**Oral Presentation:**

**Category: Early Stage Investigator**

# Pre-hospital Identification of Elevated **L**actic **A**cid Levels and **S**epsis **R**elated Outcomes (The LASR Study)

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*Introduction/Hypothesis:* Lactic acid (LA) is an indicator of severe sepsis in patients who present with infection. Typically, fluid resuscitation is based upon LA levels obtained in the emergency department. Guidelines suggest that resuscitation be initiated for LA ≥4.0 mmol/L, and that outcomes are impacted by the rate in which LA normalizes. This study evaluates the utility of fluid resuscitation during pre-hospital transport in patients with suspected infection and a LA of >2.0 mmol/L.

*Methods:* The intervention group was comprised of patients transported to Charleston Area Medical Center via Kanawha County Emergency Ambulance Authority between November 2014 and December 2015. Intervention group patients received LA testing and fluid resuscitation during transport when LA was >2.0mmol/L. These patients with a confirmed diagnosis of sepsis were compared to randomly selected controls who presented with sepsis and a LA >2 mmol/L, but did not receive LA level directed fluid resuscitation during pre-hospital transport. Outcomes were compared with p-values of ≤0.05 considered significant.

*Results:* There were 108 patients (n=216) in each study group. There were no significant differences in baseline demographics, initial LA, or vital signs. There was a 17.6% decrease in hospital mortality in the intervention group (26.9% vs. 9.3%, p=0.001). Time to correction of elevated LA, vasopressor use, and hospital length of stay were also significantly lower in the intervention group.

*Conclusions:* Pre-hospital initiation of fluid resuscitation in patients with suspected infection and a LA >2.0 mmol/L resulted in a significant decrease in morbidity and in-hospital mortality. Expanding this practice to additional pre-hospital providers should be entertained.

**Oral Presentation:**

**Category: Early Stage Investigator**

## Dual Use of Cigarettes and Smokeless Tobacco: Product Use and Nicotine Exposure

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**Introduction:** Smokeless tobacco (SLT) products have been marketed to smokers as a means to reduce risk of tobacco-related diseases or to use in situations where smoking is prohibited. An (un)intended consequence of such marketing may be that smokers supplement rather than replace their cigarettes with SLT. **Purpose:** To compare product use and nicotine exposure on days when only cigarettes are smoked (single use) versus when both cigarettes and SLT (dual use) are used.

**Methods:** Thirty dual users (≥ 5 cigarettes per day for ≥ 1 year, and ≥ 2 SLT uses per day on ≥ 4 days per week for ≥ 6 months) recorded their product use daily for two weeks via ecological momentary assessments using a mobile device. They also collected a saliva sample and butts from all cigarettes smoked each day during the two-week period.

**Results:** Participants reported use of both cigarettes and SLT on 80% of study days. The number of cigarettes recorded was significantly correlated ($p < .01$) with the number of cigarette butts collected. Levels of cotinine (nicotine metabolite) were significantly higher on dual versus single use days (mean±SEM = 357.5±12.3 ng/ml versus 289.4±18.1 ng/ml, respectively; $p < .05$), though the number of cigarettes recorded did not differ between these days (10.2±0.4 versus 10.0±0.8 cigarettes, respectively; $p > .05$).

**Conclusions:** Smokers used SLT to supplement rather than replace cigarettes, consequently exposing themselves to greater levels of nicotine. Study findings can inform policies surrounding harm reduction strategies and indoor smoking laws.

**Oral Presentation:**

**Category: Early Stage Investigator**

# Medical and psychosocial correlates of Dumping Syndrome in adults after Bariatric Surgery: Preliminary findings from the 'Bari-Aware' Study

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*Background*: Dumping Syndrome (DS) is a common complication following bariatric surgery (BS). It is characterized by rapid gastric emptying after a meal and unpleasant gastrointestinal and vasomotor symptoms. The purpose of this study is to evaluate the surgical/medical, psychosocial, and eating-related variables hypothesized to play a role in DS.

*Methods*: Adult patients (n=25) who underwent Roux-en-Y gastric bypass surgery (RYGB) or sleeve gastrectomy (SG) ≥ 30 days to ≤ 180 days postoperatively at WVU BS Center were included in this study. REDCap™ was used to obtain self-reported demographic data, surgical/medical, and psychosocial/eating-related behaviors associated with DS. Descriptive statistics are reported for this ongoing study.

*Results*: Adults were 96.0% White, 80.0% female with $M_{BMI}$ = 39.4 kg/m$^2$ and $M_{age}$= 47.9 years. Forty-four percent underwent RYGB, and 56% underwent SG. Those with SG had higher BMIs ($r$=.45, $p$<.05). Prior to surgery, the following medical/psychiatric conditions were endorsed: sleep apnea (64%), acid reflux (52%), psychiatric diagnosis (40%), diabetes (36%), and gastroparesis (4%). After BS, patients experienced DS: not at all (44%), rarely (44%), and moderately/often (12%). Most who experienced DS (56%) found it somewhat/moderately upsetting (40%) and very/extremely upsetting (12%). Patients with DS were more likely to avoid eating altogether *(*$r$=.40, $p$<.05), in addition to those who were upset by it ($r$=.60, $p$<.05).

*Conclusions*: While some patients tolerated DS, there is a subset that is symptomatic and found it distressing enough to avoid eating altogether. These preliminary findings suggest that patient education and tailored interventions are needed to prevent or alleviate DS and its effects.

**Oral Presentation:**

**Category: Student / Resident / Fellow**

**A Novel Rat Discitis Model Using Bioluminescent Staphylococcus Aureus**

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*Introduction:* Development of novel treatment methods for spondylodiscitis are needed to improve outcomes and decrease reliance on traditional antibiotic therapy. The purpose of this study was to develop a rat model for spondylodiscitis that permits *in-vivo* surveillance of infection intensity.

*Methods:* The bioluminescent *Staphylococcus aureus* strain XEN36 was prepared in concentrations of $10^3$, $10^5$, and $10^7$ CFU/ml. Eighteen rats were divided into 3 experimental groups, and injected with bacterial inoculum in the most proximal intervertebral tail segment. The third most proximal intervertebral tail segment was injected with saline as a control. Bioluminescence was quantified using the In-Vivo Imaging System (IVIS) at baseline and weekly for a total of 6 weeks. Radiographic imaging, micro CT, and histological analysis was performed. ANOVA was used to compare luminescence between groups.

*Results:* Bioluminescence peaked at day three and returned to baseline by 21 days. Bioluminescence was significantly higher for the group injected with the most concentrated bacterial solution ($10^6$ CFUs) on days 0, 3, and 7. Radiographic and micro CT analysis revealed disc space destruction and osseous bridging. Saline injected segments exhibited retained height. Histologic analysis of experimental discs revealed inflammatory infiltrates, complete destruction of the intervertebral disc, obliteration of vertebral endplates, and reactive bone formation.

*Conclusion:* Injection of XEN36 into the intervertebral disc of a rat tail is a viable animal model for spondylodiscitis research. This preclinical model allows for real time, *in-vivo* quantification of infection intensity, and decreases the number of animals required for infection studies of the intervertebral disc.

**Oral Presentation:**

**Category: Early Stage Investigator**

## Sepsis Triggers Release of Plasma Microvesicles (MVs) Containing Epigenetic Regulators Impact Autophagy

**Duaa Dakhlallah**, Jon Wisler, Tierra Ware, Amal Amer, Yijie Wang, Amy Gross, Joyce Obeng, Ahmad Dakhlallah, Timothy D. Eubank, Clay Marsh.

*Rationale:* Sepsis is a disease with high mortality and morbidity. Systemic immunosuppression associated with severe sepsis is a poor acute prognostic marker and predispose survivors to increased morbidity and mortality. Recent studies suggest that epigenetic regulation of key inflammatory mediators including TNF-α. Although global suppression of myeloid proinflammatory gene expression occurs, no mechanism has been identified. We observed that septic microvesicles production is increased, carry miRNA, mRNA and proteins. we found that autophagy pathway is halted in Sepsis due to hyper-methylation of autophagy key genes. we hypothesized that DNMT mRNA transcripts may be transferred from MVs to naïve monocytes and endothelial cells resulting in reducing inflammatory gene expression and repressing autophagy pathway through increase in DNA methylation.

*Results:* MVs from septic patients had significantly increased mRNA for DNMTs compared to MV from critical illness without sepsis patients Additionally, naïve monocytes and primary endothelial cells treated with MVs from patients with sepsis demonstrated increased expression of DNMTs, dramatically decrease in autophagosome formation and reduced TNF-α expression at 24 hours. on the other hands, treating cells with 5aza and rapamycin decrease DNMTs expression, and demethylate TNFα, and autophagy genes resulting in the increase autophagy process and restore hemostasis of the cells treated with septic MVs.

*Conclusions:* epigenetic regulators including DNMTs are highly expressed in plasma MVs in patients with sepsis and can cause proinflammatory cytokine gene and autophagy pathway silencing. Treating with DNMTs inhibitors and autophagy activator may be a therapeutic strategy in Sepsis.

**Oral Presentation:**

**Category: Senior Investigator**

## Permeability Across the Blood-Brain Barrier and Blood-Tumor Barrier; A Novel In Vitro Model on a Chip

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*Background:* The lack of efficient, reliable, and easily replicable in vitro blood-brain barrier (BBB) and blood-tumor barrier (BTB) models poses a formidable challenge. Because of the complex network and intrinsic nature of the BBB, there are few options for in vitro studies.

*Methods:* In this study, we characterize the permeability of 3 passive permeability markers, and one marker subject to efflux (with and without inhibitors) in a microfluidic BBB chip model. Human umbilical vein endothelial cells were grown and maintained under shear stress conditions, co-cultured with CTX-TDR2 rat brain astrocytes (BBB) or Met-1 metastatic murine breast cancer cells (BTB), grown and maintained under static conditions, across a porous interface in the outer and central compartments, respectively.

*Results:* The permeability of Sulforhodamine 101 acid chloride was significantly ($p < 0.05$) different in the presence of astrocytes ($2.52 \pm 0.29 \times 10^{-3}$, n=6) when compared to Met-1s ($13.1 \pm 1.31 \times 10^{-3}$, n=4) and the unrestricted diffusion $k_{in}$ of this model. A decrease was observed in the BBB models in comparison to BTB with Texas Red 3 kDa, Texas Red 70 kDa, and Rhodamine 123, however significance was not achieved due to the heterogeneous nature of the BBB and BTB, compatible to what is observed in vivo. With the addition of Verapamil to Rhodamine 123, a 14 fold increase of Rhodamine 123 permeability was observed ($14.7 \pm 7.49 \times 10^{-3}$, n=3) while an eight fold increase of Rhodamine 123 permeability was observed with the addition of Cyclosporine A ($8.77 \pm 1.76 \times 10^{-3}$, n=3) in the BBB model with compared to control Rhodamine 123 ($0.56 \pm 0.13 \times 10^{-3}$, n=4).

*Conclusions:* This dynamic microfluidic in vitro BBB model is the first commercially available model to exhibit comparable shear stress, permeability, and efflux properties to *in vivo* data with real-time visualization and quantification.

**Oral Presentation:**

**Category: Student / Resident / Fellow**

**Traumatic Brain Injury and MicroRNAs: A Novel Approach for Treating Neurotrauma**

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*Background:* traumatic brain injury continues to present a significant clinical challenge in terms of neurosurgical management and pharmacologic treatment options. A primary unknown research area is how acute injury alters the underlying biochemical landscape to affect long-term outcomes. Recent evidence suggests that microRNAs are master regulators that control multiple inflammatory and cell injury pathways. We sought to investigate these master regulators following TBI, and whether microRNA levels can be successfully targeted pharmacologically.

*Methods:* serum samples were collected from adult traumatic brain injury patients prior to any intervention. MicroRNA was isolated and quantification was done with rtPCR. An air-acceleration TBI model was used to produce moderate injury (50 PSI) exposure in young-adult male Sprague Dawley rats. A time course of serial blood draws was performed post-injury. One group received bryostatin 0.5mg/kg and the other group received saline. MicroRNA was isolated from the serum and quantified by PCR. Prior to sacrifice, rats were tested on the elevated plus maze. The brains were removed at time of sacrifice and grouped for western blot and IHC.

*Results:* A significant increase in the *let7* microRNA family was seen in both the human TBI samples as well as the rats exposed to TBI. The *let7* microRNAs are master regulators of microglia related neuroinflammation. Interestingly, bryostatin significantly influenced the *let7* microRNA family. Bryostatin significantly reduced ($p<0.01$) the biphasic peak in *let7*a when given five minutes post injury. This was correlated with reduced blood brain barrier disruption assessed with CD31 staining and decreased microglia activation measured with IBA-1. Furthermore, bryostatin improved behavioral outcomes when measured with elevated plus maze.

*Conclusion:* microRNAs play an important but poorly understood role in neural injury. Understanding how microRNAs regulate cellular cascades following neurotrauma may offer novel approaches for pharmacologically treating patients. Further research is warranted to improve patient outcomes prior to and following intervention.

**Oral Presentation:**

**Category: Senior Investigator**

## Determining the Molecular Basis for Increased Mortality in Late Stage Central Appalachian Head and Neck Squamous Cell Carcinoma Patients

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Head and neck squamous cell carcinoma (HNSCC) is an aggressive cancer afflicting the upper aerodigestive tract. Over the past decade, HNSCC is one of only four cancer types with increased incidence in Appalachia, where male patients display higher mortality than the national average. Causative agents include tobacco consumption and human papillomavirus (HPV) infection, both abundant in Appalachia. Advanced late stage (Stage IV) disease is the most lethal form due to high mutational loads. Epidemiological analysis of WV HNSCC patients treated at Ruby Memorial Hospital between 2004-2012 indicates that WV male Stage IV node/metastatic positive (N/M+) patients have significantly worse overall survival compared to other Appalachian and non-Appalachian regions. To identify the factors driving the Stage IV outcome disparity, we have established ex- and in-vivo model systems for functional and genomic analysis. An organotypic culture system for drug efficacy on tumor growth and stromal invasion has been optimized using established cell lines. Primary tissue from WV male Stage IV N/M+ patients has used to generate mouse PDXs. These systems will be used in conjunction with primary tissue to obtain the genomic landscape of Stage IV WV HNSCC. Gene expression levels, mutations and chromosomal abnormalities identified by bioinformatic analysis will be compared with control cohorts to determine the extent and degree of the male Stage IV mutational load. This work will provide the first steps towards the development of new therapeutic approaches and assignment to existing or future clinical trials to combat this worst HNSCC subtype within a chronically underserved population.