

*The Second Annual  
Carey, Marshall, Thorner  
Scholars' Day  
April 28, 2007*



*The Department of Medicine*

## Schedule of Events

- 11:30 Lunch available in Jordan 2 ABC
- 12:00 *Medical Grand Rounds*  
*Jordan Hall Auditorium*  
**Control of the Onset of Puberty –  
Implications for the Evolution of PCOS**  
Dr. John C. Marshall, MD, PhD  
Professor of Medicine  
Director, Center for Research in Reproduction  
Division of Endocrinology and Metabolism
- 1:15 – 2:30 *Oral Presentations*  
*Jordan Hall Auditorium*
- 2:30 *Coffee Break*  
*Auditorium lobby area*
- 3:00 -4:00 *Oral Presentations*  
*Jordan Hall Auditorium*
- 4:15 *Poster viewing and reception*  
*Jordan Hall, Room 2ABC*

## Oral Presentations

(The name of the presenter is underlined)

- 1:15 **Statin Therapy Improves Long-Term Outcome in Patients with Reduced Renal Function and Left Ventricular Dysfunction**  
Clay A. Cauthen, Michael J. Lipinski, Antonio Abbate, Michael J. Cowley, Evelyne Goudreau, George W. Vetrovec
- 1:30 **BAI1 on macrophages facilitates recognition of apoptotic gastric epithelial cells during phagocytosis**  
Soumita Das, K.A. Ryan, A.T. Hawley, D. Park, K.S. Ravichandran, S.E. Crowe, and P.B. Ernst
- 1:45 **Fluoroquinolone resistance correlates with Clostridium difficile intestinal inflammation, independent of the tcdC deletion, binary toxin genes, and REA type**  
Pawlowski SW, Archbald-Pannone L, Carman RJ (TechLab, Inc., Blacksburg, VA), Alcantara-Warren C, Lyerly D (TechLab, Inc., Blacksburg, VA), Genheimer CW (TechLab, Inc., Blacksburg, VA), Gerding DN (Hines VA, Hines, IL, Loyola University Chicago Stritch School of Medicine), Guerrant R
- 2:00 **Dynamic Non-linear Analysis of Heart Rate Series can be Used to Distinguish Atrial Fibrillation from Normal Sinus Rhythm.**  
Deeptankar Demazumder, Paul C. Iazzetti, Yuping Xiao, Doug Lake, and J. Randall Moorman
- 2:15 **Precursors to PCOS: Associations among obesity, hyperandrogenemia, hyperinsulinemia, and the hypothalamic-pituitary axis in adolescent girls**  
JS Collins, CR McCartney, JC Marshall

- 2:45 **Innate and Adaptive Immune Responses in Kidney Ischemic Preconditioning.**  
Gilbert R. Kinsey, Liping Huang, Amy L. Vergis, Li Li and Mark D. Okusa.
- 3:00 **Macrophages adapt to oxidative tissue damage by sensing oxidized phospholipids via TLR2 and Nrf-2**  
Alexandra Kadl, Florian Gruber, Monica Lee, Poonam Sharma, Michael R. Elliott, Ricardo Molero-Bravo, Brian Wamhoff, Norbert Leitinger
- 3:15 **Nitric oxide-induced natriuresis requires extracellular transport of cyclic GMP via an intact microtubulin network in the rat kidney**  
Jennifer Park, Brandon A. Kemp, Nancy L. Howell, John J. Glidea, Susanna R. Keller and Robert M. Carey
- 3:30 **Anti-Hypoxic Inhibition of Prostate Cancer Bone Metastases: Skeletal Effects in Combination with other Prostate Cancer Therapies**  
Vu H. Duong; Khalid S. Mohammad; John M. Chirgwin; Lauren A. Kingsley; Larry J. Suva and Theresa A. Guise
- 3:45 **Incremental prognostic value of exercise capacity in the context of chronotropic response and SPECT myocardial perfusion imaging**  
Benjamin H. Holland, Jamieson M. Bourque, Denny D. Watson, Hasan K. Kabul, George A. Beller

# Poster Presentations

(The name of the primary author is underlined)

## Gastric Th Cells Express CD39 and CD73 Leading to Adenosine Accumulation that Suppresses Helicobacter-induced Gastritis by Modulating Th Cell Function

Mohammad S. Alam, Courtney C. Kurtz, Brain K. Reuter, Soumita Das, Joel Linden, Sheila E. Crowe, Peter B. Ernst

## Divergent Roles of Sphingosine Kinases in Kidney Ischemia-Reperfusion Injury

Amandeep Bajwa, Ph.D., Sang-Kyung Jo, M.D., Ph.D., Hong Ye, Amy Vergis, M.S., Alaa S. Awad, M.D., Yugesh Kharel, Ph.D., Kevin R. Lynch, Ph.D. and Mark D. Okusa, M.D.

## Use of Synthetic Polymers to Block Adherence of *Entamoeba histolytica* to Colonic Epithelium

Tracy Bercu, M.D.

## Global and Regional Left Ventricular Function: Relationship Between Gated SPECT, Cardiac Magnetic Resonance, and Coronary Angiography

A.R. Patel, D.D. Watson, V.Arora, J.M. Christopher, D.L. Segalla, H. Bhardwaj, G.A. Beller, C.M. Kramer

## Helicobacter pylori-mediated acetylation of apurinic/apyrimidinic endonuclease-1/redox factor-1 (APE-1/Ref-1) suppresses bax transcription

Asima Bhattacharyya, Ranajoy Chattopadhyay, Kishor K. Bhakat, Sankar Mitra (University of Texas Medical Branch), Sheila E. Crowe.

## Off-label use of recombinant Factor VIIa: Clinical experience and predictors of outcomes

Jonathan S. Bleeker, B. Gail Macik

## The Biology of 5-HETE in Human Non-small Cell Lung Cancer Tissues.

Lamia Boric, MD, Halim Hanna, M.D., Mary Saprito, B.A., Dorothy Bunyan, B.A., Y. Michael Shim, MD.

## A Novel Nourished Neonatal Murine Model of Enteroaggregative *Escherichia Coli* Infection

Cabal AS, Roche JK, Sevilleja JE, Guerrant RL

## Quantifying the incretin response: the incretin sensitivity index

Shivam A Champaneri, Marc D Breton, Alice Chan, Stacey M Anderson, Boris P Kovatchev

## Inhibitory role of apurinic/apyrimidinic endonuclease (APE)-1/redox factor (Ref)-1 in *H. pylori* mediated gastric epithelial cell apoptosis

Ranajoy Chattopadhyay, Asima Bhattacharyya, Sankar Mitra and Sheila E. Crowe

## Outcome of catheter-related bloodstream infections (CRBSI) following catheter replacement

Chaudhry O, Angle J, and Hall K

## Opportunities for palliative care intervention in hemodialysis patients

Rene Claxton, MD; Jean Holley, MD; Leslie Blackhall, MD

## Hemoglobin Level at the Initiation of Antiretroviral Therapy Predicts Mortality in a Haitian Cohort

Matthew Crist, MD Rebecca Dillingham, MD

## What are Kinetic Measurements at 37°C Trying to Tell Us about Ligand-receptor Interaction?

Deeptankar Demazumder and James P. Dilger.

## Difficulty Identifying Patients At Risk For Hereditary Nonpolyposis Colorectal Cancer (HNPCC)

Frantz, David J.; Powell, Steven M.

**NZM2328.R27, a congenic strain derived from the lupus-prone NZM2328 with an 8Mb C57L/J (a non-lupus strain) fragment on distal chromosome 1, has non-anti-dsDNA immune complex mediated acute glomerulonephritis without the progression to chronic renal failure and early mortality.**

Yan Ge, Chao Jiang, Amy Morris, Felicia Gaskin, Sun-Sang Sung, Harini Bagavant and Shu Man Fu

**Resistance Testing in Treatment Naive HIV infected patients in a Rural Virginia Community. To Test or Not to Test.**

Jaime Green MD, Bram Wispelwey BS, Brian Wispelwey MD

**A comparison of changes in BMI of diabetic and non-diabetic patients in the first year after bariatric surgery.**

Raymond F. Grenfell III M.D., Anthony L. McCall M.D., Christopher J. Northup M.D., and Joel M. Schectman M.D.

**Literature Review of Gait Devices Used in Parkinson's Disease**  
Courtney Hall, MD

**Spontaneous superficial thrombophlebitis: A Systematic Review**

Hsu Blatman K, Becker DM, Philbrick JT

**Application of a Novel MRI Technique for Assessment of Atherosclerotic Plaque in the Superficial Femoral Artery of Diabetic Patients with and without Coronary Artery Disease**

Jamie L. W. Kennedy, M.D., Jamieson M. Bourque, M.D., M.H.S., Brian J Schietinger, M.D, John M. Christopher RT, Angela M. Taylor, M.D., M.S., Colleen A. McNamara, M.D., Christopher M. Kramer, M.D.

**Variations in serum calcium, phosphorous, and parathyroid hormone levels in patients on chronic hemodialysis.**

Helena Levitt MD; Mitchell Rosner MD; Kenneth Smith MD

**Medicine resident's perception of bias in the management decisions of their attendings while in a supervisory role.**

Jason J. Lewis, MD, Diane W. Farineau, Gerald R. Donowitz, MD, Farah H. Morgan, MD

**Recovery of Thrombocytopenia in Post-Liver Transplantation Hepatitis C Patients**

Christine Lin, MD; Timothy Pruett, MD; John Densmore, MD.

**A Prospective Comparison of Endoscopic Ultrasound versus MRI for Staging of Pancreatic Cancer.**

Michelle Loch

**Temporary Placement of Fully Covered Self-Expandable Metal Stents (CSEMS) in Benign Biliary Strictures (BBS): Preliminary Data**

Mahajan, Anshu; Ho, Henry C.; Brock, Andrew S.; Shami, Vanessa M.; Ellen, Kristi; Berg, Carl L.; Schmitt, Timothy M.; Kahaleh, Michel

**Validation of a Scale to Identify Hypoactive Delirium vs. Depression vs. Dementia**

Kurt Miceli, M.D., Anita Clayton, M.D., Bettina Joi Isaac

**Inhibition of Staphylococcus biofilm formation by the investigational drug SNT-2**

Florence. Tchouaffi-Nana, Paul S. Hoffman, Costi D. Sifri.

**Prognosis of Patients with Microsatellitosis in Malignant Melanoma**

Mark Pajeau, William Grosh

**Effect of Methylphenidate hydrochloride on the length of stay of chronically ventilated patients in the Intensive Care Unit.**

A Clayton, S Ramamurthy, S Rau.

**Evaluating the Role of Head CT Scans in the Management of Fulminant Hepatic Failure**

Jim Richter, Patrick Northup

**Assessing Mortality Prediction Models in the ICU**

Katherine Schafer

**Anti-Alpha 8 Integrin Immunoliposomes: Vehicles for Specific Delivery to Glomerular Mesangium**

Y.M.Scindia, U.S.Deshmukh, P.R. Thimmalapura, H.Bagavant

**Diarrheal Illness at the Onset of ART initiation**

Satu Shah, M.D., Rebecca Dillingham, M.D.

**Simultaneous Detection of *Entamoeba histolytica*, *Cryptosporidium parvum* and *Giardia lamblia* in stool samples using a single enzyme immunoassay.**

Cynthia B. Snider M.D. M.P.H.

**Gastric bypass patients remain Vitamin D insufficient despite increased supplementation.**

Rodney Snow M.D., Gregory Clines M.D., Jerry Nadler M.D., Honkun Wang PhD, Joseph Northup M.D.

**Hand-off of care practices among a group of Internal Medicine residents at the University of Virginia Health System.**

Stephen Turner, MD; Tracey Hoke, MD

**Characterization of Different Stages of Renal Disease in Systemic Lupus Erythematosus by Glomerular Transcriptional Profiles**

Hongyang Wang, Harini Bagavant, Umesh Deshmukh, Carol Kannappel, Shu Man Fu

**Obstructive atherosclerosis found in apical ballooning syndrome (Takotsubo cardiomyopathy)**

David E. Winchester MD, Angela Taylor MD

## Abstracts for Oral Presentation

### **Statin Therapy Improves Long-Term Outcome in Patients with Reduced Renal Function and Left Ventricular Dysfunction**

Clay A. Cauthen, Michael J. Lipinski, Antonio Abbate, Michael J. Cowley, Evelyne Goudreau, George W. Vetrovec  
University of Virginia Health System, Charlottesville, VA / Virginia Commonwealth University Health System, Richmond, VA

**Introduction:** Patients with chronic kidney disease (CKD) and heart failure (HF) have been shown to be at high risk for adverse cardiovascular events. Blood urea nitrogen (BUN), estimated glomerular filtration rate (eGFR) or combinations have been used to assess renal function. Recently, BUN has been shown to be a powerful predictor in decompensated heart failure. Statins may exhibit pleiotropic effects beyond lipid regulation (See Figure 1). Statin therapy has been shown to improve survival in patients with HF and Left Ventricular Systolic Dysfunction (LVSD), but uncertainty exists regarding the role of statins in patients with various stages of CKD. The goal of this study was to validate the benefits of statins in patients with LVSD and to investigate the impact of statins on long-term survival in patients with CKD and LVSD.

**Methods:** Our retrospective analysis included patients undergoing PCI with a calculated LVEF $\leq$ 50% between May 1996 and December 2005. Exclusion criteria: patients with a calculated EF $>$ 50% or with technically inadequate left ventriculograms and on dialysis. GFR was estimated using the simplified MDRD equation. Medications were recorded at discharge. Mortality data was retrieved using the US Social Security Death Index. Means are provided with SD and p-values  $<$ 0.05 were considered significant.

**Results:** Our population included 444 patients with a mean EF of 38 $\pm$ 10%, mean age of 59 $\pm$ 11 years, median BUN was 14 mg/dL, 21% had a GFR $<$ 60 ml/min/1.73 m<sup>2</sup>, 31% had Class C HF, 68% were male, and 33 were on a statin at the time of discharge. ROC curves were generated and reasonable values were determined for BUN and eGFR. Survival curves demonstrated that the lack of statin therapy, elevation of BUN (BUN $\geq$ 17mg/dL), and reduction of eGFR (GFR $\leq$ 69) were significantly associated with increased long-term mortality. Statin therapy improves long-term survival in patients regardless of BUN and eGFR. Proportional hazard regression analysis demonstrated that BUN was independently associated with increased mortality and statin therapy was significantly associated with decreased mortality, whereas eGFR was not independently associated.

**Discussion:** The main findings of this study are: 1. Survival curves demonstrate that elevated BUN and reduced eGFR are associated with increased mortality in patients with LVSD. 2. Statin therapy is associated with reduced mortality in patients with LVSD. 3. BUN and statin therapy are significant independent predictors of mortality. 4. Statin therapy reduces mortality of patients with LVSD

and concomitant renal dysfunction. Elevated BUN concentrations are not only in response to diminished GFR, but also reflect the aberrations of fluid volume, neurohormonal activities and hemodynamics. BUN serves as a more encompassing biomarker issuing the interplay between cardiovascular and renal dysfunction in patients with LVSD. Statins may exert a beneficial effect on patients with renal dysfunction through one, if not all, of the proposed pleiotropic effects: improving LV function leading to increased forward flow, anti-inflammation and decreased atherosclerosis.

**Conclusion:** Patients with LVSD and reduced renal function, evident by reduced eGFR or elevated BUN, have increased risk for short- and long-term all cause mortality. Statin therapy reduces this risk of mortality.

**Study Limitations:** This is a retrospective study. Some patients were on statin therapy prior to admission. Lack of follow-up for future cardiac endpoints and revascularization.

### **BAI1 on macrophages facilitates recognition of apoptotic gastric epithelial cells during phagocytosis**

Soumita Das, K.A. Ryan, A.T. Hawley, D. Park, K.S. Ravichandran, S.E. Crowe, and P.B. Ernst

**Introduction:** H. pylori resides in the mucous layer of the lumen, yet elicits a robust immune response in the lamina propria. It is known that gastric epithelial cells (GECs) undergo apoptosis in response to H. pylori infection, and phagocytosis of infected epithelial cells could be a mechanism for clearance of apoptotic GECs. The presence of phosphatidyl serine (PS) on the surface of apoptotic cells is believed to be an "eat me" signal that initiates phagocytosis. We assessed the ability of macrophages and one of its novel PS receptor BAI1 in the recognition and binding of apoptotic GECs prior to engulfment.

**Methods:** Apoptosis was induced in GEC using H. pylori or camptothecin. Control or apoptotic AGS gastric epithelial cells were co-cultured with phagocytes consisting of peripheral blood-derived macrophages or PMA-differentiated THP-1 cells. Fluorescent microscopy was used to assess binding of phagocytes to target cells after 1 hr. Phagocytosis was also confirmed using confocal microscopy. Involvement of BAI1 in this pathway was determined after inhibiting BAI1 expression by siRNA and blocking PS recognition using the soluble, functional domain of BAI1 (TSR-BAI1).

**Results:** Macrophages preferentially bound GECs undergoing apoptosis after exposure to H. pylori or camptothecin. Diminished binding of macrophages to GECs after Annexin V pretreatment indicated apoptosis and PS exposure was necessary for recognition by the macrophage. THP-1 derived macrophages expressed the PS receptor BAI1. TSR-BAI1 pretreatment or BAI1 downregulation in THP-1 derived macrophages impaired recognition and binding of apoptotic AGS.

**Conclusion:** Apoptotic, but not healthy, GECs bind to and get internalized by phagocytes. Recognition of the apoptotic cells depends on the external PS and BAI1 on macrophages. This process may be a novel mechanism whereby inflammatory response to *H. pylori* infection are regulated.

### **Fluoroquinolone resistance correlates with *Clostridium difficile* intestinal inflammation, independent of the *tcdC* deletion, binary toxin genes, and REA type**

*Pawlowski SW, Archbald-Pannone L, Carman RJ (TechLab, Inc., Blacksburg, VA), Alcantara-Warren C, Lyerly D (TechLab, Inc., Blacksburg, VA), Genheimer CW (TechLab, Inc., Blacksburg, VA), Gerding DN (Hines VA, Hines, IL, Loyola University Chicago Stritch School of Medicine), Guerrant R*

**Background:** Mortality associated with *C. difficile* has increased recently, thought to be due to the newly recognized hypervirulent BI strain, having the characteristics of binary toxin production, an 18-bp deletion in the *tcdC* gene, and high level fluoroquinolone resistance. Further studies indicate that a single nucleotide deletion at position 117 of the *tcdC* gene correlates with increased TcdA and TcdB in vitro. The purpose of our study was to determine the incidence of the strain at UVH and its association with intestinal inflammation.

**Methods:** *C. difficile* positive fecal specimens by TcdA and TcdB ELISA were cultured. Isolated *C. difficile* organisms underwent PCR for *gdh*, *tcdA*, *tcdB*, *cdtA/cdtB*, and the 18-bp *tcdC* deletion, as well as testing for fluoroquinolone resistance. Fecal specimens containing isolated *C. difficile* were tested for lactoferrin levels. Available isolates underwent REA typing to determine clonality among strains. We then sequenced the *tcdC* gene of a select number of isolated BI organisms to identify the presence of single nucleotide bp deletions.

**Results:** 34 *C. difficile* organisms were isolated from 52 fecal specimens. 18 (53%) contained the *tcdC* deletion and were BI group. 19 (56%) contained *cdtA/cdtB*. Fluoroquinolone resistance, independent of the presence of the *tcdC* deletion, *cdtA/cdtB*, and REA type, was associated with higher fecal lactoferrin ( $p=0.009$ ). The *tcdC* genes of 12 BI isolates were sequenced, and all contained a single nucleotide bp deletion at position 117.

**Conclusion:** The newly recognized BI strain is present at UVH. UVH BI isolates also contain the same single nucleotide bp deletion at position 117 of the *tcdC* gene, which is thought to be responsible for increased TcdA and TcdB production in vitro. Interestingly, fluoroquinolone resistance, independent of the *tcdC* deletion, binary toxin genes, and REA type, was significantly associated with higher fecal lactoferrin, suggesting that high level fluoroquinolone resistance is associated with intestinal inflammation. It also reinforces concerns about the acquisition of antimicrobial resistance, and the need for novel, non-antimicrobial therapies.

### **Dynamic Non-linear Analysis of Heart Rate Series Can Be Used to Distinguish Atrial Fibrillation from Normal Sinus Rhythm.**

*Deeptankar Demazumder, Paul C. Iazzetti, Yuping Xiao, Doug Lake, and J. Randall Moorman*

**Background:** Atrial fibrillation (AF), the most common sustained cardiac rhythm disturbance, results in significant morbidity and mortality. The University of Virginia (UVA) Heart Station maintains a clinical AF database of more than 1000 patients. An accurate estimation of the “AF burden” (proportion of time spent in AF) can help guide treatment decisions (e.g., need for anticoagulation). Sample entropy is a nonlinear analysis method, involving a series of R-R intervals that arise from a complex combination of both deterministic and stochastic physiological processes, is deduced from approximating the Kolmogorov entropy of a process and has its roots in nonlinear dynamics and Chaos theory.

**Introduction:** Our hypothesis is that the coefficient of sample entropy (COSEn), a parameter optimized for AF detection, discriminates adult heart rate data between normal sinus rhythm and AF. Our specific goals are to develop improved algorithms for detection of AF using COSEn, and to develop and make public the UVA AF database of 24-hour electrocardiogram (ECG) records with clinical correlates.

**Methods:** 94 consecutive 24-hour Holter ECG recordings (weighted toward AF, which is ordinarily ~10% of the overall data) from the UVA Heart Station were manually read for comparison with the COSEn analysis results to establish confidence intervals of COSEn in very short segments and to determine the optimal parameters for AF detection. Sample entropy is the conditional probability (CP) that 2 sequences of heart beat intervals of length  $m$  that match within a tolerance  $r$  will also match at the next point, and is the negative natural logarithm of the CP ( $-\ln CP$ ) that a short epoch of data, or template, is repeated during the time series.  $-\ln CP$  is calculated for each possible template and the results are averaged. If the data are ordered, then templates that are similar for  $m$  points are often similar for  $m+1$  points, CP approaches 1, and  $-\ln CP$  and entropy approaches 0. We vary  $r$  until the number of matches of length  $m+1$  (that is, the numerator of the CP fraction) is equal to the record length. We also normalize the entropy estimate for the heart rate. These modifications, normalized for the value of  $r$  and for the mean heart rate, constitute COSEn.

**Results:** The COSEn frequency distributions were well fit by a single exponential Gaussian function for each of the AF and NSR data. For AF, the peak frequency ( $11571\pm 40$ ) was positioned at  $-0.36\pm 0.0014$  with a width of  $0.506\pm 0.002$ . For NSR, the peak frequency ( $7316\pm 44$ ) was positioned at  $-2.44\pm 0.004$  with a width of  $0.889\pm 0.006$ . Both the empirical cumulative distribution measure and COSEn had receiver-operating characteristics curve areas  $> 0.95$  for diagnosing AF when compared to manual readings. Our analysis suggests 97% sensitivity and positive predictive accuracy in detecting episodes of AF.

**Conclusions:** COSEn is an effective method for detecting AF. Because COSEn can detect AF in very short records of heart rate, COSEn may be used to address important clinical questions about AF, reduce inappropriate shocks in implantable cardioverter-defibrillators, and help guide AF treatment protocols. The UVA AF ECG database will be an important resource for funding future work on detecting other arrhythmias.

### Precursors to PCOS: Associations among obesity, hyperandrogenemia, hyperinsulinemia, and the hypothalamic-pituitary axis in adolescent girls

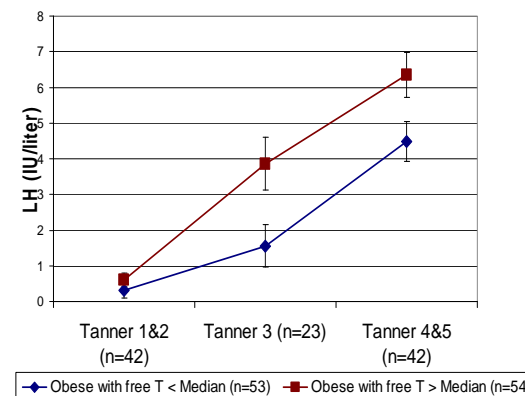
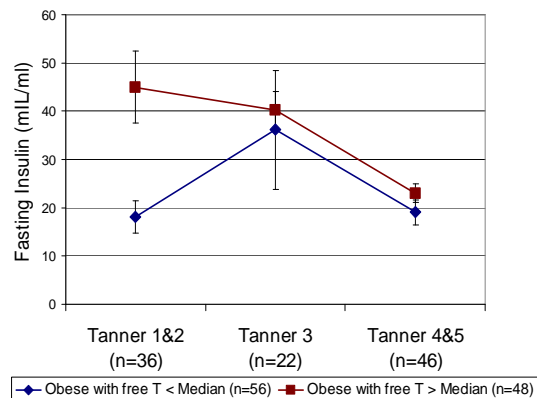
*JS Collins, CR McCartney, JC Marshall*

**Background:** Obesity is associated with hyperandrogenemia (HA) in women with and without the polycystic ovarian syndrome (PCOS). Recent data also suggest that peripubertal obesity is associated with HA, which may increase the risk of developing PCOS. However, many important questions remain. For example, it is unknown whether mild to moderate obesity is associated with HA in peripubertal girls, or whether the prevalence of HA is increased only with more extreme obesity. Additionally, not all obese girls have HA, and the etiology of obesity-associated HA is unknown: discovering why some obese girls demonstrate HA while others do not may help uncover etiological determinants of adolescent PCOS. Lastly, limited data suggests that HA in adolescent PCOS increases the risk of metabolic syndrome, but firm conclusions await confirmatory studies.

**Methods:** We analyzed fasting blood samples (~0700 h) in girls across the pubertal spectrum (n = 82 pre-menarcheal, n = 78 post-menarcheal). To assess for a possible threshold adiposity above which androgens increase, we evaluated total testosterone (T), SHBG, and free T among pre- and post-menarcheal girls in four groups: normal weight (NW; BMI-for-age-percentile [BMI%] < 85), overweight (OW; BMI% ≥ 85 but < 95), obese (OB; BMI% ≥ 95 but < 99), and extremely obese (EX-OB; BMI% ≥ 99). To investigate potential etiologies of obesity-associated HA across different stages of puberty, we compared obese girls (BMI % ≥ 95) with free T values ≥ the median (for obese girls) to obese girls with free T values < the median (for obese girls). In this regard, we focused on fasting insulin and LH, as these are known to contribute to HA in established PCOS. To examine a putative association between HA and metabolic parameters, similar comparisons were also made for selected components of the metabolic syndrome (i.e., fasting glucose and lipid parameters).

**Results:** Free T (pmol/L) was  $6.4 \pm 1.6$  (mean  $\pm$  SEM) in the NW pre-menarcheal group. Free T was markedly higher in both OB ( $17.2 \pm 3.7$ ;  $p < 0.005$  vs. NW) and EX-OB pre-menarcheal girls ( $26.9 \pm 4.6$ ;  $p < 0.0001$  vs. NW;  $p = NS$  vs. OB). Free T was  $14.0 \pm 1.7$  in NW post-menarcheal girls, but  $29.2 \pm 4.0$  in OB ( $p < 0.05$  vs. NW) and  $41.4 \pm 4.7$  in EX-OB post-menarcheal girls ( $p < 0.01$  vs. NW;  $p < 0.05$  vs. OB). No free T differences were demonstrable between

NW and OW girls. Significant differences of free T were related to increases of total T and decreases of SHBG with increasing adiposity (data not shown). When comparing obese girls with free T ≥ median to those with free T < median, fasting insulin was significantly higher in Tanner stage 1 and 2 girls with free T levels ≥ median ( $p < 0.001$ ), while LH levels were significantly higher in Tanner stage 3 ( $p < 0.05$ ) and Tanner stage 4 and 5 ( $p < 0.05$ ) girls with free T levels ≥ median (figures below). No significant differences were observed for fasting glucose, total cholesterol, triglycerides, LDL-C, or HDL-C.



**Conclusions:** These data suggest that HA is associated with mild to moderate obesity in peripubertal girls, and that androgens increase further as degree of obesity increases. These elevations of free T appear to be related to both increased androgen production and increased bioavailability. In pre- and early pubertal (Tanner 1 and 2) obese girls, higher free T was associated with fasting hyperinsulinemia; but this was not observed in more mature girls. In contrast, LH was elevated in later pubertal obese girls with high free T. This suggests that hyperinsulinemia drives obesity-associated HA in early puberty, but LH becomes an increasingly important determinant of obesity-associated HA as puberty

progresses. Our data did not reveal a clear association between HA and selected components of the metabolic syndrome in obese adolescent girls.

### **Innate and Adaptive Immune Responses in Kidney Ischemic Preconditioning.**

*Gilbert R. Kinsey, Liping Huang, Amy L. Vergis, Li Li and Mark D. Okusa.*

**Research Objective:** Acute kidney injury (AKI) induced by ischemia-reperfusion injury (IRI) is becoming an important cause of end stage renal disease and is associated with high morbidity and mortality. A major mediator of AKI is enhanced immune cell infiltration and activation in the injured kidney. The kidney has the ability to be preconditioned by a non-lethal period of ischemia, rendering the kidney refractory to further ischemia-induced dysfunction (ischemic preconditioning: IPC). Neutrophils, macrophages and invariant natural killer T (*i*NKT) cells contribute to the innate response and T cells contribute to the adaptive response following IRI. Few studies have examined their role in IPC and no studies have examined the role of regulatory T cells (Treg). Tregs are an intrinsic anti-inflammatory T cell subset that inhibit the immune response through anti-inflammatory cytokines and cell mediated contact inhibition. We hypothesized that IP induces accumulation of kidney Tregs and inhibits renal inflammatory leukocyte accumulation and activation.

**Materials/Methods:** We developed a model of IP by subjecting C57Bl/6 mice to 24 min of bilateral IRI or sham surgery on day 0, then 28 min IRI or sham on day 7. Renal function was assessed by measurement of plasma creatinine (PCr) and renal H&E staining. Kidney leukocytes were measured by 5-color flow cytometry at 24 hr after the second surgery in the IPC model and at 3, 5, 8 and 14 days after 24 min IRI.

**Results:** Bilateral renal pedicles were clamped for 24 min followed by 7 days of reperfusion. PCr at 24 hr was significantly increased and returned to sham levels by 96 hr. Twenty-four hr after 28 min IRI, the non-preconditioned mice (sham/IRI) developed AKI (PCr = 1.36±0.28 mg/dL vs. sham/sham = 0.33±0.03, p<0.01) whereas the preconditioned mice (IRI/IRI) were completely protected (PCr = 0.37±0.02 mg/dL, n.s. vs. sham/sham). IPC blocked the majority of accumulation of neutrophils (CD11b<sup>+</sup>/GR-1<sup>+</sup>), macrophages (Ly6C<sup>+</sup>/F4/80<sup>int</sup>) and *i*NKT cells in the kidney induced by IRI. Furthermore, IP reduced the percentage of IFN- $\gamma$  producing *i*NKT cells. In contrast, IRI resulted in a 2-fold increase in the number of kidney Tregs (CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup>) by 72 hr of reperfusion. We observed accumulation of Tregs in the kidney beginning at day 3 after 24 min IRI which peaked around day 8.

**Conclusion:** These results demonstrate that IRI causes accumulation of Tregs in the kidney and IPC largely prevents the accumulation of neutrophils, macrophages and activated *i*NKT cells induced by IRI. We conclude that targeting components of the innate and adaptive immune response may mimic IP and mediate kidney tissue protection following IRI.

### **Macrophages adapt to oxidative tissue damage by sensing oxidized phospholipids via TLR2 and Nrf-2**

*Alexandra Kadl, Florian Gruber, Monica Lee, Poonam Sharma, Michael R. Elliott, Ricardo Molero-Bravo, Brian Wamhoff, Norbert Leitinger*

Macrophages change their phenotype and biological functions depending on the microenvironment. Consequently, these cells can contribute to propagation as well as to resolution of inflammation. Oxidative tissue damage occurs in chronically inflamed tissues, however, it is not known how macrophages recognize and respond to oxidatively modified molecules. Using gene array analysis we show that macrophages develop a novel phenotype upon encountering oxidized phospholipids. This phenotype (M-ox) is strikingly different from the classically, Interferon  $\gamma$  and LPS or TNF- activated M1 or the alternatively, IL-4- activated M2 macrophage. While it has been shown that M1 macrophages play a role in host-defense and promotion of Th1-responses, and M2 macrophages propagate immunoregulation, promotion of tumor growth and promotion of Th2-response, the function of M-ox has yet not been recognized.

Here we describe in detail the characteristics of M-ox macrophages, and we show that a subset of pro-inflammatory M1 markers, such as COX-2 are upregulated in M-ox via the long chain fraction of oxidized phospholipids, and that this regulation is dependent on TLR2, but not on TLR4. Interestingly, the regulation of a subset of M-ox specific genes, such as HO-1 and certain redox-regulating genes are mediated by Nrf-2, a major transcription factor that regulates the expression of detoxifying and antioxidant genes. The upregulation of Nrf-2-dependent genes leads to a prolonged survival of M-ox. Moreover M-ox show reduced phagocytotic capacity, when incubated with acetylated LDL or apoptotic cells, as a result of downregulation of scavenger receptors. Finally, using flow cytometry and immunofluorescence, we present evidence that M-ox are present at sites of chronic inflammation, such as atherosclerotic lesions and inflamed adipose tissue from mice on a high fat diet.

Taken together, these data show that oxidized phospholipids induce a novel macrophage phenotype that is present at sites of chronic metabolic inflammation.

### **Nitric oxide-induced natriuresis requires extracellular transport of cyclic GMP via an intact microtubulin network in the rat kidney**

*Jennifer Park, Brandon A. Kemp, Nancy L. Howell, John J. Glidea, Susanna R. Keller and Robert M. Carey*

Previous studies from our laboratory have shown that extracellular guanosine cyclic 3', 5'-monophosphate (cGMP) inhibits Na<sup>+</sup> transport in proximal tubule cells and that renal interstitial (RI) cGMP induces natriuresis. We have also shown that nitric-oxide (NO) induced natriuresis is inhibited when cGMP is prevented from being transported outside its synthesizing cells *in vivo* using

probenecid, an organic anion transporter inhibitor. In this study, Sprague-Dawley rats (N=33) were anesthetized and uninephrectomized. A renal cortical micro-infusion catheter and a microdialysis probe for measuring RI cGMP were implanted into the remaining kidney. Rats were studied for a 1 h control period in which vehicle (5% dextrose) or nocodazole (NOC), a microtubule disruptor, was infused, after which they received a RI infusion of NO donor S-nitroso-N-acetylpenicillamine (SNAP; 0.12 nmol/kg/min; N=12) or SNAP combined with NOC (3 µg/kg/min; N=7), or NOC alone (N=6), or vehicle (V; time control; N=8) for two consecutive h followed by a 1 h post-control (PC) period in which only V was infused. All RI infusions were 2.5 µl/min. Blood pressure (BP), urinary Na<sup>+</sup> excretion (U<sub>Na</sub>V), glomerular filtration rate by inulin clearance (GFR), fractional excretion of Na<sup>+</sup> (FE<sub>Na</sub>) and RI cGMP were quantified for each control and experimental period. In response to intrarenal infusion of SNAP alone, U<sub>Na</sub>V increased from a control of 0.04 ± 0.01 µmol/min to 0.09 ± 0.02 µmol/min (P<0.05) at 1 h and to 0.10 ± 0.02 µmol/min (P<0.005; overall ANOVA F = 10.2; P < 0.0001) after 2 h followed by a reduction to 0.08 ± 0.02 µmol/min in the PC period (P = NS). In contrast, when SNAP and NOC were co-infused or NOC or V were infused alone, there was no change in U<sub>Na</sub>V (P=NS). SNAP also increased FE<sub>Na</sub> (P<0.05) without change in GFR, and the increase in FE<sub>Na</sub> was abolished by NOC. SNAP alone increased RI cGMP from 5.1 ± 0.9 to 8.4 ± 1.7 fmol/ml (P<0.05) at 1 h and to 10.5 ± 2.3 fmol/ml at 2h (P<0.05; overall ANOVA F = 4.6; P < 0.01) and cGMP decreased to 6.3 ± 1.6 fmol/ml in the PC period (P=NS). NOC abolished the increase in SNAP-induced RIcGMP and NOC or V alone did not alter RIcGMP (P=NS). V infusion did not change any of the measured parameters. These data demonstrate that microtubules have an important role in transporting cGMP from the intracellular space to the RI compartment, mediating SNAP-induced natriuresis

### **Anti-Hypoxic Inhibition of Prostate Cancer Bone Metastases: Skeletal Effects in Combination with other Prostate Cancer Therapies**

*Vu H. Duong; Khalid S. Mohammad; John M. Chirgwin; Lauren A. Kingsley; Larry J. Suva and Theresa A. Guise*

**Introduction:** Bone metastasis is a source of significant morbidity for many prostate cancer patients, causing severe bone pain, pathologic fractures, and hypercalcemia of malignancy. While the process of metastasis in bone is not completely understood, a variety of tumor factors appear to be responsible for a vicious cycle that perpetuates osteoblastic metastases. Hypoxia Inducible Factor 1a (HIF-1a) activates multiple genes including endothelin 1, adrenomedullin, PTHrP, TGF-β, and VEGF and is therefore postulated to act as a central regulator of bone metastasis. 2-Methoxyestradiol (2ME2) has both antiangiogenic and antitumor effects and has been shown to inhibit HIF-1. In this study, 2ME2 was used alone and in combination with two established treatments for bone

metastases, zoledronic acid and atrasentan. Effects of treatments on bone, tumor burden, and tumor angiogenesis were assessed.

**Methods:** A total of 120 nude mice were divided equally into eight groups (n=15/group) and were treated for a total of 24 weeks with the following agents either alone or in combination: Atrasentan (20mg/kg/day), Zoledronic acid (5ug/kg three times a week), and 2ME2 (150mg/kg/day). There was one vehicle control group, three single-treatment groups, three double-treatment groups and one triple-treatment group.

Mice were followed by Faxitron and Piximus for bone mineral density every month. Histomorphometry for trabecular bone volumes (TBV) and osteoblast number was performed on slides of hind limbs and the spine. Tartrate resistant acid phosphatase (TRAP) stain was used to identify osteoclasts.

**Results:** Atrasentan had no substantial effect on bone parameters when used alone or in combination with ZA or 2ME2. Zoledronic acid increased bone mineral density at all sites and increased TBV. Across all single, double and triple treatment groups, 2ME2 increased bone mass dramatically. Histomorphometry showed increased trabecular bone volume and more bone-resorbing osteoclasts in 2ME2-treated mice. Micro-CT showed a similar increase in TBV and a parallel increase in trabecular thickness and reduction in trabecular separation. The groups in which 2ME2 and ZA were combined showed additive effects on bone mass.

**Conclusions:** 2ME2 has positive effects on a variety of skeletal parameters and can act additively with zoledronic acid. Experiments are underway to test whether these additive effects also extend to the reduction of tumor burden in bone in mice treated with 2ME2 and zoledronic acid. This preclinical study will indicate if 2ME2 is selectively active against bone metastases and if it is more effective in combination with zoledronic acid, the approved standard of care for patients with bone metastases, than either agent alone.

### **Incremental prognostic value of exercise capacity in the context of chronotropic response and SPECT myocardial perfusion imaging**

*Benjamin H. Holland, Jamieson M. Bourque, Denny D. Watson, Hasan K. Kabul, George A. Beller*

**Background -** Exercise capacity (EC) and achievement of target heart rate (HR) are established prognostic factors in cardiovascular disease. Large populations of patients present with a wide spectrum of cardiovascular risk factors and physical conditioning. Non-invasive imaging is an effective diagnostic tool that provides proven markers of prognosis for a diverse population. The goal of our study was to evaluate the incremental prognostic value of metabolic equivalents (METs) in conjunction with chronotropic response (target HR achievement) when compared with myocardial perfusion imaging in a group of patients referred for nuclear imaging for suspected or proven coronary artery disease.

## *Abstracts for Poster Presentations*

**Methods** – We studied consecutive patients between January 31<sup>st</sup>, 2006 and February 1<sup>st</sup>, 2007 referred for exercise stress testing with standard Bruce protocol with subsequent <sup>99m</sup>Tc-sestamibi SPECT myocardial perfusion imaging were included in our analysis. Visual and quantitative scan analysis was used to determine the prevalence of regional perfusion and function abnormalities.

**Results/Discussion** – Our population included 1056 patients with a mean age of 57 years, 59% were male, 59% had hypertension, 58% had hyperlipidemia, 17% had diabetes mellitus, 15% had prior myocardial infarction, 7.2% had prior coronary artery bypass grafting, and 15.3% had prior percutaneous coronary intervention. Myocardial ischemia was present on perfusion stress imaging in 22.9% of patients. All patients that achieved greater than 10 METs had excellent overall cardiac function. Only 5.4% had an ejection fraction <50%, and 11.0% had an end systolic volume index >25. Subgroup analysis includes those who achieved greater than 85% MPHR and less than 10 METs (>85<10) and those who achieved less than 85% MPHR and greater than 10 METs (<85>10). Surprisingly, more patients had a history of CABG, MI and PTCA in the <85>10 group (85.4%, 32.9%, 32.9% resp.) compared to the >85<10 group (9.4%, 15.2%, 14.4% resp.). Unsurprisingly, the patients with a lower heart rate had a higher prevalence of beta blocker use, 50.0% compared to 27.1%.

**Conclusion** - These findings suggest that the ability to achieve a high workload is a surrogate marker for normal cardiac function and low ischemic burden. The incremental prognostic value of increased exercise capacity should be substantial and may out-weigh abnormal myocardial perfusion imaging. Patients achieving 85% MPHR but less than 10 METs may be at high risk, reflecting de-conditioning with a high prevalence of left ventricular dysfunction. In contrast, achieving 10 METs but less than 85% MPHR may represent a population taking beta blockers for existing or suspected cardiovascular disease and will likely impact the value of chronotropic response in predicting cardiovascular outcomes. Moreover, the low rate of abnormal findings in this cohort may indicate that obtaining goal heart rate is not necessary for adequate interpretation. The results of one year follow-up to assess clinical outcomes are pending.

### **Gastric Th Cells Express CD39 and CD73 Leading to Adenosine Accumulation that Suppresses Helicobacter-induced Gastritis by Modulating Th Cell Function**

*Mohammad S. Alam, Courtney C. Kurtz, Brian K. Reuter, Soumita Das, Joel Linden, Sheila E. Crowe, Peter B. Ernst*

Background. Helicobacter pylori is a life-long infection in humans that causes chronic gastritis and gastric cancer. Adenosine is an anti-inflammatory mediator that limits tissue damage during inflammation. The surface enzymes CD39 and CD73 synthesize adenosine. We studied expression of CD39 and CD73 in human Th cells and evaluated the role of CD73 in regulating Helicobacter felis-induced gastritis. Methods. Human Th cells from blood and gastric tissue were assayed for expression of CD39/CD73 mRNA and protein. The function of CD73 was investigated by examining its effects on proliferation and cytokine production as well as its effect on H. felis infection in CD73<sup>-/-</sup> mice. Results. CD73 was expressed on Treg from peripheral blood and gastric tissue, usually in association with CD39. Activation of Th cells increased CD73 with little effect on CD39 which remained associated with Foxp3<sup>+</sup> Th cells. Gastric Treg had highest expression of CD39/CD73. CD73 antagonists partially reversed Treg suppressive ability in vitro and increased IFN- $\gamma$  production. H. felis infection exacerbated gastritis, increased production of pro-inflammatory cytokines and reduced bacterial load in CD73<sup>-/-</sup> mice. Conclusion. These data indicate that CD73 expression by Th cells contributes to adenosine accumulation and attenuates gastritis. Adenosine accumulation may impair immunity to Helicobacter infection and favor persistence.

### **Divergent Roles of Sphingosine Kinases in Kidney Ischemia-Reperfusion Injury**

*Amandeep Bajwa, Ph.D., Sang-Kyung Jo, M.D., Ph.D., Hong Ye, Amy Vergis, M.S., Alaa S. Awad, M.D., Yugesh Kharel, Ph.D., Kevin R. Lynch, Ph.D. and Mark D. Okusa, M.D.*

Sphingosine-1-phosphate (S1P), a bioactive sphingolipid that is mainly produced by sphingosine kinase 1 (SphK1) and sphingosine kinase 2 (SphK2), is a ligand for a family of specific G-protein coupled receptors. SphKs can be activated by a variety of external stimuli, such as growth factors and cytokines, and mediate phosphorylation of sphingosine, producing S1P. Despite their similarity in amino acid sequences, SphK1 and SphK2 have different kinetics of expression during development as well as different subcellular localization, suggesting that these two isoforms serve different functions. The purpose of this study was to determine the role of SphK1 and SphK2 following kidney ischemia-reperfusion

injury (IRI) as well as the role of SphK2 in mediating the tissue protective effect of FTY720, a non-receptor-selective sphingosine analog. Following 32 minutes of ischemia and 24 hours of reperfusion, both kidney SphK1 mRNA expression and activity increased significantly whereas SphK2 mRNA and activity changed minimally. However, disruption of SphK1 gene (*SphK1<sup>-/-</sup>*) did not alter renal function following IRI, whereas disruption of SphK2 gene (*SphK2<sup>tr/tr</sup>*) was associated with worse renal function and histological damage following IRI. As expected, FTY720 failed to provide protection in SphK2 null mice. These data suggest that the constitutive expression of SphK2 contributes importantly in cell survival following IRI and as well as in mediating the protective effect of FTY720.

### **Use of Synthetic Polymers to Block Adherence of Entamoeba histolytica to Colonic Epithelium**

*Tracy Bercu, M.D.*

**Background:** Intestinal amebiasis is caused by Entamoeba histolytica, a protozoan parasite that affects approximately 40 to 50 million people worldwide with over 40,000 deaths annually. This parasite exists in two forms, the cyst stage, which causes infection, and the trophozoites stage, which causes invasive disease. Adherence of the amebic trophozoites to the colonic epithelium and invasion of the intestinal mucous layer has been shown to be mediated by the galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc)-specific lectin. We conducted a study to determine if galactose polymers could reduce the adhesion of E. histolytica trophozoites when compared to use of galactose. This is a novel approach since delivery of galactose to the colon is usually limited due to absorption in the small intestine, which could be overcome through use of synthetic, unabsorbable galactose polymers.

**Methods:** We tested the inhibition of amebic adherence to Chinese Hamster Ovary (CHO) cells in vitro comparing galactose, a known adherence inhibitor, and a synthetic galactose polymer versus controls. We were interested in determining if use of a nonabsorbable galactose synthetic polymer designed to adhere to the *N*-acetyl-D-galactosamine (Gal/GalNAc)-specific lectin would be a more effective inhibitor of adhesion of E. histolytica trophozoites to colonic epithelium than galactose.

**Results:** Use of a synthetic galactose polymer did reduce adhesion of E. histolytica trophozoites to CHO cells when compared with controls; however galactose consistently produced a greater reduction in adherence in vitro. Use of galactose at concentrations of 50 mM consistently reduced adherence of E. histolytica trophozoites to CHO cells to 0%, while use of the synthetic galactose polymer reduced adherence to an average of 53% at all concentrations. An average adherence rate of 77% was seen in controls. After reviewing the structure of the synthetic galactose polymer, it was noted that the position of the synthetic polymer backbone was such that it could potentially be blocking

adherence to the *N*-acetyl-D-galactosamine (Gal/GalNAc)-specific lectin which would account for the difference in adherence in the polymer versus galactose.

**Conclusions:** Currently used galactose polymers may reduce adherence of E. histolytica trophozoites to CHO cells in vitro, however, a change in the molecular structure of the polymer is likely to increase adherence to the *N*-acetyl-D-galactosamine (Gal/GalNAc)-specific lectin. Since use of a synthetic galactose polymer could potentially be ingested to prevent E. histolytica infection as well provide a novel, targeted approach to delivery of medications used to treat E. histolytica through binding of these medications to a synthetic galactose polymer, further study using reconfigured polymers are warranted.

### **Global and Regional Left Ventricular Function: Relationship Between Gated SPECT, Cardiac Magnetic Resonance, and Coronary Angiography**

*A.R. Patel, D.D. Watson, V.Arora, J.M. Christopher, D.L. Segalla, H. Bhardwaj, G.A. Beller, C.M. Kramer*

**Background:** Gated SPECT is an integral part of the nuclear stress test. Reduced left ventricular ejection fraction (LVEF) identifies patients with a poor prognosis. Regional thickening function (%TF) distinguishes attenuation artifact from myocardial scar. We sought to better define the correlation between LVEF and regional %TF calculated with gated SPECT to those measured with cardiac magnetic resonance (CMR) and also to complications of coronary artery disease. **Methods:** Forty patients with abnormal SPECT myocardial perfusion imaging (MPI) were recruited to undergo CMR. Thirty also underwent late gadolinium enhanced CMR and x-ray angiography (XA). LVEF, LVEDV, LVESV, and regional %TF for each of the 17 standard AHA segments were measured by gated SPECT using standard edge tracking and partial volume brightening fraction methods. Contiguous short axis CMR cine images spanning the LV were used to measure LVEF, LVEDV, and LVESV. A modified 17-segment model (apical segment excluded) was used to measure regional %TF and to quantify myocardial scar burden using late gadolinium enhancement (LGE) images. XA was performed using standard clinical methods. All coronary artery stenosis (CAS) >70% were assigned to the appropriate region of the modified 17-segment model. Linear regression analysis compared LVEF, LVEDV, LVESV, and %TF as measured by SPECT and CMR.  $\chi^2$  analysis was performed to define the relationship between regional wall motion abnormalities and myocardial scar and upstream CAS >70%.

**Results:** The mean age of the cohort was 61±12. LVEF measured by SPECT was 63±12%, LVEDV (137±59ml), and LVESV (55±39ml): all correlated significantly with CMR (58±11%, 141±37ml and 62±31ml; r=0.76, 0.66, and 0.85, respectively, p all <0.001). Regional %TF correlated weakly between SPECT and CMR (r=0.22, p <0.001). In subgroup analysis, LGE was present in 41% of subjects. An abnormal %TF was significantly related to the presence of

myocardial scar ( $\chi^2 = 19.8$ ,  $p < 0.001$ ) and an upstream CAS  $>70\%$  ( $\chi^2 = 10.9$ ,  $p < 0.001$ ).

**Conclusions:** LVEF, LVEDV, and LVESV correlated well between gated SPECT and CMR. A weaker correlation was also seen for quantitative regional %TF. Improved co-registration may enhance the correlation between SPECT and CMR measures of %TF. Regional wall motion abnormalities by gated SPECT are significantly related to the presence of both myocardial scar and severe upstream coronary artery stenosis.

### **Helicobacter pylori-mediated acetylation of apurinic/aprimidinic endonuclease-1/redox factor-1 (APE-1/Ref-1) suppresses bax transcription**

*Asima Bhattacharyya, Ranajoy Chattopadhyay, Kishor K. Bhakat, Sankar Mitra (University of Texas Medical Branch), Sheila E. Crowe.*

**Background:** APE-1/Ref-1 repairs oxidative DNA damage and reductively activates transcription factors. A third, less known function is negative gene regulation by lysine-acetylated APE-1/Ref-1 (ac-Ref-1). APE-1/Ref-1 is acetylated by transcriptional co-activator p300 activated by calcium. ac-Ref-1 downregulates parathyroid hormone and renin expression by interaction with negative calcium response elements (nCAREs) in their promoters. Our previous data indicate that *H. pylori* infection increases expression of APE-1/Ref-1 in gastric epithelium. It is also known that infection alters gastric epithelial growth and survival with altered expression of various genes regulating cell cycle and apoptosis such as bax. This study assesses the effect of *H. pylori*-mediated increase of intracellular calcium ion concentration  $[Ca^{2+}]_i$  on acetylation of APE-1/Ref-1 in AGS cells and its interaction with an nCARE in the bax promoter. **Methods:** The effect of *H. pylori*-induced increased  $[Ca^{2+}]_i$  (measured by fluorescent dye) on ac-Ref-1 and total APE-1/Ref-1 was determined by western blotting. p300's role in APE-1/Ref-1 acetylation was assessed by immunoprecipitation. APE-1/Ref-1 stably suppressed AGS via shRNA were transiently transfected with empty vector, wild type APE-1/Ref-1 or ac-deficient construct and cotransfected with a bax-luciferase reporter containing the nCARE. **Results:** *H. pylori*-induced  $[Ca^{2+}]_i$  increase caused APE-1/Ref-1 acetylation involving p300 which was suppressed by cytosolic  $Ca^{2+}$ -chelator BAPTA-AM. Both wild type and ac-deficient APE-1/Ref-1 enhanced bax-luciferase activity. *H. pylori* infection reduced luciferase activity in cells with basal APE-1/Ref-1 and to an even greater extent in cells overexpressing wild type APE-1/Ref-1. However, infection did not have a significant inhibitory effect in cells expressing mutant APE-1/Ref-1 suggesting that *H. pylori*-mediated repression of transcription is due to acetylation of APE-1/Ref-1. **Conclusion:** Our data indicate that *H. pylori* infection induces acetylation of APE-1/Ref-1 which represses bax transcription via nCaRE. These novel results further implicate APE-1/Ref-1 in

mediating the effects of *H. pylori* in gastric epithelial cells. (Supported by RO1 DK61769, AGA Funderburg Award)

### **Off-label use of recombinant Factor VIIa: Clinical experience and predictors of outcomes**

*Jonathan S. Bleeker, B. Gail Macik*

**Introduction:** Recombinant factor VIIa (rFVIIa) was approved in the U.S. in 1999 for the treatment of bleeding in patients with hemophilia who have inhibitors against factor VIII (FVIII) and factor IX (FIX). Since its introduction, rFVIIa has been used with great success in this population with a good safety profile. It has also been routinely used in an off-label fashion for both bleeding prophylaxis and treatment of refractory bleeding, with the majority of its use now in this population. This project aims to review all cases of administration of rFVIIa for off-label indications at the University of Virginia since 1999 in an effort to determine independent predictors of outcomes and adverse events in this population.

**Methods:** Patients receiving rFVIIa were identified using the UVA Clinical Data Repository (CDR) as well as an independent UVA pharmacy database tracking rFVIIa use. After patients receiving rFVIIa were identified, patients receiving rFVIIa for hemophilia with factor inhibitors were excluded. A retrospective, uncontrolled chart review was then performed with charts being analyzed for demographic data, premorbid conditions as well as a variety of pre-rFVIIa administration laboratory variables.

Charts were then analyzed for both clinical efficacy of rFVIIa as well as adverse outcomes potentially linked to rFVIIa administration. The adverse outcomes which were evaluated included thromboembolic events, MI, PE, CVA, line thrombus, mesenteric ischemia or DIC occurring within 7 days of rFVIIa administration. Mortality at 7 and 30 days post-administration was also determined when possible.

**Results:** Data collection is ongoing. Preliminary data reveals that 88% of off-label use is for bleeding cessation, with the remaining 12% used for peri-procedural prophylaxis. The most common off-label indication for rFVIIa use is refractory peri-procedural bleeding (43%). Use in intracerebral hemorrhage/hematoma comprised 35% and use in GI bleeding 8.6% of all off-label usage.

Use for refractory bleeding was found to be clinically effective in 78% of cases and prophylactic use was 100% effective in preventing clinically apparent bleeding. The adverse event rate was 11% in the patients reviewed thus far. The cohort reviewed to date is too small to delineate any potential pre-administrative factors which may predict these adverse events.

**Conclusions:** Use of rFVIIa for off-label indications is common and continues to grow. These data suggest that off-label rFVIIa use is generally effective in treating refractory hemorrhage and extremely effective when used

prophylactically. The adverse event rate seen in this small cohort is consistent with that found in larger case series evaluating rFVIIa use. Further data collection will allow for expansion of the cohort being analyzed and a more effective analysis on potential risk factors for adverse events.

### The Biology of 5-HETE in Human Non-small Cell Lung Cancer Tissues.

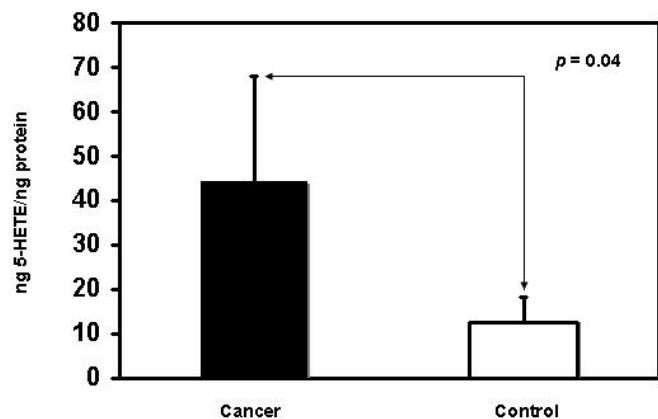
Lamia Boric, MD, Halim Hanna, M.D., Mary Saprito, B.A., Dorothy Bunyan, B.A., Y. Michael Shim, MD.

**Introduction:** 5(S)-hydroxyeicosa-6E, 8Z, 11Z, 14Z-tetraenoic acid (5-HETE) has been implicated in lung carcinogenesis. However, little is known about the biology of 5-HETE in lung cancer subjects. The aim of this proposal is to investigate the biology of 5-HETE in non-small cell lung cancer tissues obtained from human subjects.

**Methods:** Lung cancer tissues were obtained from 17 patients who were diagnosed with non-small cell lung cancer (adenocarcinoma n = 7, squamous n = 10) and who also underwent lung resection. Cancerous tissues and paired surrounding non-cancerous tissues from same subjects were homogenized and analyzed with reverse-phase high performance liquid chromatography to quantify the amounts of 5-HETE. Comparison of the amounts of 5-HETE between the cancerous and the matching non-cancerous surrounding tissues were performed by two tailed paired t-Test.

**Results:** Increased amounts of 5-HETE were found in cancer tissues compared to matched controls (44.19 ng 5-HETE per ng whole lung protein vs. 12.57 ng 5-HETE per ng whole lung protein) with *p* value of 0.04.

**Conclusion:** Exaggerated amounts of 5-HETE are found in non-small lung cell human cancer tissues compared to the matched controls.



### A Novel Nourished Neonatal Murine Model of Enteroaggregative *Escherichia Coli* Infection

Cabal AS, Roche JK, Sevilleja JE, Guerrant RL

Enteroaggregative *Escherichia coli* (EAEC) is an emerging enteric pathogen that causes acute and chronic diarrhea among children, adults and those that are immuno-compromised especially in the developing part of the world and even in the developed countries. Diarrhea is a major cause of malnutrition and even growth impairment among children who develop chronic enteric infection. We have evaluated the use of neonatal C57Bl/6 mice as a model of EAEC infection that mimics the human infection.

**Methods:** Two cohorts of 6 day-old mice were infected via oral inoculation with  $10^8$  cfu/ml of 042 wild type (WT) strain; 042 AggR<sup>-/-</sup> which carries a mutation on its AggR regulon; HS strain which is non pathogenic to humans; and Dulbeccos Modified Eagles Medium (DMEM) as control. Mice were observed for 3 weeks after infection. One cohort was undisturbed and was used to follow growth and for measurement of bacterial shedding in stool using quantitative real time PCR. The remaining cohort were also observed for growth and bacterial shedding in stool but 2 mice from each infection group were euthanized on Day 6, 9, 12 and 15 post-inoculation for histology and for detection of intestinal colonization using quantitative real time PCR.

**Results:** Our results show that mice infected with EAEC 042 WT strain have an impaired growth compared to DMEM treated mice from Day 1 post infection up to the last day of observation which was Day 21 post infection ( $p < 0.001$ ). When we compared mice infected with 042 WT to HS, 042 WT-infected mice's growth was still impaired at Days 1, 3-4, 6-10 and 15-21 post infection ( $p = 0.001-0.039$ ) and also when 042 WT-infected mice's growth was compared to 042 AggR<sup>-/-</sup> infected mice there was still a significant difference at Day 6 and 16 post-infection ( $p < 0.04$ ). Interestingly, we were able to detect infection from stool shedding using quantitative real-time PCR and it shows that all mice were chronically infected and shed the study organism up to 3 weeks after infection. Histology among mice infected with 042 WT shows mild inflammation with a very low number of neutrophils but very pronounced vacuolated Goblet cells that have emptied their mucus content into the luminal area. In addition to that, we also observed the presence of the bacteria inside the mucous-containing vacuoles of goblet cells themselves in the colon.

**Conclusion:** Based on these results we suggest that the neonatal C57BL/6 mouse may provide the first animal model sufficiently similar to human disease to allow study of the biology of and therapeutics for infection caused by Enteroaggregative *Escherichia coli*.

## Quantifying the incretin response: the incretin sensitivity index

Shivam A Champaneri, Marc D Breton, Alice Chan, Stacey M Anderson, Boris P Kovatchev

**Introduction:** The major challenge to a reliable external closed-loop control based on continuous glucose monitors and subcutaneous insulin administration remains the development of optimal control algorithms. The two major system disturbances are meal and exercise. We studied the differences in  $\beta$ -cell function in health observed between intravenous glucose tolerance tests (IVGTT) and oral glucose tolerance tests (OGTT) and mathematically explained these differences by modifying the C-peptide minimal model (CPMM) to include the action of GLP-1.

**Methods:** Twelve healthy nondiabetic male subjects age 21-35 were enrolled. After an overnight fast, each subject underwent OGTT followed by IVGTT. Serial measurements of plasma glucose, total insulin, c-peptide, and GLP-1 were obtained. Mathematical analysis was performed via deconvolution of c-peptide data to establish insulin secretion curves; the use of existing models for insulin secretion and beta cell function were then utilized with the incorporation of GLP-1 data to adapt a model to account for differences in  $\beta$ -cell function between IVGTT and OGTT.

**Results:** Of the 12 patients, 10 were able to participate fully. Of those, 7 had adequate insulin, c-peptide, GLP-1 and glucose data for both OGTT and IVGTT. CPMM worked well for IVGTT, but failed for OGTT as the fasting basal insulin secretion ( $h$ ) increased, and the amplitude of static sensitivity ( $\beta$ ) increased four-fold in the oral state. Incorporating  $\beta$  and  $h$  as functions of GLP-1 reconciled the differences. This new incretin model was a significantly much better fit than the original Breda CPMM ( $p=0.02$ ).

**Conclusion:** This new incretin minimal model can potentially serve as background for future large-scale research, which, in addition to its theoretical significance, would also have a practical clinical application into the algorithmic development of closed-loop systems for the optimal control of diabetes.

## Inhibitory role of apurinic/aprimidinic endonuclease (APE)-1/redox factor (Ref)-1 in *H. pylori* mediated gastric epithelial cell apoptosis

Ranajoy Chattopadhyay, Asima Bhattacharyya, Sankar Mitra and Sheila E. Crowe

**Background and Aims:** APE-1/Ref-1 is a multifunctional protein whose actions include activation of transcription factors including c-Jun, c-Fos, C-Myb, CREB, p53 and pax5 as well as base excision DNA repair. We have shown that *H. pylori* infection induces apoptosis and increases APE-1/Ref-1 expression in human gastric epithelial cells (GEC). APE-1/Ref-1 is known to inhibit apoptosis and both its DNA repair function and its transcriptional regulatory function after lys6/lys7 acetylation are required for cell survival. The goal of this work was to

determine whether levels of APE-1/Ref-1 modulate GEC proliferation and apoptosis associated with *H. pylori* infection and establish the specific roles of DNA repair and transcriptional regulatory functions of APE-1/Ref-1 in the process.

**Methods:** Retrovirus-mediated shRNA was used to stably downregulate APE-1/Ref-1 in AGS cells (shRNA) and empty vector stable cells (pSIREN) were used as control. After *H. pylori* treatment (MOI 1:100) for various times, western blotting for active caspase 3, cleaved PARP, active caspase 9, cytosolic cytochrome C, active caspase 8, FLIP-L and FLIP-S, Bcl-X<sub>L</sub> and Bcl-X<sub>S</sub> was performed and values were normalized to  $\alpha$ -tubulin. shRNA were transfected separately with APE-1/Ref-1 or DNA repair negative or acetylation negative mutants and after *H. pylori* infection active caspase 3 was measured by western blot. Fas immunoprecipitation to detect death-inducing signaling complex (DISC) bound proteins was compared in pSIREN and shRNA after *H. pylori* infection.

**Results:** After *H. pylori* infection, levels of active caspase 3 and cleaved PARP significantly increased in a time-dependent manner in shRNA compared to pSIREN. Substantial increases of caspase 9, cytosolic cytochrome c and Bcl-x<sub>s</sub>/Bcl-x<sub>L</sub> ratio after *H. pylori* infection in shRNA compared to pSIREN suggest a role for APE-1/Ref-1 in the mitochondria-dependent apoptotic pathway. Further studies demonstrating a two-fold induction of active caspase 8 and a rapid decrease of antiapoptotic FLIP-S in shRNA compared to pSIREN implicate APE-1/Ref-1 as an early molecule inhibiting the onset of apoptosis.

Overexpression of wild type APE-1/Ref-1 in shRNA was associated with decreased active caspase 3 whereas neither mutant had this effect, suggesting that both repair and regulatory functions of APE-1/Ref-1 modulate cell survival.

**Conclusions:** Our results indicate that APE-1/Ref-1 regulates multiple pathways involved in programmed cell death of GEC resulting from *H. pylori* infection. In addition, both DNA repair and transcriptional regulatory functions of APE-1/Ref-1 play a role in the inhibition of *H. pylori* induced apoptosis (Supported by R01 DK61769, AGA Funderburg award).

## Outcome of catheter-related bloodstream infections (CRBSI) following catheter replacement

Chaudhry O, Angle J, and Hall K

**Background:** Patients with CRBSIs often have the 1st infected central venous catheter (CVC1) removed followed by placement of a 2<sup>nd</sup> CVC (CVC2). Variables affecting outcome after placement of CVC2 are not well established. We sought to identify the conditions that predict repeat BSI after placement of CVC2.

**Methods:** A retrospective review of medical records at one university hospital identified 1175 people who had  $\geq 2$  CVCs placed by Interventional Radiology. 72 cases had a CRBSI and subsequent removal of CVC1, replacement by CVC2

within 30 days, and at least 60 days of follow-up (f/u). A CRBSI was defined as a CVC tip with >15 cfu by semiquantitative culture, a positive peripheral or CVC-drawn blood culture for the same organism, and no other apparent source.

**Results:** 5 of 72 cases who had a CRBSI of CVC1 had a repeat BSI with the same organism within 60 days of placing CVC2 (range 1-58 days). When CVC1 was a PICC the rate of repeat BSI was 13.8% (5 of 36) versus 0% (0 of 36) for other catheter types ( $p=0.05$ ). If CVC1 was placed as an inpatient the rate of repeat BSI was 9.43% (5 of 53) versus 0% (0 of 19) if placed as an outpatient ( $p=0.32$ ).

The rate of repeat BSI with *C. albicans* was 28.5% (2 of 7), coagulase negative staphylococcus was 12.5% (2 of 16), and *S. aureus* was 3.57% (1 of 27). Cases of repeat BSI received appropriate antibiotic therapy for a mean of 2.4 days prior to CVC2 placement versus 5.6 days for those without repeat BSI ( $p=0.20$ ). When negative f/u blood cultures existed, the rate of repeat BSI was 7.54% (4 of 49) and 3 of these 4 cxs were taken prior to CVC2 placement.

**Conclusions:** After a CRBSI of the 1<sup>st</sup> CVC and placement of a 2<sup>nd</sup> CVC the rate of repeat BSI appears to be higher when the 1<sup>st</sup> CVC is a PICC or if the pathogen is *C. albicans*. These findings need to be confirmed in prospective studies with larger sample sizes.

### **Opportunities for palliative care intervention in hemodialysis patients**

*Rene Claxton, MD; Jean Holley, MD; Leslie Blackhall, MD*

**Introduction:** Dialysis patients' symptom burden equals that of cancer patients and negatively impacts their health related quality of life but symptom management in this population has been rarely studied. Moreover, since dialysis patients' mortality is higher than most with cancer, advance care planning is appropriate in this population, suggesting that palliative care intervention should be integral to the care of dialysis patients. We evaluated symptom burden and management as well as evidence of advance care planning in a single center hemodialysis population to assess opportunities for palliative care intervention.

**Methods:** All adult hemodialysis patients dialyzing at The University of Virginia outpatient Kidney Center Hemodialysis Unit were asked to participate by completing a 31 question survey based on the Dialysis Symptom Index (Weisbord et al J Pain Symp Manag 27:226-240, 2004). Participants were also asked if they were prescribed medication for specific symptoms and if so, who prescribed the medication. Questions regarding end-of-life decision making as well as patient demographics were included. A chart review for medication lists and evidence of advanced care planning was also performed. Patients successfully completed a CLOX test as a screen for cognitive function as well as informed consent prior to enrollment. Comparisons were analyzed with Chi-square, Fischer's exact and Student's t-tests.

**Results:** 87 patients were approached for enrollment; 17 refused, 1 did not speak English, 7 failed CLOX screening; thus 62 patients formed the study group. 58%

were black, 40% white, 55% men, 39% diabetic on dialysis a mean of 4.4 yrs with a mean age  $59 \pm 15$  yrs. Fatigue was the most common symptom encountered (71%) followed by dry skin (65%), bone or joint pain (53%), pruritus (53%), sleep disorder (48%), lack of interest in sex (44%), dry mouth (44%), cough (44%), impaired sexual function (39%), cramps, restless legs, and numbness in the feet (each 35%). Worry (37%), irritability and sadness (each reported by 31%), anxiety (26%) and nervousness (23%) were also common. The most severe symptoms were pain from a non bone or joint source (mean 3.2/4), headache and worry (both 2.9/4) followed by sadness, bone and joint pain, and restless legs (all 2.8/4). Most patients reported receiving no treatment for their symptoms; 45% of patients who reported pain reported a medication prescribed for the symptom compared with 26% and 13% of patients with headache and worry respectively. On chart review, 58% of patients who reported pain had documentation of a prescribed pain medication. There was no significant difference in reported symptoms or the proportion of patients prescribed medication for their symptoms based on ethnicity, age, or diabetic status. Although 56% of patients reported they had an advance directive, only 37% had documented advance care wishes. White patients were more likely to report an advance directive (18/25, 72% vs 16/36, 44% of blacks,  $p = 0.03$ ) and a power of attorney (19/23, 76% vs 19/36, 53% of blacks,  $p = 0.02$ ) than blacks. **Conclusion:** We conclude that although dialysis patients suffer a large symptom burden, they are often under treated. Less than half of patients who reported symptoms in the most severe and most frequent symptom classes were prescribed medications for their symptoms, and primary care physicians were more likely to treat symptoms than nephrologists. Although most patients reported completion of an advance directive, documentation of the directive was present in only 37%. Thus, there are opportunities for palliative care intervention both for symptom management and for advance care planning in this population.

### **Hemoglobin Level at the Initiation of Antiretroviral Therapy Predicts Mortality in a Haitian Cohort**

*Matthew Crist, MD Rebecca Dillingham, MD*

**Background:** Studies of clinic based cohorts in resource poor countries have not shown as dramatic of a reduction in mortality during the HAART era as seen in developed nations. This discrepancy in mortality is most prominent in the first 90 days after initiation of therapy. If independent intervenable factors could be identified at initiation of treatment that predict mortality, a significant impact could be made by screening for and treating these factors. This study examines hemoglobin as a potential predictor of mortality.

**Methods:** A retrospective cohort study was conducted of antiretroviral-naïve, non-pregnant, adult patients with AIDS who were initiated on antiretroviral therapy (ART) at the Gheskio clinic in Port au Prince, Haiti. 736 patients were followed for a total of 8773 person-months. Initial clinical and laboratory data,

including hemoglobin, were recorded at initiation of therapy and throughout the study period. The relationship between hemoglobin and the outcome of death was evaluated as well as the effect of multiple covariates.

**Results:** Hemoglobin was found to be a strong predictor of mortality for the overall study period. Univariate analysis showed a significant relationship ( $p < 0.01$ ) between hemoglobin levels at the initiation of ART and mortality. When stratifying the hemoglobin into categories of normal and mild, moderate, and severe anemia according to the World Health Organization values, a trend is seen of increasing mortality as anemia becomes more severe. A significant difference is seen in the moderate and severe groups compared to those with normal hemoglobin levels. Men and women were analyzed separately as the normal values differ and the effect was present in both men and women. The relationship between hemoglobin and mortality was preserved when the covariates of CD4 count, ART regimen, age, level of education, socioeconomic status and the initial presence of chronic diarrhea, tuberculosis, and wasting were all controlled in the analysis ( $p < 0.01$ ).

**Conclusion:** Anemia is independently associated with mortality after initiation of ART in this resource limited setting. Early detection and treatment of anemia could be beneficial in improving outcomes in these populations.

### What are Kinetic Measurements at 37°C Trying to Tell Us about Ligand-receptor Interaction?

*Deeptankar Demazumder and James P. Dilger.*

**Background:** Detailed information about the ligand binding site of receptors (e.g., on ligand-gated ion channels) has emerged from structural and mutagenesis experiments. However, these approaches provide only static images of ligand-receptor interactions. Equilibrium measurements of protein function provide little insight into physiological processes because the free ligand, ligand-receptor bound and free receptor states are not at equilibrium in vivo. Kinetic measurements of changes in protein function are needed to develop a more dynamic picture. Moreover, little is known about the temperature dependence of ligand-gated ion channel function. Human and animal studies suggest selective and variable sensitivity to temperature. In vivo studies are difficult to interpret, however, because temperature affects many physiological processes.

**Introduction:** There are no in vitro reports on the energetics of competitive antagonism for any ligand-gated ion channel. There are no in vitro reports on the kinetics of competitive antagonism at physiological temperatures for the nicotinic acetylcholine receptor (nAChR), the prototypical ligand-gated ion channel.

**Methods:** We used a novel electrophysiological assay to measure association and dissociation rate constants for competitive inhibition of current through nAChR at 25°C and 37°C. We used rapid solution exchange protocols to determine equilibrium and kinetics of inhibition of acetylcholine-activated currents in outside-out patches by (+)-tubocurarine (the prototypical competitive antagonist)

and by other clinically used antagonists, such as pancuronium and cisatracurium. We used thermodynamics to estimate the energetics of competitive antagonism. **Results:** Kinetic rates as high as 600/sec were resolved by our technique. Binding was primarily enthalpy and not entropy driven. The 12°C increase in temperature decreased Equilibrium antagonist binding by 1.7- to 1.9-fold. In contrast, association and dissociation rate constants increased 1.9- to 6.0-fold. Activation energies for dissociation were  $90 \pm 6$ ,  $106 \pm 8$  and  $116 \pm 10$  kJ/mol for cisatracurium, (+)-tubocurarine and pancuronium, respectively. The corresponding apparent activation energies for association were  $38 \pm 6$ ,  $85 \pm 6$  and  $107 \pm 13$  kJ/mol. **Conclusions:** The higher activation energy for association of (+)-tubocurarine and pancuronium compared with cisatracurium is notable. This may arise from either a more superficial binding site for the large antagonist cisatracurium compared to the other ligands, or from a change in receptor conformation upon binding of (+)-tubocurarine and pancuronium but not cisatracurium. Our results demonstrate that the combination of kinetic and thermodynamic measurements on the nAChR reveals dynamic and energetic information that cannot be deduced from Equilibrium measurements. The temperature-dependence of equilibrium inhibition alone obscures the dramatic changes in the underlying rate constants and the fundamental differences among the antagonists. Although electrophysiological measurements at elevated temperatures may be technically difficult, they are necessary for realistic modeling of physiological processes.

### Difficulty Identifying Patients At Risk For Hereditary Nonpolyposis Colorectal Cancer (HNPCC)

*Frantz, David J.; Powell, Steven M.*

**Background & Aims:** Hereditary Nonpolyposis Colorectal Cancer (HNPCC) often affects individuals younger than the recommended age for screening colonoscopies. The genetic mutations responsible for most cases of HNPCC are known. Clinicians must determine which individuals are at risk of HNPCC and should receive genetic testing. A retrospective study was undertaken at the University of Virginia (UVA) to quantify the ability of clinicians to identify high risk patients through family history using current disease definitions and guidelines.

**Methods:** Internal Review Board (IRB) approval was obtained to perform a retrospective chart-review of all patients under the age of fifty who received their first diagnosis of colon cancer within the past three years. The patient's family history, diagnosis, and the results of genetic testing were recorded in a secure database. The family history was used to determine which individuals met screening criteria as defined by the Bethesda or Revised Bethesda Criteria. It was also used to determine if patients met the disease definitions outlined in Amsterdam, Modified Amsterdam or Amsterdam II Criteria.

**Results:** 114 Patients met the inclusion criteria. 41 patients were excluded due to an alternative diagnosis. Of the remaining 73 patients, 100% (73 pts) met Modified Bethesda Criteria, and 77 % (56 pts) met Bethesda Criteria for screening. 7% (5pts) met Amsterdam Criteria, 10% (7 pts) met Amsterdam II Criteria and 22% (16 pts) met Modified Amsterdam Criteria. Only 15% (11 pts) of the total population was sent for further testing. Of these eleven patients, none had documented results of genetic tests.

**Conclusions:** This study demonstrates a systematic failure to screen patients for HNPCC. 100% of the included patients met the Modified Bethesda for screening. In practice, only 15% (11 pts) were sent for testing, and none had documented proof of completed tests. Monetary concerns, lack of communication between hospitals, and loss to follow-up contributed to the failure to complete testing. The remaining 85% (62 pts) were never identified by their physicians as at risk. Failure to properly identify patients at risk for HNPCC obviated the proper genetic counseling and testing for the other members of the at-risk families. The data presented in this study reinforces the need to establish a systematic screening program in tertiary care centers to screen for HNPCC.

**NZM2328.R27, a congenic strain derived from the lupus-prone NZM2328 with an 8Mb C57L/J (a non-lupus strain) fragment on distal chromosome 1, has non-anti-dsDNA immune complex mediated acute glomerulonephritis without the progression to chronic renal failure and early mortality.**

*Yan Ge, Chao Jiang, Amy Morris, Felicia Gaskin, Sun-Sang Sung, Harini Bagavant and Shu Man Fu*

**Purpose:** NZM2328 mice develop severe proteinuria, glomerulonephritis (GN) and early mortality with a female bias and are used as a spontaneous murine SLE model in our laboratory. A previous linkage backcross study [NZM2328 X C57L/J)F1 X NZM2328] identified two loci *Cgnz1* and *Agnz1* on distal mouse chromosome 1 for chronic GN and acute GN, respectively. The phenotypes of the congenic strain NZM2328.C57L/Jc1 confirmed the results from the linkage study. These studies and others in our laboratory let us to hypothesize that acute and chronic GN are under separate genetic control, and that acute GN need not be mediated by anti-dsDNA antibodies and need not progress to chronic GN. In this study, we have successfully generated a recombinant line of NZM2328, named NZM2328.R27 with serological and histological evidence to validate the aforementioned hypothesis.

**Methods:** Multiple recombinant congenic lines were generated by the cross of NZM2328.C57L/Jc1 X NZM2328 and identification of heterozygotes with intrachromosomal recombination. Homozygous lines were generated by further breeding with the heterozygous mice. One such line was identified as R27. Nine R27 female mice were followed up to 12 months of age. Additional mice were followed at various ages. Proteinuria was examined every two weeks and kidney

histology of R27 females was examined when the mice were sacrificed at 12 months of age. ANA production and immune complex deposits were determined by indirect immunofluorescence. Anti-dsDNA antibodies were determined by ELISA with plasmic DNA as substrate. In addition, normal kidney sections were stained with R27 sera to see if there were antibodies against kidney present. R27 kidney sections were stained with various monoclonal antibodies to characterize infiltrating cells.

**Results:** The recombinant congenic strain R27 was shown to contain an 8Mb C57L/J chromosome 1 fragment on NZM2328 background. *Cgnz1* was within this 8Mb fragment, but *Agnz1* was not. The cohort of nine R27 female mice was shown to be negative for ANA and anti-dsDNA antibodies. None of these mice had severe proteinuria by the presence of 300mg/dL protein in their urine on two consecutive determinations. The initial cohort of nine mice survived up to 12 months when they were sacrificed. Histological examination showed that two thirds of them developed acute GN with hypercellularity, mesangial proliferation, and dilated capillary loops, but no signs of chronic GN were observed. More interestingly, the feature of dilated capillary loops was only observed in those R27 mice with acute GN, but not in NZM2328 strain. Immune complex deposits including IgM, IgG, IgA and C3 were detected in all kidneys of 6 to 12-month-old R27 mice. In order to determine the earliest time when immune complexes were detectable, R27 females at various ages were studied. Immune complex deposits were detected in some mice at 10 weeks of age. Sera of 5 to 12-month-old R27 mice stained normal kidney sections, indicating the presence of antibodies against kidney in R27 serum. Indirect immunofluorescence showed macrophages (F4/80+) and IA+ cells in the kidney cortex. However, these cells were located outside the glomeruli.

**Conclusions:** The present observations of R27 mice are consistent with the genetic data in that acute GN and chronic GN are under separate genetic control. In addition, they validate the hypothesis that acute GN need not proceed to chronic GN. Thus, acute GN and chronic GN are mediated by two distinct mechanisms. These results suggest that immune complexes are not sufficient to induce fatal lupus nephritis. They add further credence to the thesis that T cell mediated response is responsible in part for the progression to chronic GN as indicated by our previous published data. The lack of ANA and anti-dsDNA antibodies further reinforces our previous conclusion that these antibodies are not required for either diagnosis or participation in the pathogenesis of lupus nephritis. The presence of nephrophilic autoantibodies in young R27 indicates that further studies of the specificities of these antibodies are warranted. Thus far, it is apparent that R27 strain is a valuable model for lupus nephritis and it will yield further information on the pathogenesis and genetics of lupus nephritis.

## **Resistance Testing in Treatment Naive HIV infected patients in a Rural Virginia Community. To Test or Not to Test.**

*Jaime Green MD, Bram Wispelwey BS, Brian Wispelwey MD*

**Background:** The role of resistance testing in chronically infected HIV patients is still being established. In 2003, cost benefit analysis and consensus guidelines recommended resistance testing on all chronically infected HIV patients if the prevalence of resistance was greater than 5% in the community. In a more recent study in 2005, cost effectiveness for resistance testing was established if greater than 1% resistance exists in the population. The majority of studies on resistance testing in treatment naive patients come from inner city populations. These studies estimate resistance to be between 8-20% and 5-6% in acute and chronically infected patients respectively.

Genotypic testing in a chronically infected patient has many caveats. At the time of infection, a multitude of HIV sub or quasi species is transmitted. Resistant quasi species must represent 20% of the virus in order to have adequate detection (for the testing used in this study). Resistant species persist for years after acquisition, but may eventually wane or revert back to the wild type/more fit virus. This further complicates interpretation of resistance and resistance testing in chronically infected patients.

The goal of this study was to characterize the patient population in a rural Virginia community and assess the need for resistance testing in individuals who are treatment naive and chronically infected with HIV.

**Methods:** Genotypic resistance testing was conducted on chronically infected treatment naive HIV positive patients at the time of diagnosis or entrance into the clinic. Retrospective chart review was performed to further characterize the patient population including age, race, gender, and method and location of acquisition. CD4 and viral load testing was also performed and followed over time.

**Results:** A subgroup analysis of virologic suppression and immunologic recovery was performed on patients whose disease progressed to require anti-retroviral therapy. Data collection is still in progress at the time of this abstract submission but to date 83 patients meet criteria. The average age is 38.2 years with 70%(58)M, 30%(25)F. 42%(35) white, 35%(29) black, 18%(15) Hispanic, 5%(2) other. Fifty-four percent 54%(42) heterosexual, 9%(7) intravenous drug use(IVDU), 31%(24) men who have sex with men(MSM). Average CD4 count was 283 and viral load 180K. There was 4.8% resistance at the time of diagnosis, and 42% presented with AIDS. Subgroup analysis on treatment response is still in progress.

**Conclusions:** In our patient population in a rural Virginia community, the resistance in treatment naive HIV infection is just shy of the 5% criteria for resistance testing. The authors of this abstract believe that resistance testing in treatment naive patients is beneficial. For one, it is necessary to continue to monitor the prevalence of HIV resistance. Also, the risk of prolonged achievement of virologic suppression and treatment failure with HIV resistance

far outweighs as the risk of not using resistance testing. A subgroup analysis did show the trend of obtaining appropriate virologic suppression once ARV's were initiated despite the caveats of testing in the chronically infected patient. The large portion of patients in this community that are diagnosed with HIV at the presentation of AIDS exemplifies the need to change local outreach into the community for HIV testing and diagnosis.

## **A comparison of changes in BMI of diabetic and non-diabetic patients in the first year after bariatric surgery.**

*Raymond F. Grenfell III M.D., Anthony L. McCall M.D., Christopher J. Northrup M.D., and Joel M. Schectman M.D..*

Bariatric surgery has been used for many years as a successful means to obtain dramatic weight loss. Besides the obvious benefit of the substantial weight loss that can be obtained from gastric bypass surgery, multiple studies have demonstrated that many of the complications that result from obesity can also be controlled and possibly cured through bariatric surgery. One of the most dramatic of these is the changes that can be seen in the control and possible cure of diabetes after bariatric surgery even in the first year after bariatric surgery. It has been proposed that some of this effect is a result of the effects that gastric bypass surgery has on the enteroinsular axis. There are many changes in hormonal levels that have been detected after gastric bypass surgery that may contribute to the significant weight loss and improved glycemic control that is seen in patients. Two of the most important may be altered incretins, specifically, GLP-1 and GIP. In Type 2 Diabetics, GLP-1 secretion by the L-cells is decreased. In regards to GIP, the secretion is not suppressed in Type 2 Diabetics but the pancreatic response appears to be decreased as a result of down-regulation of its beta cell receptors. Gastric bypass surgery has been shown to increase the production of GLP-1 by early arrival of undigested food to the ileum and to decrease the production of GIP which results in an up-regulation of their receptors on the beta cells. It was hypothesized that possibly as a result of the effects that gastric bypass has on these hormones and potential early satiety, diabetic patients would have a greater amount of weight loss in the first year post-op compared to non-diabetic patients. Using a database of patients that had undergone gastric bypass surgery at the University of Virginia between the years 1990-2004, the BMIs of 246 diabetic and 616 non-diabetic patients were compared to see if there was any difference in the amount of weight they lost in the first year after their surgery. An unpaired, two tailed t-test was used to compare the BMIs of diabetic patients and non-diabetic patients at 6 months and 12 month follow up visits. The average mean (with confidence interval; CI and standard deviation;SD) change in BMI at 6 months for the non-diabetic and diabetic patients was 14.4 (CI=14.1-14.8, SD of 4.0) and 13.7 (CI=13.1 – 14.2, SD of 3.9) respectively. The mean change in BMI over 12 months for the non-diabetic patients and diabetic patients was 19.1 (CI=18.6-19.6, SD of 5.9) and

17.7 (CI=16.9-18.5, SD of 5.8) respectively. The final results actually showed that the non-diabetics patients had a 1.39 (CI=0.43-2.3, SD of 5.7, p= 0.0047) greater decrease in their BMI in comparison to the diabetic patients at 12 months post-op. While the changes in the enteroinsular axis may be involved in the improved glycemic control that is observed in diabetic patients in the first year after bariatric surgery, it does not appear that these changes result in a more dramatic weight loss in diabetic patients. Further prospective studies are needed to determine the exact etiology of the sudden improvement in glucose control that is seen in the first year after gastric bypass surgery.

## **Literature Review of Gait Devices Used in Parkinson's Disease**

*Courtney Hall, MD*

While Parkinson's disease (PD) may be straightforward to diagnose when patients present with typical symptoms including tremor, bradykinesia, and rigidity, the diagnosis can be quite challenging when patients present early or with atypical symptoms. The diagnosis of Parkinson's disease is a clinical one and even among movement disorder specialists, the error in diagnosis may be as high as 24%. I performed a literature review using Medline with the terms "Parkinson's Disease," "gait," and "diagnosis" which generated 36 articles. Of these, 15 articles utilized gait analysis devices. While my literature review was designed to review the studies that utilized devices to diagnose PD, I found no studies that actually used devices as initial diagnostic tools. I did find many different devices that are being used to analyze gait patterns in patients with known PD. My review revealed 2 devices using accelerometers, 2 using EMG electrodes, 1 using gyroscopes, 3 using cameras to analyze motion patterns, 1 with a computerized walkway, and the remainder used devices attached to the subjects feet. I summarize the studies including the purpose of the study, the subjects, the devices used and the results in my analysis. The type of data that has been collected with these devices is quite diverse, but many of the studies focus on cadence, gait speed, double support time, and step lengths. The data to date suggests reduced cadence, gait speed, and step length in PD patients versus controls and increased double support time. With similar results in these gait parameters based on multiple studies in patients with known PD, one might conclude that a device that analyzes the above parameters could be a useful adjunct in the diagnosis of PD.

## **Spontaneous superficial thrombophlebitis: A Systematic Review**

*Hsu Blatman K, Becker DM, Philbrick JT*

**Context:** There is uncertainty about prognosis and magnitude of risk of spontaneous superficial thrombophlebitis and when it is treated.

**Objectives:** To review the methodologic strength of the literature, estimate the risk of lower extremity superficial thrombophlebitis, and development evidence-based recommendations on prevention and treatment.

**Data sources:** Studies identified from MEDLINE from 1966 through December 2005, and supplemented of the Cochrane Central Registry of Controlled Trials.

**Study selection:** To clarify the prognosis of spontaneous superficial venous thrombophlebitis in patients without any suspected/documented systemic disorder and to determine which clinical factors influence its sequelae, we systematically reviewed the English-only literature published on this subject since 1990. Exclusion criteria included pregnancy, Trousseau's syndrome (malignancy), Behcet's disease, septic thrombophlebitis, Mondor's disease, upper-extremity disease, hypercoagulable states, Thromboangiitis obliterans (Buerger's Disease), peri-operative cases and Klippel-Trénaunay syndrome (venous malformations). We also only included studies where there were follow-up evaluations, either clinically or radiographically, after diagnosis of superficial thrombophlebitis.

**Data analysis:** Two reviewers reviewed each study independently to assess inclusion criteria, classify research design and rate methodologic features.

**Conclusion:** Final results pending

## **Application of a Novel MRI Technique for Assessment of Atherosclerotic Plaque in the Superficial Femoral Artery of Diabetic Patients with and without Coronary Artery Disease**

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**Introduction:** Peripheral arterial disease (PAD) is one manifestation of the systemic disease atherosclerosis. The prevalence of PAD, defined by ankle-brachial index (ABI) < 0.90, has been estimated at 4.3% for US adults age 40 and over, and 10.8% among diabetics. Diabetics with PAD have a significantly increased risk of cardiac events compared to those with diabetes alone (102 vs. 28 events per 1000 person years). Increasing severity of PAD correlates with increased risk of cardiovascular events and mortality, particularly for patients with critical limb ischemia. Aside from the risk of cardiovascular events, PAD limits activity, even in asymptomatic patients, and may lead to a cycle of progressive disability.

MRI is an emerging technique for the evaluation of atherosclerosis. Unlike angiography, which assesses the presence and degree of luminal narrowing, MRI allows direct visualization of atherosclerotic plaque. Components of atherosclerotic plaque (lipid, calcification, hemorrhage, thrombosis, and fibrous cap) have been identified on MRI and correlated with pathology specimens. This capability provides a non-invasive method to follow the evolution of atherosclerotic plaque *in vivo*. MRI has also been employed to monitor regression of aortic and carotid atherosclerotic plaques induced by statin therapy. Our research group has developed a novel method for quantitation of atherosclerotic plaque volume in the superficial femoral artery. This project applies the volumetric technique to an asymptomatic population at high risk for PAD, patients with DM with or without CAD.

**Methods:** Patients with DM, either type I or II, with or without CAD were recruited from general medicine, cardiology, and endocrinology clinics at UVA. Patients with known PAD, symptoms of claudication, or lower extremity ulcers were excluded, as were patients who have undergone prior evaluation for PAD. Patients with contraindications to MRI, such as claustrophobia or metal implants, and patients unable to give informed consent were also excluded. Age-matched control subjects without DM, CAD, or PAD, were recruited from the UVA faculty. ABIs were checked for each subject, and subjects with abnormal ABIs were excluded. Clinical information was collected from the subjects and the medical record. Based on preliminary results, we calculated a need for 25 subjects in each arm (DM and DM+CAD) and 15 controls to achieve a power of 95% with alpha of 0.05. Subjects underwent MRI of their lower extremities on a 1.5 T Siemens Avanto MR scanner using a multi-slice fast spin echo sequence with flow and fat suppression. Image collection began at the origin of the superficial femoral artery and extend distally 15-20 cm, depending on leg length, with contiguous 3 mm thick slices. Images were analyzed using VesselMass software to determine the mean wall thickness (WT) and total wall volume indexed to total vessel volume (IWV), to adjust for size variation of the blood vessel.

**Results:** Recruitment of patients is not yet complete. Data has been analyzed for 25 subjects with DM+CAD and compared to 13 age- and gender-matched controls. The DM+CAD subjects had significantly a higher incidence of hypertension and dyslipidemia, a higher average BMI, and an increased incidence of tobacco use (prior or current) than the controls. The mean wall thickness and the mean indexed wall volume were both significantly larger in subjects with DM+CAD than controls ( $p < 0.001$ ).

**Conclusions:** Subjects with DM+CAD have more atherosclerotic plaque than age- and gender-matched controls, despite lack of symptoms of PAD and normal ABIs. A second study arm including subjects with DM but without known CAD is still in progress, as is recruitment of 2 additional controls to meet our planned study power.

This technique may become an important means to assess overall atherosclerotic burden. Comparison to carotid intimal-medial thickness by

ultrasound or coronary CT calcium scoring may be warranted. The approach may also be an attractive one for measurement of plaque regression. The large size of the superficial femoral artery translates to large plaque volumes, so even small percentage changes produce substantial changes in the wall thickness and indexed wall volume. As an additional advantage, this MRI technique is non-invasive and does not require gadolinium contrast, and therefore poses minimal risk to the patient, unlike other methods which have been employed to monitor plaque regression such as intravascular ultrasound or coronary CT angiography.

### **Variations in serum calcium, phosphorous, and parathyroid hormone levels in patients on chronic hemodialysis.**

*Helena Levitt MD; Mitchell Rosner MD; Kenneth Smith MD*

**Background:** Control of serum calcium and phosphorous levels is important in the management of chronic hemodialysis patients. Elevated levels of serum phosphorous and calcium promote vascular calcification. Further, hyperphosphatemia from reduced renal phosphate clearance leads to secondary hyperparathyroidism and renal osteodystrophy. Hemodialysis centers typically measure each patient's pre-dialysis phosphorous and calcium levels once monthly. We make medication and dialysate adjustments accordingly. The timing of these measurements has been based on the assumption that random calcium and phosphate testing is a reliable measure of overall calcium and phosphorous balance. However, this routine practice has never been substantiated.

**Methods:** We enrolled 17 subjects who receive three dialysis sessions per week at the UVA outpatient dialysis center. Prior to enrollment, eligible patients were identified through a review of the Kidney Center Clinic database of ESRD patients. We collected serum calcium, phosphorous, and PTH levels from each patient over a month prior to every hemodialysis session. Each patient served as his/her own control because we investigated the variability in a lab parameter. During the study month, adjustments were not allowed for either Vitamin D analogs or phosphorous-binding medications.

**Results:** We determined the mean, standard deviation, standard error, and coefficient of variation for each patient's calcium, phosphorous and PTH values collected over the month. The interpretation of our data findings is pending further statistical analyses.

**Conclusions:** Based on our statistical analyses, we hope to determine the usefulness and validity of random calcium, phosphorous, and PTH testing in hemodialysis patients.

## **Medicine resident's perception of bias in the management decisions of their attendings while in a supervisory role.**

*Jason J. Lewis, MD, Diane W. Farineau, Gerald R. Donowitz, MD, Farah H. Morgan, MD*

**Introduction:** During residency training, exposure of trainees to pharmaceutical representatives is regulated by their training programs. Content of the attending/resident relationship on rounds or in the clinic however is not. While we currently require invited speakers to disclose potential financial conflicts of interest for resident conferences such as Medical Grand Rounds, we do not require disclosure of individual faculty members while in a supervisory role on the wards or in the clinics.

**Methods:** We surveyed 105 Internal Medicine Residents at four different sites in the state of Virginia (UVA, MCV, EVMS, Carilion) via an anonymous web based survey. The focus was on identification of bias in the individual's personal interactions with pharmaceutical representatives and the perception of bias in the management/decision making abilities of their attending physicians while in a supervisory role.

**Results:** One-hundred and one residents out of 105 (96%) had an interaction with a pharmaceutical representative within the last academic year. Thirty-three (33%) percent of residents surveyed felt the interaction with the pharmaceutical rep had improved the quality of care their patients received. In examining the role of personal bias; 24% felt a conflict of interest receiving small gifts (pen/flashlight) and 26% felt a conflict of interest in receiving a paid lunch from a pharmaceutical rep in exchange for their time listening to a presentation. In ascertaining perceived bias in the interactions with their attendings while in a supervisory role, 17% of residents have had an interaction with an inpatient attending that caused them to question whether that attending had a relationship with a pharmaceutical company that might be influencing their treatment decisions while 15% had a similar concern in the outpatient or ambulatory setting. Forty-eight (48%) percent of residents felt it would be helpful to have secure access to a list disclosing an attending's relationships with pharmaceutical companies.

**Conclusion:** There is a cohort of Internal Medicine Residents trained in the State of Virginia who felt a conflict of interest in their personal interactions with pharmaceutical representatives under the current guidelines. More concerning was a cohort who perceived a bias in the management decisions of their supervising attending physicians. These results highlight the need to investigate educational initiatives to instruct Internal Medicine Residents on strategies that will lead to a more productive relationship with the pharmaceutical industry in both training and practice. Consideration should be given to requiring faculty physicians to disclose any relationships which may pose a conflict of interest when in a supervisory role with trainees, similar to what is required for Medical Grand Rounds. Regulation of exposure at the resident level to pharmaceutical representatives will only be effective if there is the assurance of transparency of

all members of the health care system including the faculty intimately involved in the education of trainees.

## **Recovery of Thrombocytopenia in Post-Liver Transplantation Hepatitis C Patients**

*Christine Lin, MD; Timothy Pruett, MD; John Densmore, MD.*

**Introduction:** Thrombocytopenia is a common complication of chronic liver disease due to various suspected mechanisms. Liver transplantation has been shown to resolve the thrombocytopenia to normal platelet levels by approximately two weeks post-transplantation. Hepatitis C (HCV) is a virus causing infection that evolves into a chronic state in approximately 85% of patients. Chronic HCV infection has been associated with the development of thrombocytopenia through suspected mechanisms of HCV-induced autoantibody production against platelet glycoproteins, infection of platelets and megakaryocytes by HCV, decreased thrombopoietin production, and hypersplenism. Recurrent HCV is considered to be almost universal following liver transplantation for HCV. The study team would like to create a database for research purposes to evaluate the recovery of low platelet counts in post-transplantation HCV patients and compare it to platelet count recovery of patients with other etiologies of liver disease. Our hypothesis is that HCV patients have a decreased recovery of platelet counts post-liver transplantation compared to non-HCV post-transplant patients due persistent HCV virus infection post-transplantation.

**Methods:** All patients who underwent orthotopic liver transplantation at the University of Virginia Hospital from 01/01/01 through 02/18/07 were included in this retrospective study. Subjects' MELD scores, pre-transplantation splenic sizes, disease processes leading to indication for transplant, post-transplantation immunosuppression medications, and platelet counts pre and post-transplantation were collected and compared.

**Results:** Two-hundred eight patients were included in the study with 78 HCV patients and 128 non-HCV patients. The HCV group had lower pre-transplantation platelet counts, post-transplantation platelet count nadir, post-transplant days until platelet normalization, and major bleeding events. No statistical differences in MELD score, post-transplant days until platelets return to pre-transplantation levels, spleen size were found between the two groups.

**Conclusions:** In HCV patients, there is a more severe thrombocytopenia pre-transplantation and a decreased recovery of platelet counts post-liver transplantation compared to non-HCV post-transplant patients due possibly to persistent HCV virus infection post-transplantation.

## A Prospective Comparison of Endoscopic Ultrasound versus MRI for Staging of Pancreatic Cancer.

Michelle Loch

**Background:** Preoperative detection and staging of pancreatic cancer is crucial to determine proper therapy. Stage II and earlier pancreatic cancers are referred for surgery, while stage III and above are referred for induction therapy with hope of resection or palliative systemic therapy. Staging can be performed by EUS-FNA, CT and/or MRI. We report the first study that compares EUS vs. MRI prospectively.

**Methods:** 127 patients undergoing EUS-FNA and MRI for the workup of pancreatic cancer were captured in a prospective database. All patients underwent EUS-FNA by a dedicated pancreaticobiliary endoscopist. Final surgical stage was also recorded in patients who went to surgery. We compared EUS-FNA and MRI for the detection, staging, and resectability of pancreatic cancer.

**Results:** There were 71 men with a mean age of  $66 \pm 11.4$  years. Of the 127 patients with pancreatic cancer, 42 patients were surgically explored; and of these, 22 (52%) underwent pancreaticoduodenectomy. EUS and MRI agreed that 70/127 (55.1%) of cases were stage II or below, while there was only a 24/127 (18.9%) agreement in patients that were stage III or above. MRI was more likely to report metastatic disease or arterial involvement. The overall correlation between EUS and MRI was marginal ( $k=0.42$ , 95% CI=0.25-0.58). Of the 42 that were surgically explored, with surgical pathology as the gold standard, 10/42 (24%) were incorrectly understaged by MRI while 0/42 were overstaged. 11/42 (26%) were incorrectly understaged by EUS while 1/42 (2%) were incorrectly overstaged. EUS and MRI had a sensitivity of 30/31 (96.7%) and 31/31 (100%) respectively for stage II and earlier tumors.

**Conclusion:** In the staging of pancreatic cancer, EUS and MRI had marginal correlation, especially in the more advanced tumors. Since patients understaged by EUS were different than those understaged by MRI, perhaps both modalities should be performed in the staging of pancreatic cancer. Obviously, EUS has the added advantage of adding tissue acquisition to staging in patients with pancreatic cancer.

## Temporary Placement of Fully Covered Self-Expandable Metal Stents (CSEMS) in Benign Biliary Strictures (BBS): Preliminary Data

Mahajan, Anshu; Ho, Henry C.; Brock, Andrew S.; Shami, Vanessa M.; Ellen, Kristi; Berg, Carl L.; Schmitt, Timothy M.; Kahaleh, Michel

**Background and Aim:** Benign biliary strictures (BBS) traditionally have been endoscopically managed with the placement of multiple plastic stents. Placement of uncovered metal stents has been associated with mucosal hyperplasia, while placement of partially covered self-expandable metal stents has been associated

with migration and stricture recurrence. Recently, fully CSEMS with anchoring fins became available. We set out to analyze the efficacy and complication rates of these stents in the treatment of BBS.

**Materials and Methods:** CSEMS (10 mm diameter, Viabil, Conmed) were placed in 33 patients with BBS (25 men, mean aged  $56 \pm 16$  years). Pre-procedure diagnoses included chronic pancreatitis (CP) (n=19), gallstone-related strictures (n=10), post liver transplant BBS (n=3) and autoimmune pancreatitis (n=1). CSEMS were left in place until adequate biliary drainage was achieved, confirmed by resolution of symptoms, normalization of LFTs, and imaging. Removal was performed with a snare or rat tooth. Endpoints were efficacy, morbidity and clinical response.

**Results:** Of the 33 patients, CSEMS were removed from 24 (73%). The median time of CSEMS placement was 103 days (range: 62-178). Resolution of the BBS was confirmed in 18/24 patients (75%) after a median post removal follow-up time of 44 days (range: 0-204). All 6 patients failing therapy had biliary strictures secondary to CP; 5 of these patients underwent repeat stenting. Of the 9 patients who did not undergo stent removal, 3 expired from unrelated etiology, 3 had severe pancreatitis preventing repeat procedures, 1 had hepatocarcinoma, 1 underwent stent revision without removal and 1 was lost to follow-up. Technical difficulty during insertion included 2 stent deployments proximal to the distal stricture, repositioned endoscopically. Complications associated with placement (15%) included post-ERCP pancreatitis (n=3, with one severe), post-sphincterotomy bleeding (n=1) and pain (n=1). Complications associated with removal (13%) included post-ERCP pancreatitis (n=2), and pain (n=1). One patient had proximal biliary migration requiring CSEMS removal and one patient had CSEMS duodenal migration with resolution of the stricture. A logistical regression analysis of factors potentially predictive of success (e.g: age, previous stenting, CSEMS length and etiology), showed that BBS secondary to chronic pancreatitis is associated with failure (p value 0.008).

**Conclusion:** Temporary placement of CSEMS for BBS might offer an alternative to plastic stenting. The subset of patients with BBS secondary to CP appears to be refractory to stenting with CSEMS. Comparative long term studies using these stents are needed to confirm sustained success.

## Validation of a Scale to Identify Hypoactive Delirium vs. Depression vs. Dementia

Kurt Miceli, M.D., Anita Clayton, M.D., Bettina Joi Isaac

**Background:** Symptoms of hypoactive delirium, depression, and dementia may overlap and lead to diagnostic confusion for the inpatient clinician. Such errors in diagnosis can prolong length of hospital stay, increase cost, and worsen outcomes. We propose a scale for the primary medical provider to differentiate between delirium, depression, and dementia.

**Methods:** Using a newly devised six question scale (testing for agnosia, orientation, sleep/wake cycle disturbances, fatigue, memory impairment, and alterations in mental status), we sought to differentiate between hypoactive delirium, depression, and dementia in hospitalized, medically ill adult patients. Our study looked to validate this scale by comparing its clinical findings to that of the Axis I diagnosis made by the psychiatry consult service.

**Results:** In all, 27 patients were included in this study. Nine patients (33%) were not fully evaluated by the scale administrator for various reasons (eg: unresponsive, patient off the floor). All patients were seen by the psychiatry consult service. Of the 18 patients seen by both the scale administrator and the psychiatry team, clinical agreement was found in 9 (50%) cases. From those 9 patients in which the scale differed from clinical diagnosis, 4 were found to have conditions outside the parameters of the study (eg: adjustment disorder with anxiety, relational & occupational problems, acute stress disorder, and bipolar disorder, manic). The remaining 5 patients differed in diagnostic assessment of depression versus hypoactive delirium. For all 18 patients evaluated by the scale administrator, a diagnosis of dementia was not found by either the scale or psychiatry consult service.

**Conclusions:** Our scale highlights the diagnostic difficulty clinicians have in clearly diagnosing hypoactive delirium, depression, and dementia. The waxing and waning nature of delirium alone makes the diagnosis difficult for both the scale and the clinician. Additionally, using a short six question scale to make a diagnosis of depression does not take into full account the varied nature of the illness. Less objective measures such as assessment of fatigue contribute to the scale's variability. Moreover, an assessment of mental status changes is difficult to make when a patient is only seen once by the scale's administrator. In such instances the role of the clinician remains vital.

### **Inhibition of Staphylococcus biofilm formation by the investigational drug SNT-2**

*Florence. Tchouaffi-Nana, Paul S. Hoffman, Costi D. Sifri.*

**Background:** Coagulase negative staphylococci (CoNS) have emerged as important opportunistic hospital pathogens. These bacteria, which constitute a major portion of the normal skin and mucosal microflora, are the leading cause of catheter and indwelling device-associated infections. The capacity of CoNS to cause disease primarily relies the ability to adhere to polymer surfaces and form thick, multilayered, cellular agglomerations known as biofilm. Biofilm formation is important not only for the development of infection but also contributes to antibiotic failure and resistance to host defense responses. In the present ongoing study, we explore the ability of a new drug, SNT-2, to inhibit biofilm formation in staphylococci.

**Methods:** Laboratory and clinical strains representing different species of CoNS were cultured under microaerophilic conditions in tryptic soy broth (TSB) alone and in TSB in the presence of SNT-2 at different concentrations (5, 10, 25 µg/ml) in a polystyrene microtiter plate. After washing, staining with crystal violet, and extracting dye with ethanol, absorbance was read at 570 nm. Bacterial growth was also measured under aerobic (shaking) and microaerophilic (static) conditions in the absence and presence of SNT-2. To determine whether SNT-2 affects the production of biofilm associated polysaccharide intercellular adhesion (PIA), a modified Kirby-Bauer-type congo red assay was done with various concentrations of SNT-2, vancomycin, ampicillin and DMSO. Dye binding by bacteria was visually observed after 24hr incubation at 37°C.

**Results:** SNT-2 significantly inhibited biofilm formation and congo red binding by representative strains of *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* in a dose-dependant fashion. However, SNT-2 did not impede bacterial growth over the same range of SNT-2 concentrations.

**Summary:** The investigational drug SNT-2 at subinhibitory concentrations has anti-biofilm activity against CoNS. To identify the mechanism of action of the drug, our working hypothesis is that the drug is probably targeting the expression of the *ica* operon which is responsible for production of the polysaccharide intercellular adhesin (PIA).

### **Prognosis of Patients with Microsatellitosis in Malignant Melanoma**

*Mark Pajeau, William Grosh*

**Background:** Clinical and pathologic factors predicting outcome for melanoma patients have been studied for the last 35 years. Clark et al initially described the correlation between level of invasion of the primary tumor and patient survival. In 1970, Beslow established tumor thickness as a major determinant of survival. In 1981, Day et al described the prognostic significance of microsatellites on patient prognosis. Since that time, there have been a handful of studies investigating the prognostic significance of microsatellitosis. Most of these studies have found that the presence of microsatellites correspond to decreased five year survival. Microscopic satellites are described as nests of melanoma cells separated from the main body of tumor mass by a layer of collagen or subcutaneous fat. The American Joint Committee on Cancer Melanoma Staging System has characterized microsatellitosis as Stage III disease. These patients demonstrated 10 year survival rates of 20-40%.

However, in 2005 Shaikh et al published a study indicating microsatellites were associated with locoregional relapse, but neither distant metastasis nor overall survival. It was the belief of these authors that differences in defining and measuring of this pathological factor the reason for the discrepancies.

**Methods:** 326 patients with malignant melanoma when identified that were treated at the University of Virginia from 1996 to 2001. Variables studied were:

age, sex, location of primary tumor, site of first occurrence, site of distant metastases, presence or absence of microsatellites, date of relapse, date of death, histological staging including superficial type (nodular vs. lentigo maligna vs. acral lentiginous vs. other), tumor thickness (< 1 mm, 1-2 mm, 2-4 mm, > 4 mm), mitotic rate (< 5 mm<sup>2</sup> vs. > 5 mm<sup>2</sup>) ulceration (present vs. absent), vascular involvement (present vs. absent), tumor vascularity (present vs. absent) and lymph node involvement at time of first diagnosis.

Survival curves will be calculated by the method of Kaplan and Meier. Statistical comparisons of survival curves will be done with the use of the Mantel-Cox log-rank test. Multiple regression procedure will be used in a multivariate analysis of prognostic factors.

**Results:** Our hypothesis is that microsatellitosis in malignant melanoma is a more aggressive pathological type of disease. Patients with microsatellites have more rapid relapse of disease, a more rapidly spreading disease, and a poorer five year survival rate.

**Conclusion:** Currently, microsatellitosis in malignant melanoma is considered Stage III disease. The data indicated that these patients have a poorer prognosis. This study hopes to further examine this finding to confirm the hypothesis that these patients have a poorer prognosis.

### **Effect of Methylphenidate hydrochloride on the length of stay of chronically ventilated patients in the Intensive Care Unit.**

*A Clayton, S Ramamurthy, S Rau.*

In the intensive care unit (ICU), long and short term mechanical ventilation is required for patients with life-threatening illnesses. Some of these patients will have prolonged ICU stays secondary to difficulty weaning off mechanical ventilation. Although multiple physical factors are involved in prolonged ventilation, fatigue, depression and over sedation may often be overlooked. Case studies have shown that methylphenidate hydrochloride in selected patients may be helpful in the process of weaning from the vent. This study proposes that treatment of selected chronically ventilated ICU patients (greater than or equal to 5 days on the ventilator) with methylphenidate hydrochloride will decrease the total number of days on the ventilator and in the ICU versus patients receiving no additional treatment.

This is a single-site study which will randomize difficult to wean patients in the UVA ICU to receive increasing doses of methylphenidate hydrochloride or no additional treatment which is the current standard of care. The primary outcome measures are length of stay of the patient in the ICU as well as time to wean from the vent. CES-D depression screens will be performed throughout the patient's participation in the study on days 0, 3, 6, 10, 12 and 14. Due to the significant expected dropout rate, we will employ intent-to-treat (ITT) analysis techniques. According to the principles of ITT analysis, all subjects will be analyzed for

reduction (or increase) in time of ICU stay according to the groups for which they were originally assigned. Results for this study are pending.

### **Evaluating the Role of Head CT Scans in the Management of Fulminant Hepatic Failure**

*Jim Richter, Patrick Northrup*

**Background:** Cerebral edema is a serious and potentially fatal complication in patients with fulminant hepatic failure (FHF). Cerebral herniation has been implicated as the cause of death in 30% to 50% of patients with FHF. [1-4] Classic signs of elevated intracranial pressure (ICP) such as headache, bradycardia, and papilledema are often absent in the setting of FHF. [5] As a result, much attention has been focused on the early identification of those individuals with cerebral edema who have progression from grade 2 encephalopathy to grade 3. Important issues in the care of these patients include the prevention of brainstem herniation before liver transplantation, the selection of patients for liver transplantation who have the potential for neurological recovery and the maintenance of cerebral perfusion preoperatively and intraoperatively. The role of head computed tomographic (CT) scans in this patient population is controversial, and further studies are needed to examine its clinical utility in patient management.

**Objective:** To evaluate the role of CT scans of the brain in the management of patients with FHF.

**Methods:** We performed a retrospective review of patients admitted with FHF at the University of Virginia from November 1996 to November 2006. FHF was defined as the onset of hepatic encephalopathy within 2 months after diagnosis of liver disease (the presence of coagulopathy (prothrombin time > 15 sec or international normalized ratio  $\geq$  1.5). and any grade of hepatic encephalopathy within 26 weeks of the first symptoms in a patient with acute liver injury and no previous liver disease). [6] Patient data obtained from the time of admission unit until the time of hospital discharge, death, or liver transplantation were reviewed. Initial head CT scans were reviewed independently by two neuroradiologists who were blinded to ICP measurements, treatment, patient outcome, and each other's interpretation. CT scans of the brain were evaluated and the severity of cerebral edema was classified using predefined criteria. The relationships between CT findings, ICP measurements, and patient outcomes were then explored.

**Results:** Pending

**Conclusions:** Pending

## Assessing Mortality Prediction Models in the ICU

*Katherine Schafer*

**Introduction:** The use of “report cards” to compare physicians and hospitals is becoming an increasingly popular idea. These regular formal assessments for quality improvement often use mortality data as a marker of quality of care and patient safety. The results are not only used for internal review but are increasingly accessed by consumers, other healthcare practitioners, and third party payers. Presumed discrepancies between the health of patients at academic hospitals when compared to community hospitals pose a significant pitfall of the “pay for performance” and “report card” methodologies. In order to compensate for this problem, prediction models have been created to standardize patients across different care settings. These prediction models may be imperfect and the various models have not been compared against each other. Our project’s focus is to compare ICU quality measures, specifically the APACHE IV and the University Health System Consortium (UHC). APACHE IV has been validated in critically ill patients across ICUs internationally. We questioned whether the UHC methodology applied to critically ill patients.

**Methods:** A prospective observational study was conducted in the University of Virginia’s Porter Medical Intensive Care Unit (MICU) over a three month period (April 30, 2007 to July 31, 2007). Data was collected on all new admissions to the MICU in order to calculate predicted mortality using the APACHE IV calculator. UHC data is routinely collected through an administrative database and is accessible to the university to compare our case mix and adjusted mortality with other academic institutions. The scores were then compared using an intraclass correlation coefficient. The accuracy of the model was also compared with actual inhospital and post discharge thirty day mortality.

**Results:** Preliminary data of the first 26 patients suggest UHC methodology predicts a 50% lower mortality than APACHE IV. This is based on a linear association between APACHE and UHC prediction, where APACHE scores were plotted on the x-axis and UHC predicted mortality scores were plotted on the y-axis. The preliminary results show a correlation coefficient ( $R^2$ ) of 0.2983, meaning the APACHE and the UHC models agree approximately thirty percent of the time.

**Conclusion:** We expect the APACHE IV and UHC prediction models to correlate but given fundamental differences in their respective parameters we anticipated magnitude differences in predicted mortality. The UHC model is more heavily weighted by comorbidities while the APACHE IV accounts for some comorbidities but weighs physiologic parameters more heavily. Additionally, the UHC model does not account for severity of illness on admission which we believe to be a significant factor in determining mortality. A larger sample size is needed to definitively conclude that these differences in magnitude are real. If so, the implication to published report cards is substantial.

## Anti-Alpha 8 Integrin Immunoliposomes: Vehicles for Specific Delivery to Glomerular Mesangium

*Y.M.Scindia, U.S.Deshmukh, P.R. Thimmalapura, H.Bagavant*

**Objective:** Activation and proliferation of mesangial cells resulting in immune cell recruitment and altered extracellular matrix production is important in the pathogenesis of lupus nephritis. We hypothesized that regulation of end organ responses, specifically of mesangial cells, can modulate tissue injury. Targeting of therapeutic agents to the mesangial cells has been hampered by the lack of unique cell surface molecules on mouse (and human) mesangial cells. In this study, we describe the successful development of immuno-liposomes (ILs) that can specifically target the mesangial cells in mouse models.

**Methods:** Alpha 8 ( $\alpha 8$ ) integrin was identified as a target molecule on the surface of mesangial cells. Two strains of mice, NZM2328 and (NZM2328xNOD)F1, that develop spontaneous lupus nephritis were studied.

Expression of  $\alpha 8$  integrin in different tissues was studied by immunofluorescence staining. A mixture of different lipids and polyethylene glycol was dried and rehydrated to form stable liposomes. DiI, a red fluorescent dye was incorporated into the liposomes. The liposomes were passed through filters to obtain unilamellar vesicles of ~100nm diameter and then chemically conjugated to an anti- $\alpha 8$  integrin antibody to make anti- $\alpha 8$ ILs. Six-month-old female mice were injected (i.v.) with anti- $\alpha 8$ ILs and studied 18 hours later. Unconjugated liposomes or rabbit IgG conjugated liposomes were used as controls. Cellular uptake and tissue distribution of injected liposomes was studied by fluorescence microscopy and flow cytometry.

**Results:** Immunofluorescence staining showed expression of  $\alpha 8$  integrin predominantly on mouse mesangial cells.  $\alpha 8$  integrin was also detected in mice with glomerular immune complex deposits in early lupus nephritis. Glomeruli of mice injected with DiI labeled anti- $\alpha 8$ ILs showed red fluorescence indicating DiI+ cells. This was not seen in controls. Immunofluorescence microscopy did not show enrichment of DiI+ cells in other organs (liver, lung, spleen). Analyses of single cell suspensions from brain, liver, spleen and lung by flow cytometry showed 0.09 -5% DiI+ cells in these organs indicating that the anti- $\alpha 8$ ILs primarily target the glomerular mesangium.

**Conclusion:** The anti- $\alpha 8$ ILs are able to preferentially target the glomerular mesangium. Size of the liposomes permitted their migration through the fenestrated glomerular endothelium into the mesangial space. In addition, it prevented the liposomes from going into the urinary space between the podocyte processes. These anti- $\alpha 8$ ILs will be used to delivery therapeutic agents for regulation of mesangial responses to investigate their effect on progressive glomerular disease.

## Diarrheal Illness at the Onset of ART Initiation

Satu Shah, M.D., Rebecca Dillingham, M.D.

**Background:** Based on internal, unpublished data at GHESKIO (Haitian Study Group on Kaposi's Sarcoma and Opportunistic Infections), patients that presented with diarrhea at the initiation of Anti-Retroviral Therapy (ART) were almost twice as likely to die within one year than those who presented without diarrheal symptoms. Also, weight loss has a demonstrable effect on mortality, as even minor wasting is a risk factor for death in HIV patients even when accounting for ART initiation. Diarrhea and wasting are closely linked. It has been suggested that malabsorption of anti-retrovirals could be one factor involved in the higher mortality exhibited by HIV patients presenting with diarrhea at the onset of ART.

**Methods:** A cohort study of HIV patients beginning ART at GHESKIO was undertaken to compare and examine differences between patients with persistent diarrhea and those without diarrhea at initiation. 28 patients with diarrhea and 27 patients without diarrhea completed the study. Attempts were made to match patients for age, sex, etc. Major outcomes assessed were change in weight at 2 weeks and at 4 weeks after initiation of ART. Other secondary laboratory measures included CD4 count change from 0 to 24 weeks, presence of diarrheal pathogens at initiation, anti-retroviral (ARV) serum levels at 2 and 4 weeks, D-Xylose levels in urine specimens at 2 & 4 weeks, and viral loads at 0, 2, 4, & 24 weeks.

**Results/Conclusions:** Data analysis has yet to be formally completed, as some data points are being actively tracked down, but the database may be completed over the next 2 to 3 months. Preliminary data analysis suggests that patients with diarrheal illness at the onset of ART actually had higher ARV serum levels, which would be an unexpected finding, as malabsorption was thought to play a role in the worse outcomes experienced by those subjects with diarrhea. A possible cause of increased ARV absorption could include more porous tight junctions in the epithelium lining the GI tract arising from diarrheal injury. Data that could potentially be compiled by April or June may be an examination of the pathogens seen in stool specimens of the two groups (*E. Coli*, *Giardia*, *Cryptosporidium*, *Clostridium Dificile*, etc.)

## Simultaneous Detection of *Entamoeba histolytica*, *Cryptosporidium parvum* and *Giardia lamblia* in stool samples using a single enzyme immunoassay.

Cynthia B. Snider M.D. M.P.H.

**Background:** Diarrheal infections account for 21% of all deaths of children under the age of 5 worldwide despite large public health efforts for the improvement of sanitation, clean water supplies and introduction of oral rehydration solution. The traditional screening test, the microscopic ova and

parasite (O&P) exam of stool, suffers from poor sensitivity and specificity, and requires expertly trained personnel to interpret results. Improved diagnostic methods specific for enteric pathogens that could be easily applied in resource poor countries would be valuable in the management diarrheal illnesses. The detection of parasite antigen in stool by enzyme-linked immunoassay (ELISA) is the current diagnostic method of choice. However, real-time PCR tests (RT-PCR) for the enteric parasites are not yet practical or cost effective as screening assays. In this study, a prototype of a fecal ELISA screening test designed to simultaneously detect *Entamoeba histolytica*, *Cryptosporidium spp.* and *Giardia sp.* was field tested against the gold standard of individual ELISA to evaluate the feasibility of a single ELISA screening test having comparable or greater sensitivity in the detection of these enteric parasites.

**Methods:** Stool specimens were obtained from a cohort of children and adults from Dhaka, Bangladesh where *E. histolytica* is endemic. The Tri-Combo ELISA provided by TechLab, Inc (Blacksburg, VA) is a conventional two-step ELISA format with HRP-conjugated detecting antibodies for colorimetric development was designed to simultaneously screen stool specimens for *Giardia lamblia*, *Cryptosporidium spp.* and *E. histolytica* using a single assay well. For those samples with discrepancy results between the Tri-Combo versus individual specific antigen test, RT-PCR analysis was conducted.

**Results:** Based upon 235 stool specimens tested, the Tri-Combo ELISA has 99% Sensitivity and 96% Specificity, Positive Predictive Value 95% and Negative Predictive Value 99.2%. The Tri-Combo ELISA has a 97% correlation to individual parasite ELISA and RT-PCR.

Table 1: Clinical evaluation of the Tri-Combo ELISA's ability to detect *Giardia*, *Cryptosporidium* and *E. histolytica* in human stool.

<i>Tri-Combo ELISA Results</i>	Individual ELISA and RT-PCR Results		
	Positive	Negative	Total
Positive	95*	5	100
Negative	1	133	134
Total	96	138	234

\* 1 stool specimen was excluded secondary to insufficient quantity for further testing

**Conclusion:** This preliminary data of the Tri-Combo ELISA for simultaneous detection of *Giardia sp.*, *Cryptosporidium spp.* and *E. histolytica* reveals similar test characteristics as the currently FDA approved individual parasite ELISAs. This diagnostic method represents a potential cost savings tool in the detection of enteric parasite infections.

## **Gastric bypass patients remain Vitamin D insufficient despite increased supplementation.**

*Rodney Snow M.D., Gregory Clines M.D., Jerry Nadler M.D., Honkun Wang PhD, Joseph Northup M.D.*

**Background:** Vitamin D insufficiency is a major unrecognized health problem and has been reported in approximately 36% of otherwise healthy young adults (ages 18-29), 41% of outpatients (ages 49-83), 57% of general medicine inpatients (ages 18-95) and more than 50% of postmenopausal women currently taking medications for osteoporosis in the US. In addition to the general population's risk factors for vitamin D insufficiency (inadequate sun exposure, lack of dietary vitamin D, latitude, older age), the obese population is believed to intentionally expose less skin to the sun by remaining indoors, consistently maintain a vitamin D poor diet and encounter sequestration of ultra violet light-generated vitamin D in excess adipose tissue. Along with the increasing prevalence of obesity, the total number of bariatric surgeries for obesity has increased from 13,386 procedures in 1998 to 121,055 in 2004. Currently, there are no national guidelines for monitoring and/or treatment of vitamin D inadequacy in patients after gastric bypass surgery.

**Objective:** Assess if the recommended intake of vitamin D in a group of UVA GBSRY (Gastric Bypass Surgery with Roux-en-Y) patients (either 800 or 1000 IU of Vitamin D) was sufficient to prevent vitamin D insufficiency at one year post operation.

**Design:** This was a retrospective study of patients undergoing GBSRY from September 2004 through April 2006 at UVA (n = 288). This analysis examined the bioactive levels of vitamin D and intact parathyroid hormone before GBSRY, and then post-operatively at 3, 6 and 12 months. A clinical nutritionist counseled patients at each clinic visit and recorded if the patients were compliant with their recommended supplements. Based on vitamin D levels in the "normal" or "below normal" ranges (as reported by the UVA laboratory vitamin D assay) patients were asked to consume 800 IU of Vitamin D (400 IU from diet plus 400 IU from multi-vitamin), or to consume 1000 IU of cholecalciferol (D3) supplement per day (Nature's Way), respectively. Due to a change in the vitamin D assay, and thus new "normal" values, half-way through the analysis period, a "percent of upper limit of normal" was used to compare all vitamin D levels over time for trends.

**Results:** The PULN of vitamin D values for this patient population did significantly increase over the course of one year (p=0.0073). However, of the 118 patients whose vitamin D values included both vitamin D2 and D3 (new assay only) throughout the observed time period, 84 patients (71%) had values below the recommended 30ng/ml.

**Conclusions:** Overall, this population of GBSRY patients showed an increase in the vitamin D levels, but a subset analysis showed that a significant majority (71%) of patients remained below the recommended value of 30 ng/ml. This would suggest that most GBSRY patients at UVA who are taking the

recommended dose of vitamin D did not attain target levels. More aggressive supplementation is necessary to ensure that high-risk patients such as GBSRY attain adequate vitamin D levels.

## **Hand-off of care practices among a group of Internal Medicine residents at the University of Virginia Health System.**

*Stephen Turner, MD; Tracey Hoke, MD*

**Introduction:** The handoff of patients from one health care provider to another is a ubiquitous part of medical practice. On average, a resident working in today's academic hospital will take part in 300 patient handoffs per month. Local custom, tradition, and access to technology dictate how residents currently handoff patients to one another rather than accepted standards or formal training. Within the Department of Medicine at the University of Virginia Health System, for example, handoff of patients occurs in a two step process: 1) physician to physician communication on the phone or face-to-face and 2) a transfer note written by the sending team to the accepting team. In 2006, The Joint Commission on Accreditation of Healthcare Organizations (JCAHO) commissioned a multidisciplinary team of 20 health care professionals from across the United States to develop best practice recommendations for handoff communication. Eight key elements were identified as essential to handing off a patient from one physician to another. The final product is offered as a guide to hospitals implementing change in order to comply with the JCAHO National Patient Safety Goals. Their expert recommendation were used as a benchmark to compare to our current practices at UVA.

**Methods:** A survey was developed to assess physician to physician communication and congruence with JCAHO's recommendations. Ten residents staffing the General Medicine I and II wards anonymously completed surveys for each patient transferred to their respective team from the Medical Intensive Care Unit (MICU) over a one month period. In addition, transfer notes written by the MICU team were audited for the presence or absence of the gold standard items.

**Results:** 23 surveys were completed by upper level residents and 23 transfer notes were evaluated over a one month period. Based on survey data, five out of the eight gold standard items were communicated 90% of the time, including patient name, other patient identifier, current condition, opportunity for questions, and contact name and number of referring MD. 30% of handoffs lacked information regarding anticipated changes in the patients' condition or treatment plan, and 25% were missing what to watch out for in the next interval of care. 17% did not adequately address the patients' current condition. 43% of residents surveyed felt as if information was missing from the handoff. In addition, extraneous information not required by JCAHO was often communicated such as medications and lab results (52% and 47%, respectively).

Preliminary audit of the transfer notes demonstrated that the majority (89%) did not include anticipated changes and what to watch for in the next interval. 55% of the notes did not address the patients' current condition on transfer. Notes were inconsistent in content with 66% listing medications, 33% including lab results, and 25% listing allergies and code status-all items not required by JCAHO. 30% of accepting residents did not read the transfer note.

**Discussion:** The results demonstrate that Internal Medicine residents are doing a fair job at handing off patients. During face-to-face communication, key elements recommended by an expert group are often included. However, the survey and transfer note audits demonstrate that emphasis is not being placed on anticipated changes and what emergencies to watch out for in the next interval of care. Most transfer notes neglected to address this, and nearly a fifth to one third of the time, this information was not communicated verbally. JCAHO and other groups have emphasized the importance of this information as critical to insuring a safe handoff between physicians. Patients transferred out of a tertiary care MICU, in particular, are often at risk of complications and clinical change. This project identifies areas for improvement in resident handoff practices.

### **Characterization of Different Stages of Renal Disease in Systemic Lupus Erythematosus by Glomerular Transcriptional Profiles**

*Hongyang Wang, Harini Bagavant, Umesh Deshmukh, Carol Kannappel, Shu Man Fu*

Systemic Lupus Erythematosus (SLE) is complex autoimmune disease affecting multiple organ systems. Renal involvement is the frequent complication of SLE. Although an autoimmune response is the primary mediator of renal injury in SLE, the intrinsic susceptibility or resistance of the end organ and its response to immune injury are important in the development of this disease. We investigated inbred strain of lupus prone mice-New Zealand Mixed (NZM) 2328. These mice spontaneously develop autoantibodies and progressive glomerulonephritis (GN). Females have preponderance in development of renal failure. Three different pathological stages can be clearly identified in female NZM2328 mice as normal, acute and chronic GN. Both female and male mice develop immune complex deposit and proliferative GN featured by mesangial expansion and glomerular cellularity. Female mice progress from acute to chronic GN characterized by glomerulosclerosis, fibrosis, tubular atrophy and fatal renal failure. Purpose of Study: To characterize lupus glomerulonephritis in different development stages at molecular and transcriptional level. Methods: Glomeruli were collected by Laser Microdissection (LMD) system from cryosections of normal (10 weeks old), acute GN (26 weeks old) and chronic GN (40 weeks old) NZM2328 female mice. Affymetrix array (Mouse Genome 430.2.0) hybridizations were performed using LMD-derived total RNA samples. Statistical method, Local-Pooled-Error (LPE), was applied to identify genes with significant difference using  $p$  value  $< 0.001$  and fold change  $> 1.5$ . Data validation

was performed using Q-PCR from LMD-derived total RNA and immunofluorescence staining. Results: Compared with normal glomeruli, acute GN was associated with changes in 880 genes. Of them, 373 genes were down-regulated in AGN, whereas 507 genes were found highly expressed at acute GN stage. Up-regulated genes include innate immune response genes like Toll-like receptors (TLR2, TLR4), interferon response genes (ifl203, ifl205 and mx1), pro-inflammatory chemokines, cytokines and their receptors (IL-1b and IL-10), genes related to myeloid cells infiltration (Fpr1 and Fprs2). Down-regulated genes contain kidney related genes (WT-1 and podocin), oxidoreductase (Akr1b7 and Fabp3), and other enzymes involved in metabolism. In progression from acute to chronic GN stage, genes involved in tissue repair and fibrosis (TGF beta) were found to be highly expressed at chronic GN stage. Whereas, genes related to immune response and infiltrated cells were decreased. Conclusion: Gene expression profiles from this study show differential gene expression at different stages of glomerulonephritis i.e. aGN and cGN. Genes involved in progression from normal kidney through aGN to cGN are identified. These data may reveal novel targets for intervention in the progression of lupus nephritis and help us in identification of unique diagnostic markers in lupus development and disease progression.

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### **Obstructive atherosclerosis found in apical ballooning syndrome (Takotsubo cardiomyopathy)**

*David E. Winchester MD, Angela Taylor MD*

**Background:** First described in 1991, apical ballooning syndrome (ABS, or takotsubo cardiomyopathy) is becoming more commonly recognized. Etiology is not well understood, but patients present with symptoms similar to those of acute coronary syndrome (ACS) including chest pain and shortness of breath. Cardiac biomarkers are elevated and systolic ventricular wall motion abnormalities are present. Diagnostic criteria are not universally agreed upon, however most researchers believe that patients must be free of coronary atherosclerosis, or at most, concurrence is rare.

**Methods:** We used the University Clinical Data Repository to identify patients hospitalized between 1996 and 2007 with ICD-9 codes consistent with a diagnosis of ABS. 31 patients were identified based on predetermined diagnostic criteria.

**Results:** 28 of the patients were female. There was documentation of a stressful event preceding onset of symptoms in 68% of patients, roughly half were stressful medical events and half were stressful social events. Peak troponin values ranged from zero to 9.9. Quantitative coronary angiography was performed noting that 61.3% had evidence of coronary artery disease (CAD) and

6.5% had at least one obstructive lesion (of greater than or equal to 70% stenosis).

**Discussion:** Previous studies of ABS have used evidence of atherosclerosis as an exclusion criterion for the diagnosis of ABS. Our design allowed for identification of patients in which the diseases coexist. Though limited as a retrospective analysis, this study provides strong evidence that ABS and CAD occur together frequently. Evidence applied from other studies of CAD provide a framework for understanding ABS as a possible manifestation of early microvascular dysfunction.