**Title:** TARGETING KRAS PATHWAYS IN NON-SMALL CELL LUNG CANCER

Valsamo Anagnostou, MD-PhD, **Mentors:** Dr Roy Herbst, MD-PhD and Dr Peter Koo, PhD

**Background:** NSCLC is a major cause of cancer-related death and the prognosis for metastatic NSCLC patients remains dismal. New personalized treatment strategies have incorporated targeted therapies however high resistance rates remain an unresolved challenge. KRAS is frequently mutated in NSCLC especially in adenocarcinomas and in tumors of patients with smoking history; KRAS G12C/V is the predominant mutation found in tumors of patients with a smoking history, whereas KRAS G12D is more commonly found in non-smokers. Mut-KRAS mediated signaling through the Raf-MEK-ERK cascade has been found to abrogate the effects of many targeted agents including EGFR, VEGF and PI3K inhibitors. Preclinical results from the BATTLE-1 study indicated that different KRAS amino acid substitutions produce varying effects on KRAS-activated signaling and more importantly result in different response to targeted therapies. KRAS interacts with its different downstream effectors by undergoing large conformational changes in the switch I and II regions that surround amino-acids corresponding to codons 12 and 13. Molecular dynamic and protein–protein docking studies showed higher affinity of mut KRAS G12C for Raf, whereas mut KRAS G12D has higher affinity for PI-3-K/AKT. This is consistent with findings of transcriptome microarray studies of human tumor samples and reverse-phase protein array studies of NSCLC cell lines with known substitutions in KRAS showing that cell lines with mut KRAS G12D had activated PI-3-K and MEK signaling, whereas those with mut KRAS G12C/G12V had activated Raf signaling and decreased AKT activation. Furthermore, P70 S6 kinase (p70S6K) is activated and exerts feedback inhibition on growth factor receptor-activation of AKT with a weak inhibition observed by mutant KRAS G12D, moderate inhibition by wild-type KRAS, and strong inhibition by mutant KRAS G12C.

**Specific Aim:** Study the effect of specific mut-KRAS signaling on response to inhibitors of downstream signaling pathways, identify rational combinations of molecularly targeted therapies that potentially will mitigate the effects of mut-KRAS in NSCLC as well as develop molecular signatures for mut-KRAS-mediated sensitivity and resistance

**Hypothesis:** We hypothesize that specific KRAS mutations lead to different structural changes in the KRAS protein and result in activation of specific downstream signaling pathways resulting in different response to targeted therapies.

**Methods:** The inhibitory effect of the AKT inhibitor MK2206, MEK inhibitor AZD6244, RAF/VEGFR2/PDGF receptor sorafenib and their combinations was determined on luminescence-based cell viability assays for H1792 (mut-KRAS G12C), H441 (mut-KRAS G12V) and A549 (mut-KRAS G12S) cell lines. Whole cell lysates from treated and untreated (control) cells were used for western blot analysis to detect differential expression of pAKT (S473), pMEK (S217/221), pSTAT3 (Y705), Bim and p4EBP1 (Thr37/46).

**Results:** Single agent therapy with either AZD6244, MK2206 or sorafenib resulted in modest inhibition of cancer cell growth. The AZD6244-MK2206 combination resulted in a synergistic effect on cell growth for A549 cells compared to single treatment, an effect that was not seen in H1792 or H441 cells. Western blot analysis revealed that the AZD6244-MEK2206 combination enhanced Bim overexpression and apoptosis and effectively inhibited pAKT expression whereas no difference was seen in expression of the translation repression protein p4EBP1. Interestingly, synergy between AZD and sorafenib was observed for H1792 cells compared to H441 and A549 cells.

**Conclusions:** We observed that the effect of AKT, MEK and RAF inhibitors and their combinations varied among cell lines with different KRAS mutations. Our findings suggest that different KRAS mutations lead to different cell signaling and regulate response to targeted therapies; further studies are required to identify novel predictive biomarkers.
Title: Emergency department use among HIV-positive, recently-released jail detainees in two cities in Connecticut

Resident: Andrew Boyd, Advisor: Dr. Rick Altice

I. Specific aims and hypothesis: I used data from baseline interviews and six-month follow-up interviews with members of a research cohort of HIV+ jail detainees in Connecticut to track emergency department (ED) use after release from jail. The hypotheses of this study were the following: 1) that those participants who were not retained in HIV care will use the ED more frequently, and 2) that those participants who use the ED more frequently will have increased prevalence of untreated substance use and co-morbid psychiatric conditions, a less-stable housing situation, and more poorly-controlled HIV disease.

II. Research methods: For all study participants (n=109), medical records from four area hospitals were reviewed for emergency department visits in the year following release from jail. Primary outcomes were the rate ratio for ED use in the twelve months following release from jail, reason for ED visits, and investigation of covariates with ED use such as subject demographics, retention to HIV care and buprenorphine treatment, substance use status, psychosocial well-being, HIV disease markers, and perceived health-related quality of life.

III. Results: When compared with people who used the ED less frequently, those who used it more frequently were more likely to be female, have an uncontrolled HIV viral load, and have a higher severity of lifetime alcohol use. Those subjects that had early retention to HIV treatment, defined as seeing an HIV specialist within three months of leaving jail, had less ED use.

IV. Conclusion: The period immediately following release from jail is a very unstable and vulnerable time for people living with HIV/AIDS and returning to the community, and this study, in which poorer HIV control is associated with more ED use, reflects that vulnerability. In addition, the study demonstrates the importance of early referral to HIV primary care for this population, since being seen by an HIV provider within the first three months was associated with less ED use, an association not borne out in those subjects who first saw a provider after three months post-release.
Proposal Title: Characterization of PSGL-1 expression on peripheral blood plasma cells in rheumatoid arthritis

Jacqueline Cook, MD and Joseph Craft, MD

Background: Rheumatoid arthritis (RA) is a chronic, immune-mediated disease characterized by chronic inflammation with synovial hyperplasia, leading to progressive cartilage and bone destruction. B cells contribute to both disease initiation and perpetuation; conversely, B cell depletion, using anti-CD20 antibodies, has been used a therapeutic option in patients who have failed remission therapy with TNFα blockade. Long-lived antibody-secreting cells may survive therapeutic intervention in RA, producing autoantibodies and perpetuating the pathogenic process; therefore, elimination of these cells is a key therapeutic goal in treatment of B cell-perpetuated diseases including RA. Nonetheless, autoantibodies often return, despite their initial depletion, suggesting that long-lived plasma cells (or antibody-secreting cells) are not sensitive to such interventions and require neither T cells nor the continuing presence of antigen to survive. Persistence of plasma cells correlates with poor responsiveness to anti-CD20 therapy, and long-lived CD20-negative plasma cells that secrete autoantibodies are difficult to eliminate with current therapies. Thus, a strategy to eliminate these cells, potentially by interfering with their trafficking to niches that otherwise ensure their survival, would be a therapeutic advance. PSGL-1 is a marker that plays an important role in plasma cell trafficking to the bone marrow, a niche for plasma cell survival in autoimmunity. We will determine if PSGL-1 expression on plasma cells correlates with disease activity in RA, as a further step to validate PSGL-1 as a therapeutic target.

Specific Aim: Assess PSGL-1 expression on plasma cells from patients with RA.

Hypothesis: We will determine if PSGL-1 expression on plasma cells correlates with disease activity in RA, as a further step to validate PSGL-1 as a therapeutic target.

Methods: Peripheral blood mononuclear cells (PBMC) from patients with RA and those of healthy controls were isolated by Ficoll and stained for B cell markers including CD19, IgD, CD38, CD27, CD138, and PSGL-1. CD19+ B cells were separated into different subsets, including naïve, germinal center, memory, and antibody-secreting cells by IgD, CD27, and CD38, respectively, following strict flow guidelines. PSGL-1 expression was then compared among patients with RA and healthy controls.

Results: Unfortunately, the results of our study are limited by sample size for both RA patients (n=11) and healthy controls (n=4). Although CD19+ populations in RA patients was approximately 1.59 times that of healthy controls, there was no significant difference observed in CD38 populations nor PSGL-1 expression.

Conclusions: Based on the results of this project, there is no difference in expression of PSGL-1 on plasma cells in patients with RA as compared to that of healthy controls. Factors limiting our results include sample size, and that all patients with RA enrolled in this study were already undergoing treatments for their disease, none of which included anti-CD20 therapy. To accurately test the abovementioned hypothesis, sample sizes of approximately 30 RA patients and 30 healthy controls are needed, for which recruitment in a larger study is ongoing.
Title: Retrospective analysis of clinical characteristics of patients with Hepatitis C and Hepatocellular carcinoma at Yale New Haven Hospital 2006-2011 and (future) correlation to molecular microRNA characteristics.

Huong Do, M.D., Tamar Taddei, M.D.

Background: Hepatocellular carcinoma (HCC) is the sixth most common malignancy worldwide and it is the third cause of cancer-related mortality. The incidence of HCC has tripled in the past twenty years while the five year survival rate continues to be less than 12%. Major risk factors for the development of HCC include HBV or HCV infection, alcoholic liver disease and nonalcoholic fatty liver disease. Management of hepatocellular carcinoma is challenging because it arises in an already diseased organ. Therapy is guided not only by the stage of the cancer but by the patient’s hepatic synthetic function. Several staging systems are in use for HCC, but the practice guidelines of the American Association for the Study of Liver Diseases (AASLD) have adopted the Barcelona Clinic Liver Cancer (BCLC) classification as a staging and treatment algorithm for HCC. The BCLC staging system examines tumor stage, liver function, and physical performance status. However, it does not include molecular characteristics. In recent years, microRNAs have been extensively studied in relation to HCC. MicroRNAs are short non-coding RNAs that are able to regulate a large number of different mRNAs therefore affecting gene expression and cellular differentiation. It has already been shown that aberrant expression of miRNAs is associated with oncogenesis. Several groups have published studies identifying certain miRNAs specific to HCC that are strongly associated with survival, metastasis and even responsiveness to treatment. However the majority of these studies examined heterogeneous populations, including patients with different etiologies of cirrhosis. More granularity can be added by looking a homogenous population of patients with HCC in the setting of Hepatitis C.

Specific Aim: To identify clinical correlates to aberrant microRNA expression in a homogenous population of patients with hepatocellular carcinoma in an effort to identify a molecular marker to aid in disease management.

Methods: A retrospective chart abstraction of YNHH electronic medical records was done. Patients were included if they had HCV, HCC and available FFPE tissue from the Pathology CoPath database between January 1, 2006-December 30, 2011. Several clinical and pathologic variables indicative of prognosis, severity and time course of HCV cirrhosis and liver cancer were gathered and analyzed. FFPE specimens from biopsies, resections and explants of these patients were found in the Pathology CoPath database. Samples will be analyzed via real time PCR. A descriptive analysis will be generated based on the expression patterns and correlated with the clinical database.

Results (thus far): Among 94 patients with HCV and HCC (aged 31-90 years, with male:female ratio 4:1) the average age at diagnosis was 58.6 yrs old. Comorbidities included HIV co-infection (5%) chronic alcohol use (69%), IVDU (35%), obesity BMI >30 (26%), diabetes (31%), hyperlipidemia (17%), and ESRD on HD (5%). Many were minorities (41%). In 37% of the time the diagnosis was made using both MRI findings and tissue biopsy. Most patients at the time of diagnosis were well compensated with a Child-Pugh score of A (54%) and had a BCLC stage A (61%). On average it took 143 days between diagnosis and first treatment. Only 61% responded to treatment and of those 34% (21) of patients had recurrence of disease. The average number of treatment modalities (such as TACE, RFA, chemotherapy and etc) utilized was 2.5. Tissue data is pending completion for final analysis and correlation to the clinical data.
Title: Automated Data Collection for Assessing Exposure to a Behavior Change Video Game
Tamer Fakhouri, MD, Lynn Fiellin, MD

Background A limitation of conventional behavior change interventions is the ability to accurately catalog exposure to a given intervention and the fidelity with which the intervention is delivered. Interventions may be a composite of several activities obscuring which components drive the observed outcome. Video game-based interventions are emerging as effective and compelling strategies for behavior change. They have the advantage of providing highly accurate and granular exposure information through analysis of each player’s unique game play experience. The video game software creates automatic logs (with timestamps) of all aspects of a player’s experience (button presses, game levels entered and exited, actions taken), to a level of detail allowing for the re-creation of the entire game play session.

An interactive video game grounded in a social cognitive model of health behavior change has been developed that addresses the development of behavioral skills needed to avoid HIV risk behaviors. The intervention video game, Play Forward: Elm City Stories, has a main story line comprised of “challenge stack” levels that engages the player with role-playing scenarios where decisions around risky behaviors can be made, and their consequences experienced. Players must also earn points in “mini-games” designed to build either health knowledge or behavioral skills needed to avoid HIV risk behaviors. One of these mini-games is called Refusal Power which focuses on teaching the player skills around negotiating and refusing risky situations that revolve around drugs and sex. Adolescents from the target population are being enrolled in a 6-week randomized controlled trial (total approx 16 hrs of play) of playing Elm City Stories vs. a set of control video games (as a time/attention control) unrelated to behavior change. Along with other measure, the subjects’ self-efficacy for drug use resistance will be assessed using validated instruments, collected via a secure web-based system at baseline, 3, and 6 weeks.

Specific Aim To determine if software-generated logs of player activity in a video game intervention can be used to measure exposure to specific intervention components.

Hypothesis We hypothesized that logged data from the video game software could potentially provide valuable information about the proportion of drug use-themed video game content completed by the player, and the player’s performance in the Refusal Power mini-game.

Methods Video game log files containing information from 3 weeks of game play for two subjects were uploaded into a Microsoft Access Database, totaling 23,805 logged events. Logged events containing player score on the Refusal Power mini-game, and logged events signaling completion of drug use themed levels in the game’s main storyline were collected. Each player’s total score on the Refusal Power mini-game was calculated as a percentage of the maximum possible score. Each player’s count of drug use themed levels in the challenge stacks was calculated as a percentage of the maximum number of levels accomplished.

Results Player 1 completed 80% of all possible levels of the Refusal Power mini-game, and earned a score of 24 out of a possible 30 stars, giving them an 80% of maximum star rating. They completed 50% of the levels of the Refusal Power mini-game that deal explicitly with drug use and 66% of the challenge stack levels that deal explicitly with drug use. Player 2 completed 100% of all possible levels of the Refusal Power mini-game; earned 93% of the maximum possible score for the mini-game; completed 100% of the Refusal Power mini-game levels and 83% of the challenge stack levels that deal explicitly with drug use, respectively.

Conclusions Automatically generated log files are a novel and reliable method of measuring exposure to specific components of a complex, multifaceted, behavior change intervention. Next steps include evaluating correlations between exposure to specific intervention components through game software and target outcomes to identify the most efficacious components of the intervention and pave the way for the development of improved behavior change interventions.
Association of Interventional Cardiology board certification and in-hospital outcomes of patients undergoing percutaneous coronary interventions.
Paul Fiorilli, M.D., Jeptha Curtis, M.D.

Background: Board certification plays an important role in the delivery of medical care, as it conveys a sense of clinical competence and superior training. It is of particular importance within the field of Interventional Cardiology, in which the number of interventional procedures has grown rapidly over the past two decades. PCI is a highly specialized procedure that requires a specific knowledge base and extensive clinical training to safely perform. To help standardize the process of training, the ABIM developed a qualifying exam in Interventional Cardiology that was first introduced in 1999. Although more than ten years has passed since the introduction of this certification, limited information is available regarding its impact on the care and outcomes of patients undergoing PCI. The number of physicians who do not hold board certification who perform PCI is unknown. In addition, information regarding the characteristics of these physicians, whether they never passed the exam or were “grandfathered” in, is also not available. Previous analyses have shown that board certification has been shown to be associated with better patient outcomes. Given the volume of PCI performed yearly and the high stakes nature of the procedure, it is essential to understand the impact of certification on outcomes.

Specific Aims: To determine the association between board certification in interventional cardiology and the in-hospital outcomes of patients undergoing PCI.

Hypothesis: Patient outcomes from PCI performed by board certified interventional cardiologists will be superior to those performed by non-board certified physicians, independent of volume of procedures performed as well as baseline patient characteristics.

Methods: We linked data regarding physician certification in IC from the ABIM database with patient outcomes data from the CathPCI Registry (national procedure-based PCI registry). We conducted a retrospective analysis including all PCI procedures performed in 2011 and linked patient outcomes to physician certification. Physicians were analyzed in five groups: 1. Certified (finished FS<1999), 2. Certified (finished FS>1999), 3. Never-certified (finished FS<1999), 4. Never-certified (finished FS>1999), 5. Lapsed. We excluded procedures performed on patients < 18, and restricted to the first PCI performed during hospitalization. Primary endpoints included all-cause in-hospital mortality and bleeding complications (72 hours). Secondary endpoints included emergency CABG, vascular complication requiring therapy, and a composite endpoint of any adverse event (in-hospital death, emergency CABG, bleeding, vascular complications). The results were adjusted for physician volume as well as baseline patient characteristics.

Results: A total of 5175 physicians and 502,471 procedures were included. Overall, 23.1% of physicians had not been certified by 2011, and 6.1% had lapsed certification. There were no clinically significant differences noted in procedural volume or patient characteristics across physician groups. For all-cause mortality, the never-certified (finished FS<1999), had a higher mortality rate (OR 1.13, p 0.01), as well as for CABG (OR1.29, p 0.01). No significant differences were seen with bleeding, vascular complications, or the composite endpoint.

Conclusions: In our study, the outcomes of patients undergoing PCI were similar regardless of the certification of the performing physician. Compared with physicians who were certified, physicians without interventional certification who finished fellowship before 1999 (the “grandfathered-in” group) had a higher rate of emergency CABG and all-cause in-hospital mortality. However, the differences were modest and were not seen for the outcomes of vascular complications and the composite endpoint of any adverse event. Our findings highlight a disconnect between the skills needed to pass a standardized exam and those needed to perform a procedure safely. In the absence of a strong association between certification and outcomes, efforts to obtain and maintain board certification may not be warranted.
Title: Comparing Gain- and Loss-Framed Messages for Smoking Cessation in Lung Cancer Screening Patients

Navid Hafez MD, MPH, Benjamin Toll, PhD

Background: Prospect Theory provides the foundation for understanding the psychological processes involved in the influence of messages on smoking cessation. The framing implications of Prospect Theory suggest that decision makers organize information in memory relevant to such decisions in terms of potential gains or potential losses. With respect to health behaviors, it appears that the type of behavior being promoted influences the effects of message framing. Choosing to perform prevention behaviors is a risk-averse option – it maintains good health. Because risk-averse options are preferred when people are considering benefits, gain-framed messages may be more likely to promote prevention behaviors. In numerous studies, gain-framed messages were more effective in promoting the use of certain surgical procedures, physical exercise, sunscreen utilization, and smoking cessation, all prevention behaviors (e.g., McCall & Martin Ginis). Conversely, loss-framed messages were more effective in promoting BSE, mammography, and HIV testing, all early detection behaviors (e.g., Schneider et al.) We emphasize, in line with Prospect Theory, that it is the match between a message frame (gain or loss) and the required health behavior (prevention or detection) that especially motivates behavior change. Thus, as stated above, the primary hypothesis for this pilot project is that: Exclusively gain-framed messages are more persuasive when promoting cancer prevention behaviors like smoking cessation.

Specific Aim: It has been hypothesized that lung cancer/lung nodule screening is a teachable moment for smokers. Thus, effective tobacco cessation at this teachable moment might considerably enhance the benefits of screening. Specifically, we aim to compare the efficacy of gain vs loss framed messages in promoting cancer prevention behaviors.

Hypothesis: Several preliminary studies have shown the efficacy of adding a message framing intervention to standard smoking cessation treatments. It is our team’s hypothesis that gain-framed messages will be more effective than loss-framed messages in promoting cancer prevention behaviors, in this case tobacco cessation, in a population of lung cancer screening patients.

Methods: Six 10-minute videos (3 gain-framed and 3 loss-framed) concerning smoking cessation were created for use with standard treatments for lung cancer/lung nodule screening patients. Gain-framed videos emphasized the benefits of smoking cessation on health, while the loss-framed videos emphasized the costs of continuing to smoke on health.

At the time of our Research in Residency proposal, the plan was to ensure that messages were accepted and perceived as appropriate and credible through the use of several kinds of formative evaluations, including that of a focus group of active smokers as well as expert investigators at the Center for Nicotine and Tobacco at Yale. The plan was to collect feedback data from these presentations, modify messages based on this feedback, and use the updated messages in a pilot study of message delivery. Videos were to be shown to study participants based on eligibility criteria based on the NLST criteria for lung cancer screening as described in our RIR proposal. Eligible participants were to be randomized to either: 1) standard care (i.e., counseling sessions and NRT) + gain-framed intervention or 2) standard care + loss-framed intervention as previously described. The methods called for 5 weeks of study in which participants would receive 5 additional counseling sessions based on the standard operations of the Smoking Cessation Service. At 6 weeks tobacco-dependence would be assessed by the 3-hydroxycotinine/cotinine metabolism ratio and to collect DNA for possible future uses. Breath CO will be assessed at baseline and weekly throughout the 6-week intervention as an objective measure of tobacco abstinence.

Results: Unfortunately, this project took much longer to get underway than anticipated. No patients have been enrolled as of yet. In the interim, my advisor asked me to prepare a literature review of the studies on smoking cessation specifically related to lung cancer screening projects. Subsequently, I prepared an editorial for submission supporting the hypothesis that lung cancer screening is a teachable moment for smokers.
Polysonmographic Indices and Type 2 Diabetes Mellitus

Chase Hendrickson, M.D. and Barbara Gulanski, M.D., M.P.H.

**Background:** The link between obstructive sleep apnea (OSA) and type 2 diabetes mellitus (T2DM), while studied many times, remains poorly understood. OSA is a form of sleep-disordered breathing in which recurrent upper airway collapse during sleeping causes repeated episodes of hypoxia and micro-arousals. The increase in obesity over the past several decades has been the explanation for the increased prevalence of both OSA and T2DM. However, T2DM and impaired glucose metabolism have been shown numerous times to be associated with OSA independent of obesity. Two primary explanations exist for OSA’s contribution to the development of T2DM. The first explanation is based on recurrent arousals as the underlying pathophysiology. A study has linked mediators of insulin resistance to fragmentation of sleep, supporting the hypothesis that it is the micro-arousals seen in OSA that contribute most significantly to the development of T2DM. The second explanation is that it is the hypoxia that develops during the respiratory events that is the key to the development of T2DM.

**Specific Aim:** Identify which of the pathological processes that occur in OSA is most closely associated with T2DM, utilizing measurements made in standard overnight polysomnography.

**Hypothesis:** Hypoxia is the key pathologic process occurring in OSA, and so measures of hypoxia are more closely associated with T2DM than indicators of arousal.

**Methods:** The sample consisted of 1751 subjects referred to three different VA sleep centers for overnight polysomnography for suspected sleep-disordered breathing. Utilizing standard overnight polysomnography, a database of each subject’s Apnea Hypopnea Index (AHI), markers of arousal, and measures of hypoxia was compiled. Determination of T2DM and pre-diabetes were based on measurements of hemoglobin A1C and/or fasting glucose levels. Patient characteristics were listed as means, standard deviations, and medians for continuous variables and frequency and proportions for categorical variables. Polysomnography results were treated as continuous variables. Multivariate analysis was employed to adjust for T2DM-associated characteristics.

**Results:** The average subject age was 58. Nearly 95% of the patient population was male. Nearly 16% of the patients were classified as being a “high-risk” ethnicity. The average BMI was over 35, with over % of the patients being classified as obese. Over 72% of the patients were diagnosed with OSA, with an average AHI across the sample of 27. Over 48% of the patients were classified as pre-diabetic, and over 26% of the patients were classified as diabetic. In the unadjusted analysis, a numerical increase from “normal” to pre-diabetic to diabetic subjects was seen in age, sex, BMI, obesity, and hypertension, as would be expected with risk factors and sequelae of the metabolic syndrome. In an adjusted analysis, neither of the markers of arousal was significantly associated with T2DM. However, multiple markers of hypoxia were associated with T2DM in a statistically significant fashion. Specifically, the CT 90% (percent of cumulative study time spent with a saturation less than 90%) was most significantly associated with T2DM, with a p-value of 0.0004 and a hazard ratio of 1.181 for every 0.1 unit increase in the fraction of study time spent with a saturation less than 90%.

**Conclusions:** Hypoxia is more closely associated with T2DM than are arousals. This finding suggests that the hypoxia associated with OSA is the key pathological process contributing to T2DM in subjects with OSA. While many studies have been done linking OSA and insulin resistance/glucose intolerance as well as OSA and mediators of insulin resistance, few studies specifically examined the polysomnographic abnormalities seen in subjects with T2DM. As the study group consisted of veterans and so was predominately middle-to-older-aged males, it remains to be seen if these results can be broadly generalized. With this knowledge, future studies can be designed to both further characterize the disease-causing aspects of hypoxia from OSA in T2DM, as well as exploring impact on treatment of OSA with continuous positive airway pressure (CPAP) ventilation.
The Prevalence Of Previously Undiagnosed Diabetes and the Impact of Baseline Glycemic Control Upon Outcomes In The Medical Intensive Care Unit (MICU)

Quynh Hoang, MD; Margaret Pisani, MD, MPH; Silvio Inzucchi MD; Buqu Hu, MS; Shyoko Honiden, MD, MSc

Rationale: Hyperglycemia is common during critical illness and can adversely affect outcomes. Previous studies have, however, demonstrated a differential impact of hyperglycemia, based on prior diabetes status, with non-diabetic patients having worse outcomes than those with established diabetes. The proportion of patients admitted to the MICU with stress hyperglycemia who have previously undiagnosed diabetes is unknown. We sought to determine the prevalence of undiagnosed diabetes among patients with stress hyperglycemia and the effect of baseline glycemic control on mortality.

Methods: A prospective, observational cohort study was performed in the Yale-New Haven Hospital MICU from December 2011-April 2012. Patients were included if they had a history of diabetes or developed hyperglycemia in the MICU. Hyperglycemia was defined as two fasting glucose values of ≥140mg/dl or a random glucose of ≥200mg/dl. Hemoglobin A1c (HbA1c) levels were checked and used to determine the prevalence of newly diagnosed diabetes (defined as HbA1c≥6.5%), as well as to stratify patients according to baseline glycemic control. Patients with recent blood transfusions, hemoglobinopathies and hemolysis were excluded. The primary outcome of the secondary aim was hospital mortality. Comparisons between groups were conducted using chi-square tests, student’s t-test, or 2 proportions z-test as appropriate. The relationship between HbA1c, glycemia, and diabetic status was explored using a multivariate logistic regression.

Results: We screened 742 consecutive MICU admissions. 299 patients were enrolled. During MICU admission, 13.7% of non-diabetics had HbA1c of ≥6.5. There was a significant difference in mortality between patients with HbA1c<6.5 and those with HbA1c≥6.5 (19.3 vs. 11.7, p=0.038). There was no significant difference in demographic characteristics between these two groups. The number of previously known diabetics, glucose variability and average glucose were greater in the higher HbA1c subgroup. This group was also less likely to receive corticosteroids. Multivariate logistic regression revealed HbA1c to be an independent predictor of hospital mortality, with lower HbA1c associated with higher mortality, even after adjusting for diabetic history and many other covariates (odds ratio=2.44 [1.37-4.37]p=0.003)

Conclusion: There is a significant number of MICU patients with elevated HbA1c levels who do not have a prior diagnosis of diabetes. Hyperglycemia with lower baseline HbA1c during critical illness is associated with increased mortality, even after adjusting for multiple factors. While further study is needed to discern the pathogenesis of this phenomenon, this subgroup may represent a cohort that would benefit from tighter glycemic control during acute illness.
Adherence to Quality of Care Measures in Patients with Inflammatory Bowel Disease May not Improve Patient Outcomes

Varun Kumar, M.D., Anil Nagar, M.D.

**Background:** Inflammatory bowel disease (IBD) is a chronic disorder that affects approximately 1.4 million Americans, with an annual cost of $10-18 billion. A uniform approach to patient care, such as utilization of practice guidelines, may be beneficial in reducing morbidity and improving patient outcome.

**Objective:** The aim of this study is to determine if proposed American Gastroenterological Association (AGA) quality of care guidelines can be met at a teaching program gastroenterology clinic and to determine if adherence to guidelines results in improved patient outcome.

**Hypothesis:** We hypothesize that patients with IBD who routinely receive GI subspecialty care in the West Haven VA meet criteria for quality of care guidelines as indicated by the AGA. Adherence to these guidelines leads to improved patient outcomes as measured by need for surgery and hospitalizations.

**Methods:** This is a retrospective single center study of patients with IBD. Consecutive patients were identified using pharmacy, pathology, and clinic records. 234 patients were identified as having IBD. Of the 234 patients, 126 were followed in the gastroenterology clinic and included in the analysis; the remaining patients used the VA hospital for medication only. The electronic medical record was reviewed for: adherence to the ten quality improvement measures proposed by the AGA, hospitalizations and surgeries within 3 years.

**Results:** 126 patients with IBD were included. The AGA guidelines were met as follows: Inflammatory Bowel Disease type (99.2%), disease location (92.8%), and disease activity (96%), screening/cessation counseling in tobacco abusers (98.8%), use of corticosteroid sparing therapy (97.6%), administration of inpatient veno-thromboembolism prophylaxis (89.5%), Yearly Influenza Vaccination (83%), inpatient testing for *Clostridium difficile* (74%), hepatitis B status prior to initiation of biologic therapy (73%), assessment of bone loss related to corticosteroid therapy (69%), pneumococcus vaccine administration (65%), testing for latent TB prior to initiation of biologic therapy (65%). 91 patients (72%) met 80% of the quality care guidelines. During the study period of 3 years, 17 patients were admitted for IBD related complications, 10 for non-IBD related complications and 6 patients underwent surgery. We did not find any correlation between adherence to quality of care indications and reduction in hospitalization or surgery.

**Conclusions:** Proposed quality of care guidelines can be met in a majority of patients in clinical practice. Adherence to these guidelines does not correlate with improved patient outcomes as measured by hospitalization or surgery. Further work is required in identifying clinical care guidelines that correlate with improvement in patient outcomes.
Title Multiphoton Imaging of Fresh Tissue from the Biliary Tract

Theresa Lee, M.D., Albert Mennone, M.S., Michael H. Nathanson, M.D., Ph.D.

Background: The diagnosis of cholangiocarcinoma often requires evaluation of the bile duct via ERCP in order to differentiate malignancy from benign strictures. However, even with endoscopic imaging, it can be difficult to reliably identify malignancy due to poor sensitivity of tissue biopsy. There has been increasing interest in developing probe based microscopy systems compatible with endoscopy that can inform more targeted biopsies. These systems obtain images in vivo on a cellular level, similar to histopathology. Multiphoton microscopy is one such proposed system that has the advantage over confocal endomicroscopy in using the tissue’s own natural fluorophores and second harmonic generation (SHG) for labeling, instead of relying on fluorescence from intravenous fluorescein. There has been success using multiphoton microscopy in freshly excised mucosa of the esophagus and colon and it has been shown there to provide details about mucosal architecture similar to histopathology. Multiphoton imaging may be able to yield similarly detailed information in the biliary tract.

Specific Aim: Optimize imaging of unfixed, unstained biliary tract mucosal biopsies with the multiphoton microscope.

Hypothesis: Multiphoton imaging of fresh, unfixed mucosa of the biliary tract can reliably provide high quality images similar to histopathology.

Methods: Preliminary studies were obtained using benign biliary mucosa from a healthy rat. The common bile duct was surgically dissected, placed in phosphate buffered saline, then immediately imaged with the Zeiss LSM 710 multiphoton microscope. Two predetermined sets of wavelength filters were chosen and compared, based on expected autofluorescence of intrinsic subcellular molecules. Multiphoton images were then acquired using excitation wavelengths of 710nm, 740nm, 790nm, and 840nm. Given an interest in potential confounding from pre-injected fluorescein, 0.3mg of fluorescein was injected into the IVC prior to dissection and images were obtained within 10 minutes of injection. The resulting images were reviewed for qualitative evaluation of resolution quality.

Results: Of the tested combinations of applied wavelengths and filter sets, an excitation wavelength of 790nm provided the best spatial resolution and separation of autofluorescence, fluorescein, and SHG signals using the emission filter set: <360nm, 360-410nm, 420-500nm, 500-550nm. By using this combination, we were able to avoid any significant interference with fluorescein as it typically fluoresces in the 500nm range, higher than the expected autofluorescence of the subcellular structures of interest.

Conclusions: Acquiring multiphoton images using an excitation wavelength of 790nm and using emission channels of <360nm, 360-410nm, 420-500nm, and 500-520nm permits high resolution imaging of SHG and autofluorescence structures in freshly excised biliary mucosa in rats, even in the presence of fluorescein. With this strategy, autofluorescence of intrinsic molecules like NADH and flavins, and SHG imaging of collagen provides details about subcellular architecture. Given the limited population of patients available for study and that they are likely to be co-enrolled in both confocal and multiphoton studies at our institution, we have designed an imaging strategy that will not be confounded by fluorescein. The findings suggest that we can proceed to use the strategy designed here, with good resolution, for obtaining images of freshly biopsied human biliary mucosa obtained during ERCP in the future.
Title: Reasons for Readmission in a High-Risk Population

Authors: Theodore Long, MD; Inginia Genao, MD; Leora I. Horwitz, MD, MHS

Background: Hospital readmissions are a substantial healthcare cost and a disproportionate burden for the underserved, but there is a paucity of qualitative data describing what happens to patients after they are discharged from the hospital.

Objective: To gather qualitative data to elucidate the reasons for readmissions in a high-risk population of underserved patients.

Design: We created an instrument with 27 open-ended questions based on current interventions.

Setting: Yale-New Haven Hospital.

Patients: Patients at the Yale Adult Primary Care Center (PCC).

Measurements: We conducted semi-structured qualitative interviews of patients who had four or more admissions in the previous six months and were currently readmitted to the hospital.

Results: We completed 17 interviews and identified themes relating to risk of readmission. We found that patients went directly to the Emergency Department (ED) when they experienced a change in health status without contacting their primary provider. Reasons for this included poor telephone or urgent care access and the belief that the PCC could not treat acute illness. Many patients could not name their primary provider. Conversely, every patient except one reported being able to obtain medications without undue financial burden, and every patient reported receiving adequate home care services.

Conclusions: These high-risk patients were receiving the formal services that they needed, but were making the decision to go to the ED because of inadequate access to care and fragmented primary care relationships. Formal transitional care services are unlikely to be adequate in reducing readmissions without also addressing primary care access and continuity.
Title: Predictors of outcomes in inpatient admissions for patients with decompensated cirrhosis requiring ICU transfer: retrospective analysis
Jennifer Y. Pan, M.D. and Michael L. Schilsky, M.D.

Background: Management of patients with decompensated cirrhosis presents a unique challenge given the complexity of medical issues that arise, especially in the inpatient setting, as these patients often require higher levels of care. Reasons for requiring ICU transfer after admission to a medical service vary and are often not due directly to liver failure, but rather from infectious complications or failure of other organ systems related to the underlying liver disease.

Aims/Hypothesis: We performed a retrospective study of patients transferred from an inpatient liver service to the ICU to determine if current scoring models can be used to predict ICU outcome. Our hypothesis was that differences in current indices for predicting mortality in patients with liver disease (MELD) and patients entering the ICU (SIRS, SOFA, APACHE-II, and SAPS-II) can predict ICU stay outcome prior to transfer, with (a) primary outcome of ICU survival and (b) secondary outcome of time period and predictability of transfer.

Methods: Study Design and Patient Population - Our study group consisted of patients admitted to the inpatient (Klatskin) liver service who were transferred to either the SDU or MICU, for a 6-month period from January 1 - June 30, 2011. Each admission and transfer was considered an individual event. In total, 104 patients had 121 individual admissions. Of these, 24/104 patients were transferred to the ICU/SDU, for a total of 37 transfers. Consecutive patients admitted to the service from Jan 1 – Feb 28, 2011, but who did not require transfer of care, were used as the control population in comparing admission data. ICU stay outcomes were distributed as: back to floor (70.3%), death (27.0%), discharge from ICU (2.7%), and transplant (2.7%). Statistical Analysis - For primary outcome analysis, transplant was included in the death [D] group. The ICU survival [S] group also included a small subcategory (N=6) of patients who were discharged to either hospice or died on the floor after transfer out of the ICU. Scores were calculated at admission, at transfer, and the highest score for each 24 hour period (between 48h prior to transfer to 72h after transfer) was recorded. Student’s t-test was used for analysis of primary outcomes and for comparison of study vs control populations.

Results: Differences at admission were noted between the control and study populations with several of the indices: MELD (Δmean 10.31, p=0.0033), SOFA (Δmean 2.82, p=0.0026), APACHE-II (Δmean 3.74, p=0.0026), and SAPS-II (Δmean 9.92, p=0.0001), but not between the [S] and [D] groups. SOFA [S] vs SOFA [D] was found to be significant in all time-periods, from 48h pre-transfer (Δmean 2.33, p=0.0304, Sn 78%, Sp 67%) to 72h post-transfer (Δmean 3.78, p=0.0414, Sn 75%, Sp 32%). Differences in MELD [S] vs MELD [D] were noted as early as 48h pre-transfer (Δmean 13.05, p=0.0005, Sn 100%, Sp 57%) but were no longer statistically significant by 48 post-transfer (Δmean 5.55, p=0.1617). Conversely, differences in SAPS-II [S] vs SAPS-II [D] were noted as early as 24h pre-transfer (Δmean 7.55, p=0.0106, Sn 56%, Sp 84%) up to 72h post-transfer (Δmean 21.73, p=0.0004, Sn 88%, Sp 55%). A composite score of MELD+SAPS-II (modified acute physiology score for liver [MAPS-L]) showed most statistical significance throughout the study period from 48h pre- (Δmean 18.79, p=0.0010, Sn 78%, Sp 81%) to 72h post- (Δmean 30.30, p=0.0008, Sn 63%, Sp 60%) transfer. While APACHE-II score had no significant difference in means, high specificity (95%) was noted at 48h prior to transfer. No scores were statistically significant at the point of transfer.

Conclusions: Of the different indices evaluated, MELD, SAPS-II, SOFA have the highest predictability of both the need for ICU transfer, as well as overall ICU survival. While present organ system model scores can be used to predict the need for transfer, our MAPS-L score gives even higher predictive value for ICU survival than current indices alone. These results will need to be further evaluated in a prospective manner.
Title: Localization of angioectasias in the small bowel using capsule endoscopy

Resident's Name: Eileen Plotkin, MD

Mentor's Name: Avlin Imaeda, MD, PhD

Background, aims, hypothesis: Obscure gastrointestinal bleeds cause significant morbidity and mortality. Debate exists as to the best endoscopic approach to be used in patients with overt obscure bleeding. We hypothesized that in patients older than 50 most overt obscure bleeding can be managed with push enteroscopy rather than deep enteroscopy due to non-random localization of angioectasias in the small bowel (SB). The study aim was to evaluate the localization of angioectasias in the SB using capsule endoscopy.

Methods: We conducted a retrospective review of all capsule endoscopy studies done in the West Haven, CT Veterans Hospital. The database contained 428 inpatient and outpatient studies from 2005-2012. All studies were included. Angioectasias were localized by both lead mapping and SB transit time. The duodenal sweep, horizontal duodenum and region of the ligament of Treitz (LOT) in the left upper quadrant can typically be identified on the lead map. Beyond this there are no identifying landmarks until the capsule reaches the cecum. Lesions in the sweep or horizontal area were identified as duodenal. Lesions located in the left upper quadrant (LUQ) just after the duodenal passage labelled LOT. Lesions after movement from the LUQ to 50% transit were labelled jejunum. Beyond 50% were labelled ileum. Statistical analyses were performed using the chi-square test of equality of proportions.

Results: A total of 428 studies were identified in the capsule database. 72 studies on 70 patients identified SB angioectasia. 64% of patients had a single angioectasia, 36% of patients had multiple. Lead mapping showed a significant difference in the location of angioectasias (26.77% duodenum, 40.16% LOT, 23.62% jejunum and 9.45% ileum; p < 0.0001). The proportion of angioectasias in the duodenum versus the LOT was not significantly different (40% vs 60%; p=0.065), however the percentage of angioectasias in the LOT was significantly greater than the proportion in the jejunum (62.96% vs 37.04%; p=0.0196) and the ileum (80.95% vs 19.05%; p<0.0001). Analysis using percent of small bowel transit time also showed a significant difference in the location of angioectasias (78.3% were in 0-25% of the SB transit; 11.32% in 26-50%; 4.72% in 51-75% and 5.66% in 76-100%; p<0.0001). Comparing those located in 0-25% to the remainder of the SB was also significant (78.3% vs 21.7%; p<0.0001).

Conclusions: The majority of angioectasias are located in the proximal SB, before or near the LOT or within the first 25% of the SB. These angioectasias are likely within reach of push enteroscopy. Therefore, obscure overt GI bleeds in most patients with angioectasias in whom endoscopy is the chosen therapy can be managed with push enteroscopy. This is important since many patients require multiple endoscopies to identify symptomatic lesions and deep enteroscopy is not widely available.
Model to Predict Death or Re-infarction for Patients Suffering from STEMI who Undergo Stent Implantation

Resident: Charles Rouse, MD. Mentor: Alexandra Lansky, MD

Background: Adoption of newer therapies for the treatment of myocardial infarction has lead to changes in the expected long-term mortality for patients who present with such events. Treatment of ST-segment myocardial infarction (STEMI) has undergone significant changes since the 1980s, when the ISIS-2 trial demonstrated improved survival in patients who undergo treatment with aspirin and thrombolytics1. The introduction of percutaneous coronary intervention (PCI) further reduced early and late mortality resulting from acute myocardial infarction2. With these developments, several risk models based on demographic, electrocardiographic, and angiographic data have been developed to aid clinicians in prognosticating mortality for patients who present with STEMI 3 4 5 6. The introduction of stents as an adjuvant to primary angioplasty has been proven to improve survival in patients with STEMI and has become standard of care in patients for whom primary PCI is appropriate 2 7. Even with these measures, there continues to be variability in outcomes for patients who undergo these procedures. A risk model to predict adverse outcomes would have important implications for patient care, healthcare resource utilization, and clinical research.

Specific Aim: Given the lack of guidance on prognosis for patients who present with STEMI and undergo stent therapy, this study aims to develop a risk model that will predict death or reinfarction (primary end point) for these patients.

Hypothesis: Baseline characteristic, angiographic data, and hospitalization associated variables will reliably predict rates of mortality or reinfarction for patients who present with STEMI who undergo target vessel revascularization with stents.

Methods: The model for this sub-study is derived from the Harmonizing Outcomes with Revascularization and Stents in Acute Myocardial Infarction (HORIZONS-AMI) trial. This was a prospective, open label, randomized, multicenter trial that compared bivalirudin alone with heparin plus a glycoprotein IIb/IIIa inhibitor in 3,602 patients with ST-segment elevation myocardial infarction who were undergoing primary PCI8. Initial candidate variables were selected based on statistical significance for the primary endpoint at three years during the univariable analysis. These variables were then subject to a bootstrap analysis for consideration into the final multivariable model. Variables that appeared in at least 15% of the bootstrap models were then subjected to a multiple logistic regression. Independent variables were evaluated using stepwise logistic regression with entry/stay criteria of 0.1/0.05. This yielded an odds ratio for death and reinfarction for each variable.

Results: After performing the multivariable analysis nine variables were found to be significant predictors of three year mortality or reinfarction: insulin dependent diabetes (OR 1.75 95%CI[1.04,2.96] p = 0.04), history of prior MI/PCI (OR 2.39 95%CI[1.71,3.32] p<0.0001), history of PVD (OR 2.09 95%CI[1.21,3.61] p = 0.01), site reported baseline LVEF < 40% (OR 2.11 95%CI[1.52,2.92] p<0.0001), baseline hematocrit < 30% (OR 2.22 95%CI[1.47,3.34] p=0.0001), any post procedure platelet count drop from baseline >25% (OR 1.62 95%CI[1.22,2.19] p=0.0009), post procedure peak creatinine (per 1 mg/dl increase) (OR 1.50 95%CI[1.29,1.75] p<0.0001), baseline number of plaques >50%DS (OR 1.10 95%CI[1.02,1.18] p=0.01), and number of stents implanted (OR 1.30 95%CI[1.12,1.51] p = 0.0005).

Conclusions: The purpose of this study was to develop a risk model that predicts death or reinfarction in patients suffering from acute STEMI who undergo stent based target vessel revascularization. Using data from the HORIZONS-AMI trial, the largest trial available that evaluated STEMI patient undergoing stent implantation, nine variables were identified that reliably predict this adverse event. This prognostic information should have important implications for patient care, medical resource utilization, and clinical research.
The Role of Cigarette Smoke Exposure in T Helper Cell Type 1 (Th1) Cytokines, Interleukin-15 and Chitinase-like Proteins During Respiratory Infections in the Pathogenesis of COPD

Sudipa Sarkar, MD, Wei Liu, MD, Anil Ghimire, MD, Nathaniel Andrews, Jonathan Siner, MD, Jack Elias, MD, Charles Dela Cruz, MD, PhD

Background: Cigarette smoking (CS) is a risk factor for COPD. A minority of smokers are diagnosed with COPD suggesting that factors such as respiratory viral infections affect the pathogenesis of COPD. However, how CS interacts with the immune system to cause an increased inflammatory reaction has not been delineated until recently. In mouse models, our laboratory implicated T helper 1 (Th1) cytokines and interleukin-15 (IL-15) in COPD pathogenesis. Chitinase-like proteins like YKL-40 were also shown to be important in COPD, but their role in the setting of respiratory virus infection and CS exposure is not known.

Aim: To measure circulating serum levels of Th1 cytokines (IFN-γ, IL-12), T helper 2 (Th2) cytokines (IL-5, IL-4, IL-13), IL-15, YKL-40, and chitinase activity in patients with a current respiratory virus infection and to compare these levels among never smokers (NS) and ever smokers (ES), which includes both former smokers (FS) and current smokers (CS).

Hypothesis: CS exposure results in an enhanced Th1 response and increased IL-15 cytokine levels, YKL-40 levels and chitinase activity in patients infected with respiratory viruses.

Methods: Participants were enrolled from patients admitted to Yale New-Haven Hospital. Virus infection was indicated by a positive direct immunofluorescence assay or PCR on a nasopharyngeal sample. Serum was obtained after informed consent and smoking history was ascertained by questionnaire and chart review. Cytokines were measured by a fluorescence bead based cytokine multiplex system, and IL-15 and YKL-40 were measured by ELISA. Chitinase activity was determined using a fluorogenic substrate. Significance of the associations between smoking status and cytokines, proteins, and chitinase activity were analyzed using SAS statistical program.

Results: 222 patients infected with respiratory viruses who were admitted to the hospital were included in our study. 145 patients (65.3%) were classified as ES and 77 (34.7%) as NS. There were no significant differences between ES and NS in age, sex, or race. 27.4% of the patients were infected with influenza virus, 23.9% with respiratory syncytial virus (RSV), and 29.27% with rhinovirus. The rest of the patients were infected with human metapneumovirus, parainfluenza virus, and adenovirus. Among all patients, there were significantly higher levels of IFN-γ (p<0.0001), IL-5 (p<0.004), and TNF-α (p=0.0022) among ES than NS. There were no significant differences between the levels of YKL-40 and chitinase activity between ES and NS. Among patients infected with influenza virus, there were significantly higher levels of IL-2 (p=0.03) and IL-15 (p=0.02) among ES than NS. CS had higher levels of IFN-γ (p=0.0205) and IL-15 (p=0.0321) compared with NS who were infected with influenza virus. Among patients infected with RSV virus, there were significantly higher levels of IFN-γ (p=0.04), IL-5 (p=0.02), IL-15 (p=0.03), TNF-α (p=0.03), and IL-12 (p=0.02) among ES than NS. Among patients infected with rhinovirus, there were significantly higher levels of IFN-γ (p=0.03) and TNF-α (p=0.009) among ES than NS. Interestingly, when we compared infected patients with no known lung disease compared to COPD, we found that IL-15 level was significantly elevated in the COPD group.

Conclusions: The different major respiratory viruses are associated with unique cytokine profiles in ES and NS. Interestingly, there appears to be a predominant IL-15 and Th1 response pattern in ES infected with respiratory viruses. Our study shows an association between CS exposure, respiratory virus infection, especially influenza virus and RSV, and increased levels of IL-15 and Th1 cytokine levels. IL-15 was significantly elevated in patients with COPD who were infected with respiratory viruses. This suggests an important link between CS exposure and these cytokines during viral infection, potentially implicating a role for IL-15 and Th1 cytokines in the pathogenesis of COPD.
Diagnosing Cirrhosis among Patients with Chronic Hepatitis C

Wagahta Semere, M.D. and Guadalupe Garcia-Tsao, M.D.

Background: Hepatitis C virus is the leading cause of chronic liver disease and with approximately 3% of the world’s population infected presents a significant global health burden. In general, 20% of HCV carriers will go on to develop cirrhosis with an estimated 5-7% related mortality. It is well known that the absence of systemic screening is independently associated with poorer outcomes among HCV infected patients. Hepatitis C patients who develop cirrhosis but in whom the diagnosis is not correctly made will not be screened for typical complications. This group of HCV patients is at risk for presenting with late stage untreatable HCC or life threatening variceal hemorrhage.

Specific Aims: To identify West Haven Veterans Hospital patients with chronic hepatitis C who have a platelet count less than 100, data strongly suggestive of cirrhosis. To determine whether these patients have an ICD-9 diagnosis for cirrhosis in the CPRS Veterans Hospital electronic medical record system. Secondarily, we identify factors including primary care versus specialist involvement and patient compliance that may lead to increased or decreased rates of appropriate cirrhosis diagnosis.

Hypothesis: Based on preliminary data using CCR we hypothesize that roughly half of patients with chronic Hepatitis C that meet criteria for cirrhosis are not correctly identified in the medical record as having a diagnosis of cirrhosis.

Methods: Using the Clinical Case Registry at the West Haven Veterans Hospital Tertiary Care Center we designed a cross-sectional study to identify all patients who are anti-HCV positive and HCV PCR positive as documented from 2002-2008. We identified patients with platelet count <100K and therefore with strong suspicion for cirrhosis. Next we determined whether these patients had an ICD 9 chart diagnosis of cirrhosis in CPRS. For the patients in whom a diagnosis of cirrhosis was not made we investigated the medical chart for explanations that suggest a diagnosis was missed or correctly omitted.

Results: During the study period 3,629 patients were confirmed as having Hepatitis C and included in CCR database. 605 patients were identified as having a platelet count less than 100 at some point during the study period. Of this group, 384 had an ICD 9 Cirrhosis Code and the remaining 221 did not. Among 100 analyzed in this cohort, 79 had an alternate etiology for their thrombocytopenia; 10 had a chart mention of cirrhosis though no specific ICD 9 Code; 11 did not have a clear alternate explanation for their thrombocytopenia and no mention in the chart notes or coding of cirrhosis. 3 of these patients had concern for complications of cirrhosis; 5 had some contact with a liver specialist; 5 had inconsistent contact with a primary provider.

Conclusion: We identified that 21% of patients with Hepatitis C and significant thrombocytopenia did not have an ICD-9 diagnosis for cirrhosis and 11% had no chart mention of cirrhosis. Further, 3 of these patients had concern for complications of cirrhosis. Future studies are needed to illicit provider perceptions of diagnosing cirrhosis in the setting of significant thrombocytopenia. Also important is an understanding of the support structure for patients with new Hepatitis C diagnosis who often struggle with substance abuse and general adherence.
The Association of Opioid Use and Sleep Disordered Breathing
Husham Sharifi, Mentor: Klar Yaggi,

Background: Opioids do not appear to suppress respiration significantly during wakefulness, and limited data exist regarding the impact of opioids during sleep. Sleep apnea is associated with all-cause mortality, as is opioid use. In a well defined cohort of patients referred for the evaluation of sleep disordered breathing, we examine the association of opioid use, sleep disordered breathing and all-cause mortality, including a dose response analysis.

Specific Aims and Hypotheses: Among patients with sleep-disordered breathing, we examined the independent association between opioid use and the risk for mortality after adjustment for medical comorbidity. We hypothesized that opioid use is associated with measures of sleep-disordered breathing and all-cause mortality and that this association exhibited a dose-response relationship.

Methods: A retrospective analysis of 3,300 patients was performed on patients referred for overnight polysomnography at three Veterans Hospital Administration sleep centers from 01/01/00 to 12/31/04. Eligible patients included those referred for suspected sleep-disordered breathing, with least one history and physical documented in the chart, and who underwent at least two hours of full polysomnography. Patients were excluded if they were referred for reasons other than the evaluation of sleep-disordered breathing, had the study performed with uncovered tracheostomy, or were using airway pressure of any form during the study. A final analytic cohort of 2,200 was obtained and refined to 344 patients with sleep apnea who are also on opioid medications for any amount of time. Independent variables include the index variable of morphine dose equivalent, calculated per consensus conversions produced in the literature, and all risk adjustment variables.

Results: Opioid use did not increase sleep disordered breathing in the opioid group versus the control group as measured by AHI (22.2 versus 26.5, p value 0.08.) It did show correlation with some changes in sleep architecture, including increases in sleep latency (23.3 vs 16.3 minutes, p value 0.015) and more upstage shifts from deep sleep to light sleep (22.6 vs 20.0, p value 0.0396.) Notably, there is an increase in mortality when opioid use is analyzed as an unadjusted variable (OR 1.52, p value 0.0192,) but this effect is attenuated with adjustment by cardiovascular and cerebrovascular risk factors as represented by the Charlson comorbidity index. There is no dose-response relationship between opioid use and mortality when assessed in morphine equivalents. As seen in previous studies, worsened sleep-disordered breathing is associated with higher rates of mortality.

Conclusions: We hypothesized that opioid use would increase sleep disordered breathing but unexpectedly found that it did not. There is an observed association between opioid use and mortality, and much of this association is explained by coexisting medical comorbidity. There is no association between opioid dose and mortality, as assessed by a dose-response analysis.
The True Chitinase Chitotriosidase is an Essential Mediator of Cell Death in Bacterial Pneumonia through Regulation of the Inflammasome Complex

Avraham Sofer MD, Wei Liu MD, Jack Elias MD, and Charles S. Dela Cruz, MD, PhD

Background: The 18 glycosyl hydrolase family of chitinases is an ancient gene family expressed by a variety of organisms. True chitinases such as chitotriosidase (chitinase 1, Chit1) are expressed in the lung and other organs. Elevated levels of Chit1 are seen in a variety of infections, yet the mechanism of Chit1 action remains unclear. Previous data from our laboratory have shown that mice deficient in Chit1 have increased airway inflammation and cell death in lung tissue and points to pyroptosis as a potential mechanism for this robust inflammatory response. During pyroptosis, activated caspase-1 promotes the proteolytic maturation and secretion of pro-inflammatory cytokines which in turn lead to membrane breakdown and exposure of intracellular host and pathogen moieties to extracellular mediators of immunity. Caspase-1 activation is ultimately controlled by tightly-regulated intracellular multi-protein complexes known as inflammasomes.

Specific aim: To elucidate the role of Chit1 in mediating inflammatory and cell death pathways in macrophages infected with the bacterial pathogen *Pseudomonas aeruginosa*.

Hypothesis: Chit1 is an important mediator of cell death in response to bacterial infection through regulation of the inflammasome complex as well as several caspases involved in cell death.

Methods: Wild-type (WT) and Chit1-null mice were injected intraperitoneally with 2.5mL of 3% thioglycollate. After five days mice were euthanized and ascitic macrophages were collected and plated. Macrophages were then infected with 25 uL of a 5 x 10^5 fresh stock of vector or *P. aeruginosa* (PA), generously provided by B. Kamierczak from Yale. Protein lysis buffer with protease inhibitors was used for collection of cell lysates, with protein concentrations determined by Bradford Assay. 25-40 μg of lysate were run on 4-12% Tris-glycine gels in SDS-PAGE reducing buffer. Western blotting, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining and lactate dehydrogenase (LDH) cytotoxicity assays were performed according to standard protocols.

Results: Cell death as measured by TUNEL staining was two-fold higher in uninfected Chit1-deficient macrophages compared to WT cells. We also observed increased cytotoxicity, as measured by LDH release, as well as increased apoptosis after 16 hours in Chit1-deficient cells infected with PA. Chit1-null macrophages had increased basal levels of the inflammasome adaptor molecule apoptosis-associated speck-like protein containing a carboxy-terminal CARD (ASC). We also observed significantly higher basal levels of caspase-3, 6, 8, 9 and 11 in Chit1-deficient cells in addition the pro-apoptotic protein Bcl2-associated X-protein (Bax). Infection with PA led to increased cleavage of caspase-1, 3, 8, 9 and 11 in Chit1-null as compared to WT cells.

Conclusions: We demonstrate that Chit1-deficient cells have increased basal levels of the inflammasome molecule ASC and several key caspases, suggesting that Chit1 is a key regulator of inflammatory and cell death pathways in macrophages. This has important functional consequences, since cells deficient in Chit1 have increased cytotoxicity and apoptosis both at baseline and with infection. Our data therefore suggest that Chit1 may not only provide an anti-apoptotic signal in uninfected cells, but may also be involved in limiting the excessive tissue inflammation and cell death seen in bacterial infections. These data have important clinical applications, as Chit1 levels may correlate with the degree of tissue inflammation and injury seen in bacterial infections. Further studies are needed to elucidate the precise mechanism by which Chit1 regulates the cellular response to bacterial infection.
Title: Clinical and Histological Predictors of Progression of Fibrosis in Patients with Hepatitis C

Authors: Brandon Sprung, Antonio Galvao Neto, Xuchen Zhang, Tamar Taddei

Background: Hepatitis C infection is a leading cause of chronic liver disease worldwide and results in significant morbidity and mortality related to associated complications such as cirrhosis and hepatocellular carcinoma. Much of disease progression from the development of chronic viral infection through the end stages of disease relate to underlying changes in liver pathology, including the development of progressive hepatic fibrosis and inflammatory changes. It has been demonstrated that worse clinical outcomes are associated with more advanced hepatic fibrosis. The ability to reliably identify clinical and histological factors associated with progression of fibrosis and therefore significant clinical outcomes has major implications on prognosis and treatment. Previous studies have such focused on determination of numerous clinical and pathologic factors associated with underlying fibrosis and subsequent disease progression, including advanced age, duration of infection, alcohol consumption, low albumin and platelets, elevated transaminases and high BMI. However, the predictive sensitivity and specificity of these clinical factors are highly variable across such studies and are highly dependent upon patient population, the type of study used, and the methods used for quantifying fibrosis. The population at the Veterans Affairs Medical Centers represents a unique group of patients with hepatitis C in which the prevalence of hepatitis C infection is greater than that of the general population and there is a high prevalence of co-morbidities.

Specific Aim: We aim to identify clinical and histological predictors of progression of hepatic fibrosis in a Veteran Administration population with hepatitis C who have had two separate liver biopsies.

Hypothesis: Patients who have earlier stage fibrosis and less inflammation on the first biopsy, and those with lean body mass and fewer co-morbidities (e.g. diabetes, hypertension, and hyperlipidemia) will have less progression of fibrosis on their subsequent biopsy.

Methods: This study retrospectively analyzed the clinical charts of 44 Veteran Administration patients with known active HCV infection using data available on the Computerized Patient Record System (CPRS) at the VA Connecticut Healthcare System. This study was approved by the Institutional Review Board. Both demographic and clinical data were collected as previously obtained for routine clinical care. All patients had at least two interval liver biopsies, and those with HIV infection or other forms of chronic liver disease were excluded. Stage of hepatic fibrosis as well as histological markers of injury and inflammation on liver biopsy specimens (e.g. steatosis, inflammation, iron, acidophil bodies, and Mallory bodies) were interpreted by the study pathologists according to the Metavir scoring system. Those subjects who had an increase in fibrosis by at least one stage were defined as progressors.

Results: A total of 42 subjects were analyzed utilizing univariate analytical methods. One subject did not have sufficient follow-up and one did not have sufficient tissue. There were at total of 26 progressors (62%), with 19 progressing by one stage, 6 by two stages, and 1 by three stages. Of the progressors, 4 progressed to cirrhosis. There were no significant differences in initial age at first biopsy, initial fibrosis stage, BMI, presence of medical co-morbidities, antiviral treatment status, or time between biopsies among progressors vs. non-progressors (62.7 vs. 73.7 months, p=0.73). No differences were observed in surrogate markers of synthetic dysfunction or portal hypertension. Progressors had significantly higher initial AST (mean 39 vs. 73, p=0.01) and a higher change in INR between biopsies (p=0.01) compared with non-progressors. Progressors had significantly higher ferritin levels (mean 232 vs. 503, p=0.04), but had significantly less stainable iron on biopsy (p = 0.02) than non-progressors. Among subjects with stainable iron, there were no significant differences in the hepatic distribution of iron (hepatocytes vs. Kupffer cells) between the groups. There were no other significant differences for the other histological variables.

Conclusions: High AST and ferritin at the time of first liver biopsy significantly predict progression of hepatic fibrosis at the time of subsequent liver biopsy in Veteran Administration patients with HCV. In this cohort, medical co-morbidities were not predictive of fibrosis progression, nor were the presence of steatosis or inflammation on initial biopsy.
Changes in Inflammation-associated Cytokines in Myeloma Patients treated with Pomalidomide

Jamie Stratton, M.D. & Madhav Dhodapkar, M.D.

Background: Multiple myeloma (MM) is a hematopoietic malignancy involving growth of transformed plasma cells. The common pathologic features required for diagnosis include: a bone marrow plasmacytosis (>10% plasma cells) and an M protein spike in serum or urine. In the bone marrow, cytokines are one of the many different factors that play a role in progression of multiple myeloma. Cytokines are signaling proteins that are involved in inflammatory responses and the growth, development and activation of the immune system. Researchers have been trying to determine which factors play a role in the development and/or progression of multiple myeloma in hopes of deriving focused therapies against these signaling proteins. Immunomodulatory drugs (IMIDs) have been shown to influence cytokines that are present in the myeloma micro-environment and that are thought to play a role in cancer progression. Pomalidomide (Pom), a thalidomide analog, has been shown to have multiple effects on the bone marrow milieu.

Specific Aims: 1. To measure the level of a panel of inflammation-associated cytokines in patients with refractory MM enrolled in clinical trial evaluating pomalidomide. 2. To compare cytokine expression patterns in patients with MM vs. healthy donor controls and pre/post treatment with Pom.

Hypothesis: Changes in cytokines before/after therapy in the sera of MM patients undergoing therapy with pomalidomide may provide insights into biologic effects of this therapy in vivo.

Methods: Luminex multiplex arrays were performed on subject samples from patients enrolled in a clinical trial of pomalidomide in relapsed/refractory myeloma. Serum from 15 subjects was assessed at two time points, pre and post treatment (day 0 and day 7). In addition, 4 healthy donor controls were also examined. Expression of 20 different cytokines was assessed; IL-1B, IL-2, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17A, IL-17E, IL-17F, IL-21, IL-22, IL-23, IL-27, IL-28A, IL-31, IL-33, INFγ, TNFα and MIP3A. Concentrations used for analysis were equal to or greater than the minimal detectable concentration (MDC) of the Luminex assay. A chart review was performed on the 15 subjects enrolled in the clinical trial to determine response to treatment.

Results: Of the panel tested, MM subjects had increased levels of TNFα (p = .007) and decreased IL-6 (p = .002) when compared with controls. When evaluating MM patients pre vs. post treatment there was a decrease in IL-12 and IL-21 (p = .09 and p = .08). Of the 14 MM subjects, 7 were found to be pomalidomide responders (defined as a decline in M protein or serum FLCs for ≥ 120days) and 7 were pomalidomide non-responders. Responders had a significant decrease in IL-21 after treatment (p = .02). Non-responders saw an increase in IL-10 after treatment (p = .08).

Conclusions: The finding of increased levels of TNFα in MM plasma is consistent with prior studies and its putative role in promoting MM growth. The finding that MM cohort had low mean IL6 levels is somewhat surprising but appeared to be driven by only 2 outliers. A preliminary result that needs further follow-up relates to reduction in IL21 following therapy with pomalidomide (which approached statistical significance) and its potential correlation with response to therapy. IL-21 plays an important role in regulating plasma cell differentiation and may serve as a growth factor in myeloma. These data therefore support further studies to ascertain the effect of Pom on IL21, the mechanism underlying Pom-mediated inhibition of IL21 and its possible contribution to the anti-MM effects of pomalidomide.
Use of Remote Monitoring of Implantable Cardiac Defibrillators: insights from the Patient Related Determinants of ICD Remote Monitoring (PREDICT RM) study

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**Background:** Implantable cardiac defibrillator (ICD) therapy has been shown to improve survival of patients with reduced ejection fraction. Despite efficacy of these devices, they require close monitoring to ensure proper functioning. Current guidelines recommend that ICDs be evaluated every three to six months. Remote patient monitoring (RPM) has been proposed to reduce the burden of out-patient follow-up. Recent data has shown RPM to be non-inferior to outpatient office visits and to decrease the rate of ICD-related hospitalizations. Consequently, the Heart Rhythm Society and European Heart Rhythm Association have advocated for the routine use of RPM. Despite universal availability and support from professional societies, use of RPM has neither become universal nor routine. Use of RPM depends on two essential steps: 1) Enrollment of the patient into a RPM system, and 2) Activation of RPM by the enrolled patient.

**Specific Aim:** This project seeks to identify patient-related, physician-related, and institution-related determinants for utilization of RPM.

**Hypothesis:** Patient-related socio-economic status and co-morbidities would be the primary determinant of the use of RPM.

**Methods:** This study made use of the National Cardiovascular Data Registry – ICD registry and the Boston Scientific Corporation ALTIITUD database. 39,158 patients met the inclusion criteria. Patients were categorized into three groups: 1) patients with remote monitoring-capable devices not enrolled in RPM; 2) patients who were enrolled in RPM but who had not activated RPM; 3) patients enrolled in RPM and who had activated RPM. The primary outcome was activation within 180 days of device implantation. Patient, physician, hospital, and regional characteristics were compared between the three groups. The chi-square test and t-test were used for categorical variables and continuous variables respectively. Pre-specified covariates were applied to multivariate logistic regression analyses to find determinants for RPM enrollment and then RPM activation.

**Results:** 39,158 patients met inclusion criteria for this study. 15,045 (38%) were not enrolled in RPM. 24,113 (62%) were enrolled in RPM. Of those enrolled, 18,289 (76%) activated their device. Significant determinants for **enrolling** in RPM included implantation-site (OR 3.43), Electrophysiology board-certified physicians (OR 2.32), physicians who used Boston-Scientific devices (OR 1.54), black race (OR 0.69), Hispanic race (OR 0.63), patients receiving public aid (0.72), and patient location -- those who live in mid and south Atlantic states (OR 0.51), pacific states (OR 0.55), or rural locations (OR 0.77). Patients receiving cardiac resynchronization devices were more likely to be enrolled in RPM than those receiving single chamber ICDs (OR 1.34). Co-morbidities such as lung disease (OR 0.84), chronic kidney disease (OR 0.72), and atrial flutter were also associated with lower enrollment (OR 0.90). Significant determinants for **activation** also included implantation-site (OR 1.69), patients with relatively preserved ejection fraction (OR 1.27), diabetes (OR 0.834), chronic kidney disease (OR 0.79), and hemodialysis (OR 0.69). Other factors included patients living in pacific states (OR 0.63), and patients living greater than 100 miles from the implanting facility (OR 1.40).

**Conclusions:** Despite universal applicability, use of RPM remains low -- mainly due to lack of enrollment (38% of patients). Despite socio-economic factors and patient co-morbidities being significant determinants for both enrollment and activation of RPM, implantation-site is the dominant determinant of RPM use.