Disagreement in middle ear volume values between tympanometry and 3-dimensional volume reconstruction
David Carpenter, Debara L. Tucci, David M. Kaylie and Dennis O. Frank-Ito

OBJECTIVES/SPECIFIC AIMS: Middle ear volume (MEV) is a clinically relevant parameter in the treatment of many common conditions including otitis media, tinnitus, and conductive hearing loss. A growing number of studies have shifted from using tympanometry to 3-dimensional volume reconstruction (3DVR) to calculate MEV; however, MEV values between these methodologies have never before been directly compared. Here, our objective is to characterize agreement between MEV measurement methods across disease states and middle ear sizes. METHODS/STUDY POPULATION: Middle ears were identified from 36 patients ranging 18–89 years of age who underwent tympanometry testing during preoperative workup for tympanic membrane (TM) perforation, up to 1 month prior to a standard-of-care temporal bone computed tomography (CT) between October 15, 2005 and October 15, 2015. MEV values calculated by both tympanometry and 3DVR were analyzed for agreement using Bland and Altman plots. A correction factor was calculated where ear canal volumes were available for contralateral middle ears without TM perforation (n = 12), and was applied to a second Bland and Altman plot in the corresponding patient subgroup. MEV agreement was characterized across MEV quartiles (1 = smallest; 4 = largest) and across increasing states of middle ear disease using Kruskal-Wallis and Wilcoxon testing with Bonferroni correction. RESULTS/ANTICIPATED RESULTS: A Bland Altman plot demonstrated significant disagreement of MEV differences as compared to a priori clinical thresholds. Absolute MEV difference was significantly greater in the average MEV fourth to first quartile (p = 0.0024), fourth to second quartile (p = 0.0024), third to first quartile (p = 0.0048), and third to second quartile (p = 0.048). Absolute MEV difference was not significantly different across varying states of middle ear disease (p = 0.44). DISCUSSION/SIGNIFICANCE OF IMPACT: Statistically evident and clinically significant disagreement was demonstrated across tympanometric and 3DVR MEV estimates. This lack of agreement was most pronounced at higher average MEV and was persistent yet not appreciably different across varying severities of middle ear disease. These findings may limit the generalizability of studies of the middle ear that differ in MEV estimation methodology, particularly in pathophysiological states where MEV is increased.

Defining peripheral B cell tolerance in pemphigus vulgaris
Nina Ran, Christoph Ellebrecht, Eun Jung Choi and Aimee Payne

OBJECTIVES/SPECIFIC AIMS: Pemphigus vulgaris (PV) is a potentially fatal blistering disease caused by autoantibodies to the keratinocyte adhesion protein desmoglein 3. Several other autoimmune diseases have defective B cell tolerance checkpoints, resulting in the accumulation of self-reactive and polyreactive B cells. METHODS/STUDY POPULATION: The present work aims to determine whether PV patients develop normal tolerance to self-antigens other than desmoglein 3, as a potential "first hit" in the development of autoimmunity. We use FACS to isolate single B cells at 4 developmental stages from 8 PV patients. We perform single-cell RT-PCR to amplify each B cell receptor, produce monoclonal antibodies, and screen these for autoreactivity using ELISA/fi to several self-antigens. At each B cell stage, we compare the frequencies of self-reactive and polyreactive B cells to those found in healthy controls. RESULTS/ANTICIPATED RESULTS: We anticipate similar frequencies between PV patients and controls, suggesting that the B cell repertoire in PV patients develops normally at early checkpoints. DISCUSSION/SIGNIFICANCE OF IMPACT: The absence of generalized reactivity would distinguish PV from other autoimmune diseases and would show that PV arises from a specific break in tolerance to a single self-antigen (desmoglein 3) during late B cell maturation. Such a result would further support PV as an ideal candidate for targeted immunotherapy.

Estimation of HIV viral load using quantitative measurement of HIV-p24 on lateral flow immunoassays
Joseph A. Conrad, Kelly Richardson, Anna Bitting, Spyros Kalams and David Wright

OBJECTIVES/SPECIFIC AIMS: High-sensitivity diagnostics for early infant diagnosis (EID) of HIV at the point of care (POC) are not widely available. Lateral flow immunoassays (LFA) can detect HIV-p24, but are not sensitive enough in practice. With improvements, LFA are a compelling platform for POC in EID. We used functionalized magnetic beads and immunocomplex dissociation to improve sensitivity of HIV-p24 LFA. Here, we evaluate the utility for LFA to quantitatively report HIV-p24 concentration and estimate HIV viral load. Using purified p24 protein and virion constructs, we determined the limits of detection for HIV-p24 using LFA rapid tests. Using measurements from HIV-p24 ELISA, laboratory-developed RT-PCR, droplet digital PCR, and gold standard clinical viral load, we further characterized the relationship between HIV-p24 concentration, HIV genomic RNA, and LFA test line signal. METHODS/STUDY POPULATION: We measured HIV-p24 concentration by ELISA (R&D Systems) and LFA (Alere Determine HIV-1/2 Ab/Ag Combo). An LFA reader instrument was used to image test lines and measure test line signal on the LFA. HIV viral loads were measured using RT-PCR and droplet digital RT-PCR protocols adapted in our lab. We obtained gold standard viral load measurements using the Roche Cobas TaqMan system at Vanderbilt University Medical Center. Data analysis was performed using Prism 7 and Stata 14. RESULTS/ANTICIPATED RESULTS: A LFA signal in a predictable, dose-dependent manner and correlates with concentrations of purified HIV-p24 with a linear range between 50 and 1000 pg/mL (Spearman r = 1; p = 0.0004). We compared p24 concentration (ELISA). We evaluated the utility of LFA to quantify HIV-p24 from virions suspended in human plasma, which increased the limit of detection for HIV-p24 to 100 pg/mL. We then increased the limit of detection to 100–10,000 pg/mL (Spearman r < 0.77; p < 0.001). To evaluate the relationship between HIV-p24 concentration and concentration of HIV RNA, we employed 3 molecular techniques. The LFA is capable of detecting HIV-p24 concentrations that correspond to a range of viral loads between 653,000 and 336,000 copies/mL.

The role of platelet factor-4 (PF4 or CXCL4) in B cell differentiation
Sara Blick, Craig Morrell, Sara Ture and David J. Field

Vanderbilt University, Nashville, TN, USA

OBJECTIVES/SPECIFIC AIMS: To investigate the role of platelet factor-4 (PF4) in B cell differentiation and development strategies to better modulate B cell differentiation in vitro and in vivo. METHODS/STUDY POPULATION: We use tissue culture and flow cytometry to examine the role of PF4 in B cell differentiation. We use wild type (WT) and PF4−/− mice on a C57Bl6j background. PF4−/− mice have reduced in vivo B cell differentiation responses. RESULTS/ANTICIPATED RESULTS: We anticipate that our studies will demonstrate that PF4 promotes B cell differentiation in the bone marrow microenvironment. DISCUSSION/SIGNIFICANCE OF IMPACT: The significance of this project may be valuable in developing efficient methods and strategies to increase or limit B cell numbers in vitro and in human disease.
The effect of skeletal muscle lipoprotein lipase overexpression on energy expenditure during weight loss maintenance and weight regain

David M. Presby, Rebecca M. Foright, Julie A. Houck, Ginger C. Johnson, L. Allyson Checkley, Vanessa D. Sherk, Michael C. Rudolph, Robera M. Olijira, Matthew R. Jackman and Paul S. MacLean

University of Colorado Anschutz Medical Campus, Aurora, CO, USA

OBJECTIVES/SPECIFIC AIMS: Obesity is a rapidly growing epidemic and long-term interventions aimed to reduce body weight are largely unsuccessful due to an increased drive to eat and a reduced metabolic rate established during weight loss. Previously, our lab demonstrated that exercise has beneficial effects on weight loss maintenance by increasing total energy expenditure above and beyond the cost of an exercise bout and reducing the drive to eat when allowed to eat ad libitum (relapse). We hypothesized that exercise’s ability to counter these obesogenic-impetuses are mediated via improvements in skeletal muscle oxidative capacity, and tested this using a mouse model with augmented oxidative capacity in skeletal muscle.

METHODS/STUDY POPULATION: We recapitulated the exercise-induced improvements in oxidative capacity using FVB mice that overexpress lipoprotein lipase in skeletal muscle (mLPL). mLPL and wild type (WT) mice were put through a weight-loss-weight-regain paradigm consisting of a high fat diet challenge for 13 weeks, with a subsequent 1-week calorie-restricted medium fat diet to induce a ~15% weight loss. This newly established weight was maintained for 2 weeks and followed with a 24-hour relapse. Metabolic phenotype was characterized by indirect calorimetry during each phase. At the conclusion of the relapse day, mice were sacrificed and tissues were harvested for molecular analysis.

RESULTS/ANTICIPATED RESULTS: During weight loss maintenance, mLPL mice had a higher metabolic rate (p = 0.0256) that was predominantly evident in the dark cycle (p = 0.0001). Furthermore, this increased metabolic rate was not due to differences in activity (p = 0.2877) or resting metabolic rate (p = 0.4881). During relapse, mLPL mice ingested less calories and were protected from rapid weight regain (p = 0.0235), despite WT mice exhibiting higher metabolic rates during the light cycle (p = 0.0421). DISCUSSION/SIGNIFICANCE OF IMPACT: These results highlight the importance of muscular oxidative capacity in preventing a depression in total energy expenditure during weight loss maintenance, and in curbing overfeeding and weight regain during a relapse. Moreover, our data suggest that the thermic effect of food is responsible for the differences in metabolic rate, because no differences were found in activity or resting metabolic rate. Additional studies are warranted to determine the molecular mechanisms driving the ability of oxidative capacity to assist with weight loss maintenance.

BIOMEDICAL INFORMATICS/HEALTH INFORMATICS

Two EMR query strategies to assess prevalence of adrenal incidentaloma

Michio Taya, Viktoriya Paroder, Linda Haramati and Eran Bellin

OBJECTIVES/SPECIFIC AIMS: To compare methods of ascertaining prevalence for adrenal incidentalomas METHODS/STUDY POPULATION: Retrospective electronic medical record study using Looking Glass Clinical Analytics (Streamline Health, Atlanta, GA, USA) at an urban university medical center. All patients with CT or MR imaging of the abdomen between 1997 and 2014 were identified. Patients with a documented diagnosis (ICD-9 code or problem list) for any history of adrenal disease were excluded. The prevalence of adrenal incidentalomas was ascertained by 2 different detection strategies: (1) documented diagnosis of adrenal incidentaloma or (2) imaging reports containing in the same sentence “adrenal” and “nodule”, “adenoma”, or “mass,” and not containing “no” and “adrenal” in the same sentence. Adrenal pathology surprise was further established in the second approach by excluding patients having previously undergone adrenal lab testing (cortisol, aldosterone, catecholamines, adrenocorticotrophic hormone, renin) or having been registered in the cancer registry for any cancer excluding superficial skin cancers.

RESULTS/ANTICIPATED RESULTS: In total, 194,624 individuals were identified in our initial search, from which 1056 were excluded for past adrenal disease (Table 1). Detection by the documented diagnosis method yielded 1578 cases (0.8%), compared with 13,697 cases (7.1%) by the imaging report method (Figure 1). Further restricting detection to true “Adrenal Surprise” by excluding those with any past adrenal lab testing and cancer history yielded 10,568 cases (6.1%). Validation studies for the 7.1% prevalence with 100 records revealed an adrenal incidentaloma positive predictive value (PPV) of 98%. When restricted to size ≥1 cm the PPV was 84%. DISCUSSION/SIGNIFICANCE OF IMPACT: Comparing our first strategy using documented diagnoses as criterion for incidentaloma as used in a recent paper by Lopez D (Annals of Internal Medicine 2016: 165: 533–542), we found a prevalence of 0.8% in our population similar to her 0.6%. However, when searching at the level of radiology report text, we found a prevalence ten-fold greater at 7.1%. Therefore, adrenal incidentalomas are more robustly identified by searching radiologic reports.