IGG4 ASSOCIATED AUTOIMMUNE HEPATITIS: A NEW CLINICAL ENTITY?
Jeffrey Adler MD, James Boyer MD

Background: Liver infiltrating IgG4+ plasma cells have been described in various settings, however little is known about this manifestation in autoimmune hepatitis (AIH). IgG4+ plasma cells (≥5/high powered field) were described in 34% of Japanese patients with AIH and were associated with a more durable response to prednisolone. New consensus criteria have been published for IgG4+ related disease that focus on the combined presence of typical histopathological findings and increased numbers of IgG4 plasma cells, specifically ≥10 IgG4 plasma cells/HPF for liver biopsy specimens. It remains unclear (1) if this is a distinct clinical AIH subset, (2) if it exists in Western patient populations, and (3) how many patients satisfy the new consensus criteria for IgG4 related disease. Specific Aim: To analyze IgG4 associated AIH in a US cohort and determine if this group has increased responsiveness to immunosuppression.

Hypothesis: IgG4 associated AIH has a unique clinical course compared to classic AIH.

Methods: Retrospective study of stored biopsy tissue and clinical records from Yale Liver Clinic patients with AIH, AIH-PSC, and AIH-PBC overlap syndromes. Data was collected from medical records. Average daily immunosuppressant dose was calculated by dividing each patient's cumulative dose by his or her total duration of therapy. Time to remission was defined by the time from initiation of therapy to achieving normal AST and ALT. Relapse/recrudescence was defined by dose escalation of prednisone following stability or a taper. Biopsy specimens were reviewed by an expert pathologist for the total number of IgG4+ plasma cells and the largest number of IgG4 + plasma cells in any HPF. Low density (LD; <5 IgG4+/HPF) and High density (HD; ≥5 IgG4+/HPF) IgG4+ plasma cell counts were dichotomized. Mean number of relapses, time to remission, and average daily prednisone dose were compared.

Results: A total of 21 AIH patients were identified and 14 had sufficient tissue for IgG4+ staining and analysis. Of the 14 cases, only 1 case fulfilled a criterion of > 10 IgG4+/HPF for IgG4 related disease. Four were categorized as HD and 10 were categorized as LD. There were no differences in the age (47.4yrs vs 47.5yrs; p=0.9863) or AIH score (16 vs 15.4; p=0.8688) at presentation. The presenting ALT levels were significantly greater in HD cases compared to LD cases (1187 vs 241; p=0.0069). HD cases experienced more frequent relapses/recrudescence per year of follow-up than did LD cases (1.10/year vs 0.40/year; p=0.0214 / Fig.1). There was no difference in the average time to remission (6.3 vs 15.0 months; p=0.4992), average daily prednisone dose (HD 11.6mg vs LD 8.1mg; p=0.4262) or average daily azathioprine dose (HD 38.9mg vs LD 50.0mg; p=0.4438) between HD and LD cases respectively.

Conclusion: A subset of AIH patients may have a moderately increased IgG4+ plasma cell frequency, which does not meet new consensus criteria for IgG4 related liver disease but nonetheless has important and distinct clinical features. These patients may present more acutely and experience more frequent relapses/recrudescence. Larger sample sizes are necessary to validate these initial findings.

ANTIBIOTIC TREATMENT AND SMOKING EXPOSURE MODULATE LUNG IMMUNE RESPONSE IN A MOUSE MODEL OF INFLUENZA PNEUMONIA

Resident: Nicholas Arger, M.D.; Mentor: Charles Dela Cruz, M.D., Ph.D.

Background: The effects of antibiotics in acute respiratory viral infections can result in a negative shift in the normal gut and respiratory tract “microbiome” and potentially impair normal host defense. The effects of broad spectrum antibiotics on host defense in the lung in response to viral infections and cigarette smoke (CS) exposure have yet to be understood.

Specific Aims: 1) Characterize the effects of antibiotics on the host response in an influenza pneumonia mouse model; 2) Determine both the separate and concomitant effects of smoking in the same mouse model; 3) Identify immune signaling pathways mediating the immunologic changes seen due to both antibiotics and CS exposure.

Hypothesis: Antibiotic treatment decreases innate immune response to influenza pneumonia and cigarette smoke (CS) counteracts this effect.

Methods: Wild-type C57BL/6 mice were given 2 weeks of 4 oral antibiotics (neomycin, metronidazole, ampicillin and vancomycin), and subsequently infected intranasally with varying LD50 dosages of A/PR8/34 influenza virus. A similar cohort of mice had daily CS exposure for four weeks prior to influenza inoculation. Bronchoalveolar lavage (BAL) leukocyte count and differential as well as viral load via conventional plaque assay were measured on day 7 of infection. Three pro-inflammatory cytokines, TNF-α, IL-6, and IL-1β, were measured in BAL and in lung tissue via ELISA and quantitative PCR. Lung tissue was also prepared for histological analysis.

Results: Effects of antibiotics treatment on lung inflammation and viral burden depended on the dose of virus and CS exposure with the largest effects seen with the high influenza dose. Antibiotic treatment resulted in increased initial weight loss, higher BAL influenza titer, and more inflammatory infiltrate on histology. Antibiotics increased distal ileum and cecal stool volume in all mice. TNF-α increase and IL-6 decrease in BAL correlated with these negative effects. In the setting of prior CS exposure, TNF-α levels were still higher in antibiotic-treated mice, but decreased relative to non-CS exposed mice; viral load was similar to non-CS exposed controls but was also increased with antibiotics. Weight loss was greatest in the antibiotic-treated, CS-exposed mice. IL-6 was decreased with antibiotic treatment as well as with prior CS exposure. IL-1β was similar with antibiotic treatment in both BAL and lung tissue, but pro-IL-1β mRNA levels were decreased.

Conclusions: Broad spectrum antibiotics resulted in worse phenotype in influenza-infected mice and correlated with increased TNF-α response and decreased IL-6. Prior CS smoke exposure decreased inflammatory and cytokines responses, but still increased weight loss and viral load. In the setting of antibiotics and prior CS exposure, there was also increased TNF-α and decreased IL-6.
Transitions of Care at a Community Hospital - Sharing the Voices of Readmitted Patients through Qualitative Interviews

Manik Chhabra, MD and Janet Ho, MD; Donna Windish, MD, MPH

Background: With the implementation of the Patient Protection and Affordable Care Act, measures of high-value, patient-centered healthcare delivery now include 30-day readmission rates. Most research on alterable readmission risk factors has explored the healthcare provider’s viewpoint, but there has been little research assessing the patient’s. We conducted a qualitative study of readmitted patients to elucidate the patient experience when transitioning from hospital to home.

Methods: We completed semi-structured qualitative interviews of patients readmitted to a medicine service within 30 days of discharge at Waterbury Hospital, a 357-bed, private, community teaching hospital in Waterbury, Connecticut, using a sample of convenience. Exclusion criteria included the inability to pass a Mini-Cog screen, speaking a language other than English or Spanish, or chart evidence of advanced cognitive impairment. All interviews were conducted and taped while patients were in the hospital, then transcribed by an independent company. Three investigators independently generated codes from the primary data and developed a final code list using the constant comparative method, from which themes were identified. Interviews will conclude once theoretical saturation is achieved.

Results: Of the 25 patients interviewed, the mean age was 66 years. Twelve were female, 16 had a high school education or less, and 11 had only Medicare and/or Medicaid coverage. The mean Charlson Comorbidity Index was 5.4, an estimated 10-year survival probability of 10%. In the past year, the mean number of hospitalizations was 4 and emergency room visits was 5.7. The median time to readmission was 10 days, with 13 patients (52%) having the same admission diagnosis as last discharge.

Patients infrequently reported obstacles obtaining medications, problems accessing a primary care provider (PCP), difficulty receiving in-home services (e.g. visiting nurses), or confusion managing health conditions. Many felt they were discharged too soon; however, most felt positively about the discharge process. Overall, patients reported good relationships with their PCP but perceived a limited scope of practice. Patients rarely utilized after-hours care and often self-traiged to the emergency room with a change in health status.

Discussion: Previous literature suggests correlations between increased readmission risks and low socioeconomic status (SES), with discharge interventions often targeting medication reconciliation and education. We found, however, that despite a low SES, our patients infrequently reported financial obstacles to obtaining medications or confusion about medication instructions. Instead, Emergency Department visits and readmissions were commonly the result of patients making their own triage decisions that bypassed potential intervention from a PCP. We also found that this self-triage decision often resulted from a patient’s perception that the medical problems a PCP could address were limited. As we continue to interview our readmitted patients, we hope to reveal more patient-driven themes to guide potential interventions reducing rates of unnecessary readmissions.
Title: Use of Echocardiography to Identify Potential Candidates for ICD

Moulin Chokshi, M.D., Robert McNamara, M.D., M.H.S., Rachel Lampert, M.D.

**Background:** Based on the strength of numerous randomized controlled trials, the 2013 ACCF/AHA guidelines recommended implantable cardioverter-defibrillator (ICD) therapy for “patients with nonischemic (dilated cardiomyopathy) or ischemic heart disease at least 40 days post-MI with LVEF of 35% or less and NYHA class II or III symptoms” as well as ICDs for patients with Class IV ambulatory symptoms who had QRS>120 milliseconds. However, widespread underutilization has been well documented, with failure to refer to Electrophysiology (EP) as the most common reason eligible patients do not receive ICDs.

**Specific Aim:** To determine if placing a reminder statement in echocardiogram reports for patients with an EF ≤ 35% will increase rates of EP referrals and ICD implantation for appropriate patients.

**Hypothesis:** Placing a reminder statement into echocardiogram reports for appropriate patients will increase referral rates to EP as well as rates of ICD implantation at a large academic hospital.

**Methods:** To establish a historical control group, all patients who had an echocardiogram performed between March and August 2012 with an EF ≤ 35% at Yale-New Haven Hospital (YNHH) were reviewed. In late 2012, a reminder statement was created that would automatically be inserted into the body of all echocardiogram reports with an EF ≤ 35%. The attending cardiologist reading each individual echocardiogram had the option to delete this reminder if he or she felt it was inappropriate. From January 2013 to June 2013, all echocardiograms with an EF ≤ 35% that had the reminder statement present were reviewed to establish an intervention group. Patients were excluded from either the control or intervention group if they had a preexisting ICD, repeat echo within 6 months of index echocardiogram that showed an EF>35%, expired within 6 months, end stage medical disease, malignancy, uncontrolled psychiatric disease, dementia, refused an ICD in the past, did not want to be resuscitated if they were to have a cardiac arrest, or were lost to follow-up. The remaining patients were considered eligible for ICD consideration. Charts were reviewed for the 6 months after the index echocardiogram for referral to EP and/or placement of an ICD. Using Fisher’s exact test, the rates of EP referral and ICD implantation for eligible patients were compared between the control and interventions groups.

**Results:** In the control group, a total of 363 unique patients had an echocardiogram performed with an EF ≤ 35%. Of these patients, 117 (32.2%) already had an ICD, 143 (39.4%) patients were considered inappropriate for ICDs, and 21 (5.8%) patients were lost to follow-up, leaving 82 eligible patients. In the intervention group, 343 total patients had an echocardiogram done with an EF≤ 35%, 109 (31.8%) had an ICD, 156 (45.5%) patients were considered inappropriate, and 18 (5.2%) were lost to follow-up, leaving 60 eligible patients. In the control group, 50 out of 82 patients were referred to EP compared with 48 out of 60 in the intervention group (61.0% versus 80.0%, p = 0.02). Follow-up data show that 36 out of 82 eligible patients in the control arm received an ICD compared with 32 out of 60 in the intervention arm (43.9% versus 53.3%, p = 0.31).

**Conclusions:** A simple reminder statement on select echocardiography reports lead to a significant increase in EP referrals. Although an absolute increase in ICD placement was also seen, the difference was not statistically significant. Given only a small number of patients were eligible for ICDs in both the control and intervention groups, this study may have been underpowered in its ability to show a difference in ICD implantation. A follow-up study comparing larger cohorts of patients before and after the implementation of a reminder statement may be able to more accurately assess whether this intervention increases ICD implantation and should be universally used as a screening tool.
Title: The permissive effect of BV on the Acquisition of Chlamydia or Gonorrhea
Aletheia Donahue, M.D.; Dana Dunne, M.D.

Background: The microbiom of the healthy human vagina is characterized by low pH, and the presents of lactic acid producing lactobacillus. In vitro studies have shown that lactic acid has a bactericidal and virucidal effect and thus may be protective against sexually transmitted infection including gonorrhea and chlamydia. Bacterial vaginosis (BV) is a clinical syndrome of vaginal dysbiosis resulting in a reduction of lactobacillus and often an increase in Gardnerella vaginalis. There is some evidence that the presents of BV or the lack of lactic acid producing bacteria increases the risk for acquiring gonorrhea or chlamydia. Brotman1 et al showed in 2010 showed that BV diagnosed with Amsel criteria was associated with a 25% increase in Chlamydia, Gonorrhea or Trichomonas. It is thought that up to 50% of women are asymptomatic when they meet clinical criteria for BV. Current clinical standard of care is not to treat asymptomatic Bacterial Vaginosis, however, if it was shown that presents of bacterial vaginosis increases the risk of acquiring sexually transmitted disease it may be of benefit to treat asymptomatic bacterial vaginosis in high-risk individuals.

Specific Aim: To determine the permissive effect of asymptomatic BV on the acquisition of chlamydia or gonorrhea infection.

Hypothesis: Presence of bacterial vaginosis increases the likelihood of acquiring chlamydia or gonorrhea in sexually active women.

Methods: This was a retrospective analysis of data gathered from sexually transmitted infection (STI) clinics housed in health departments in New Haven, Hartford and Bridgeport. Clinicians, nurse practitioners and medical doctors, have routinely collected and entered into a digital database for every clinic visit continually starting in year 2000 to the present. The records of women who visited one of the three STI clinics and who presented as partners exposed to chlamydia or gonorrhea were included in the analysis. Women were identified using a query written by Wayne Goldman of the entire de-identified female data base. Diagnosis of BV was made based on Amsel’s criteria; diagnosis of Chlamydia or Gonorrhea was determined by DNR PCR of vaginal sample acquired at the same visit. We compared the women with a clinical diagnosis of BV to the women without a clinical diagnosis of BV, on the basis of infection with Chlamydia or Gonorrhea. An odds ratio was determined. We adjust results to control for condom use, and number of sexual partners. We performed a small subgroup analysis for women that had 2 or more visits to the health department. In this group we found women with a diagnosis of BV at a previous visit that was untreated and asymptomatic and compared these women to age matched controls without BV on the basis of the presents of Gonorrhea or Chlamydia at a subsequent visit.

Results: Pending data analysis.

A Clinical Risk Score Predicting Heart Failure and Cardiomyopathy after Adjuvant Trastuzumab Therapy for Breast Cancer
Ghideon Ezaz\textsuperscript{1}, Jessica B. Long\textsuperscript{1,2,3}, Cary P. Gross\textsuperscript{1,3}, Jersey Chen\textsuperscript{2}

\textsuperscript{1}Section of General Internal Medicine, Department of Internal Medicine, Yale School of Medicine, New Haven, CT
\textsuperscript{2}Mid-Atlantic Permanente Research Institute, Kaiser Permanente Mid-Atlantic States, Rockville, MD
\textsuperscript{3}Cancer Outcomes, Public Policy, and Effectiveness Research Center, Yale School of Medicine and Yale Comprehensive Cancer Center, New Haven, CT

\textbf{Introduction}
Adjuvant trastuzumab improves survival for HER2+ breast cancers. However, trastuzumab, independently and in combination with anthracyclines, increases the risk of heart failure and/or cardiomyopathy (HF/CM), particularly in older patients. Clinical risk stratification tools would be useful to guide monitoring and treatment decisions regarding choice of chemotherapy.

\textbf{Methods}
We used the linked SEER - Medicare database to study older women with stage I-III breast cancer who were treated with adjuvant trastuzumab +/- chemotherapy following surgery. Major exclusion criteria included pre-existing HF/CM or other cancers. The study outcome was HF/CM within 3 years of adjuvant therapy as identified by ICD-9 codes. We divided the study sample into derivation and validation cohorts. In the derivation cohort, we estimated a Cox proportional hazards model, and we used significant risk factors to construct a risk score. Risk scores were then calculated for each subject in the validation cohort, and the risk of HF/CM was classified into low, medium, and high strata.

\textbf{Results}
In the full cohort of 1,664 women, ages 67 to 94, 318 (19.1\%) developed HF or CM during 3 years of follow up. The risk score model included the following risk factors: concurrent chemotherapy (anthracycline regimen vs. non-anthracycline regimen vs. none), age, and history of coronary artery disease, atrial fibrillation or flutter, diabetes, hypertension, and renal failure. Individual risk score totals ranged from 0 to 9. The risk score allowed classification of 3-yr HF/CM risk in the validation cohort from low (0-3 points) at 16\%, to medium (4-5 points) at 26\%, to high (6-9 points) at 39\%, as seen in Table 1.

\textbf{Conclusion}
This risk score can stratify patients for risk of HF/CM after adjuvant trastuzumab therapy. While limited by use of administrative data, this approach demonstrates proof-of-concept that a clinically useful risk score can be developed in the future using primary clinical data.

\textbf{Table 1: Risk Categories and Incidence of HF/CM}

<table>
<thead>
<tr>
<th>Risk Category</th>
<th>Derivation</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Patients</td>
<td>HF/CM in 3 Years after Diagnosis</td>
</tr>
<tr>
<td>Low (0-3)</td>
<td>595</td>
<td>14%</td>
</tr>
<tr>
<td>Medium (4-5)</td>
<td>195</td>
<td>26%</td>
</tr>
<tr>
<td>High (6-9)</td>
<td>42</td>
<td>43%</td>
</tr>
</tbody>
</table>
Title: Chase ICU (Chase Outpatient Center Intensive Care Unit) – An Intensive, Team-based Approach to Ambulatory Care in a Resident Clinic

Faye Farber, MD; Manik Chhabra, MD; Janet Ho, MD; Megan Lemay, MD; Nora Segar, MD; Steven Holt, MD, MSc; Julie Rosenbaum, MD; Sarita Soares, MD; Tracy Rabin, MD, SM

Background: Primary care delivery can be fragmented and difficult to navigate, particularly for patients with low health literacy and socioeconomic status. In response, The Patient Centered Medical Home (PCMH) model aims to improve patient access and coordination of care, inspiring a separate “Ambulatory Intensive Care Unit (ICU)” model. The Ambulatory ICU model is a team-based, interdisciplinary approach toward outpatient interventions for high-risk patients through proactive self management education and support, improved access to specialized care and team members, and customized care plans. Previous Ambulatory ICU models have been associated with improved patient health outcomes, decreased emergency room visits, and decreased cost of care.

Specific Aim: We proposed to create a team-based, interdisciplinary model in the outpatient setting called the Chase Ambulatory ICU (Chase ICU) at the training clinic for the Yale Primary Care Residency Program. The Chase ICU aimed to provide high-quality, team-based care to our highest risk patients as well as to improve resident satisfaction with the clinic experience.

Hypothesis: We hypothesized that the intervention cohort would improve continuity of care, better scores on markers of core measure compliance as measured by chart review, and improve patient and resident satisfaction.

Methods: We proposed a prospective cohort study to compare outcomes for patients receiving Chase ICU care with patients receiving usual care. Minimum Chase ICU intervention included 1) a goal setting exercise between patient and provider 2) scheduled visits with the clinic’s advanced nurse practitioner 3) weekly phone calls from team members 4) improved access to the physicians by way of an on-call pager system and 5) offer of a home visit assessment.

Results: At this time, 15 patients have been enrolled in the project. Although we are still collecting data, initial results suggest that for some patients, enrollment in the Chase ICU improved continuity of care through more frequent follow up and a comprehensive sign out process among covering residents. We have some patients who achieved their goals, such as decrease in hemoglobin A1c. Challenges with the project included the loss of our clinic APRN, which further disrupted continuity of care, as well as difficulties in resident scheduling resulting in individual residents being away from clinic for prolonged periods of time.

Conclusions: Team-based care may improve outcomes for the complex patients cared for in residency clinics. Additionally, grassroots, resident-organized teams have the potential to improve outcomes even in the absence of interdisciplinary support and full-time clinic staff.
Increased bone density and increased skeletal remodeling in a patient with an allelic variant in SATB2

Resident: Sitala Gadiraju, MD, Mentor: Karl Insogna, MD

Background: A 51 year old woman, in her teens, was incidentally found to have dense bones on skeletal radiographs. Densitometric studies confirmed increased bone mass, which was accompanied by high serum markers of bone turnover. However turnover is almost always associated with low bone mass. Genetic and biochemical studies excluded mutations in LRP5, OPG or SOST as the etiology of this syndrome. It was presumed that the patient must have an unknown genetic mutation explaining her rare phenotype.

Aims & Hypothesis: A novel mutation is responsible for the phenotype of increased bone density with increased bone turnover. We will use whole exome sequencing to identify candidate genes and use in vitro and in vivo studies to test this hypothesis.

Methods:

Genetic Sequencing - DNA samples were collected from the patient and both her parents and used for whole exome sequencing at the Yale Center for Genome Sequencing.

Data Base Queries - Data base queries were used to evaluate which allelic variants were relevant to the phenotype of interest. The data set was filtered for genes known to be associated with skeletal phenotypes. SATB2 was identified as a candidate gene since it plays a role in osteoblast function, and loss of function mutations cause low bone mass.

In Vitro Studies – Primary osteoblast cells were obtained from calvaria of neonatal mice and subjected to sequential digestion with 0.1% bacterial collagenase and 0.2% dispase. Digests were then pooled and plated. Lenti-X Vectors containing the gene of interest were then co-transfected into Lenti-X 293T cells in order to assemble infectious virions, which were then infected into the osteoblast cells. However there was difficulty infecting primary osteoblasts so the murine osteoblast cell line MC3T3E1 was used instead.

Mouse Model – A targeting vector containing the SATB2 allelic variant was engineered and electroporated into embryonic stem cells. ES cell clones were then screened to identify those with successful recombination. One clone was identified and used to create knock in mice (i.e. heterozygous for the SATB2 allelic variant). Animals with germline transmission heterozygous for the mutation will have bone density analyzed and serum turnover markers measured to assess if the mutation replicates the human phenotype.

Results & Conclusions: SATB2 is known to be a positive regulator of osteoblast differentiation and acts by binding DNA at matrix attachment regions. It is presumed that the SATB2 allelic variant in our patient represents a gain of function mutation. This allelic variant occurs in the CUB domain of SATB2, a highly conserved region. Available data indicate that mutations in this domain reduce DNA binding. However, the allelic variant in our patient resulted in a lysine to asparagine change in the protein sequence. This lysine is conserved back to flies. Lysine is a target for ubiquitination and if the allelic variant in this patient prevents ubiquitination but does not affect DNA binding, it could still result in a gain of function mutation. In-vitro studies using the MC3T3E1 cells over-expressing mutant SATB2 did not show increased mineralization, but showed impaired mineralization. It was thought that might reflect the fact that the cells were overexpressing the mutant allele in the presence of two normal SATB2 alleles, which does not replicate the in vivo phenotype. We currently have 3 mice with germline mutations. If the phenotype is not replicated in the knock in mice, then one thought would be that the mutation is in a gene that is not known to be related to bone physiology and thus was filtered out in the initial whole exome screen.
**Title:** Dyrk1B Mutation enhances its adipogenic effect.
Ali R. Keramati M.D., Arya Mani M.D.

**Background:** Coronary artery disease (CAD) is the leading cause of morbidity and mortality worldwide. A cluster of highly heritable risk factors known as metabolic syndrome is a major and growing predisposing factor for development of CAD. Recent development of next generation sequencing has provided a potent genetic tool for identification of rare variants with large effects on disease risk. We identified 3 kindreds, all notable for a high prevalence of early onset coronary artery disease and metabolic syndrome. Using combined linkage analysis and whole-exome sequencing, we have identified a founder mutation in Dyrk1B which substitutes cysteine for arginine 102 in the highly conserved kinase-like domain of Dyrk1b. Morbid obesity was a highly penetrant and specific trait among mutation carriers. The role of Dyrk1B in the adipogenesis has not been previously established. Dyrk1B expression steadily increases during adipocyte differentiation. We hypothesized that over expression of Dyrk1B_{R102C} induces pre-adipocyte differentiation and enhances the protein expression of major adipogenic proteins during the differentiation process.

**Specific aim:** Identify the role of Dyrk1B_{R102C} mutation in adipogenesis.

**Hypothesis:** Dyrk1B_{R102C} mutation enhances adipogenic differentiation through increased expression of adipogenic genes including CEBP, PPARγ1/2 and PGC1α.

**Method:** The permanent 3T3L1 preadipocyte expressing Dyrk1B_WT, Dyrk1B_{R102C} and empty vectors were generated and the expression of Dyrk1B was confirmed by western blot and RT-PCT. The 3T3L1 pre-adipocytes were differentiated into adipocytes with traditional differentiation protocol. The adipocytes differentiation was followed for a total of 9 days. The differentiating cells were studied at days 2, 5, 7 and 9 by oil red O staining and expression levels of CEBP, PPARγ1/2 and PGC1α were examined by western blotting. All in vitro experiments were carried out in quadruples. Data will be expressed as means ± SE.

**Result:** Adipogenesis is tightly regulated by a cascade of transcription coactivators and suppressors. Dyrk1B expression dramatically rises during adipogenic differentiation, we hypothesized that the Dyrk1B promotes adipogenesis.

We examined the effects of Dyrk1B_WT, Dyrk1B_{R102C} on adipogenic differentiation in 3T3L1 cells and the time-courses of Gli-2, CEBPα, PPARδ1/2 and PGC1α protein expressions.

Adipogenic differentiation started roughly 5 days earlier in 3T3L1 cells expressing Dyrk1B_WT and Dyrk1B_{R102C} compared to vector alone and was significantly greater in cells expressing Dyrk1B_{R102C} than Dyrk1B_WT or vector alone. Accordingly, the expression levels of CEBPα, PPARδ1/2 and PGC1α were highest and those of Gli-2 and p27kip were lowest in preadipocytes expressing Dyrk1B_{R102C} followed by Dyrk1B_WT and empty vector.

**Conclusion:**
Dyrk1B is a ubiquitously expressed protein with dual kinase activities. Very little is known about the biological function of this gene and its potential involvement in human disease. The novel finding of our study is induction of adipogenesis by Dyrk1B and its augmentation by Dyrk1B_{R102C} mutation. These effects were associated with increased expression of adipogenic genes, CEBPα, PPARδ1/2 and PGC1α.

In conclusion, our study characterizes Dyrk1B as a regulator of adipogenesis and associates its altered function to enhanced adipogenic differentiation. These investigations strongly implicate Dyrk1B as a promising target for novel therapeutics directed against obesity.
Role of Adjuvant Radiation in Patients with Oral Cavity Squamous Cell Carcinoma

**Resident:** Steve Maron, M.D.  
**Mentor:** Daniel Morgensztern, M.D.

**Background:** Oral cavity cancers represent eleven percent of head and neck malignancies. Despite current treatment, five year overall survival in the national SEER database from 2002 to 2008 was only 61.5 percent among all oral cavity and oropharynx cases and 57.3 percent among those with lymphatic spread. As with most malignancies, AJCC staging is guided by the TNM algorithm, which takes tumor size, nodal involvement, and metastases into account in order to stratify patients by stage and to guide both treatment and subsequent analysis of similar populations.

Current guidelines recommend surgical resection with neck dissection followed by radiation with or without chemotherapy if extra-capsular spread, perineural invasion, positive margins, pT3, pT4 or extensive nodal disease is present during pathologic examination. Adjuvant radiation with or without chemotherapy is supported by large randomized trials of all head and neck cancers, as well as those specific to oral cavity cancer. However, no studies have specifically evaluated stage III oral cavity cancer survival and treatment needs.

**Specific Aim:** To explore survival disparities amongst stage III TNM subsets while secondarily assessing the effects of treatment modality, age, gender, race, tumor site, and tumor grade.

**Hypothesis:** Disparate survival between subtypes will necessitate variable treatment strategies.

**Methods:** Study data was obtained from the Surveillance Epidemiology and End Results (SEER) database of the National Cancer Institute between 2004 and 2009. Cases were limited to oral cavity squamous cell cancers based on the International Classification of Diseases for Oncology 3rd Edition, excluding those lacking surgical or radiotherapy status (n=26). Data collected included patient age, gender, race, site, staging, extra-capsular extension, and treatment modalities used. Analysis was performed in SAS using a Cox proportional hazard model to account for patient variables. Overall (OS) and disease-specific survival (DSS) rates were estimated by the Kaplan-Meier method and compared using log-rank testing.

**Results:** Among the 1,051 patients meeting eligibility criteria, the study population included 246 (23.4%) T1N1, 352 T2N1 (33.5%), 334 (31.8%) T3N0, and 119 (11.3%) T3N1 cases. Five-year OS ranged from 33.3% in T3N1 to 52.8% in T1N1. Median survival was 66 months in T1N1, 41 months in T2N1, 39 months in T3N0, and 24 months in T3N1 by Kaplan-Meier estimates. Although imbalances existed in the distribution of sex, race, and site, no significant overall survival differences were noted for these characteristics in univariate or multivariate analysis. Compared to surgery alone, the addition of radiation therapy improved 5-year OS in the entire cohort from 39.5% to 51.1% (HR 0.70; 95% CI, 0.55-0.88, p=0.003). This benefit, however, was only significant for stage T3N0 (p=0.006) with a trend towards improvement in the T3N1 group (p=0.11). No significant benefit was observed in T1N1 or T2N1 disease.

**Conclusions:** If confirmed in prospective studies, further subdivision of stage III OSCC may be necessary, and the indication for RT may be restricted to patients with T3 disease.
Resident Reflection Rounds: The Association Between Resident Reflection Rounds and Resident Emotional Distress
Kristen A. Marrone, M.D., Shin Lin, M.D. and Kathleen Akgün, M.D.

Background: Medical residents are integral to inpatient care delivery in academic teaching hospitals. Residents frequently discuss changes in disease trajectories with patients and their families, including end of life (EOL) decision-making. Formalized debriefing for residents on these experiences has been largely overlooked in medical education. Debriefing may alleviate symptoms of resident emotional distress (RED). RED can lead to depersonalization, lack of fulfillment and fatigue, which are core features of burnout. Burnout is associated with higher perceived medical errors and negatively affects physician quality of life and retention.

Resident Reflection Rounds (RRR) were introduced to the Internal Medicine (IM) postgraduate year (PGY)-1 class at Yale-New Haven Hospital (YNHH) in 2012-2013. Informal feedback of RRR indicated this may be an unmet need. We asked whether expansion of RRR to all PGY classes in the 2013-2014 academic year would affect RED.

Specific Aim: Determine whether RRR changes prevalence of symptoms associated with RED.

Hypothesis: RRR will change RED. Improvements in RED will vary by post-graduate year.

Methods: All YNHH IM Residents were invited to enroll in an observational cohort study. The study and surveys were approved by the Yale University School of Medicine’s Institutional Review Board. Participating IM residents gave informed consent prior to enrollment. Surveys were distributed via the IM Residency Program listserv at baseline (October 2013) and after 6 sessions of RRR (March 2014). RRR are 1-hour sessions held during noon conferences at YNHH and the West Haven Veterans Administration (WHVA) hospital. RRR were offered to all IM residents, regardless of survey completion. RRR are moderated by volunteer faculty members and fellows who receive detailed instruction about the goals of the sessions in advance. The format of RRR is intentionally informal. Emails were sent to remind participants to focus on difficult patient interactions and EOL discussions along with intermittent suggested readings.

Results: Of 128 residents on the listserv, 45 (35%) completed the baseline survey. Participants were 51% female and most frequently PGY-2 (18/45 (40%)). At baseline, 27/45 (60%) of participants reported prior exposure to debriefing. However, debriefing with attending physicians following important discussions (family meetings, code status and informing next of kin of patient’s death) was infrequent, reported less than 30% of the time. Almost half (47%) of respondents felt that debriefing was an unmet need. More than two-thirds of participants felt unprepared (31/45 (69%)) and more than half (56%) felt overwhelmed when asked about their prior experiences with difficult discussions and EOL conversations at baseline. Debriefing with peers was valued by 67% of participants and 73% felt that debriefing with faculty members would be helpful.

Conclusions: Medical residents bear a significant emotional burden during their clinical training related to difficult interactions and EOL decision-making. Trainees frequently experience symptoms of RED. Debriefing may represent an unmet need of the YNHH IM residents’ medical education. We are currently collecting surveys following 6 sessions of RRR to determine whether RRR affect RED prevalence. Preliminary results will be available for the YNHH Internal Medicine Research in Residency Day on May 1, 2014. Addressing RED may decrease medical errors, improve resident satisfaction during training and promote long-term retention.
RESIDENT: John McGinniss, MD
MENTORS: Praveen Mannam, MD and Patty Lee MD.
TITLE: MKK3 regulates mitochondrial turnover and susceptibility to sepsis.

AIMS/HYPOTHESIS:
Dysfunctional mitochondria are potent mediators of inflammatory responses in sepsis. MAP kinase kinase 3 (MKK3) is an upstream kinase of p38; both are ancestral components of innate immune responses that may predate the canonical Toll signaling pathways. We recently found LPS-exposed MKK3 deficient mice and cells were protected against death with less inflammation and mitochondrial ROS generation compared to wild-type (WT). Hence, we sought to examine the link between MKK3 and mitochondrial turnover. We hypothesize that MKK3 deficiency protects against sepsis by up-regulation of mitochondrial turnover via both augmented biogenesis (formation of new mitochondria) and mitophagy (removal of damaged mitochondria). In a pilot study we assessed whether cellular bioenergetics and mitochondrial health could be measured in readily accessible, peripheral blood mononuclear cells (PBMCs).

METHODS:
We performed quantitative real-time polymerase chain reaction (qRT-PCR) in MKK3/- primary mouse embryonic fibroblasts (MEFs). We used a mitochondrial targeted mKeima probe to measure mitophagy. Mice underwent cecal ligation and puncture (CLP) and survival was measured. We enrolled patients admitted to the Yale-New Haven Hospital Medical Intensive Care Unit (MICU). MKK3 levels were assessed in PBMCs using AlphaScreen Surefire kinase assay (Perkin Elmer). PBMCs from healthy volunteers were analyzed using the XF Extracellular Flux Analyzer (Seahorse Bioscience).

RESULTS:
We found higher expression of Pink1 and Parkin in MKK3/- MEFs compared with WT. MKK3/- cells showed a higher level of mitophagy as measured by mKeima. Additionally, biogenesis increased with higher mitochondrial mass as measured by relative DNA quantification and expression of PGC-1α and TFAM. In a mouse CLP sepsis model, MKK3/- mice had better survival compared to WT mice. We found clinical relevance of our data in higher MKK3 activation in isolated peripheral blood mononuclear cells (PBMCs) from septic patients compared to non-septic controls. LPS exposure caused impaired mitochondrial function as shown by reduced the oxygen consumption rate and spare respiratory capacity of PBMCs.

CONCLUSIONS:
M KK3 has a role in regulating mitochondrial biogenesis and mitophagy. M KK3/- mice have a survival advantage in CLP-induced sepsis and M KK3 is elevated in PBMCs of septic MICU patients. Our findings reveal a critical role of mitochondria in the pathogenesis of sepsis and a new function of M KK3 in mitochondrial turnover and quality control. These results provide evidence of novel roles for M KK3 and mitochondrial function as potential therapeutic targets against sepsis. We are developing a novel methodology for measuring mitochondrial bioenergetics in patients. Assessment of the mitochondrial metabolic function and correlation with M KK3 levels in PBMCs from patients can give us insights into the bioenergetics during critical illness such as sepsis.
Title: Incidence and Burden of “Missed Primary Prevention ICDs” Among Out-of-Hospital Cardiac Arrest

Resident: Chirag Shah, MD

Mentor: Rachel Lampert, MD

Background:
While the high societal burden of primary prevention ICDs is discussed, the burden of failure to implant appropriate primary prevention ICDs has not been investigated. The percentage of cardiac arrests that could have been prevented by primary prevention ICD implantation, and how the resource burden of arrest due to “missed primary prevention” (“MPP”) compares to elective implantation, is unknown.

Specific Aim:
Identify the incidence of out-of-hospital cardiac arrests (OHCA) between 2000 and 2012, and of those OHCAs, to identify the percentage that could have been prevented with an appropriate use of an ICD as per the MADIT-II and SCD-Heft Criteria. Additionally, we want to compare the utilization of resources between an OHCA versus elective implantation of an ICD.

Hypothesis:
Of the patients that suffered an OHCA, and were admitted alive, there will be a significant rate of missed primary prevention and the utilization of resources will be greater in the patients that suffered a cardiac arrest versus those that had an elective ICD implantation.

Methods
Charts were reviewed of patients presenting to Yale-New Haven Hospital with an out-of-hospital cardiac arrest (OHCA) and of patients undergoing elective primary prevention ICD implantation for standard indications, between 6/09 and 3/12. “MPP” cases were defined as OHCA due to pulseless VT, VF, or asystole, and documented pre-arrest EF≤35%.

Results
Of 121 OHCA, 94 had VT/VF/asystole. Of these, 12 (13%) had a known pre-existing EF≤35%, meeting the definition of MPP. Elective ICD implantation was performed in 153 patients. There was no significant difference between the MPP-OHCAs vs electives in age, (68±3.5 vs 64±0.9), EF (30±1.5 % vs 28±0.4%) or NYHA (mean 2.4±0.2 vs 2.3±0.05). More MPP-OHCAs were female (53% vs 25%, p <0.02).
The average length of stay for MPP-OHCAs was 9.8±0.5 days (12.4±0.5 days for those discharged alive), vs 1.2±0.1 days for electives (p <0.0001 vs all MPP-OHCAs). Of the MPP-OHCAs, 100% had a left heart catheterization, transthoracic echocardiogram, and head CT, 50% had a hypothermia protocol, 92% mechanical ventilation, 50% EEG, 67% central venous access, 58% arterial line, 17% temporary pacing, and 75% pressor support. Acute kidney injury occurred in 42%, with 17% requiring hemodialysis and 33% suffering sustained renal injury. Recurrent arrest occurred in 33%, AF in 42%, and ICU delirium in 42%. Infections included 42% aspiration pneumonias, 17% ventilator-associated pneumonias, and 17% UTIs.Disposition included 42% to home, 33% nursing facility, and 25% died. None of these procedures or complications occurred in the electives.

Conclusion
From 2009-12 at this tertiary care center, 13% of arrhythmic OCHAs admitted alive could have been prevented through appropriate primary prevention ICD implantation. LOS, complications, and use of procedures and therapies were high in MPP-OHCAs compared to elective implants.
Title: Mastering the art of deprescribing: An interprofessional outpatient polypharmacy program for older veterans using the shared medical appointment model

John Thomas, M.D., Anne Hyson, M.D., M.Sc.

Background: Polypharmacy is common in older patients and is associated with negative health outcomes and poor adherence. Shared medical appointments (SMAs) have shown promise as a care model for older patients with chronic conditions, but have not been applied in the context of polypharmacy.

Specific Aim: Our objective was to pilot a polypharmacy intervention involving an SMA for older veterans as a performance improvement project.

Hypothesis: An interprofessional outpatient polypharmacy program using the SMA model will result in safe medication reduction and improvement in both cognitive and physical function.

Methods: In an outpatient interprofessional team training program of a Veterans Affairs Hospital, we invited patients to participate in a two-part polypharmacy intervention consisting of an SMA and an individual provider visit. Eligible patients were ≥65 years old, with ≥10 prescribed medications, and referred by their provider as appropriate for an SMA. For one of the three pilot sessions, eligible patients were additionally co-prescribed a narcotic and a benzodiazepine. For all three pilot sessions, the SMA was co-facilitated by a physician, pharmacist, health psychologist, and nurse practitioner, and consisted of interactive discussions about patients’ experiences with and beliefs about medications, as well as an educational component about the concept of polypharmacy and safe medication disposal. The individual visit included standard geriatric assessments such as the Saint Louis University Mental Status Exam (SLUMS), activities of daily living (ADLs), independent activities of daily living (IADLs), and orthostatics; a medication reconciliation; shared decision making regarding medication changes; and a detailed follow-up plan. Discussions were aided by a compilation of medication discontinuation guidelines based on the BEERS criteria and selected disease-based guidelines.

Results: Thirteen of the 15 scheduled patients attended. The average age was 76 and the average number of medications was 19. In the SMA, many patients agreed they did not understand the indications for all their medications, but they trusted their providers’ judgment of medication appropriateness. Patients exchanged ideas about how to increase adherence. In the individual visit, the average SLUMS exam score was 23/30 (range 13-29), the average number of dependent ADL or IADLs was 2 (range 0-7), and one patient was orthostatic. Two patients were newly diagnosed with dementia. An average of 2.5 medications per patient were discontinued, and an average of 1 medication per patient was decreased in dose or frequency. We identified instances of hazardous prescribing in four patients, notwithstanding those co-prescribed a narcotic and a benzodiazepine. Among the latter patients, one agreed to discontinue his benzodiazepine outright, while three others agreed to seek further treatment for anxiety and/or insomnia prior to tapering their benzodiazepine. Referrals were made as appropriate to geriatrics, health psychology, home-based primary care, and other pertinent resources.

Conclusions: We successfully executed three pilot sessions as a polypharmacy intervention for older veterans. Patients expressed limited understanding of their medications, and instances of unnecessary or inappropriate prescribing were identified in all patients. We plan to continue enrolling patients, to perform 6-month follow-up assessments for all patients after the initial intervention, and to evaluate the trainee experience. Preparations are being made for potential expansion of the program to other outpatient settings.
Risk Factors and Outcomes in Spontaneous Bacterial Peritonitis Patients with Initial Non-Response to Antibiotic Therapy, and Proposal of a Novel Scoring Algorithm to Predict Initial Non-Response

Uyen To, Mentor: Dr. Garcia-Tsao

1. Restatement of the specific aim and hypothesis: Our goal was to characterize factors that predict lack of response to first line antibiotic therapy for spontaneous bacterial peritonitis (SBP) in patients with cirrhosis. We hypothesize that the lack of response to first line antibiotic therapy for SBP will be related to multidrug resistant bacteria and fungal organisms. Non-response will likely be higher in cirrhotic patients who have received prophylactic fluoroquinolones, have a history of nosocomial infections, and recent admissions.

2. Brief review of methods actually used in the project: A retrospective analysis of all cirrhotic patients admitted to the Klatskin service between the years of 2009-2013 was performed. 856 records were reviewed and all patients with SBP (defined as a diagnostic paracentesis with >250 PMN) were identified. We then expanded our data collection to include demographics and lab data 24 hours prior to the diagnostic tap, hospital course, and antibiotic regimen. We evaluated the lack of response to first line treatment for SBP by analysis of the cell count from the ascites fluid on the day of diagnosis and 48-72 hours later. Lack of response to first line antibiotic therapy was defined by <25% decrease in PMN count from pretreatment levels, or mortality related to sepsis prior to repeat paracentesis. Preliminary data analysis was initially done with SPSS for univariate and then will be formally done by SAS. Variables found significant on univariate analysis will be extrapolated to multivariate analysis.

3. Description of Results: A total of 136 patients were found to have SBP in the years of 2009-2013. 24 patients were excluded for secondary bacterial peritonitis or no diagnostic tap after 48-72 hours. Of the 112 remaining patients, 64 were initial responders and 48 were non-responders. When evaluating the data, we had to re-define our group of non-responders as those who did not have a decrease of >25% PMN from their initial diagnostic tap and those who had ascites fluid PMN count that was >250 on subsequent taps after 48 hours, necessitating antibiotic adjustment. Of the categorical and continuous variables that were collected, preliminary univariate data analysis showed that NASH, hepatitis B cirrhosis, positive ascites fluid culture, WBC, minimum body temperature, pre-tap MELD, serum lactate and serum hemoglobin obtained 24 hours prior to tap were significant on univariate analysis. On analysis of the antibiotics that patients received in the first 24 hours of SBP diagnosis, 52% received 1st line therapy with a 3rd generation cephalosporin while 48% received broad spectrum antibiotics. On review of the microbiological data, gram-negative bacteria composed 65% of the bacteria that were isolated. Resistant organisms were seen in 20% of the cases, of which 11% were gram negative resistant bacteria and 9% were gram positive resistant bacteria. Fungal organisms were seen in 15% of the cultures. Finally, preliminary analysis of clinical deterioration showed that those with initial non-response had statistically significant increased morbidity and mortality.

4. Conclusion: Further data analysis is required for the groups who received first line antibiotics vs. broad-spectrum antibiotics and the variables that predict non-response in each group. Depending on the variables that are significant on repeat univariate analysis, they will be extrapolated to multivariate analysis to ultimately create an algorithm that will predict non-response for decompensated cirrhotics.
Title: CELA2A Mutation in a Family with early coronary artery disease

Ephraim Weiss M.D, Arya Mani M.D

Background: Coronary artery disease (CAD) is the leading cause of death worldwide. Despite significant progress no major risk factors have been identified since the Framingham study. Identification of CAD causing genes may lead to further understanding of the pathophysiology and establishing a cause and effect of the disease. Exome sequencing can reveal novel mutations that may cause CAD.

Specific Aim: To identify a disease causing mutation with large effect in an Irish-American family with early onset CAD

Hypothesis: An Irish- American family with a strong predisposition to early CAD was identified. Evaluation of the kindred revealed a high likelihood of a Mendelian inheritance pattern of disease. Exome sequencing was carried out in both affected and unaffected members.

Methods: We identified one kindred with early onset CAD. This case was ascertained via a 28 year old female who presented with exertional angina. Perfusion stress imaging revealed an area of anterior ischemia and subsequent coronary angiography revealed an LAD stenosis. She underwent bare metal stenting with subsequent improvement of her angina. Detailed family history and clinical data was obtained for all other available family members. Members of the family were characterized by invasive or CT angiography for having CAD. 21 of 31 blood relatives were diagnosed with CAD, 17 of which had a coronary event before the age of 60. Exome sequencing from five affected family members revealed a novel mutation on Chromosome 1. DNA and serum were then obtained from several other family members to detect if the mutation was present and to determine if there was any phenotype.

Results: Exome sequencing seven family members, six of which had premature CAD, revealed a missense mutation D121N at amino acid 121 in the CELA2A gene, located on Chromosome 1. The mutation converts an aspartic acid to an asparagine, a mutation predicted to be damaging according to PolyPhen2 and SIFT. The mutation is also significant as it alters a charge relay site. This gene plays a functional role in the relationship of elastase to elastin which has been shown to play a role in atherosclerosis. Analysis of unaffected family members’ DNA did not show the presence of the mutation. Serum elastase was measured in all available members with a statistically lower level of elastase detected in affected members (p<0.05)

Conclusion: A novel mutation was detected in the CELA2A gene in a family with premature CAD. Functional elastase levels were significantly lower in affected members. Further analysis is required to determine the role of this gene and elastase in development of premature CAD.
Using SPECT/CT to investigate peripheral vascular disease (PVD)

Resident: Wunan Zhou, MD
Mentor: Albert Sinusas, MD

Specific aim and hypothesis: PVD is a major health problem but despite its prevalence, approximately 75% of patients with PVD are undiagnosed as current diagnostic tools are not highly sensitive, particularly in diabetic patients with microvascular disease. We aim to use $^{99m}$Tc-Tetrofosmin SPECT/CT to evaluate lower extremity perfusion for detection of microvascular and macrovascular disease in specific vascular territories to improve detection and guide therapy. Additionally, we aim to correlate indices of physiological stress with changes in perfusion during exercise in evaluation of patients with possible microvascular disease and/or claudication. We hypothesize that lower extremity perfusion will be reduced in patients with PVD and/or microvascular disease at rest and during exercise.

Methods: Five controls patients and 3 patients with PVD who were referred for clinically indicated cardiac stress perfusion studies were recruited and underwent additional SPECT/CT imaging of both calves at stress and/or rest. Patient had ankle brachial indexes (ABIs) measured. Under the imaging protocol, subjects had 12 lead ECG and blood pressure monitoring for the cardiac stress test. In order to gauge exercise capacity, parameters such as percent age-predicted heart rate goal and metabolic equivalents (MET) were used. Volumes of interest (VOIs) over 5 major calf muscle groups were then drawn on CT slices using Osirix in accordance with the VH Dissector Pro anatomy atlas. The VOIs were then superimposed onto the SPECT images for quantification of radiotracer uptake. Total radiotracer activity per volume was calculated using Osirix for each muscle group. The radiotracer activity per volume was then adjusted for injected dose (Act/Vol/ID) for each of the 5 specific muscles groups.

Results: In control patients, $^{99m}$Tc-tetrofosmin Act/Vol/ID increased from 55.3 ± 6.1 at rest to 149.2 ± 40.4 during stress (p = 0.17). This increase was not as pronounced in patients with PVD, as act/Vol/ID only increased from 92.1 ± 11.4 at rest to 106.5 ± 35.3 during stress. (p = 0.78) There was a good, although not statistically significant correlation, between calf $^{99m}$Tc-tetrofosmin Act/Vol/ID both with the age predicted heart rate (r = 0.71) and METs (r = 0.71). The lack of significance is due to the small sample size of this pilot study. The changes in activity under stress were not more pronounced within a specific muscle group. In the PAD patients with indeterminant ABIs, the observed impairment in stress perfusion with exercise did not correlate with ABIs and may reflect microvascular disease.

Conclusion: $^{99m}$Tc-tetrofosmin SPECT/CT may be a useful imaging approach for evaluation of both microvascular and macrovascular disease when combined with stress cardiac imaging. Physiologic indices of the level of exercise correlated to changes in perfusion in our population as hypothesized. Although our patients with PVD did not have angiography data, future studies will use angiographic data to correlate muscle perfusion with specific vascular territory defects.