which have been linked to the pathogenesis of AAD, in the BAL and lung tissue of chronic versus acute HDM-exposed mice (p < 0.01). In addition, chronic HDM exposure was associated with a 3-fold increase in frequency of IL-10- and TGF-β+ producing AMs. Mean fluorescence intensity (MFI) of IL-10 was also significantly elevated in BAL and lung tissue of chronic HDM-exposed mice when compared to control and acute HDM-exposed mice. Further analysis revealed a significant decrease in MHCIi expression on chronic HDM-exposed AMs in BAL (p < 0.05) and lung tissue (p < 0.01) when compared to acute HDM-exposed AMs. Together, these findings suggest that chronic HDM exposure induces a transition of AMs from a pro-inflammatory (i.e. AAD-promoting) to a regulatory phenotype with subsequent development of immunological tolerance to this allergen.

Conclusion: Chronic HDM exposure stimulates AMs to transition from pro-inflammatory (i.e. AAD promoting) to regulatory (IL-10+TGF-β+) cells. This suggests that AMs may play a crucial role in orchestrating tolerance to commonly inhaled allergens and may thus serve as a potential therapeutic target for patients with asthma.

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FINAL ID: 143
DIVERGENT GENOME-WIDE TRANSCRIPTIONAL PROFILES FROM IMMUNE CELL SUBSETS ISOLATED FROM LUPUS PATIENTS WITH DIFFERENT ANCESTRAL BACKGROUNDS
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Background/Purpose: Systemic lupus erythematosus (SLE) is a complex multi-system autoimmune disease of uncertain etiology. Different ancestral backgrounds demonstrate different clinical manifestations and autoantibody profiles. In this study we examined genome-wide transcriptional patterns in major immune cell populations across different ancestral backgrounds.

Methods: Peripheral blood was collected from 21 African-American (AA) and 21 European-American (EA) SLE patients, 5 AA controls, and 5 EA controls. CD4+ T-cells, CD8+ T-cells, monocytes and B cells were purified by flow sorting. Each cell population from each subject was run on an Illumina HumanHT-12 V4 expression BeadChip array (n=208 arrays). Differentially expressed genes (DEGs) were determined by comparing cases and controls of the same ancestral background.

Results: The overlap in DEG lists between different cell types from the same ancestral background was very modest (<1%). Typically between 5-10% of DEGs were shared when comparing the same cell type between different ancestral backgrounds (for ex. CD20 AA vs. CD20 EA). Quantitative measurement of global IFN-induced gene expression revealed that AA subjects demonstrated more concordance across all studied cell types. In EA subjects, a subset of patients demonstrated increased IFN-induced gene expression in all lymphocyte populations but not monocytes, and another subgroup demonstrated IFN-induced gene expression in monocytes but not in CD4 or CD8 T-cells.

Conclusion: We find striking differences in gene expression between different immune cell populations and between ancestral backgrounds in SLE patients. The IFN signature is diverse, with different transcripts represented in different cell populations, and signature-positive cell subsets differed in EA vs. AA patients.