

**CASE WESTERN RESERVE UNIVERSITY
SCHOOL OF MEDICINE**

**IRWIN LEPOW
STUDENT
RESEARCH DAY**

THURSDAY, JANUARY 8, 2009

10:00 A.M. – 5:30 P.M.

WOLSTEIN RESEARCH BUILDING LOBBY – POSTER SESSION

BIOMEDICAL RESEARCH BUILDING - ROOM 105 SPEAKERS

AGENDA

Thursday, January 8, 2009

LOBBY OF WOLSTEIN RESEARCH BUILDING

- | | |
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| 10:00 – 2:00 p.m. | Poster Session |
| 11:00 – 12:30 p.m. | Poster Review by the Judges |
| 12:30 p.m. | Buffet Lunch
For student participants, judges and invited guests |
| 1:00 – 2:00 p.m. | Judges meet |

BIOMEDICAL RESEARCH BUILDING ROOM 105

- | | |
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| 2:00 – 4:00 p.m. | Introductory remarks from Dr. Michael Lamm
Oral Presentations by Students |
| 4:00 – 5:00 p.m. | Dean Pamela Davis' talk |
| 5:00 – 5:30 p.m. | Presentation of awards to students |
| 5:30 | Reception in the lobby |

Oral Presentations
2009 Irwin H. Lepow Medical Student Research Day
January 8, 2009 at 2:00 pm
BRB 105

2:00 – 2:30: Introductory remarks by Robert L. Haynie, M.D., Ph.D., Associate Dean for Student Affairs, and Michael Lamm, M.D., Professor, Department of Pathology, both of the Case Western Reserve School of Medicine

Oral presentations by students, 2:30 – 4:00

2:30: George Anesi. HIV-1 negative factor (Nef) protein in Kaposi's sarcoma.

Mentor: Ethel Cesarman, M.D., Ph.D., Department of Pathology and Laboratory Medicine, Weil Cornell Medical College, New York, NY

2:45: Alex Davis. Investigation of the biomechanical consequences of sub-fracture damage of cancellous bone.

Mentor: Christopher J. Hernandez, Ph.D., Department of Mechanical and Aerospace Engineering, Case Western Reserve University

3:00: Candida (Didi) Desjardins. A remote and non-contact measurement of the blood pulse waveform with a laser Doppler.

Mentors: Lynn Antonelli, Ph.D., Jennifer Cummings, M.D., and Edward Soares, Ph.D., Naval Undersea Warfare Center (Division Newport, R.I.), The Cleveland Clinic Foundation, UMASS Memorial Hospital

3:15: James Gatherwright. Augmentation of the regeneration of peripheral nerve defects with the transplantation of donor-derived bone marrow stromal cells

Mentor: Maria Siemionow, M.D., Ph.D., D.Sc., Department of Plastic Surgery, The Cleveland Clinic Foundation

3:30: Sungho Lee. Mouse model of Alzheimer's disease deficient for fractalkine receptor exhibits reduced beta-amyloid deposition.

Mentor: Bruce T. Lamb, Ph.D., Department of Neurosciences, The Cleveland Clinic Foundation, and Department of Genetics, Case Western Reserve School of Medicine

3:45: Aaron Lindsay. Inner ear protein networks and biomarkers in a mouse model for deafness in Usher Syndrome 1F and DFNB23.

Mentor: Kumar Alagramam, Ph.D., Center for Proteomics and Mass Spectrometry and Department of Otolaryngology, Case Western Reserve School of Medicine

4:00: Pamela B. Davis, M.D., Ph.D., Dean of the School of Medicine, Case Western Reserve University. "Thinking Small: Nanoparticles for Gene Therapy."

5:00 – 5:30: Awards Ceremony

5:30 on: Reception, BRB lobby

SALIM-TAMUZ ABBOUD

Reproducibility of Serial Optical Coherence Tomography Without Pharmacologic Pupillary Dilatation

Salim Abboud

Department of Neurology, The Cleveland Clinic, Mellen Center

Background:

Optical Coherence Tomography (OCT) is a technique proposed for longitudinal monitoring of multiple sclerosis, and as an outcome measure in clinical trials. However, little is known about the precision of serial measurements when implemented without the use of pharmacologic pupillary dilatation (PPD). Quantification of the variability of serial measurements is necessary for sample size calculations in planning clinical trials.

Methods:

Peripapillary retinal nerve fiber layer thickness (RNFLT) and macular volume (MV) were serially measured in ten consecutive healthy volunteers (20 eyes) using the Zeiss Stratus OCT system by "Fast RNFL" and "Fast macular thickness" scan protocols without PPD. In each subject, two serial measurements were obtained at least one week apart by a single operator. A third set of measurements was acquired using the "repeat" scan registration function to evaluate its reproducibility compared to serial independent measurements. Only signal strengths of 6 and above were accepted for each scan. The relationship between signal strength and reproducibility was evaluated.

Results:

Mean RNFLT in the group was 96.65 μ m. Mean macular volume was 6.81mm³. Coefficients of variation (COV) for independent serial measures was 2.86% for RNFLT and 1.90% for MV. COV for RNFLT and MV using the repeat function were 3.14% and 1.16%, respectively. Median signal strength for RNFLT was 8 (range 6.5-10), and for MV was 9 (range 6.5-10). The correlation between OCT signal strength and individual COV for serial independent measure of RNFL approached significance ($r=-.41$, $p=0.07$).

Conclusions:

Serial measurements of RNFL and MV are sufficiently precise to employ as outcome measures in clinical trials when implemented without PPD. Despite a trend for higher signal strengths to provide more precise data, signal strengths greater than 6 are easily achievable and highly precise. Reproducibility may be lower in patients with MS who potentially have impairment of visual acuity and ocular motility.

Supported by the Crile Fellowship

GEORGE ANESI

HIV-1 negative factor (Nef) protein in Kaposi's sarcoma

George L. Anesi, BS, MS-II, Ethel Cesarman, MD, PhD

Department of Pathology and Laboratory Medicine
Weill Cornell Medical College, New York, NY

Infection with the human immunodeficiency virus-1 (HIV-1) predisposes patients to Kaposi's sarcoma (KS), a vascular tumor caused by the Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV-8). Independent KSHV infection confers far less risk of developing KS versus KSHV coinfection with HIV-1 which shows a dramatically increased risk of tumor development reported at up to 50% at 10 years. This increased risk is beyond what would be expected with immunodeficiency alone. It seems to not be KSHV infection alone that leads to KS but rather also an environment conducive to KSHV replication and transformation provided by HIV-1. KS cells contain the KSHV genome, but not the HIV genome, so an effect would have to be indirect or produced by a secreted HIV protein. HIV-1 negative factor (Nef) protein is produced by HIV-1 and required for viral replication and plays a significant, though not entirely understood role, in its attack on the host. Specifically, Nef is protective for infective cells against cytotoxic T lymphocyte attack and apoptosis. Infected cells release Nef into the extracellular environment and it has been shown that Nef is taken up by B cells. We hypothesize that Nef could play a role in the transformation of KSHV-infected cells and as such could contribute to the dramatic increase in risk of KS development in HIV-1/KSHV coinfection versus KSHV infection alone. We analyzed skin and lymph node tissue biopsies of KS lesions from HIV-1-positive patients for the distribution of the HIV-1 Nef protein. We used immunofluorescence to demonstrate the presence of HIV-1 Nef in cells positive for the KSHV protein latency-associated nuclear antigen (LANA), currently unreported in the literature. LANA-positivity indicates that these Nef+ cells are indeed KSHV infected and further analysis confirmed these cells were also CD34+ suggesting endothelial derivation consistent with the vascular, spindle cell lesions of KS. Further research must be done to evaluate what regulatory role HIV-1 Nef plays in KSHV-infected cells and in KS development.

GEORGE L. ANESI

Autonomy and Beneficence in Conflict: Weighing Patient Confidentiality and a Duty to Warn Relatives about an Inherited Cancer Risk

George L. Anesi, BS, MS-II and Georgia Wiesner, MD

Department of Bioethics and Department of Human Genetics
Case Western Reserve University School of Medicine and University Hospitals Case Medical Center

The expectation of an agreement of confidentiality is central to the patient-physician relationship. Such an agreement is based on practical, ethical, and legal principles. Confidentiality, however, is not by default infinite. Challenges to patient confidentiality have arisen in the fields of infectious diseases and psychiatry where the health status of patients—a dangerous and transmissible infection or a violent state of mental instability, respectively—could potentially threaten the health or lives of third parties. In such cases, a potential “duty to warn” was seen in which physicians might seek or be required to breach confidentiality in an effort to avert harm to a threatened third party. This dilemma has arisen anew in the field of genetics, where the detection of a genetic abnormality in many situations immediately and automatically reveals information about potential health risks faced by family members of the proband. Nowhere is this new challenge to confidentiality more important—and indeed becoming increasingly more so—than in the testing for and treatment of inherited cancers. We now have the capacity to test for a number of cancer-associated alleles and identify carriers who are at far higher risk of developing a given malignancy. The knowledge of carrier status can allow for utilization of a number of extremely important prevention and treatment strategies that may lead to significant improvements in morbidity and mortality. We have sought to investigate the question of whether or not a physician has a duty to warn relatives about an inherited cancer risk against a patient’s wishes and in doing so, breach patient confidentiality. The dilemma will be investigated on legal, professional, practical, and ethical grounds, in effort to provide clinicians with guidance in navigating this and related issues of confidentiality and third-party risk.

GEORGE L. ANESI

Noise Reduction in the Neonatal Intensive Care Unit

George L Anesi, BS, Lucas Donovan, BA, Monica Reddy, BA, Jason Young, BA, Emily Hull, BA, Audrey Choi, BA, Tristan Klosterman, BA, Anne Newcomer, BA, King Ogbogu, BA, Thomas T Lai, MD, Cynthia F Bearer, MD, PhD, Michele C Walsh, MD, MS

Pediatrics, Division of Neonatology
Rainbow Babies and Children's Hospital, Cleveland, OH; University of Maryland, Baltimore, MD

In the intensive care unit, noise and other external stimuli have been documented to have adverse effects on patients. The Yacker Tracker is a continuous sound meter without recording capabilities that may provide a visual cue for caregivers and family members to reduce their noise levels in the NICU. We hypothesized that with use of the Yacker Tracker, the number of events where noise levels exceeded 70 dB, the maximum sound levels (Lmax), would be reduced by 20%. Sound levels were recorded in two different 6 bed nurseries for 24 hrs continuously at baseline with a SoundPro DL sound level meter. Average sound level (Lavg), maximum sound level (Lmax), and the highest sound pressure level (Lpk) were recorded at 60 second intervals. Lavg and Lmax were weighted using the slow A scale. After baseline measurements, a Yacker Tracker was placed in each nursery in a central location. Caregivers were then informed of the project and the Yacker Tracker was set to 70 dB for 24 hrs as an introductory period before being set to 60 dB. After 1 week, post intervention measurements were made continuously for 24 hrs and compared to baseline measurements. Both nurseries had significant decreases in the amount of time Lavg was above 60 dB. Nursery A also had a significant decrease in the amount of time Lmax was above 70 dB, and nursery B had a significant decrease in the amount of time Lmax was above 60 dB. The Yacker Tracker is an inexpensive tool to help decrease noise in the NICU. In conjunction with caregiver education, the Yacker Tracker significantly reduced noise levels in the NICU after 1 week. With longer use, a greater noise reduction may be made.

This research was supported in part by the Department of Pediatrics at University Hospitals Case Medical Center and by The Mary Ann Swetland Center for Environmental Health at Case Western Reserve University School of Medicine. Yacker Trackers were provided by Learning Advantage, Inc., Timnath, CO. The authors wish to thank the following individuals for their assistance in the project's conception: Daniel Wolpaw, MD and Jerry Strauss, PhD.

Canaan Baer

Active Tuberculosis and HIV Case Finding

Canaan Baer, Mary I. Huang and Dr. Christopher Whalen

Department of Epidemiology and Biostatistics
Case Western Reserve University School of Medicine, Makerere University School of Public Health

BACKGROUND: Transmission of tuberculosis is diminished within days of initiating appropriate treatment; most disease spread occurs before treatment is started. The development of a cost-effective approach to actively identify cases of tuberculosis is necessary to reduce transmission.

OBJECTIVE: To address Uganda's TB/HIV burden, we are testing the effectiveness of a community-based chronic cough survey to actively screen for TB/HIV.

DESIGN: In the Rubaga Division of Kampala, Uganda, we are conducting chronic cough surveys among residents greater than 15 years old who can communicate in Luganda or English and plan to reside in the household for >1 week. Identified chronic coughers (cough ≥ 2 weeks) are further evaluated for TB and HIV with the Tuberculin Skin Test (TST), sputum microscopy, and HIV testing. If indicated, a physical exam and chest radiography are performed, and referral is given to appropriate care. Effectiveness of the chronic cough survey is determined by the number of subjects needed to screen to identify a case of TB/HIV.

RESULTS: 4202 subjects were surveyed from January to October 2008. The prevalence of tuberculosis in this population was 0.67%. 148 cases (5.4%) experienced chronic cough. Among the chronic coughers, 71 subjects (48%) had latent TB infection, and 64 (43.2%) were HIV seropositive. Of the chronic coughers, 29 cases (19.6%) had active tuberculosis; 25 of these cases had a positive AFB sputum smear ($n=20$) or culture ($n=5$). HIV infection was present in 8 cases, giving a prevalence of 5.4% among chronic coughers and a prevalence of 29.6% among those identified with active disease.

CONCLUSION: Our survey, based on self-reported cough of 2 weeks or more, effectively identifies members of a community with high likelihood of having active tuberculosis; one would need to evaluate only 5 chronic coughers to find an additional case of TB. HIV rates were also high among cases of TB.

Supported by the National Institute of Health (T32) Training Grant in Pulmonary Host Defense Infectious Disease Society of America Medical Scholars Program

Jason Balkman

Dual Energy Subtraction Digital Radiography Improves Performance of a Commercial Computer-aided Detection Program

Jason Balkman and Robert C. Gilkeson

Department of Radiology, University Hospitals

Skeletal structures are a significant source of anatomic noise on a chest radiograph, making them a major limiting factor for the detection of subtle lung nodules for both physicians and computer-aided detection (CAD) programs. Dual energy subtraction (DES) enables the acquisition of a soft tissue only chest radiograph and has shown potential to improve physician performance in the detection of subtle cancers. Few studies have used DES to examine its effect on CAD performance, which is often poor because of difficulties distinguishing bony structures. The purpose of this study was to apply a commercial CAD program to the analysis of both standard posteroanterior (PA) and DES chest radiography, and compare the sensitivity and number of false-positive marks achieved by the CAD system in both cases.

One hundred and two patient records were retrospectively identified as having DES radiographs and pulmonary nodules confirmed by CT. Those patients with biopsy proven lung carcinoma ($n=45$) were selected and the panel was narrowed to identify patients with lung nodules 8-30 mm in size ($n = 36$) to satisfy the search criteria for the CAD system. The final panel of 36 patients with a total of 48 nodules was evaluated.

The sensitivity of the CAD program with the standard PA was 46% (22 of 48 nodules) compared to 67% (32 of 48 nodules) using the DES soft tissue, or bone-subtracted view ($P=0.064$). The average number of false positives per image (FPPI) identified by CAD was significantly lower using DES (FPPI_{ST}=1.64) when compared to the standard PA chest radiograph (FPPI_{PA}=2.39) ($P<0.01$).

Our results demonstrate the potential of DES to improve CAD performance on subtle lung cancer lesions, particularly those concerned with overlaying bone shadows on the standard PA chest radiograph. The decrease in false-positives should improve reader performance and radiologist acceptance of the CAD technology.

Supported by Ohio State Grant

Jennifer Bauer

Gross Anatomical Study of Lumbosacral Vertebrae

Jennifer Bauer and Dr. Allison Gilmore

Pediatric Orthopaedics, Univeristy Hospitals Rainbow Babies and Children

Purpose: Lumbosacral transitional vertebrae are believed to cause lower back pain, but their prevalence is disputed. Past studies used radiographic images of pre-selected populations to catalogue the anomaly into categories originally laid out by Castellvi. The sum of the anomalies in each study ranged from 4.6% to 30%, with as many as 6 other percentages found. By performing a gross anatomical study on disarticulated skeletons of a nonselected population, we will determine a prevalence void of selection bias and radiologic inconsistency. This will better determine its importance in a lower back pain differential diagnosis.

Methods: Using the Hamann-Todd Osteological Collection of the Cleveland Museum of Natural History, we examined 2990 skeletons. Exclusion criteria included sacra missing, damaged, or younger than 12 years old. Abnormal sacra were photographed and classified into Castellvi categories IIa-IV.

Results: In a sample size of 2,865 sacra and lumbar vertebra were examined an anomaly was seen in 392 sacra. 168 are easily categorized into Castellvi categories, and 224 have intermediate characteristics similar to both normal and transitional vertebra. The questionable sacra do not immediately fit into a category, but depending upon their eventual classification, prevalence may range from 5.8% to 13.6%.

Conclusion: The close anatomical study allowed an appreciation of a wider range of anomalies than the few categories of Castellvi's. The variations make classification too subjective for only one researcher's observation. Further studies are underway for each anomalous sacra to be independently classified by each of 4 different orthopaedic surgeons, with a sub-sample blinded re-check.

Significance: Castellvi categories used by past studies are not inclusive of the spectrum of anomalies seen at the L5/S1 joint. There is a potentially higher prevalence of lumbosacral transitional vertebrae in the general population than in the populations presenting with pain. If true, many transitional vertebrae must be asymptomatic.

Supported by Crile Foundation Paul Curtiss, M.D. Award, UH Orthopaedic Department

Joshua Bear

The Role of Angiogenesis in Tumor Maturation: Oxygen Delivery or Waste Elimination? Joshua Bear, Dr. Hanping Wu and Dr. John Haaga

Department of Radiology, University Hospitals Case Medical Center

Although the process of angiogenesis in tumor growth has been defined and studied for decades, recent advances in our understanding of the process are being realized through the use of computed tomography (CT) to study blood perfusion patterns. New questions regarding the actual role of angiogenesis in the life cycle of tumors have stimulated further research to investigate whether the role of tumor angiogenesis is to provide the lesions with nutrients or merely to offer a way to dispose of metabolic wastes. This study examines the changes in tumor perfusion over four weeks of growth using a rabbit model to support concurrent studies exploring the role of tumor angiogenesis.

Tumors were injected into the livers of nine rabbits and allowed to grow for five weeks. Blood perfusion measurements using a high-resolution CT scanner were taken every week. The measurements were analyzed using Siemens Medical Solutions' *syngo*® MultiModality Workplace to calculate perfusion values in the following regions of interest (ROIs): aorta, tumor center, tumor ring, adjacent liver, remote liver. The data were entered into Microsoft Office Excel 2008 in order to calculate the time to start (T0), time to perfusion (TP), and tissue blood ratio (TBR).

Although the study is ongoing, current analyses demonstrate that the slope of enhancement for both the tumor ring and the tumor center decreases over time while the slope of washout for both ROIs increase over time ($p < 0.05$). In contrast, the enhancement and washout for both adjacent and remote liver controls do not change over time.

The results obtained are inconclusive by themselves, but lend support to the hypothesis that tumor angiogenesis is more important as a means to eliminate wastes than as a means to obtain nutrients. Concurrent and future studies are underway to elaborate on this hypothesis.

Student research funded by the National Institutes of Health (NIH) T35 grant.

Candice A. Bookwalter

Multiple Overlapping k-space Junctions for Investigating Translating Objects

Candice A. Bookwalter and Mark A. Griswold and Jeffrey L. Duerk

Department of Biomedical Engineering and Department of Radiology, Case Western Reserve University

INTRODUCTION: Magnetic Resonance Imaging (MRI) is a useful clinical imaging tool in both diagnostic and interventional radiology. However, MR images are susceptible to corruption by motion such as bulk motion from an uncooperative patient or respiratory motion which may obscure useful clinical information. Traditional methods for motion artifact correction including respiratory gating and navigator echoes undesirably increase imaging time. We describe a novel method called Multiple Overlapping k-space Junctions for Investigating Translating Objects (MOJITO) which is a k-space (i.e., MRI raw data) based self-navigated method without significantly increasing acquisition time. The MOJITO method requires a trajectory (i.e., order of acquiring raw data in k-space) which has multiple intersections.

METHODS: This study investigates the performance of MOJITO in the presence of confounding factors such as noise and field inhomogeneities when BOWTIE trajectory intersections are used. Multiple calculated phase differences ($\Delta\phi$) and known k-space locations (k_x and k_y) are used to calculate a time-dependent representation of motion (Δx and Δy) occurring throughout a BOWTIE acquisition using the equation $\Delta\phi = \Delta x k_x + \Delta y k_y$. Simulations, phantom experiments, and in vivo experiments were used to determine the effects of signal-to-noise ratio (SNR) and off-resonance.

RESULTS/DISCUSSION: Noise simulations showed that an SNR of 12 was sufficient for 1 mm accuracy in both in-plane directions. Off-resonance simulations showed a small drift and offset error in Δx and a discontinuity in Δy . Phantom and in vivo data matched simulations results where Δx is detected with good fidelity, while Δy demonstrated a severe discontinuity. The phantom and in vivo images corrected with only Δx showed excellent results for motion in the x-direction. Unlike conventional motion artifact correction techniques, MOJITO provides artifact correction without the loss of efficiency seen in traditional methods. The MOJITO motion artifact correction method will afford new efficiency in correcting 2D rigid body translational motion.

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Adriane Boyle

Do donor registries and first person consent laws accurately fulfill donor preferences?

Adriane Boyle, Stuart Youngner, MD

Department of Bioethics, Case Western Reserve University School of Medicine

Background: Organ and tissue transplantation has the potential to improve and save many lives, but there is a significant shortage of organs available for transplantation. Two methods intended to fix the organ shortage that are in use today are donor registries and first person consent laws. Although these registries and laws have been in place for years in some states, there is not much literature examining their performance since implementation and whether they are meeting their goals.

Methods: To evaluate the effectiveness of donor registries, and examine whether donor registries and first person consent laws fulfill the concept of informed choice, we created a survey assessing knowledge and attitudes regarding organ and tissue donation. Data was collected in person or over the phone from 64 faculty members in the basic sciences departments at CWRU Medical School, and was analyzed using SPSS.

Results: Descriptive data reveal that most of the study population was in favor of donating (90.6%) but was not very knowledgeable about donor registries or first person consent laws. The majority of the study population supported including more options for donors to express their preferences when joining a donor registry. Most respondents agreed with first person consent laws in theory (79.7%), but there was a notable minority (34.4%) for whom first person consent laws conflicted with their personal preferences.

Conclusions: Further studies assessing the knowledge and attitudes of the general population regarding donor registries and first person consent laws need to be conducted. However, our study reveals that there is relatively low knowledge even among medical school faculty regarding donor registries and first person consent laws. This study also suggests several areas in which they can be improved to ensure that donor preferences are respected, and can serve as a springboard for future studies.

Supported by the Department of Bioethics

Rebekah C. Brown

Proteomic Analysis of Human Synovial Fluid, Synovium and Cartilage in Healthy and Osteoarthritic Subjects: An Investigation of the Knee Joint

Rebekah C. Brown, Reuben Gobezie MD., James Crish Ph.D., Eldra Daniels, Eric Rodriguez, Mark Chance Ph.D., Gurkan Bebek Ph.D., Tim Henderson, Serguei Ilchenko Ph.D., Giri Gokulrandan Ph.D., Patrick Leahy Ph.D. and Chunbiao Li

University Hospital Department of Orthopedics, Case Center for Proteomics and Bioinformatics, The Gene Expression and Genotyping Core Facility, Case Comprehensive Cancer Center, Case Western Reserve University

Osteoarthritis is a multifactorial degenerative joint disease characterized by pathophysiologic changes to synovial joints. (e-medicine) Osteoarthritis (OA) of the knee joint is a huge problem affecting, approximately, 27% of people over the age of 45. **[MD Consult Epidemiology of Osteoarthritis Rheumatic Diseases Clinics of North America - Volume 34, Issue 3 (August 2008)]** The question to ask is how do we mitigate OA and its effects? Through proteomics, “[t]he study of the structure and function of proteins, including the way they work and interact with each other inside cells.”

[www.cancer.gov], we can answer key questions; namely, what is the proteome of an arthritic individual’s synovium, synovial fluid, and cartilage; what are the biological pathway alterations between these tissues and disease states; and what is the molecular level of alteration(s) defining osteoarthritis? These questions were explored using several proteomic techniques and microarray analysis. The synovial fluid, synovium and cartilage samples were prepared from five individuals for 1-D PAGE electrophoresis with subsequent in-gel digestion and Western Blot analysis. The in-gel digestion products were analyzed by FT/LTQ and OrbiTRAP mass spectral analysis, MASCOT and MASRAN (MASCOT Results Analyzer) within the Case Center for Proteomics and Bioinformatics. Western Blot analysis was performed targeting the proteins Gelsolin and Afamin. RNA isolation procedures were performed in the Gobezie lab for microarray analysis at The Gene Expression and Genotyping Core Facility, Case Comprehensive Cancer Center. Analysis showed that there are proteomic differences between disease states. In synovial fluid, the proteome of the early osteoarthritis (EOA) group is greater than the healthy group, which was greater than the late osteoarthritis (LOA) group. The LOA proteome is greater than the EOA proteome in synovial tissue and cartilage. Additionally, within the same individual, the proteome differs between tissue types. The proteome is larger in synovium versus that of synovial fluid and cartilage. Preliminary microarray analysis results substantiate these proteomic results. Results are still pending regarding the biological pathway analysis. So far, it is evident that there are discrete protein alterations between healthy and osteoarthritic individuals. This knowledge of proteomic changes associated with OA can be translated into obtaining definitive diagnoses and earlier detection of the disease. Focusing on a proteomic approach to study osteoarthritis, such as biological pathway alteration, can potentially yield very insightful information about the arthritic process.

Supported by NIH Heart, Lung and Blood Institute Grant: Ruth L. Kirschstein National Research Service Award Short-Term Institutional Research Training Grants (T35)

Lauren Cao

Evaluation of cardiovascular disease risk profile in psoriasis patients by the use of pro-inflammatory surrogate markers of cardiovascular disease in CT scan, ultrasound and serum findings

Lauren Cao, B.S., Rivka Feig and Neil Korman, MD/PhD

Department of Dermatology, University Hospitals Case Medical Center

Psoriasis is a recrudescing immune-mediated disease that affects 2% of U.S. population, with costs exceeding \$1 billion. A growing literature suggests association of psoriasis with cardiovascular diseases (CVDs). The skin-driven vascular inflammation, propagated by elevated levels of pro-inflammatory S100A8/A9 and VEGF released from psoriatic plaques, may contribute to increased CVD risk seen in psoriasis. This cross-sectional study will assess the propensity of psoriasis patients to develop CVD compared to properly-selected controls, as measured by coronary artery calcification scoring (CACs) CT scan; carotid intima-media thickness (CIMT) and flow-mediated dilation (FMD) ultrasound results. We are stratifying by disease severity (moderate-severe, mild, control), age (≥ 40 , < 40), matching for sex and BMI ($\pm 3\text{kg/m}^2$). Our sample size is 132, but we will perform a preliminary analysis before January 2009, once we have data on 5 patients per strata. After adjusting for co-morbidities which also increase CVD risk, we expect that moderate-severe psoriasis patients will have higher CACS and CIMT than mild psoriasis patients, who will have higher levels than controls ($p < .01$). Moderate-severe psoriasis patients will have less dilation in their brachial artery during FMD than mild psoriasis patients, who will have less dilation than controls ($p < .01$). Moderate-severe psoriasis patients will have higher serum levels of S100A8/A9 and VEGF than mild psoriasis patients, who will have higher levels than controls ($p < .01$). We expect that this study will also demonstrate other trends, which merit future studies: Moderate-severe psoriasis patients will have higher numbers of plaques in their carotid arteries, higher serum levels of hsCRP, myeloperoxidase, leptin and resistin, and higher skin levels of S100A8/A9 and VEGF, epidermal thickness, and dermal inflammatory infiltrates than mild psoriasis patients, who will have higher levels than controls. Moderate-severe psoriasis patients will have lower serum levels of paraoxonase and adiponectin than mild psoriasis patients, who will have lower levels than controls.

Supported by Murdough Family Center for Psoriasis (University Hospitals Case Medical Center)

Shelley Chang

Skin and Environmental Contamination by Patients With Methicillin-Resistant Staphylococcus aureus (MRSA) Occurs Before Admission PCR Results Become Available

Shelley Chang, Curtis J Donskey, Usha Stiefel, Jennifer L. Cadnum, and Ajay K Sethi

Department of Epidemiology and Biostatistics,
Case Western Reserve University School of Medicine, Louis Stokes VA Medical Center

Background: Active surveillance to detect patients colonized with MRSA is increasingly practiced in healthcare settings. However, inpatients may have already become sources of transmission before appropriate precautions are implemented.

Objective: We examined the frequency of MRSA contamination of commonly touched skin and environmental surfaces before patient carriage status became known.

Methods: We conducted a 6-week prospective study of patients colonized with MRSA at a hospital where active surveillance is performed via nasal PCR screening on admission. Skin and environmental contamination were assessed within hours of PCR completion.

Results: In April-May 2008, 83/113 patients identified via positive admission PCR for MRSA were enrolled. Overall, 38/74 (51%) and 37/83 (45%) patients had skin and environmental contamination, respectively. 75% of samples were collected within 7 hours after PCR completion, and 88% were collected before PCR result notification. By 25 and 33 hours post-admission, at least 18% and 35% of MRSA patients had contaminated their environments, respectively. Among the 32 (39%) patients who had previously shared a room, 13 (41%) had contaminated their environment. Median time from admission to PCR completion and from result to notification were 20 hours (interquartile range (IQR) [18, 23])) and 23 hours (IQR [21-28]). Nasal MRSA density >500 colony-forming units was also associated with skin or environmental contamination (76% vs 40%; $P=0.005$, and 71% vs 33%; $P=0.002$).

Conclusions: By the time precautions are implemented, many screened patients have already contaminated their skin and environment with MRSA. The first few hours post-admission represent important opportunities to reduce risk of cross-transmission. Strategies to reduce delays, to preemptively identify patients at high risk for disseminating MRSA, or to improve universal precautions are needed.

Support by This study was supported by the Department of Veterans Affairs and in part by the Geriatric Research Education and Clinical Center, Cleveland Veterans Affairs Medical Center, Cleveland, Ohio

Shelley Chang

Skin and Environmental Contamination with Methicillin-Resistant *Staphylococcus aureus* in Carriers Identified Clinically Versus Only Through Active Surveillance

Shelley Chang, Ajay K. Sethi, Brittany C. Eckstein, Usha Stiefel, Jennifer L. Cadnum, Curtis J. Donskey i

Department of Epidemiology and Biostatistics
Case Western Reserve University School of Medicine, Louis Stokes VA Medical Center

Background. Controversy exists regarding the recommendation that healthcare facilities perform active surveillance to detect patients colonized with methicillin-resistant *Staphylococcus aureus* (MRSA), as it is uncertain whether patients identified only through active surveillance represent a significant risk for transmission.

Objectives. To determine whether MRSA carriers identified only by active surveillance have a low frequency of skin and environmental contamination when compared with patients with MRSA infection or positive clinical cultures, and to identify factors associated with contamination.

Methods. We enrolled inpatients with MRSA nares colonization from June 2007 to June 2008. The density of nares colonization and the frequencies of skin and environmental contamination and hand acquisition after skin contact were compared among carriers identified only by active surveillance versus those with MRSA infection or positive clinical cultures. Log-binomial regression was performed to determine predictors of contamination.

Results. Of 115 MRSA carriers, 57 (50%) were detected only by active surveillance. For carriers detected by active surveillance versus clinically, the frequencies of skin and environmental contamination (47% vs. 50%, $P = 0.75$) and hand acquisition (38% vs. 45%, $P = 0.43$) were equivalent. Bedridden status (adjusted prevalence ratio [aPR], 2.31; 95% confidence interval [CI] 1.52-3.54), increased nares density (aPR, 1.90; 95% CI 1.37-2.65), age above 65 (aPR, 1.55; 95% CI 1.09-2.20), and MRSA bacteremia (aPR, 3.91; 95% CI 1.61-9.46) were independently associated with skin and environmental contamination. However, even ambulatory MRSA carriers age 65 or younger identified by active surveillance had a 22% frequency of contamination.

Conclusions. Half of MRSA carriers in our institution were identified only by active surveillance. These individuals were as likely to have skin and environmental contamination as those identified clinically, suggesting that strategies to limit MRSA transmission must address colonized as well as infected patients.

This study was supported by the Department of Veterans Affairs and in part by the Geriatric Research Education and Clinical Center, Cleveland Veterans Affairs Medical Center, Cleveland, Ohio.

Connie Chen

Early Release of HMGB1 After Severe Trauma in Humans: Role of Injury Severity and Tissue Hypoperfusion

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Objective: High mobility group box nuclear protein (HMGB1) is a DNA nuclear binding protein that has recently been shown to be an early trigger of sterile inflammation in animal models of trauma-hemorrhage via the activation of the Toll-like-receptor 4 (TLR4) and the receptor for the advanced glycation end-products (RAGE). However, whether HMGB1 is released early after trauma-hemorrhage in humans and is associated with the development of an inflammatory response is unknown and constitutes the aim of the present study.

Design, Setting and Patients: A prospective cohort study of severe trauma patients admitted to a single Level 1 Trauma center.

Measurements and Main Results: Two hundred-eight patients were studied. Blood was drawn within 10 minutes of arrival to the Emergency Room before the administration of any fluid resuscitation. HMGB1, TNF- α , IL-6, von Willebrand Factor (vWF), Angiopoietin-2 (Ang-2), Prothrombin time, (PT), prothrombin fragments 1+2 (PF1+2), soluble thrombomodulin (sTM), protein C (PC), plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator (tPA) and D-Dimers were measured using standard techniques. Base deficit was used as a measure of tissue hypoperfusion. The results show that plasma levels of HMGB1 were increased within 45 minutes after severe trauma in humans and correlated with the severity of injury, tissue hypoperfusion, early posttraumatic coagulopathy and hyperfibrinolysis as well with a systemic inflammatory response and activation of complement. Non-survivors had significantly higher plasma levels of HMGB1 than survivors. Finally, patients who later developed organ injury, (acute lung injury and acute renal failure) also had higher plasma levels of HMGB1 early after trauma.

Conclusions: The results of this study demonstrate for the first time that HMGB1 is released into the bloodstream early after severe trauma in humans. The release of HMGB1 requires severe injury and tissue hypoperfusion and is associated with posttraumatic coagulation abnormalities, activation of complement and severe systemic inflammatory response.

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Lauren Chmielewski

Barriers to Hypertension Management in an Urban Population in Santo Domingo

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BACKGROUND: Hypertension is a major public health concern and a profound risk of coronary artery disease, stroke, heart failure, and renal disease. Elevated blood pressure is often asymptomatic until acute cardiovascular complications arise. Thus, screening for hypertension is a critical aspect of preventative medicine. Awareness of blood pressure combined with patient education about modifiable, lifestyle changes can improve management of blood pressure and lead to better health outcomes. Blood pressure is not routinely checked at the FEDOPO clinic in Santo Domingo. In the economically disadvantaged population served by the FEDOPO clinic, the consistent practice of monitoring blood pressure could represent a cost-effective strategy with the potential for significant reductions in morbidity and mortality. **OBJECTIVE:** To improve the monitoring and management of blood pressure within the population served by FEDOPO. **METHODS:** First, to assess FEDOPO's blood pressure monitoring protocol. Second, to implement an intervention consisting of providing necessary equipment and education to the FEDOPO health care staff and patients. Finally, to reassess blood pressure monitoring post-intervention and compare to previous FEDOPO protocol. **RESULTS:** Initially, blood pressures were not routinely taken during patient encounters. It was also noted that patients were not aware of their blood pressure or how to manage it. There was no way to record or follow a patient's blood pressure over time, due to a paucity of medical record keeping. Once appropriate equipment and education was provided, blood pressure assessment became routine. Record-keeping cards for the patients with the date and their blood pressure reading were provided with the hope of improving the management of blood pressure over time. **CONCLUSIONS:** The lack of blood pressure monitoring at FEDOPO was due to the lack of necessary equipment for assessing blood pressures. Managing blood pressure requires having the appropriate equipment and record keeping by the health care worker and patient.

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Audrey Choi

Dynamics of Antioxidant Profiles of Bovine Antral Follicles: Correlation with Follicle Size, Follicle Dominance and Stages of Estrus Cycle

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The follicular fluid environment, including the antioxidant capacity of this fluid, surrounding the oocyte plays an important role in the oocyte quality, fertilization potential, and subsequent embryo development potential. The aim of this project is to characterize the follicular fluid antioxidant profile of bovine oocytes in progressive stages of follicular development and estrus cycle. The bovine model is a well-studied and recognized model for studies on human ovarian physiology.

The translational value of this research will be its application in assisted reproductive technologies (ART), such as in vitro maturation (IVM) of oocytes, as the manipulation of gametes for ART carries the risk of gamete exposure to supraphysiologic levels of reactive oxygen species (ROS). With information on normal antioxidant levels during maturation, we will be better equipped to supplement IVM culture media with antioxidants to combat oxidative stress during ART procedures.

Because no study has ever been performed on this topic, we cannot exactly hypothesize what the trend in antioxidant levels will be during the oocyte maturation process. However, we can postulate that there will be a trend over the course of maturation in the two antioxidant parameters measured.

We are measuring the catalase activity and total antioxidant capacity of follicular fluid taken from oocytes of various developmental stages. Measurements were performed using colorimetric assay kits from Cayman Chemical and analyzed by ELISA plate reader.

To date, the experiment is on going. The results, analysis and conclusion components will be forthcoming when all the samples have been tested and the data has collected and analyzed.

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Matthew Clark

Body mass index trends in normal and overweight children pre- and post-puberty

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Background: No longitudinal studies have investigated how body mass index (BMI) progresses in children from the pre-pubertal to post-pubertal period.

Purpose: We studied BMI trends in a culturally diverse cohort of urban children to investigate those factors which may be associated with BMI percentile changes post-puberty.

Methods: A retrospective chart review was conducted on electronic medical records. 1,314 subjects were identified as having at least two outpatient Pediatric Department visits: one in 1999-2000 (Time 1, T1) while 6-11 years old and one in 2006-2007 (Time 2, T2). From this initial set, 554 (42%) were ineligible (73% had missing BMI data or a BMI <5th percentile and 27% had already entered puberty). Data collected included demographic information, BMI percentiles and provider diagnosis of obesity and weight-related co-morbidities. Statistical Analysis Software (SAS, version 9.3) was used for frequency counts, univariate analyses, and regression analysis. A p-value of <0.05 was considered statistically significant.

Results: At T1, 464 (61%) subjects had a normal BMI percentile ($\geq 5^{\text{th}}$ and $< 85^{\text{th}}$ percentiles) and 296 (39%) had an overweight or obese BMI percentile ($\geq 85^{\text{th}}$ percentile). There were no statistically significant differences between the normal and overweight/obese groups with respect to gender, ethnicity and age. The odds of having a normal T2 BMI was not dependent on ethnicity. 21% of those with a normal T1 BMI percentile progressed to an overweight or obese BMI percentile at T2. Furthermore, subjects with a T1 BMI percentile in the 4th quartile (72nd-84th percentile) of the normal BMI range were 4.8 times more likely to be overweight or obese at T2 than their peers in the lower 3 quartiles of the normal range (OR 4.825, CI 2.988-7.791). 84% of the overweight and obese patients at T1 remained at or above the 85th percentile at T2.

Conclusion: A pre-pubertal BMI percentile at the high end of normal appears to be a risk factor for progression to overweight or obesity status post-puberty. Better identification and intervention for overweight, pre-pubertal children could lead to higher rates of BMI percentile improvement post-puberty

Laurence Cook

Assessment of the relationship between knowledge and disease management in patients presenting to an emergency department with asthma: In search of a common misunderstanding.

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Asthma is defined as an airway hyperresponsiveness to stimuli accompanied by edema and inflammation. The self-reported prevalence of asthma has increased by 74% since 1980 and accounts for approximately 2 million ED visits annually. Treatment of asthma in the ED can be very expensive and many episodes are preventable. The advancements in understanding the pathophysiology of asthma and the efficacy of pharmacologic agents has not translated to better quality of life for patients. This is reflected in the increased number of ED visits since 1992. A patient's understanding of their disease is crucial in the effective self-management of their asthma. The complexity of asthma as an immunological condition, coupled with its intermittent nature makes it difficult for most patients to fit asthma into a classic chronic disease framework. An understanding of asthma as an intermittent chronic disease that can be managed through the identification of asthma triggers can lead to better outcomes for the patient. Poor patient understanding of their disease process and medication use truncates the effectiveness of self-management as a viable management strategy. An analysis studying the relationship between a patient's management of their own asthma and their understanding of their disease would be effective in determining the correlation between knowledge and the effectiveness of self-management.

This project utilizes a cross sectional approach to evaluate patients who come to the Metro Health ED with self-reported asthma. Patients who come to the ED and have a self-reported history of asthma have been and continue to be interviewed regarding their asthma knowledge and maintenance. The interview is conducted regardless of their reason for their ED visit is because of an asthma related complaint. The interview is designed in such a way to access the patient's knowledge of the pathophysiologic of asthma. Patients are asked if they can identify what is unique about their own asthma and its triggers. Finally, patients are asked if they understand the rational and efficacy of both home and clinical treatment strategies. The questions are a combination of true/false and open response format. The second component of the interview uses a universal set of questions used by ED visitors to assess a patient's management of their asthma. This gives the interviewer the information needed to assess how able the patient is to control their asthma.

The data gathered from these questionnaires will be used in a two-pronged approach to determine the correlation between knowledge and the effectiveness of self-management. The true/false questions lend themselves to a numerical score and will be compared to a score of their asthma management determined from the management question set. These two sets of numbers will be compared to determine correlation between knowledge and management. The free response questions will give the researchers unique insight to find common components of a patient's disease schema. These common components might lend themselves to better or worse management of asthma as a chronic disease. This vital component of the project could affectively tweeze out common misconceptions to look out for in a clinical setting. These misconceptions could then be remedied through explanations regarding their asthma.

The questions that this study addresses are ones that encompass the fields of public health and health service strategies. The questions that the project addresses are
Does better knowledge of your disease correlates with a better management of that disease? Preliminary data from the questionnaires suggests that having a good foundational knowledgebase of what asthma is as a chronic immune mediated disease gives the patient a much better chance at adequate management. Those patient's that view their disease as something that they cannot control, and a disease they cannot predict, have decreased management skills and more frequent visits to the emergency department. One of the more interesting developments from the preliminary data shows that poorer asthma knowledge correlates with your chief complaint for coming to the emergency department. If this trend in the data continues, it could mean that better patient education could not only lead to better patient outcome but also more efficient departmental resource utilization in the emergency department. This increased efficiency would stem from keeping more asthma patients out of the ED by increasing their ability to manage their own disease. Another question this project analyzes is: are their common misconception about the pathophysiology of a chronic condition that lends itself to poor disease management and more ED visits? Preliminary data from the project suggests that physicians need to do a better job helping their patients discover what their own asthma trigger is. Patients who could name their own asthma trigger seem to score much better on their management questionnaire. This is an interesting concept that will be further analyzed as the project continues.

The final question that this project poses looks at the components of a chronic condition that, when understood by the patient, empowers them to make better self-management decisions. This question will require more data, and more analysis to adequately answer and understand. This project will dissect and analyze the disease models that patients use to understand (or misunderstand) their conditions in order to identify areas where improvement would give the patient a better quality of life.

Alex Davis

Investigation of the biomechanical consequences of sub-fracture damage of cancellous bone

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To explain the observation that almost half of osteoporosis-related fractures in the spine are not related to a spontaneous loading event, it has been proposed that vertebral mechanical damage occurs due to multiple or prolonged loading events. These events are believed to lead to microscopic cracks in otherwise healthy bone and ultimately reduce bone stiffness and strength. This experiment was designed to begin investigating the characteristics of loads necessary to generate microscopic tissue damage in cancellous bone without causing an overt fracture in the cortical shell. Using caudal vertebra (7-9) from adult female Sprague Dawley rats, each vertebrae was potted in bone cement and mounted in a material testing device using custom fixtures. Once mounted in the testing device, a sinusoidal cyclic load ranging from 0N to 260N was applied at 2Hz. Loading was stopped prior to failure based on the rate of change of compliance and if this criteria was not met within 5 hours of loading. All bones were stained for microscopic damage and were embedded undecalcified in methyl methacrylate. Standard histomorphometrical analyses were used to determine bone volume fraction and amount of microscopic cracks, trabecular microfractures, and cortical shell cracks. All loaded specimens displayed considerable diffuse damage in the epiphyseal regions. Microfractures were observed in nine out of ten specimens loaded into the tertiary phase and were fewer in number in specimens loaded in the secondary phase. Very few microscopic cracks or diffuse damage were observed in the metaphyseal regions or in the cortical shell and no macroscopic or microscopic cracks were observed in the cortical bone of any of the specimens. This present investigation demonstrates that microscopic tissue damage in cancellous bone of the rat caudal vertebrae can be generated using a cyclic loading profile. The observation that trabecular microfracture was the predominant form of damage raises further questions about the failure and repair processes of trabecular bone.

Min Deng

Neural Substrates of Age-related Decline in Executive Functions: In vivo measures of gray-matter abnormalities from magnetic resonance imaging.

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Background: Executive functions are of fundamental importance for independent function in daily life, and are impaired in Alzheimer's disease, ADHD, schizophrenia and aging. Yet there is a major gap in our knowledge of the anatomical substrates associated with executive function loss.

The purpose of this study was to identify regions of cortical thinning associated with decreased performance on executive function in healthy older adults using MRI. We hypothesized that the trigger for EF decline would cause gray matter changes in neuro-anatomical regions associated with executive functioning in the elderly and these anatomical changes might be the basis for the associated clinical deterioration.

Methods: Thirty-six subjects ≥ 65 years old and free of diseases that could cause cognitive deficits were scanned with a high-resolution Siemens Avanto 1.5T MRI scanner, followed immediately by a cognitive evaluation. *A priori* cortical regions associated with executive function were established using primary and tertiary literature search. Cortical thickness measurements were calculated using FreeSurfer based on established methods. Thickness measurements in *a priori* regions were correlated with performance on two executive function tests - Trails-making test and Verbal Fluency test. Correlations were tested for statistical significance using Pearson Correlation.

Results: Cortical thinning regions associated with decreased performance on Trails-making test were found in right dorsolateral prefrontal cortex, right posterior cingulate, left supramarginal, and left rostral anterior cingulate regions ($P \leq 0.05$). Decreased performance in Verbal Fluency test correlated with thinning of right supramarginal, right parietal, and left medial orbital frontal regions ($P \leq 0.05$).

Conclusion: Decreased performance on the Trails-making and Verbal Fluency test is correlated with thinning of specific cortical regions in frontal, parietal, and cingulate regions. These findings suggest a gray-matter neuro-anatomical basis for loss of executive function.

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Min Deng

Bcl-2 photodamage and preferential killing of malignant T cells after photodynamic therapy of mycosis fungoides, using silicon phthalocyanine Pc 4

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Key Words: Cutaneous T-cell Lymphoma, Photodynamic Therapy, Pc4, Bcl-2, CD45RB, CD45RO

Mycosis Fungoides is a primary cutaneous T-cell lymphoma characterized by malignant epidermotropic-T-cells that are largely CD45RB+ and CD45RO-. In vitro studies using photodynamic therapy with silicon phthalocyanine Pc 4 indicated increased sensitivity of Jurkat T-cells to PDT-induced killing. The mechanism behind Pc 4-PDT cell killing is known to involve photodamage to the anti-apoptotic protein bcl-2. The purpose of this study was to evaluate skin biopsies from MF patients treated with Pc 4-PDT for damage to bcl-2 and preferential killing of malignant T cells. From a Phase 1 clinical trial on Pc 4-PDT, tissue samples of treated and untreated MF lesions were available from 6 patients who clinically demonstrated partial response. These biopsies were evaluated for bcl-2 expression via Western blot. Paraffin sections from the biopsies were likewise stained with antibodies to CD45RB and CD45RO, and assessed via image analysis (Image Pro Plus, Media Cybernetics). T-testing was used to determine statistical significance. Bcl-2 expression in treated lesions collected from patients who showed clinical response was significantly decreased when compared to untreated lesions. Interestingly, bcl-2 expression in treated lesions collected from patients who did not respond clinically was not different from untreated lesions. CD45RB-stained surface area was significantly reduced in treated lesions compared to corresponding untreated lesions ($p=0.0078$). Treated lesions stained with CD45RO showed marginal significance upon Pc 4-PDT ($p=0.056$). On average, the reduction of CD45RB was 2.1 times higher than the reduction of CD45RO. The ratio of reduction of CD45RB to CD45RO was marginally significant ($p=0.069$). Patients who clinically responded to Pc 4-PDT showed molecular evidence of Bcl-2 damage that was not seen in patients who did not respond to treatment. Although there was reduction in both T-cell markers, CD45RB and CD45RO, the more pronounced reduction of CD45RB compared to reduction of CD45RO suggests a trend that shows a more sensitive effect on epidermal malignant T-cells vs. benign reactive T-cells.

Candida Desjardins

A Remote and Non-Contact Measurement of the Blood Pulse Waveform with a Laser Doppler Vibrometer

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The use of lasers to detect the blood pressure waveform of humans without contact would provide a powerful diagnostic tool, particularly for burn and trauma patients. The purpose of our sensor method and apparatus invention is to remotely and non-invasively detect the blood pulse waveform of both animals and humans. The device monitors the skin in proximity to an artery using radiation from a laser Doppler vibrometer (LDV) interferometer system. This system measures the velocity (and displacement) of the pulsatile motion of the skin, indicative of physiological parameters of the arterial motion in relation to the cardiac cycle. Tests have been conducted that measure surface velocity with an LDV and a signal-processing unit, with enhanced detection obtained with optional hardware including a retro-reflective dot. The laser light reflected from the skin surface undergoes a Doppler shift due to the surface motion along the axis of the laser, is detected by an interferometer, and demodulated to obtain the velocity of the skin surface. The blood pulse waveform is obtained by integrating the velocity signal to get surface displacement using standard signal processing techniques. In this study, the continuous waveforms from dogs and humans were found to correlate with heart rate, timing of peak systole, left ventricular ejection time, and aortic valve closure. Additionally, the blood pulse waveform could be obtained from patients with potentially complicating conditions such as obesity and cardiac abnormalities. The waveforms from animals and humans along with catheterized blood pressure waveforms are being analyzed to correlate the time history of the blood pulse waveform with actual pressure values. These results demonstrate the current capabilities of the optical, non-contact sensor for the continuous recording of the blood pulse waveform without causing patient distress.

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Lynnel (Kate) Donatto

EPO Treatment for Impaired Dendritic Spine Formation Due to Placental Insufficiency

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Placental insufficiency is a complication of pregnancy that is associated with an increased risk for premature delivery and neurological and intellectual impairments including mental retardation, cerebral palsy and epilepsy. Our preliminary data suggest placental insufficiency impairs KCC2 expression in infants born preterm, compared to their peers born at term. The KCC2 transporter is a neuron-specific K-Cl co-transporter that induces the developmental switch of GABAergic synapses from excitatory to inhibitory, and it has been shown that damage to this transporter leads to a greater seizure propensity in preterm infants. In addition to regulating the intracellular chloride gradient, KCC2 is a key factor in normal neuronal branch and dendritic spine formation. We hypothesize that placental insufficiency will hinder KCC2 expression and thus dendritic spine formation, and result in a lower seizure threshold. Determining to what extent placental insufficiency results in abnormal KCC2 expression will enhance the development of effective neonatal interventions and one such treatment may be EPO. The cytokine EPO is essential for neuronal differentiation during brain development. We will evaluate whether neonatal EPO treatment can reverse this damage to dendritic spine formation.

To mimic the damage done by placental insufficiency in humans, an established rat model of systemic prenatal hypoxia-ischemia was utilized. The transient hypoxic-ischemic insult was delivered on embryonic day 18 (E18) by uterine artery occlusion. This is the time period that corresponds to the onset of synaptogenesis and dendrite development. On postnatal day 44 (P44) the rat brains were removed and stained with Golgi-Cox solution. Coronal sections that included the cortex and hippocampus were cut with a vibrating microtome, and the sections were mounted. The sensory portion of the parietal cortex and the CA1 and CA3 portions of the hippocampus were analyzed.

Neurons that have been analyzed thus far are only in the experimental and control groups without EPO treatment. They show that there is a difference in not only the spine lengths, but also in the number of spines on each neuron between the two groups. In the control groups the average number of spines has been 6.615 per 10 micron sections on a dendrite with a mean spine length of 1.734 +/-0.26 microns. The experimental groups show an average number of spines of 5.2 per 10 micron sections on a dendrite with a mean spine length of 2.299 +/-0.463 microns. In conclusion, the group that experienced placental insufficiency had less spines developed on their dendrites and spines lengths were longer and therefore less developed. Only preliminary results have been established, and more neurons from both the control and experimental groups must be analyzed. The next step is to analyze control and experimental neurons from groups treated with EPO to evaluate whether EPO is able to recover any of the damage.

Lucas Donovan

Feasibility of a Sleep Intervention for Adolescents who are Obese

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Accumulating data suggest that variations in sleep quality and duration are risk factors for obesity. Prospective studies have found short sleep duration to be a risk factor for obesity among children and adults. Irregular sleep schedules have been related to obesity among adults. Irregular sleep patterns also exist in adolescents in the form of weekend oversleep and irregular sleep. The goal of this investigation was to determine the feasibility of a sleep intervention among adolescents who are obese for increasing sleep duration and regularity. A manualized approach for improving sleep was developed based on cognitive behavioral principles (e.g., self-monitoring, problem solving and goal setting) and Motivational Interviewing strategies to increase the desire to change. The intervention with six adolescents (ages 12 to 15) and their parents consisted of three one-hour group sessions. The intervention was well received by parents (M=6.55, SD=0.3 out of a 7-point scale) with 100% attendance. Assessments were conducted at baseline (B) and four weeks later at post-intervention (P) with objective measurement of sleep and physical activity for seven days using actigraphy. Food preferences were assessed with a Likert scale. From B to P, a trend existed for a decrease in weekend oversleep (38.08 to 19.80 minutes/night, $p = 0.165$) with an improvement in 5 of the 6 subjects. Trends existed for increases in weekday daytime activity ($p=0.18$) and decreases in weekday naps ($p=0.16$) and fat cravings ($p=0.068$). No significant changes were observed for sleep duration (8:24 (hours:minutes) at B and 8:08 at P). There was no statistically significant change in BMI for this sample (2.44 B to 2.45 P, $p = 0.151$). This pilot study demonstrates the acceptability of this intervention and suggests its potential utility as a means for improving sleep schedule consistency. Continued follow up and extension with a larger sample will be needed to determine the intervention's effectiveness for short- and long-term improvements in sleep.

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Development of a multi-channel serological assay for multiple parasites

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The importance of parasitic infections in the realm of international health has already been significantly documented, but the impact on health and disability of multiple parasitic infections – polyparasitism – is only recently coming to the forefront, and thus the amount of information to be gathered is large and growing.¹ There have been significant associations seen between different helminth infections (such as between schistosomiasis and hookworm infection) and between malaria and helminth infections.^{2,3,4} This paper will describe the development of a multi-channel fluorescent antibody detection assay that assesses previous exposure to schistosomiasis, filariasis, hookworm, and malaria in the context of a large polyparasitism study in coastal Kenya. IgG4 against *Brugia malayi* antigen, BMA, was chosen as a marker for filarial exposure, because the IgG4 response against BMA is positively associated with presence and intensity of infection.^{5,6,7,8,9} IgG4 against soluble adult worm antigens, SWAP, was chosen as a marker for exposure to schistosomiasis because IgG4 levels against adult worm antigens have been shown to be associated with infection intensity in the realm of recurrent infection giving a good estimation of exposure even when controlled for age.¹⁰ Although the IgG4 level against the excretory / secretory proteins, ES, of the hookworm *Necator americanus* drops significantly after the first year, it is still present, and in areas where exposure and infection are regular, it will continually be renewed, so it is still ideal for use in assessing past exposure.^{11,12} IgG4 anti-malarial antibodies, AMA, will be used to test for previous exposure to *P. falciparum* malaria because their levels are consistent throughout ethnic populations.¹³ *Brugia malayi* have been obtained from the CDC and BMA prepared from them as well as *Schistosoma mansoni* SWAP preparation *Plasmodium falciparum* AMA preparation from within the Center for Global Health and Diseases. Beads conjugated with BMA, SWAP, and AMA are currently being adjusted in order to maximize fluorescence. ES proteins from *N. americanus* are still in the process of being obtained. Once the beads have been maximized for fluorescence, they will be tested with serum with known parasite infection history and the results will be published.

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Carey Faber

Early Outcomes using a Steroid-Avoidance Immune Suppression Protocol in Non-neonatal Heart Transplant Recipients

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Purpose: Chronic oral steroid use is a key ingredient of maintenance immune suppression (IS) in heart transplant (HT) patients (pts) and is associated with increased morbidity. In a 2-center study, we analyzed early clinical outcomes in 40 consecutive pediatric (non-neonatal) HT pts managed with a steroid avoidance protocol.

Methods: Eligible HT pts (non-sensitized pts, n=35, selected pts with mild sensitization and negative crossmatch, n=5) entered a steroid avoidance IS protocol consisting of induction therapy (thymoglobulin pre-treated with steroids) for a median duration of 5-days (3-6 days) followed by 2 drug tacrolimus-based, steroid-free IS. The primary outcome variable was freedom from moderate rejection (ISHLT 2R or antibody mediated rejection, AMR).

Results: Median age of pts was 8 yrs (1 month-22 yrs) who were followed for a median duration of 15 months (1-38 months). Indication for HT was congenital heart disease in 11 (28%) and myocardial disease in 29 (72%). Median ICU stay post-HT was 6 days and hospital stay 19 days. Moderate rejection episodes occurred in 4 pts (cellular rejection in one and AMR in 3 pts). Freedom from moderate rejection was 97% at 6 months and 89% at 1 year post-HT. Seven pts were treated for CMV antigenemia (6 asymptomatic, detected on monitoring) and one patient for post-transplant lymphoproliferative disease. In 6 pts (15%) steroids were either continued after first 5 days post-HT or restarted. One of these 6 pts received maintenance steroids post-rejection episode. Post-HT survival was 92% at 6 months and 88% at 12 and 24 months. Four deaths occurred; 3 early hospital deaths due to multi-organ failure and one 8 months post-HT due to AMR.

Conclusions: An IS protocol of induction followed by steroid avoidance was associated with low incidence of moderate rejection during the first year after heart transplant in young HT recipients.

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Steven Foy

Extensive triglyceride synthesis in epididymal adipose tissue in the absence of dietary carbohydrate: Evidence in support of glycerol-glucose cycling.

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The obesity epidemic has generated interest in determining the contribution of various pathways to triglyceride synthesis. We hypothesized that a dietary intervention would demonstrate the importance of glucose vs. non-glucose carbon sources to triglyceride synthesis in white adipose tissue. C57BL/6J mice were either fed a low-fat, high-carbohydrate (HC) diet or a high-fat, carbohydrate-free (CF) diet and maintained on 2H₂O (to determine total triglyceride dynamics) or infused with [6,6-²H]glucose (to quantify the contribution of glucose to triglyceride-glycerol). The 2H₂O labeling data demonstrate that although de novo lipogenesis contributed ~ 80% vs. ~ 5% to the pool of triglyceride-palmitate in HC vs. CF fed mice, the epididymal adipose tissue synthesized ~ 1.7-fold more triglyceride in CF vs. HC fed mice. The [6,6-²H]glucose labeling data demonstrate that ~ 69% and ~ 28% of triglyceride-glycerol is synthesized from glucose in HC vs. CF fed mice, respectively. Although these data are consistent with the notion that non-glucose carbon sources (e.g. glyceroneogenesis) can make substantial contributions toward the synthesis triglyceride-glycerol, these observations raise an important point regarding the operation of a glycerol-glucose cycle.

Jonathan Frankel & Alex Levitt

Testing of Therapeutic Agent to Prevent Hearing Loss in Animal Model of Endolymphatic Hydrops

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***These authors made equal contributions and should be considered co-first authors.**

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Meniere's Disease (MD) is associated with tinnitus, episodic vertigo, endolymphatic hydrops (ELH) and hearing loss, and MD affects ~1 in 5,000 each year. No treatment is available to delay or prevent hearing loss linked to MD. Although the etiology of MD is unknown, glutamate induced excitotoxicity of cochlear neuronal cells is well established.

We hypothesize that glutamate release blockers will prevent permanent hearing loss linked to MD.

The pharmaceutical agent Riluzole, a known glutamate release blocker, was administered intraperitoneally daily to BALB/c-Phex mutant mice, a model for ELH-associated hearing loss. Preservation of hearing function in treated mice was quantified based on auditory-evoked brainstem response (ABR) at postnatal day 21 (P21), P25, and P30. Time points were chosen based on previous work showing ABRs for BALB/c-Phex mice reliably measured after P21 with definitive hearing loss occurring at P30.

Preliminary results show that the average hearing loss in untreated mutants is greater than mutants treated with Riluzole; this difference was far greater at P21 compared to P25 or P30. Further experiments are underway to confirm these results.

These results corroborate the role of glutamate excitotoxicity in the pathogenesis of Meniere's disease and demonstrate that the progression of hearing loss can be retarded by Riluzole, a drug that inhibits glutamate release.

James Gatherwright

Augmentation of the Regeneration of Peripheral Nerve Defects with the Transplantation of Donor Derived Bone Marrow Stromal Cells: A Preliminary Report

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Purpose: Autologous nerve grafting remains the current "gold standard" for peripheral nerve defect repair following injury. However, nerve grafts are limited by donor site morbidity and availability. This study was performed to assess the regenerative potential of an epineural conduit filled with bone marrow stromal cells (BMSC). Methods: 24 epineural tubes were transplanted in 2 experimental groups (12 animals per group). Group 1 was control saline and Group 2 isogenic BMSCs. In Group 2, BMSCs were stained with PKH-dye before transplantation, to assess nerve engraftment and migration. After staining $2.5-3.0 \times 10^6$ cells were delivered directly into the transplanted epineural tube. Evaluations were performed at 6 and 12 weeks post-transplant. Sensory and motor recoveries were evaluated by Gastrocnemius Muscle Index (GMI), pinprick, toe-spread and Somato-Sensory Evoked Potentials (SSEP). Axonal countings were performed in addition to immunostaining with nerve growth factors: NGF, Laminin B2, GFAP, VEGF and Von Willebrand Factor (vWF) for the assessment of the expression of neurotrophic factors and regenerative potential of transplanted BMSCs. Results: 6 weeks post transplantation both groups scored 3 on the pin-prick test. Average toe spread were 1.7 and 2 for the Saline and BMSC groups respectively. SSEP measures showed decreased P1 and N2 latencies in the BMSC group. GMI was also slightly improved in the therapy group (0.48 vs. 0.45). Group 2 showed a higher number of regenerated axons (90.6 ± 26.9) compared to Group 1 (71.4 ± 3.0). In group 2, PKH positive cells were found to express NGF, Laminin B2, GFAP, VEGF and vWF in the transplanted tubes suggesting that BMSCs differentiated into neural tissues and expressed neurotrophic factors. Conclusion: Co-transplantation of BMSCs within epineural tubes enhanced nerve regeneration and supports the putative, regenerative potential of BMSCs in peripheral nerve repair.

Sobenna George

TOWARDS A MORE EFFICIENT METHOD FOR DETECTING *M. tb* INFECTION: A PRELIMINARY SOUTH AFRICAN STUDY

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In 2006, approximately 9.2 million people contracted and 1.7 million people died from *Mycobacterium Tuberculosis* (*M. tb*) worldwide. The tuberculosis burden was especially felt in resource-limited countries as 12 African countries made the top 15 list of countries with the highest incidence rates of the disease (1).

Early detection and treatment of *M. tb* infection are integral to the effective control of the disease. Until recently, one of the main tools used to detect *M.tb* infection was the tuberculin skin test (TST). The major drawbacks of this test are its limited specificity and sensitivity (2). T-cell based Interferon (IFN)- γ release assays (IGRAs) are relatively newer methods of detecting *M. tb* infection that provide some of the solutions to the problems of the skin test. However, limited research has been done on the performance of IGRAs in children less than 17 years old (3).

It is possible that IGRA outcomes may be affected by INF- γ gene expression. Since gene expression investigations are dependent on ribonucleic acid (RNA) extraction, this preliminary study focused on identifying a suitable method for RNA extraction from the limited blood samples that can be taken from children. One aim of this study was to test the reproducibility of the method of RNA extraction outlined by *Carrol et al.* (4) on pediatric blood samples, using the PAXgeneTM Blood RNA System (PreAnalytiX, QIAGEN, Germany). The second aim of the study was to test another easily accessible RNA extraction kit, the QIAamp[®] RNA blood mini kit (QIAgen Ltd.), also on pediatric blood samples

Neither extraction kit was optimized for the small blood volumes used as they each yielded very small quantities of RNA. Although the RNA yields from the *Carrol et al.* (4) study were not reproduced, there may still be some merit in optimizing the method for the PAXgeneTM Blood RNA System (PreAnalytiX, QIAGEN, Germany) since this kit consistently extracted larger quantities of RNA than the QIAamp[®] RNA blood mini kit (QIAgen Ltd.).

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Katrina Gipson

PHYSICIAN-PATIENT CONCORDANCE REGARDING RELEVANCE OF POSITIVE PATCH TESTS

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Background: The efficacy of patch testing may be enhanced by data allowing the physician to estimate the likelihood that results of a patch test reading are relevant to patients' dermatitis.

Objective: The goal of this study is to compare the rates of agreement between the physician's assessment of relevance at time of final reading and patient's report of whether avoidance of an allergen was needed to remain free of dermatitis.

Methods: We mailed 407 IRB-approved questionnaires to patients and 118 surveys were returned. We analyzed results for 91 patients reporting greater than 80% improvement of their dermatitis. Cross-reacting allergens tested on the same patient were combined for analysis. Cohen's kappa was used to assess inter-rater reliability.

Results: Cohen's kappa: all allergens: -0.067 (95% CI -0.24-0.10); nickelsulfate hexahydrate: -0.11 (95% CI -0.52-0.30); neomycin sulfate: -0.18 (95% CI -0.94-0.58); fragrance: -0.046 (95% CI -.044-0.36); formaldehyde and formaldehyde releasing preservatives: 0 (95% CI -1.3-1.3). For most allergens, agreement between raters was less than chance agreement excluding formaldehyde, where raters' agreement equals that of chance agreement. Sample size limits statistical significance.

Conclusion: Relevance may vary between allergens or with anatomic affected areas. Physician assessment of relevance at time of final reading is a poor measure of which allergens are responsible for the allergic contact dermatitis. This may have implications for when best to determine the relevance of certain allergens.

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Role and Regulation of CxCR4 in Ventricular Remodeling

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Introduction: Cardiovascular disease (CVD) is the leading cause of mortality in the United States. CVD is mediated by ventricular remodeling which is an alteration of tissue function that results from cardiac myocyte injury due to irreversible ischemia. Gaining a significant understanding of ventricular remodeling would be of great use in developing potential therapies to combat CVD. As such, our project focuses on the chemokine control of ventricular remodeling, specifically, the role and regulation of CxCR4 in ventricular remodeling post-MI. It is currently known that CxCR4, when activated by Stromal cell Derived Factor (SDF-1), increases cardiac myocyte survival during hypoxia. It is also known that Fibroblast Growth Factor (FGF), Hepatocyte Growth Factor (HGF), and Insulin-like Growth Factor (IGF-1) decrease the size of a myocardial infarct as well as the rate of cardiac myocyte apoptosis. Our hypothesis is that cardiac myocyte derived CXCR4 participates in ventricular remodeling post MI and is the key modulator of the effects of factors like SDF-1, PDGF, FGF, HGF and IGF-1 on ventricular remodeling post MI.

Methods: In vitro cultures of neonatal cardiac myocytes were treated with PDGF, FGF, HGF, and IGF-1 in both hypoxic and normoxic conditions. CxCR4 expression was analyzed qualitatively with immunostaining and quantitatively with mRNA – RT PCR.

Left Anterior Descending (LAD) artery ligation was performed on WT C57BI/6J mice and then FGF was injected at the infarct border. Ventricular function will be assessed using echocardiography and CxCR4 expression was analyzed qualitatively using immunohistochemistry and quantitatively using Western blot. The results will be compared to results which will be obtained from CxCR4 conditional KO mice.

Results: Preliminary results show that FGF increases CxCR4 expression in vitro in the neonatal cardiac myocytes. Other growth factors did not increase CxCR4 expression to a statistically significant level. In WT control mice that underwent LAD ligation, the peri-infarct zone shows increased CxCR4 expression as compared to areas of normal myocardium. Results from CxCR4 conditional KO mice are not yet available for comparison.

Conclusion: Current literature shows that CxCR4 activation in cardiac myocytes, post-MI, has cardioprotective effects by decreasing apoptosis of those myocytes. Our preliminary results suggest that FGF can also have cardioprotective effects by increasing the expression of CxCR4. Whether CxCR4 is required for the benefits of FGF is a question our project is still working on. If the potential of FGF is proven further in future studies, it could be a target for therapy in patients who suffer an MI.

Shipra Gupta

Development and Validation of an Instrument for Predicting a Diagnosis of Psychogenic Nonepileptic Events Versus Epilepsy in Patients Experiencing Seizures

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The purpose of this study is to help physicians, more specifically, neurologists, to distinguish between epileptic seizures and psychogenic nonepileptic seizures. It is well known that the two types of seizures are often confused and may lead to adverse effects and increased morbidity and mortality. The question addressed was whether or not certain indices included in the survey would help to determine whether or not a patient was classified as an epileptic patient or a psychogenic nonepileptic patient. In order to pursue this question, a survey was administered to adult patients admitted to the Epilepsy Monitoring Unit at University Hospitals Case Medical Center. Consent was obtained and the study was thoroughly explained to the patient and participation was completely optional. The patient was given one to two days in order to complete the survey and ask any questions if necessary. The entire survey had to be completed in order to be included in the database. The study is still ongoing and therefore conclusive results cannot be made yet. Increasing the number of patients included in the study will lead to more valid and accurate results. Therefore, the survey will continue to be administered in order to obtain the best possible result in the future.

Steven Healan

RAMBO, a novel oncolytic virus designed to modulate OV-induced changes in the tumor microenvironment of malignant gliomas

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Glioblastoma multiforme (GBM) is a fatal cancer with no current effective means of therapy. Recently, Oncolytic virus (OV) therapy has emerged as a putative treatment option. However, OV treatment of intracranial rat tumors triggers a host defense response resulting in increased and decreased secretion of pro- and anti-angiogenic factors respectively, leading to significant neo-angiogenesis and regrowth in the residual disease. We have previously shown that Brain Angiogenesis Inhibitor-1 (BAI1) expression is lost in a majority of GBMs and glioma cell lines, and that expression of its extracellular fragment (vasculostatin) results in slower glioma growth *in vivo*. To counter the pro-angiogenic change in the tumor microenvironment after OV treatment, we have engineered and tested RAMBO (rapid anti-angiogenesis mediated by oncolytic virus), which is a novel HSV-1 based attenuated OV that expresses vasculostatin. RAMBO treatment of mice bearing intracranial and subcutaneous gliomas revealed a significant increase in median survival (26 vs. 56 days for intracranial tumors and 19 vs. 41 days for subcutaneous tumors) as compared to HSVQ treatment (control). As BAI1 has been shown to be an engulfment receptor on macrophages, we propose that vasculostatin, produced by RAMBO infected cells, binds to the apoptotic marker phosphatidylserine (Pstdr) expressed on infected cells, and prevents recognition and clearance by phagocytes, allowing enhanced viral propagation. To test this, we performed synchronized infections of U251 T2 GBM cells with RAMBO and HSVQ, and visualized Pstdr expression on the cell surface at various time points using a commercially available apoptotic marker, Annexin V. Initial results confirmed OV-induced apoptosis in infected glioma cells up to 3.5 hours post infection. We anticipate that later time points will demonstrate decreased detectable Pstdr on the surface of cells infected with RAMBO compared to those infected with HSVQ, resulting in decreased recognition and clearance by phagocytes and enabling necessary OV replication

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Allison Henry

Knockdown of the Duffy Antigen in Hematopoetic Stem Cells by shRNA

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Malaria, a disease caused by the invasion of Plasmodium protozoa, is one of the most significant issues in global health. Merozoite invasion in Plasmodium vivax requires specific receptor-ligand interactions between DARC (Duffy Antigen Receptor for Chemokines) and Duffy binding protein. Individuals with the Duffy-negative phenotype are resistant to invasion by this malarial parasite. We hypothesized that silencing the expression of the Duffy antigen decreases susceptibility to P. vivax infection.

The question we raise is this: Can gene manipulation of a red blood cell precursor affect susceptibility to malaria in mature red cells? Our first goal was to develop a system by which hematopoetic stem cells can be manipulated and then differentiated in vitro. The second step is to design an shRNA construct that targets and silences the Duffy antigen and to introduce it to HSCs via lentiviral vector. After infection the stem cells will be stimulated to differentiate into mature, Duffy-negative RBCs.

My goal for this project was to lay groundwork for this extensive project. I cultured Human Erythroleukemia cells, which constitutively express DARC. I established cell culture protocols for the HEL cells as well as 293T cells. The 293T cells were used to package a lentivirus construct to determine the infectability of HEL cells. Once it is established that HEL cells can be infected by this lentivirus, an shRNA construct targeted against DARC can be designed. I designed and tested PCR primers for evaluation of DARC knockdown.

Finally, I harvested CD34+ stem cells from umbilical cord blood. These cells can now be cultured to maintain an erythropoetic stem cell population, manipulated through molecular biology techniques, and stimulated to differentiate into mature red blood cells.

By establishing these protocols and procedures I have started an extensive project that another researcher or myself will continue in the future.

Mary I. Huang

Effects of Tuberculosis on the Survival of Patients with Human Immunodeficiency Virus: A Meta-analysis

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BACKGROUND: Most experts agree that HIV accelerates the progression of TB; however, controversy exists regarding the contribution of TB to mortality in patients with HIV. Although many studies indicate that TB accelerates HIV mortality, there are others that show no effect. To date, no comprehensive review of these studies has been performed. **OBJECTIVE:** To provide a comprehensive relative risk of death in HIV patients co-infected with TB compared to HIV patients without TB. **METHODS:** 120 studies with the following pubmed MESH search criteria were included in the initial screening: AIDS-related opportunistic infections, HIV infections/complications, tuberculosis, cohort studies, tuberculosis/mortality, HIV infections/mortality, and mortality. 57 additional studies were included through a citation review of relevant articles, with a total of 177 studies screened. 160 studies were excluded because they did not involve patients who are HIV+; separate patients with and without TB infection; provide survival data; define TB by smear, culture, clinical, and/or other criteria; and/or had a longitudinal study design or cross-sectional design with a standardized mortality ratio. A total of 11 studies were included in the primary analysis. **RESULTS:** The overall estimate of risk based on univariate analyses was 1.46 (1.30-1.63), indicating a 50% increase in the risk of death associated with TB. Estimates were insignificant for heterogeneity, and did not change with the more conservative random effects analysis. **CONCLUSIONS:** Based on the results of this meta-analysis, it appears that TB can accelerate the progression of HIV. When interpreted in the light of the effect of immune activation on HIV disease progression, TB may intensify immune activation that enhances viral replication. Further analysis will be performed on the effect, if any, of AIDS, CD4, opportunistic infections, and co-morbidities on survival.

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Douglas Hutcheon

Feedback from Patients to Improve Familial Cancer Risk and Prevention Reports Created by the Genetic Risk Easy Assessment Tool (GREAT)

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Background

Family history is an important risk factor for cancer. Both the American Cancer Society and The American Society of Clinical Oncology recommend recording a three-generation family tree as part of cancer risk assessment for all patients.^{1,2} While the primary care setting is the venue for the majority of clinical preventive measures, the potential to appropriately intensify cancer preventive services for patients at increased risk has not been fully realized. A major barrier to this has been the lack of feasible and valid methods for systematically recording family history and assessing its implications for cancer risk. In response to this need, investigators at Case developed the Genetic Risk Easy Assessment Tool (GREAT), a web-based system that allows patients to enter their family history of cancer and provides them with personalized prevention and risk messages that they can then share with their physicians and potentially with other family members.

Evaluating the GREAT involves the systematic collection and analysis of feedback and impressions from a diverse group of primary care patients to the GREAT's Personal Prevention Reports. Challenges to using the GREAT, such as variations in literacy level, comprehension of genetic terms and risk messages, cultural understandings of disease processes, comfort with the Web-based format, notions about privacy and ideas about how families may be involved in cancer prevention vary among patients of differing socioeconomic and cultural backgrounds, and must be elucidated in order to maximize the accessibility of the GREAT to a broad base of primary care patients.^{3,4}

Methods

Potential participants were recruited from the University Hospital/Case Medical Center's Department of Family Medicine. Patients and their adult household members who were between the ages of 35 and 70 years and who did not have a personal history of cancer were eligible for participation. In-person interviews were conducted during which participants were shown a hypothetical Personalized Prevention Report and asked to place themselves in the role of the individual receiving the report. Participants were then asked a series of semi-structured questions in order to assess their:

- 1) Opinions about when, where, and under what circumstances they might decide to use the GREAT if offered by their medical provider, or why they might decline to use it.
- 2) Whether patients might involve relatives in recording their family history and receiving familial cancer risk assessment, and/or in family-based cancer prevention activities, and especially,
- 3) Comprehension and evaluation of hypothetical Personalized Prevention Reports created by the GREAT.

Results and Conclusions

Over a two month period, approximately forty potential participants were screened. Ultimately, nine participants completed the screening process and participated in the interview process. Reasons for screen failure included: outside the eligible age window, personal history of cancer, unwillingness to participate, and lost to follow-up after initial indication of willingness to participate.

The median age of the nine participants was 50 years (range 44-65); five were female and four were male. Seven participants identified themselves as African-American, one as Caucasian, and one as mixed race.

To date, analysis of participants' responses and impressions of the GREAT's Personalized Prevention Reports is ongoing. Initial responses have indicated consistent positive feedback with regard to the family tree created by the report as well as the report's message regarding the benefits of smoking cessation. Challenges to implementation of the GREAT that have been identified from initial participant responses include participant lack of familiarity with medical and genetic terminology and lack of computer access.

Obehioya Irumudomon

The Influence of Inflammation and Ischemia on Proinflammatory Cytokine Production and the Impact of Administering the Neuroprotective Agent Exogenous Erythropoietin

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In addition to being an important growth factor in CNS development, erythropoietin (EPO) can suppress inflammatory responses in the brain and act as a neuroprotectant. We investigated whether neonatal EPO would suppress the inflammatory response after prenatal ischemia plus inflammation in a mouse model. We anticipated that EPO treatment after prenatal brain injury would reverse the cell death response and result in neural cell survival. Cytokine levels in serum and brain samples were examined to better this phenomenon.

We studied trends in pro-inflammatory cytokine (PIC) levels at four time points in development after different types of embryonic day 18 insults: ischemia, inflammation, combined insults, and sham controls. The first important finding is the low level of cytokine crossover from the serum into the brain. Therefore, for all the PIC test results, serum levels of cytokines are much higher than brain levels, and this suggests brain cytokine response needs further investigation. EPO treatment in Pups 3-days post-partum caused serum interleukin-6 (IL-6) levels to drop in the groups with inflammation due to lipopolysaccharide (LPS). However, the levels of IL-6 remained higher compared to the other cytokines. Tumor necrosis factor (TNFa) levels also responded to the treatment with EPO with a reproducible pattern. Unexpectedly, TNFa levels in the sham control group and the LPS only group rose with neonatal EPO treatment compared to saline treatment. TNFa levels of the ischemia and ischemia plus inflammation groups dropped significantly with neonatal EPO treatment, suggesting EPO treatment administered days after the prenatal insult can still be effective. Recent studies have shown EPO-mediated neuroprotection requires signaling through a TNFa receptor. Our results suggest EPO is a promising neuroprotectant to explore in relation to the cytokine response after in-utero ischemia and combined ischemia/inflammatory injuries.

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Daniel Kelmenson

Transcriptional Regulation of KLF4

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Endothelial cells play a central role in vascular physiology, and their dysfunction is a key determinant in the development of many vascular diseases. An understanding of the factors that mediate the effect of injurious stimuli on endothelial phenotypic alteration is critical to understanding the pathogenesis of vascular disease. Kruppel-like factors (KLF) are members of the zinc-finger family of transcription factors that play essential roles in regulating cellular growth, differentiation, and phenotype. Endothelial cell KLF4 regulates endothelial activation in response to pro-inflammatory stimuli. KLF4 has anti-inflammatory and anti-thrombotic effects. KLF4 expression is induced by laminar shear stress, statins, and pro-inflammatory stimuli. There has been little elucidation of the pathways by which these stimuli regulate KLF4 expression, and my project attempted to determine the molecular mechanism of regulation of transcription of KLF4. I attempted to clone a 5kb region upstream of the KLF4 initiation site, using a commercially available bacterial artificial chromosome (BAC). I would then use this 5kb region as the parent construct to create deletion constructs to identify areas with potent effects on regulation of transcription of KLF4. Progressively shorter deletion constructs would enable me to determine which regions of the KLF4 promoter are critical for regulation of the gene (i.e. have promoter activity). Using promoter analysis to determine which transcription factors might bind to the final deletion construct, I would then create site-directed mutants to alter specific (potential) transcription factor binding sites and test these mutants for promoter activity. Cloning out a 5kb region of the promoter proved to be very difficult. Multiple rounds of PCR with varying conditions and enzymes could not produce sufficient product for further molecular cloning. Although I also performed other side projects during the summer, the main project was ultimately unsuccessful. We sent the project to SeqWright at the end of the summer so that they could perform the cloning, but they also proved unable to PCR out the 5kb fragment. Three months after I left, using a new BAC template, my lab was able to complete the 5kb cloning and move on with the project. Although I was not able to accomplish all that I wanted during the summer, I still had an enjoyable experience in the lab and the project is now able to proceed with the 5kb promoter construct. Further work will involve creating deletion constructs and site-directed mutants.

Supported by NIH T35 Grant

Hari B. Keshava

USING PEDIATRIC DONOR LUNGS FOR ADULT RECIPIENTS: FEASIBILITY AND OUTCOMES

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OBJECTIVE: There is little information describing the impact of pediatric organ use in adult lung transplantation (LTx). Importantly, sizing criteria classically applied in adult allograft sizing are less established for pediatric organs, particularly with regard to donor bronchi. We reviewed our institutional experience of pediatric organ use for adult recipients, with emphasis on feasibility, bronchial anastomotic complications, and overall patient survival.

METHODS: From 2/1990 to 1/2008, 609 adults underwent primary LTx at our institution. Of these, 43 (7.1%) underwent LTx with pediatric allografts (donor less than 16 years old), and 39 medical records were available for review. Donor, recipient, and transplant variables, including airway complications and ICU and hospital lengths of stay, were abstracted and analyzed. Institutional review board approval was given.

RESULTS: Median donor age was 14 years (range 7–16) and median recipient age 48 years (range 23–66). 24/39 (62%) underwent double LTx and 15/39 (38%) single LTx. Median length of time until extubation was 2 days (range 1–49) and ICU length of stay 5 days (range 1–62). 10/39 (26%) were noted at the time of LTx to have important size mismatch of donor and recipient bronchi (all oversized recipient bronchi). 3/39 (8.0%) patients experienced a major postoperative airway complication: 2 had catastrophic airway dehiscence (1 lethal and the other requiring reoperation), and 1 developed problematic airway stenosis requiring stenting. Kaplan-Meier survival at 30 days, 1 year, 3 years, and 5 years post-transplant was 88%, 74%, 67%, and 60%, respectively.

CONCLUSION: At our institution, it was feasible to perform adult LTx with pediatric donors, which substantially increased the donor pool. Sizing criteria for adults seem to be applicable to pediatric organs, although size mismatch of the airway was common. This may predispose to major complications, suggesting that attention must be paid to matching both parenchymal and airway size. Overall survival after LTx appears similar to that for adult donors.

Tristan Klosterman

Improving Diabetic Foot Screening in an Urban Dominican Republic Clinic

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BACKGROUND: Poor urban clinics present unique problems and opportunities in addressing health concerns through intervention. Diabetic complications of the feet are a particularly relevant concern in the Dominican Republic where chronic care access can be poor. Physicians and health care staff may also not be aware of foot exam guidelines to prevent such complications. **OBJECTIVE:** The objective was to increase the number of foot exams given in an urban shanty - town clinic (FEDOPO) in the Santo Domingo suburb of Guaricano through education of physicians and staff of the importance and necessity of the exams. **METHODS:** It was originally proposed to obtain information from thirty diabetic patient visits regarding whether or not the physician did a foot exam. Following this, educational intervention would be given to physicians and support staff through the means of a pamphlet describing the importance of exams in the prevention of morbidity and mortality. In addition, posters would be displayed in order to reinforce the information. Following the intervention, thirty encounters would again be observed to see if patients were given exams. The data would be analyzed for statistical significance. **RESULTS:** The characteristics of the clinic prevented the implementation of the project. It was discovered, to the astonishment of our adviser, that diabetic patients were referred to another location. The reasons included an inability to follow up with chronic care patients due to a lack of medical records and the incompleteness of patient encounter documentation as well as a lack of blood sugar monitoring equipment and an inability of the local lab to respond to acute emergency situations where blood sugar readings would be essential. This made the procurement of the data impossible. This information was obtained through extensive physician interviews as a modification of the project. **CONCLUSIONS:** The proposed site was not able to meet the demands of the research proposal. Faults in the organization of the clinic and miscommunications between the clinic director and the research adviser prevented the implementation as planned. However, much was obtained in regards to the barriers to research and interventions in poor urban clinics. Medical records and fundamental equipment that are available in most modern clinics may deeply impact the quality of delivered health care as well as hinder solutions and research.

Craig Lance

QI in Heart Failure for VA Cleveland Facilities: An Implementation Plan

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Objective: To improve heart failure (HF) practice quality by decreasing diuretics and increasing neurohormonal inhibition in outpatients at the VA Cuyahoga County facilities with a diagnosis of HF secondary to systolic dysfunction. Angiotensin converting enzyme inhibition (ACEI) and/or angiotensin II receptor blocker (ARB) administration is part of the evidenced-based HF Guidelines and a performance measure for HF.

Rationale: Among the arguments for controlling diuretic use is the increase in the renin-angiotensin-aldosterone system (RAAS) as a result of diuresis. Although a therapeutic goal is to achieve euvolemia, the ADHERE (acute HF) Registry has pointed to a higher mortality associated with higher diuretic use. Whether the association is truly causal is uncertain at this time. The use of ACEI/ARB for reduction in the RAAS cascade has been well documented in the literature and serves as a performance measure which is linked to a favorable outcome and quality care.

Aims: To decrease loop diuretic to the lowest necessary level in the Cleveland VAMC, Brecksville and McCafferty and institute a flexible diuretic regimen as recommended in the HF Guidelines. To maximize ACEI/ ARB use to tolerability.

Methods and Intervention: NHeFT (National Heart Failure Training Program) of Case is a multi-site, nation-wide HF training CME program. The mission of NHeFT is to disseminate best practices in HF care for health care practitioners to change practice patterns. The typical program includes ½ day of didactics followed by a preceptorship with the instructors at the attendees site seeing HF patients. The program stresses the appropriate use of Guideline-driven therapy with emphasis on RAAS inhibition and lowering diuretics to optimally needed doses with a flexible diuretic regimen. Health care providers will be enrolled in the NHeFT programs at all 3 facilities after the baseline data collection.

Results: Research is ongoing.

Supported by Health Services R&D department of the VAMC

Melissa Latigo

The potential impact of infant HIV point-of-care tests in Uganda

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In Uganda, about 80,000 infants are at risk from contracting HIV annually. Dried blood spot DNA PCR (sensitivity = 94.35%, specificity = 98%) is the gold standard in infant HIV diagnosis. However, lengthy result turnaround times decrease the probability that infant caregivers return for results and that infants receive timely treatment. Northwestern University researchers are designing a portable p24 antigen (Ag) test (sensitivity = 84.92%, specificity = 97.02%) and a bench-top DNA PCR test (sensitivity = 94.35%, specificity = 98%) that can be used in the clinic setting and turn around results in under 1 hour.

We evaluated whether it is cost-effective to diagnose infant HIV in Uganda using novel point-of-care (POC) tests versus a laboratory-based test.

The study involved a cost-effectiveness analysis that incorporated the decision model for testing asymptomatic infants at risk for HIV. The main outcome was the number of true infant HIV cases identified given that a caregiver returned for results. Probability of caregiver return with a POC test was assumed to be 100%. We collected the POC and the laboratory-based test costs related to infant HIV diagnosis. Costs were obtained from the Ministry of Health perspective.

Testing records revealed an estimated infant HIV prevalence of 18.29% (14,632 HIV-infected infants annually). The laboratory-based test would identify 7960 (54.4%) infants at \$18.70 per test. The p24 Ag and DNA PCR POC tests would identify 12,432 (84.96%) infants at \$3.00 per test and 13,824 (94.48%) infants at \$8.80 per test respectively. The DNA PCR and p24 Ag POC tests on average cost \$51.11 and \$19.31 respectively per infant HIV case identified.

POC tests are more cost-effective and would increase the average number of infants identified by about 5000 (65%). At a fixed budget, the p24 Ag POC test would be able to identify more infant HIV cases.

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Sungho Lee

Mouse Model of Alzheimer's Disease Deficient for Fractalkine Receptor Exhibits Reduced Beta-Amyloid Deposition

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Alzheimer's disease (AD) is the leading cause of dementia in the elderly. It is characterized by distinct neuropathological hallmarks including extracellular deposits of beta-amyloid peptide, which is proteolytically cleaved from the amyloid precursor protein (APP). In addition, there is considerable evidence for altered inflammation within the AD brain, including increased numbers of inflammatory cells and expression of a variety of pro-inflammatory molecules. Microglia, the primary immune effector cells in the brain, continually monitor the brain for pathological alterations and become activated in most neurodegenerative conditions including AD. Depending upon local conditions within the brain, it has been postulated that microglia can either play a neuroprotective role such as the removal of debris, or may be directly involved in the pathogenesis of neurodegenerative diseases through the production of neurotoxic cytokines. Recent studies indicate that microglia may play a direct pathogenic role in neurodegeneration via alteration in communication with neurons mediated by the microglial fractalkine receptor, CX3CR1. *Cx3cr1* deficient mice exhibit increased neurotoxicity and worsening of phenotypes in a variety of mouse models of neurodegenerative disease. To gain further insight into the role of inflammation in AD pathogenesis, we performed genetic crosses between the APPPS1 mouse model of AD and *Cx3cr1* deficient mice. As expected, transgene-derived APP and APP processing products are largely unaltered. Interestingly, transgenic mice both heterozygously and homozygously deficient for *Cx3cr1* exhibit gene dosage-dependent reduction in beta-amyloid deposition when compared to control APPPS1 animals. Furthermore, these animals demonstrate reduced microglial activation, as indicated by the decreased number of microglia surrounding each beta-amyloid deposit. These data suggest that fractalkine signaling between microglia and neurons mediate pivotal roles in AD pathogenesis, most likely with regards to beta-amyloid clearance.

Supported by American Federation for Aging Research

Aaron Lindsay

Inner Ear Protein Networks and Biomarkers in a mouse model for deafness in Usher Syndrome 1F and DFNB23.

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Introduction: This study identifies candidate biomarkers of inner ear pathogenesis in the Ames waltzer (*av*) mouse, a model for deafness in Usher syndrome 1F (USH1F). Additionally, candidate protein networks active in the degeneration process are identified with the aid of mapping software. *Methods:* Cochleae from *av* and normal mice at postnatal day 30, a time point where cochlear pathology is well established in the *av* model, were dissected and homogenized in ice-cold lysis buffer. The protein was extracted, labeled by Cydye, prefractionated by 2-D DIGE, and analyzed by mass spectrometry. DeCyder software 6.5 was used to quantify protein spots and Metacore software was used to predict protein interactions and establish statistically significant candidate networks. To validate the proteomics analysis, two protein products (p53 and caspase-3) were further analyzed by qPCR and enzyme activity assays, respectively. *Results:* 43 proteins were found to be up-regulated and 26 down-regulated in the cochleae of *av* mice compared to controls. Cochlin was identified in 20 peptide spots of both increased and decreased expression. 7 statistically significant candidate protein networks that are predicted to be active in USH1F were identified. Some key proteins predicted to be up-regulated in the networks include c-JUN, NF- κ B, p53, and caspase-3. Enzyme activity assays for caspase-3 and qPCR analysis for p53 confirmed increased activity and transcript levels, respectively, in *av* cochleae compared to controls. *Conclusions:* This study identifies candidate biomarkers and protein networks of inner ear pathogenesis in an USH1F model. Cochlin, which has been associated with other forms of hearing loss, was found to be both up-regulated and down-regulated in the USH1F model. Factors known to be associated with apoptosis and degeneration, including c-JUN, p53, and caspase-3, are predicted to be active in the degeneration of hair cells and spiral ganglion cells in the USH1F model.

This work was supported by a grant from Rainbow Babies and Children's Hospital, University Hospitals Case Medical Center, Cleveland, Ohio 44106.

Kathryn Martires

Factors that Affect Skin Aging: a Cohort-based Survey on Twins

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Introduction: Photoaging describes a phenomenon brought about by long-term sun exposure that causes inflammatory skin changes, resulting in photodamage and premature skin aging. The topic has become an issue of great importance and expense. In 2005, \$160 billion were spent on aging-reversal procedures. More importantly, the presence of photodamage is also related to the development of skin cancers, warranting investigation into the etiology and prevention of photodamage.

Objective: This study identifies environmental factors that correlate with skin photoaging, controlling for genetic susceptibility by using a questionnaire administered to pairs of twins at the annual Twins Days Festival in Twinsburg, Ohio.

Methods: The survey collected information about degree of sun exposure, sun protective behaviors, history of skin cancer, smoking and drinking habits, and body mass index from a cohort of twins. Clinicians then assigned a Fitzpatrick type and clinical photodamage score to each participant. Univariate analysis examined associations between each variable and photodamage, using the Spearman correlation coefficient and the Kruskal-Wallis test. For factors found to be significant in univariate analysis, multivariable linear regression with the forward model selection procedure was used to determine independent associations. All tests were two-sided and p-values less than 0.05 were considered significant.

Results: Factors found to positively predict photodamage include cigarettes smoked, with a correlation coefficient (r) of 1.62 ($p=0.0004$), skin cancer history with $r=1.62$ ($p=0.045$), and weight with $r=0.45$ ($p=0.008$). Factors negatively correlated with photoaging include Fitzpatrick type with $r=-0.47$ ($p=0.0004$), and sun screen use with $r=-0.31$ ($p=0.007$).

Conclusions: The study of twins provides a unique opportunity to control for genetic susceptibility in order to elucidate environmental influences on skin aging. The relationships found between smoking, weight, sunscreen use, skin cancer, and photodamage in these twin pairs may help to motivate the reduction of risky behaviors.

Supported by University Hospitals Case Medical Center Department of Dermatology

Kathryn Martires

Characteristics and Prognosis of Transected Melanomas

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Introduction: Melanoma is the most common fatal skin cancer. The prognosis and therapy of melanoma is directly related to the depth of cutaneous invasion at initial removal, referred to as “Breslow’s depth.” When melanomas are transected at diagnosis, true Breslow’s depth is difficult to ascertain. If residual melanoma is present on re-excision, the Breslow’s depth of the residual tumor is added to that of the original transected tumor. Sometimes, no residual melanoma is present on re-excision, and only the depth of the transected tumor (original Breslow’s depth) is available to guide prognosis and therapy.

Objective: The purpose of this study is to better define prognosis for this group by comparing survival rates of patients with transected melanomas that have no additional tumor on re-excision with that of melanomas of the same Breslow’s depth that are *not* transected.

Methods: This is a cohort study of patients diagnosed with melanoma at UHCMC between 1996 and 2007. The study examined the number of transected melanomas, the proportion of transected melanomas without residual tumor, and relative survival rates of transected tumors found to have no residual tumor compared with non-transected tumors of similar Breslow’s depth. Univariate and multivariate survival analyses were used, and the significance level was set at 0.05.

Results: The study identified 625 patients, and found that 178 of 625 (28.5%) melanomas were transected at diagnosis. Of those, 59.0% revealed no residual tumor on re-excision. Univariate analysis demonstrated that patients with transected melanomas with no residual tumor had poorer survival than patients with *no* transection ($p=0.0479$). The multivariate analysis trended toward this result as well ($p=0.0887$).

Conclusions: A high number of melanomas are transected at diagnosis, making appropriate staging and therapy difficult. Patients with transected melanomas with no residual tumor on re-excision may have poorer survival, and as a result, more aggressive diagnostic and therapeutic procedures may be appropriate for them.

Supported by American Dermatological Association, Derm Foundation

The Choking Game: Physician Perspectives

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Objective. To assess awareness of the choking game amongst physicians who care for adolescents and explore their opinions regarding inclusion of its dangers into anticipatory guidance for their patients.

Methods. We surveyed 865 pediatricians and family practitioners in Northeast Ohio. The survey was designed to assess the physician's awareness of the choking game and its warning signs, the suspected prevalence of their patients' participation in the activity, and the willingness of physicians to include the choking game in adolescent anticipatory guidance. Information was also collected regarding their use of general anticipatory guidance with adolescent patients and their parents.

Results. The survey was completed by 163 physicians. One-hundred eleven (68.1%) had heard of the choking game; 68 of them (61.3%) through popular media sources. General pediatricians were significantly more likely to report being aware of the choking game than family practitioners or pediatric subspecialists ($p=0.004$). Of physicians aware of the choking game, three fourths identified at least one warning sign; 52.3% identified three or more. Only 7.6% of physicians aware of the choking game reported they cared for a patient they suspected was participating in the activity and two (1.9%) reported they include the choking game in anticipatory guidance for adolescents. However, 64.9% of all respondents agreed the choking game should be included in anticipatory guidance.

Conclusions. Close to a third of physicians surveyed were unaware of the choking game, a potentially life threatening activity practiced by adolescents. We feel this represents a critical lack in awareness amongst physicians caring for adolescents. Despite acknowledging that the choking game should be included in adolescent anticipatory guidance, few physicians reported actually discussing it. To provide better care for their adolescent patients, pediatricians and family practitioners should be knowledgeable about risky behaviors encountered by their patients, including the choking game, and provide timely guidance about its dangers.

Christopher McCrum

Validation of the MicroScribe coordinate measurement system for knee kinematics

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In order to better understand the kinematics of the knee, a reliable, accurate, precise form of data collection is crucial. The R2000 robot has the ability to manipulate knee kinematics throughout the range of motion of the knee in all planes. To measure kinematics of the knee, the robot uses the technology known as a MicroScribe coordinate measurement machine to define the place in space of the knee throughout the experiment. However, the data collected by the robot has not been validated through experimentation, while in the past, measurement systems that have been validated, such as motion capture with the Qualysis MacReflex system, has been used. Hence, the hypothesis of this experiment is that there is no difference between the data collected by the robot and the data collected by motion capture system, throughout the range of motion of the knee. In order to test this hypothesis, a protocol was implemented, where the robot was ordered to move to positions defined by the MicroScribe, while this movement was being measured by the motion capture system. Such data points included a range of -10 to +10mm medial translation, -30 to +20mm posterior translation, -13 to -5mm superior translation, 0 to 10 degrees flexion, -10 to +10 degrees of valgus, -40 to +40 degrees of internal rotation, and several combined movements, all measured as the movement of the tibia relative to the femur. Analysis showed that there was not a significant difference between the root mean squares of each of the groups, and the correlations were greater than 0.97 in all planes of motion, indicating a strong relationship between experimental measurements. Thus, under these motion conditions, there is strong evidence that the kinematic data collected by the R2000 robot with the MicroScribe collects valid, reliable data.

Jacob M. McGrath

Genotyping bradykinin B2 receptor/apolipoprotein E dual knockout mice by the polymerase chain reaction

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It has previously been established that bradykinin B2 receptor (BKB2R) knockout(KO) mice are protected from thrombosis, while apolipoprotein E (APOE) knockout mice are at increased risk for thrombosis. To determine whether a double knockout will eliminate thrombosis protection seen in the BKB2R, the two strains must be cross-bred. Since both genes were disrupted by the introduction of the neomycin (*neo*) resistance gene, assaying progeny for the presence of this gene is insufficient to determine whether dual-knockouts have been produced. The 5' flanking sequence to the *neo* gene in the BKB2R knockout mice was determined, and 5' primers suitable for the polymerase chain reaction (PCR) in this region, as well as 3' primers in the *neo* gene sequence, were identified. Multiple PCRs were carried out using DNA from both BKB2R KO mice and wild type mice, and one primer pair was found to produce a distinct band unique to BKB2R KO DNA. The fidelity of this band as part of the BKB2R was determined by DNA sequence analysis, and found to correspond to the known sequence of the mouse genome at the junction of the interrupted BKB2R gene and the 5' end of the *neo* gene. Together with a similar method that has been developed for genotyping APOE knockout mice, using a 5' primer hybridizing within the coding region of the *neo* gene and a 3' primer hybridizing to the 3' flanking region of the APOE gene, this reaction can be used to assay for the presence of both KO genes in a single animal. These studies produced an incontrovertible screening method for the double BKB2R and APOE KO mouse. This assay facilitates investigations on the influence of competing thrombosis risks.

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Pritesh Mehta

PEER-REVIEW OF THE SURGICAL LITERATURE: A DOUBLE-BLINDED RANDOMIZED CONTROLLED TRIAL

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Introduction: Evidence-based surgery is predicated on the quality of published literature. We measured the quality of surgical manuscripts selected by peer-review and identify predictors of excellence. **Methods:** A random sample of 120 manuscripts about clinical therapeutics in surgery were taken from 1998 in five eminent peer-reviewed surgical and medical journals. Manuscripts were blinded for author, institution, and journal of origin. Four surgeons and four methodologists evaluated their quality using two novel, validated instruments based on subject selection, study protocol (i.e. "blinding", inclusion criteria, use of control groups), statistical analysis and inference, intervention description, outcome assessments, presentation of results, and perceived impact a decade after publication via citation index. They generated separate clinical and methodological quality scores for each manuscript. Predictors of quality were identified based on univariate and multivariate regression analyses. **Results:** Among our sample of manuscripts, oncology was the most common study subject (26%), followed by general surgery/GI (24%), and vascular, transplant, and/or cardiothoracic surgery (23%). The average number of study subjects was 417; the majority of manuscripts were from the U.S. (53%) and from a single institution (59%). Just 18% had a statistician-author. The mean number of citations was 128. Surgical manuscripts submitted to medical journals, as compared to surgical journals, had on average significantly better clinical (1.7 vs. 2.5, $p < 0.001$), methodologic (2.1 vs. 2.7, $p < 0.001$) and total quality scores (3.8 vs. 5.2, $p < 0.001$). They had more subjects (mean = 891 vs. 259, $p < .001$) and were more likely to have a statistician as a co-author (43 vs. 10%, $p < 0.001$), a multi-institutional, international collaboration (30 vs. 8%, $p < .001$), and a high citation index (70 vs. 10%, $p < .001$). They were more often from outside the U.S. (70% vs. 40%, $p < 0.001$). Manuscripts stemming from direct journal submission had better methodologic scores than those submitted for publication after presentation at surgical society meetings (2.5 vs 2.7, $p < .05$), but were comparable in clinical and total quality scores. The best scores by subject were in vascular, transplant, and/or cardiothoracic surgery ($p < 0.01$) Overall, significant independent predictors of surgical manuscript quality were having a statistician as a co-author, number of times the manuscript has been cited, and a larger number of study subjects. For all surgical manuscripts, quality assessment using our novel instrument predicted the number of citations 10 years later ($p < 0.001$). **Conclusion:** The quality of surgical manuscripts can be improved by including a statistician as a co-author. Efforts should be directed to implementing multi-institutional and interdisciplinary trials. Peer-review across journals can be standardized through the use of a validated instrument such as this one to measure the methodologic and clinical quality of manuscripts submitted for publication.

Anthony C. Merlocco

CXCR4-eGFP mice in the cuprizone model: Characterization of CXCR4 positive cells in the adult mouse brain before and during demyelination

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Background—One key pathogenic attribute of multiple sclerosis is failure of remyelination after CNS insult, which requires successful cellular signaling of glial precursors for migration and proliferation. CXCR4 is a hemopoietic chemokine receptor shown to be involved in the neuropoietic system with its sole ligand, SDF1. Using transgenic CXCR4-eGFP mice we tested the hypotheses that CXCR4 positive cells will expression stem cell markers and neurogenic markers during demyelinating cuprizone treatment.

Methods and Results—Starting at a minimum of 8 weeks of age mice were fed ad libitum 0.2% (w/w) cuprizone (Sigma-Aldrich Inc., St.Louis, MO) milled into mouse chow for 1–5.5 weeks (CXCR4-eGFP::SDF1-mRFP1) or 1-6 weeks (wild type C57BL/6) to induce chronic demyelination. Mice were anesthetized and perfused transcardially and spinal cord and brain were rapidly dissected and stored in 4% PFA at 4°C. Scanning and confocal microscopy were used to image tissues. During demyelination, CXCR4⁺ cell expression does not increase with cuprizone but individual cells appear throughout the corpus callosum. CD45 cell expression increases along corpus callosum, consistent with demyelination and GFAP positive cell populations grossly expand in hippocampus, corpus callosum, and ventricular zones. As well, CD133 expression is similar in double transgenic and WT mice during curprizone treatment.

Conclusions— In CXCR4-eGFP postnatal mice, GFAP positive cell populations may represent areas of active neurogenesis potentially as radial glial cells. During demyelination, increased CXCR4⁺ cell expression occurs sparsely in the corpus callosum and may represent neurogenesis or infiltrating peripheral blood leucocytes. CXCR4⁺/CD45⁻ cells increasing in the corpus callosum represent microglia, neurons, or astrocytes. There are a several CXCR4⁺/GFAP⁺ cells appearing in the corpus callosum at 2 weeks and 4.5 weeks of cuprisone treatment which represent astrogliosis or neurogenesis. increases along corpus callosum, consistent with demyelination and GFAP positive cell populations grossly expand in hippocampus, corpus callosum, and ventricular zones.

Wei (Cindy) Mi

CT Evaluation of Aortic Dimension, Calcification, and Pericardial Thickness in a Geriatric Population with History of Smoking

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Objective: To use a high risk cohort of geriatric subjects with history of heavy smoking to describe the physiologic ranges of aortic dimensions, aortic valve calcification, and pericardial thickening in elderly smokers.

Methods: Sixty-four participants of the National Lung Screening Trial cohort at UCLA were randomly selected for this study (mean age 69.0 ± 2.8 years, 57.8% male). All participants had minimum 30 pack-year smoking history and received one chest CT per year for the three years they were followed. Aortic dimensions were measured at the ascending aorta (AAD), descending aorta (DAD), and aortic arch (AARD). Additionally, the ratio of main pulmonary artery diameter (MPAD) to ascending aortic diameter (AAD₁), aortic valve calcification (AVC), and pericardial thickness (PT) were evaluated. Means and standard deviations were calculated based on gender and age group (65-67, 68-70, 71-74 years) at baseline and year 2. Correlation analysis was done between pack years and all cardiac measurements.

Results: We found no significant differences in cardiac measurements among age groups though there was a significant gender difference. The mean AAD at baseline was 35.72 ± 3.86 and 38.86 ± 3.50 mm in females and males, respectively ($P = 0.0012$). Likewise, DAD, MPAD, AAD₁, and AARD showed significant gender differences. Pack years was not a predictor of outcome except for MPAD/AAD₁ ($P = 0.032$). None of the cardiac measurements were found to have significant changes from baseline to year 2 though Pearson's correlation showed a significant correlation between the two measurements.

Conclusions: While it is common clinical practice to assume an increase in vessel size and calcification with increase in age, our study indicates that this assumption is unreliable, even when evaluating high risk elderly with heavy smoking history. Comparison of this cohort with the general adult and geriatric populations show no observed differences in aortic dimensions and calcification.

Supported MSTAR Program

Stephanie Michal

Establishing if accelerated atherosclerosis mediated by periodontal disease is attributed to CD36

Stephanie Michal, Maria Febbraio

Cell Biology Department
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CD36 is an integral membrane glycoprotein found on the surface of many cell types and its functions include recognition of apoptotic cells and modified lipids, uptake of fatty acids, regulation of angiogenesis and recognition of ligands that trigger an innate immune response. This summer, I work in Dr. Maria Febbraio's laboratory working on establishing a link between the expression of CD36 and the development of atherosclerosis. The scavenger receptor CD36 has been linked to a pro-thrombotic phenotype that is observed in metabolic states marked by hyperlipidemia. Yet, when a CD36 knockout mouse is created there appears to be no difference between the knockout and the wild type mice in the development of atherosclerosis. From these findings it has been hypothesized that increasing the inflammatory stimulus that is associated with the generation of reactive oxygen species that causes oxidant stress is essential for the creation of CD36 specific pro-atherogenic ligands. It has previously been proposed that there is a link between periodontal disease and atherosclerosis because periodontal disease in some patients represents a state of chronic inflammation. The objective of the experiment this summer was to determine if the atherosclerosis that is attributed to periodontal disease is CD36 dependent. In order to perform the experiment I harvested macrophages from the peritoneum of both wild type and CD36 knockout mice and plated the cells. Then four groups were formed, two wild type one with the bacteria in the media and one without and two knockouts one with bacteria in the media and one without. After 24 hours the media was removed and the macrophages were stained with oil red O (stains LDL) and fixed. The number of foam cells were then counted using a light microscope. The expected results were that the most foam cells would be found on the plate of wild type macrophages that contained media filled with bacteria however there was no statistical difference between the plates. In conclusion, I would like to acknowledge the support I received to work on this project from the T35 grant from the NIH.

Michael Moore

Piezoresistive pressure sensors in the measurement of intervertebral disk hydrostatic pressure.

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Summary of Background Data: An implantable, free-standing, minimally invasive, intervertebral disc pressure sensor would vastly improve the knowledge of spinal biomechanics and the understanding of spinal disease. Additionally, it would improve clinical indications for surgical interventions in disc-related pathology. Adaptation of current commercially-available materials, technology, and micro-fabrication techniques may now make such a device feasible to produce.

Objective: Determine if Piezoresistive Pressure Sensor (PPS) technology could be applied as the functional sensing element in an intervertebral disc microsensor. **Methods:** Commercially available PPS chips were modified, producing sensor chips measuring .8 cm² by .3 cm with an internal sensing element measuring .15cm² by .1 cm. A needle-mounted pressure sensor functionally identical to those used in discography procedures was also tested in parallel as a control. Both sensors were calibrated for hydrostatic pressure using a purpose built pressure chamber, and then tested in human Functional Spinal Units (FSU). Methods were developed to implant the sensor and measure the intervertebral disc pressure in response to axial compressive loads. **Results:** Modified commercially-available PPS elements were functionally adapted to measure intervertebral disc pressures. Both the PPS and needle-mounted sensors measured a linear increase in hydrostatic disc pressure with applied axial load. Fluctuations between the slopes of the output versus load curves were observed in the PPS sensor experimental trials. These fluctuations were attributed to the large size of our working model and its impact on the hydrostatic and mechanical properties of the disc. **Conclusions:** It is hypothesized that future miniaturization of this working model will eliminate mechanical disruption within the disc and the fluctuations in the slope of sensor output that this induces. It should be possible to construct an implantable sensor for the intervertebral disc. This may provide valuable clinical and physiological data.

Supported by Crile

Nickolas J. Nahm

Early fixation of femoral shaft fractures in multiply injured patients is associated with fewer complications

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Introduction: In seminal work examining the effects of timing of treatment of femoral fractures in multiply injured patients, Bone and colleagues concluded that early definitive stabilization is the optimal approach to managing femoral shaft fractures. However, based on studies of thoracic trauma patients, Pape and colleagues proposed an alternative tactic, known as damage control orthopaedics, in which femoral fractures are provisionally stabilized with an external fixator, then treated on a delayed basis (several days to two weeks later), tailoring treatment based on systemic injury of the patient. This study contributes to this ongoing discussion by exploring the effects of timing of femoral fracture treatment on the incidence of complications in the multiply injured patient.

Methods: Data were collected retrospectively on 511 skeletally-mature multiply injured patients with a femoral shaft fracture treated at a North American Level I Trauma Center between 1998 and 2006. Patient cohort groups were established based on timing of treatment and accompanying injuries. Rates of pulmonary, renal, and infectious complications were compared.

Results: Pneumonia and sepsis were less frequent in patients treated within 24 hours (9.7% and 0.9%, respectively, n = 422) compared to patients treated after 24 hours (21.3% and 9.0%, respectively, n = 89, (p = 0.004 and 0.0002, respectively)). Furthermore, among patients with accompanying chest injuries (n = 227), abdominal injuries (n = 141), or head injuries (n = 257), the rate of sepsis was lower in patients treated definitively within 24 hours (1.8%, 4.0%, 1.9%, respectively) compared to patients treated after 24 hours (11.5%, 15.0%, 11.8%, respectively, (p = 0.005, 0.03, and 0.005, respectively)).

Conclusions: Despite differences in age and injury severity between patients treated within and after 24 hours of injury, this study suggests that the majority of multiply injured patients may be definitively treated on an early basis with few complications. Further study may help to define specific parameters where a damage control strategy is warranted.

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James T. Nelson

Volatile anesthetics and complex I of the mitochondrial electron transport chain

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The mechanisms by which volatile anesthetics exert their clinical effects are not well understood (1, 2). Genetic studies in the nematode, *C. elegans* identified a single amino acid mutation in the gene *gas-1*, which increased sensitivity to all volatile anesthetics, conferred varied sensitivity to different stereoisomers of isoflurane, and decreased complex I-dependant mitochondrial respiration (3). RNA interference of *gas-1* gene expression in N2 Bristol wild type *C. elegans* increased sensitivity to the volatile anesthetic halothane and decreased complex I-dependant mitochondrial respiration, however, *gas-1* RNAi in wild type nematodes resulted in a significantly weaker hypomorph than the *gas-1(fc21)* allele (4). Work here presented examined whether *gas-1* RNAi in *eri-1(mg366)* *C. elegans*, a strain of *C. elegans* identified as having an enhanced RNAi phenotype, would more closely mimic the anesthetic sensitivity and mitochondrial respiration observed in the *gas-1(fc21)* mutant (5). *eri-1(mg366)* *C. elegans* were cultured using either control HT115 bacteria or RNAi feeding bacteria for K09A9.5 or W10D5.2 (Open Biosystems, Huntsville, Alabama). After a complete generation nematodes were washed and aliquots were used for 1) anesthetic sensitivity testing, 2) RNA extraction/quantitative RT-PCR, and 3) mitochondrial extraction/oxidative phosphorylation assays. *gas-1* RNAi in *eri-1(mg366)* exhibited a phenotype similar to *gas-1* RNAi in N2 Bristol *C. elegans*. Thus, identical methods for RNA inhibition in both strains produced similar results in mRNA knockdown, changes in complex I-dependent mitochondrial respiration, and had similar effects on anesthetic sensitivity. *gas-1* RNAi in *eri-1(mg366)* *C. elegans* failed to more closely approximate the phenotype observed in the *gas-1 (fc21)* mutant.

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Anne Newcomer

Assessing the Relationship between Depression and Other Exercise Predictors in Older Cardiac Patients

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Background and Objectives:

Exercise after cardiac rehabilitation improves health outcomes. Depression, problem-solving skills, exercise self-efficacy and health beliefs are all factors that influence exercise adoption and maintenance. The purpose of this study was to determine: (1) Is there an association between depression and social problem-solving, self-efficacy and health beliefs at the end of cardiac rehabilitation? (2) Does depression at the end of cardiac rehabilitation predict problem-solving, self-efficacy and health beliefs 2 and 6 months later?

Methods:

A sample of 171 older adults ($x=68.2$ years \pm 8.2; 76% male) was analyzed. All subjects were enrolled in a randomized controlled trial comparing two different theoretically-based interventions as compared to usual care to improve the adoption and maintenance of exercise after cardiac rehabilitation. The subjects completed a baseline survey at the end of cardiac rehabilitation that included measures of depression (Geriatric Depression Scale), problem solving (Problem Solving Inventory), health beliefs (Benefits and Barriers to Exercise questionnaire), barriers self-efficacy (Self-Efficacy for Overcoming Barriers to Exercise), and adherence self-efficacy, (Self-Efficacy for Adherence). Subjects completed the same battery of measures at 2 and 6 months after completion of cardiac rehabilitation. To investigate the relationship between depression and social problem-solving, self-efficacy and health beliefs scores, Pearson correlations and linear regression analyses were employed.

Results:

Mean depression score at the end of cardiac rehabilitation was 1.48 (scale of 0-15, range 0-11) and this remained constant at 2 and 6 months after cardiac rehabilitation. At the end of cardiac rehabilitation, there were negative associations between baseline depression and problem-solving ($r = -.39$, $p \leq 0.01$), barriers self-efficacy ($r = -.29$, $p \leq 0.01$), and health beliefs ($r = -.30$, $p \leq 0.01$) There was no significant relationship between depression and adherence self-efficacy at baseline. Linear regression analyses indicate that baseline depression explained 14.4% of the variance in baseline problem-solving ($F = 29.51$, $p \leq 0.01$), 8.3% of the variance in self-efficacy barriers to exercise ($F = 15.21$, $p \leq 0.01$) and 8.3% of the variance in health beliefs. ($F = 16.40$, $p \leq 0.01$). However, at two months after cardiac rehabilitation, the only significant association with baseline depression levels was problem solving ($r = -.33$, $p \leq 0.01$). Baseline depression explained 9.8% of the variance in problem solving 2 months after cardiac rehabilitation ($F = 9.43$, $p \leq 0.01$). At six months after cardiac rehabilitation, the only significant association with baseline depression levels was problem solving ($r = -.45$, $p \leq 0.01$); depression explained 18.6% of the variance in problem solving at 6 months ($F = 12.90$, $p \leq 0.01$). At 2 and 6 months following cardiac rehabilitation, depression did not predict health beliefs or self efficacy.

Conclusion:

Our data indicate that at the end of cardiac rehabilitation, higher depression levels are associated with lower social problem-solving abilities, lower perceived benefit of exercise/higher perceived barriers to exercise, and lower self-efficacy in overcoming exercise barriers. Baseline depression levels continued to predict worse problem solving skills at 2 and 6 months after cardiac rehabilitation. Baseline depression levels failed to predict self-efficacy and health beliefs 2 and 6 months after cardiac rehabilitation. These results imply that current depression levels affect current and future social problem-solving ability; however, current depression levels affect current, but not future self-efficacy and health beliefs. Treating depression pharmacologically could potentially benefit cardiac rehabilitation patients' problem-solving abilities, possibly helping them solve problems related to exercising more effectively. Beyond pharmacological treatment of depression, increasing exercise levels has been shown to improve depression levels. Previous research has also shown that a converse relationship between problem-solving and depression may exist, with problem-solving ability predicting depression levels. Interventions focusing on improving problem-solving skills may also aid in decreasing depression and increasing problem solving abilities. Other factors such as gender differences, antidepressant medication use, and adoption and maintenance of exercise might also be considered.

Mike S. Nguyen

Ex Vivo Characterization of Human Atherosclerotic Iliac Plaque Components using Cryo-Imaging

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We characterized atherosclerotic plaque components with a novel cryo-imaging system *in lieu* of standard histological methods commonly used for imaging validation and research endpoints. We aim to accurately identify plaque tissue types from fresh cadaver specimens rapidly (less than 5 hours) in three dimensions for large specimens (up to 4 cm vessel segments). A single-blind validation study was designed to determine sensitivity, specificity, and inter-rater agreement (Fleiss' Kappa) of cryo-imaging tissue types with histology as the gold standard. Six naïve human raters identified 344 tissue type samples in 36 cryo-image sets after being trained. Tissue type sensitivities are as follows: greater than 90% for adventitia, media-related, smooth muscle cell ingrowth, external elastic lamina, internal elastic lamina, fibrosis, dense calcification, and hemorrhage; greater than 80% for lipid and light calcification; and greater than 50% for cholesterol clefts. Specificities were greater than 95% for all tissue types. The results demonstrate convincingly that cryo-imaging can be used to accurately identify most tissue types. If the cryo-imaging data are entered into visualization software, 3D renderings of the plaque can be generated to visualize and quantify plaque components.

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Daniel Oberlin

Benefits of a Steroid-Free Regimen with Thymoglobulin Induction in Pancreas-Kidney Transplantation

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Introduction:

Steroid-free immunosuppressive protocols in pancreas transplantation are becoming more common due to improved patient mortality from infectious and cardiovascular disease; yet persistent concerns regarding long-term graft survival exist. Our clinical observations imply the hypothesis that steroid-free immunosuppression protocols not only offered the same graft-survival as steroid-based protocols but also have provided improved metabolic outcomes for patient.

Methods:

We performed a retrospective chart review of 110 pancreas transplant recipients—77 simultaneous pancreas-kidney transplants, 50 pancreas-after-kidney transplants, and 19 pancreas transplant alone—who underwent transplantation within the period of January 2002 to August 2007 and who received induction therapy with thymoglobulin followed by maintenance immunosuppression with tacrolimus and mycophenolate mofetil. The chart review compared one group on a steroid-free regimen with a steroid-based regimen. The two groups were analyzed for matching baseline characteristics.

Results will portray:

Analysis of difference in graft and patient survival between groups.

Analysis of acute rejection differences

Difference in hospitalization and infection

Analysis of immunologic differences measured by infection with CMV and BK virus.

Comparison of body mass index (BMI), lipid profiles, and blood glucose levels between the two groups.

Conclusion:

Statistical results will determine if a steroid-free regimen of thymoglobulin induction followed by tacrolimus and mycophenolate mofetil maintenance provide similar graft-survival and prevention of rejection. Furthermore, because steroid regimens can have significant impact on metabolic parameters which affect patient cardiovascular risk, results will conclude if steroid-free regimens provide improved clinical outcomes and overall patient health when compared to steroid-based protocols.

Daniel Oberlin

The Effect of Systemic Versus Portal Venous Drainage in Pancreas Transplantation on Metabolic Outcomes

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Introduction:

Pancreas transplantation is a therapeutic option for patient with end-stage diabetic nephropathy or diabetes mellitus with the inability to properly control blood glucose levels with insulin. Our objective is to compare portal and systemic venous drainage of pancreas transplants and assess the metabolic profiles and survival differences between the two groups.

Methods:

We performed a retrospective chart review of 110 pancreas transplant recipients—77 simultaneous pancreas-kidney transplants, 50 pancreas-after-kidney transplants, and 19 pancreas transplant alone—who underwent transplantation within the period of January 2002 to August 2007 and who received induction therapy with thymoglobulin followed by maintenance immunosuppression with tacrolimus and mycophenolate mofetil. The venous effluent of these patients was managed by either portal drainage (n=72) or systemic drainage (n=38). The two groups were analyzed for matching baseline characteristics and managed with equivalent immunosuppression regimens.

Results will portray:

The results will reflect comparisons of recipient characteristics and HLA matching
Comparison of change in body mass index (BMI), blood hematocrit, blood glucose level, lipid profiles, and creatinine between the two populations at 6 months, 12 months, 3 years, and 5 years.
Survival analysis will be performed with possible analysis as to the grade of rejection.
Multivariate analysis to determine which parameters affected rejection.

Conclusions:

Significant difference in graft survival and metabolic profiles between the two groups will allow for a comparison of clinical outcomes and influence surgical decision-making with respect to location of venous drainage. Conclusions will address observed differences from published reports and determine if significant clinical outcomes are different between portal and systemic venous drainage of pancreas allografts.

Ajitesh Ojha

A Comparison of the Incidence of Syncope in different orthostatic syndromes

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Background: There are conflicting opinions on whether POTS predisposes to syncope. We investigated this relationship.

Materials and Methods: We queried our autonomic laboratory database of over 3500 patients. POTS was defined as > 30 bpm rise in heart rate within ten minutes of upright tilt accompanied by orthostatic symptoms and further rise in heart rate during the remainder of the tilt study. Syncope was defined as an abrupt decrease in heart rate and blood pressure (less than 3 minutes in duration) requiring termination of the tilt table study. Statistical analysis utilized Fisher's exact test and student's t test.

Results: Of 810 patients referred for POTS, 185 met criteria, 329 patients met the criteria for orthostatic hypotension. 35% of POTS patients had tilt syncope while 22 % of OH patients had tilt syncope. Analysis of clinical syncope in the two groups showed 63 patients(90%) had a history of syncope in the POTS group with tilt syncope while clinical syncope in the orthostatic hypotension group with tilt syncope was observed in 29 patients(40.8%).

Discussion: Our results demonstrate that even in a large population referred for autonomic dysfunction, syncope occurs far more commonly in patients who have POTS than in patients with orthostatic hypotension. Furthermore, there was concordance between tilt syncope and clinical syncope in the POTS group but not in the orthostatic hypotension group. The high rate of correlation between POTS and syncope is striking, and suggests that these two disorders may actually share a common etiology, or may each constitute a strong predisposing factor for the occurrence of the other.

Key Words: Postural Orthostatic Tachycardia syndrome, syncope, orthostatic hypotension, tilt table test, dysautonomias

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Melvin E. Omodon

Quantifying Risk Factors For Shunt Malfunction In Pediatric Brain Tumor Patients

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Introduction: Complications associated with shunts placed in children with brain tumors are not well quantified.

Methods: 184 children who had brain tumors and required shunting were identified under an IRB-approved protocol. The average age was 72 months. The average follow-up time was 53 months. Tumor types included 50 medulloblastomas, 24 ependymomas, 70 pilocytic astrocytomas, 21 anaplastic astrocytomas, and 14 craniopharyngiomas.

Results: 31% of the shunts (56 patients) required revisions and 10% of the shunt placements (18 patients) were complicated by infections. 42% of all malfunctions occurred within the first 6 months post shunt placement. The rate for shunt malfunction was highest in patients with anaplastic astrocytoma (37%), and lowest in patients with ependymomas (25%). Highest rates of shunt malfunction was associated with supratentorial tumor location (44%), anaplastic astrocytoma histology (47%), and an age of initial shunt placement between 2-5yrs (51%). Risk factors for distal malfunction include infratentorial location (24%). Highest rate of multiple malfunction were found in patients between age of 2-5 yrs (38%).

Risk factors for infection included supratentorial tumor location (11% infection rate), ependymoma histology (13%), and age of shunt placement between 6 months to 2yrs (12%). The lowest infection rate was in patients with craniopharyngiomas (5%) and the highest in ependymomas (13%).

Conclusions: The risk factors for shunt failure in children with brain tumors can be quantified. Shunts placed in children with brain tumors have lower rates of malfunction and higher rates of infection than seen in the overall hydrocephalus population.

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Jennifer L. Pfau

Barriers to early diagnosis and treatment for endemic Burkitt Lymphoma in Kenya and Uganda

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Burkitt lymphoma (BL) was first described 50 years ago and remains the most prevalent paediatric cancer in Equatorial Africa with an annual incidence of 2 per 100,000 children. BL is extremely responsive to chemotherapy due to its high tumour proliferation index. The in-hospital survival rate for BL in Kenya and Uganda is nearly 70% and is associated with early diagnosis and treatment. However, 38% of children are admitted to hospital in late-stage disease. In this study, we sought to identify reasons for delay in early diagnosis and treatment for children with BL in Kenya and Uganda. Semi-structured key informant interviews were conducted with eight persons involved in the care of children with BL in Kisumu, Kenya and Kampala, Uganda, including physicians, nurses, and other supportive staff. This information was used to design a questionnaire administered to parents of children with BL to determine their knowledge, attitude, and health care practices regarding their child's illness. A resonating theme was that few parents had heard of pediatric cancer and many of them believed their child had been cursed or bewitched. Therefore parents initially sought treatment from traditional healers, witch doctors, or spiritualists and only came to hospital after these treatments failed. Some parents reported that clinicians at first misdiagnosed the tumor as a boil (or skin abscess) due to its rapid progression. Lack of money for transportation and hospital fees was also commonly cited as a barrier to prompt treatment. With regard to cancer, many parents believed that it was incurable. However, most parents changed their outlook as they saw their child and other children improve with treatment while in hospital. In conclusion, this investigation identified numerous factors that contributed to delays in prompt and appropriate care for children with BL. This information will in turn guide campaigns to increase pediatric cancer awareness in the community and improve early diagnosis within the health care setting. The ultimate goal is to increase the survival of children diagnosed with BL through improved recognition of this disease, especially in countries where BL is most prevalent.

Stephanie Polites

CD55 and CD59 Expression in Normal and Diseased Cardiac Tissue

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Introduction: Antibody-mediated rejection (AMR) following cardiac transplantation is associated with graft failure and decreased lifespan due to complement fixation and subsequent vasculopathy. Circulating graft tissue alloantibodies cause fixation of complement, however recent studies suggest that decay accelerating factor (CD55) and membrane inhibitor of reactive lysis (CD59) may act as innate immunoprotective factors countering the damage of AMR. Little is known about the expression profile of CD55 and CD59 within cardiac tissue or whether significant quantitative differences of their expression can exist between individuals to allow for their theoretical role in influencing susceptibility to AMR damage. The aim of this study is to describe CD55 and CD59 expression in cardiac tissue under physiologically normal and pathologic conditions so that future studies can effectively analyze the natural history of their expression in transplanted hearts.

Methods: Endocardial biopsy specimens were obtained peri-mortem and flash frozen in liquid nitrogen. Cardiac tissue was collected from both healthy donor hearts (not transplanted due to reasons unrelated to cardiac function) and diseased hearts showing histological evidence of myocarditis. Following CD55 and CD59 mRNA isolation and reverse transcription to cDNA, mRNA expression was measured using quantitative polymerase chain reaction (RT-PCR). Protein levels of CD55 and CD59 were measured by Western Blot. To evaluate the significance of donor origin and cardiac tissue type on the levels of CD55 and CD59, the non-parametric Kruskal-Wallis H-Test was performed. All analyses were performed in SPSS 16.0.

Results: Cardiac specimens from the left atrium, left ventricle, and interventricular septum of ten healthy donor hearts were used in addition to specimens from the interventricular septum of five diseased hearts. Statistical testing showed no significant difference between chambers of individual donor hearts. CD55 and CD59 expression are, respectively, consistent across heart chambers within individual donor hearts ($P > .05$ for CD55 and CD59 mRNA and protein). Expression between individuals, however, is inconsistent (Figure 2) and statistically significant differences in mRNA and protein levels of CD55 and CD59 were found from one heart to another with the exception of CD55 protein levels, which varied but did not reach significance ($P = .006$ for CD55 mRNA, $P = .002$ for CD59 mRNA, $P = .288$ for CD55 protein, $P = .046$ for CD59 protein). We also found that it is possible to measure CD55 and CD59 mRNA expression and protein levels in diseased hearts.

Conclusions: There is significant variability in both CD55 and CD59 levels between individual hearts. This has implications for future studies related to protective effects of CD55 and CD59 in AMR, as quantification of relative increases or decreases in expression will depend on individual baseline expression levels. There is not significant variability of either CD55 or CD59 across chambers of individual hearts, allowing mRNA expression and protein levels of a single endocardial biopsy to be applied to the entire heart. This study also demonstrates that methods used to quantify CD55 and CD59 levels in healthy hearts can be used in diseased hearts.

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Monica Reddy

The Unfolded Protein Response is Activated in Severe Pulmonary Artery Hypertension

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The origins of the insult(s) that serve as the genesis of PAH pathobiology have long been under intense investigation. Viruses, ingestion of diet pills, high-altitude, and chronic thromboembolic disease have all been implicated. We hypothesized that, regardless of etiology, the lung vasculature is severely stressed secondary to these conditions. In many conditions of cell or tissue stress, the unfolded protein response (UPR) is activated and plays a number of important roles in both normal and disease processes. We hypothesized that patients with severe pulmonary artery hypertension (PAH) exhibit an underlying protein folding derangement. We therefore investigated whether the UPR is activated in human tissue and in two mechanistically distinct animal models with severe PAH. Further, we sought to examine the role of acute and chronic hypoxia in the generation of UPR in pulmonary vascular cell cultures. Tissue samples from both animal models and human models were obtained and examined using immunohistochemistry, immunoblot analysis, cell cultures, and RNA analysis. Antibodies BiP, IRE-1, and ATF-6 were used as markers of UPR. We found that some groups in both rat models' tissue demonstrated tight correlation with the human tissue results with respect to expression of BiP, ATF-6, Hrd1, and IRE-1. In a hypoxic state, BiP, IRE1, ATF6, and Hrd1 are highly expressed at 48 hours of hypoxia (Figure 5) as measured by immunoblot and immunofluorescence, as well as XBP RTPCR.

David Reznick

Altered Gene Expression in RFX4-hml/hml Homozygous Mutant Mice

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Regulatory factor X 4 (RFX4) is a DNA binding protein. A mutation of this gene, hypoplasia of the membranous labyrinth (hml), was analyzed in this lab using two-dimensional differential in-gel electrophoresis (2D-DIGE) and mass spectrometry. Cochleae from Rfx4hml/hml mutant showed down regulation of two proteins, aldolase C and prohibitin, when compared to the cochleae of their wild type littermates. Aldolase is a glycolytic enzyme, where aldolase C is the isoform found in the brain. Prohibitin (Phb) is a regulatory protein within cells whose most predominant function is the inhibition of DNA synthesis. Because Rfx4hml/hml mice exhibit hearing impairment and hypoplasia of the membranous labyrinth, we hypothesized that the mutant phenotype can be correlated with a decrease in these proteins Phb and Aldolase C. To test our hypothesis and to further confirm the 2D-DIGE results, we investigated expression of these two genes at the mRNA and protein level in Rfx4hml/hml mutant and wild type littermates. Semi-quantitative RT-PCR was used to determine the level of mRNA expression of these genes in cochleae from Rfx4hml/hml mutant and wild type mice. Cochleae cryosections from the same mice were analyzed by immunofluorescence to further elucidate any alteration in prohibitin protein expression. The semi-quantitative RT-PCR assay of Rfx4hml/hml and wild type littermates showed a decreased expression of aldolase C in mutants, while prohibitin had no statistically significant change in expression. Immunofluorescence in these mouse tissues showed the presence of prohibitin in the organ of Corti with no significant alterations in expression quantity and location. In conclusion, aldolase C was found to have altered expression in the Rfx4hml/hml mice compared to wild type littermates, whereas prohibitin was not altered to statistically significant levels in the Rfx4hml/hml mice. These results regarding prohibitin are still significant as they are the first demonstration of prohibitin expression in the inner ear.

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Kelsey Rohlck

Left Upper Quadrant Laparoscopic Instrument Insertion Angle: Abdominal Anatomy Characterization By Magnetic Resonance Imaging.

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Objective The left upper quadrant (LUQ) is an alternative site for Veress needle and primary trocar placement for patients at increased risk for intra-abdominal adhesions. Instruments are placed into the abdomen at Palmer's Point, 3 cm below the left subcostal margin in the midclavicular line. In the axial plane, it is recommended that the instruments be inserted perpendicular to the surface of the abdomen. In the sagittal plane, various angles of insertion from vertical to 45° toward the lower abdomen have been recommended. However, little is known about the dimensions of the abdominal cavity beneath Palmer's point and thus the safest angle for instrument insertion is uncertain. This study was designed to determine the angle of insertion of laparoscopic instruments at Palmer's point that is least likely to injure retroperitoneal structures and to determine if this angle should be varied according to the patient's body mass index (BMI).

Methods Abdominal magnetic resonance images were reviewed for 78 women between 18 and 50 years of age. Abdominal wall thickness at Palmer's point and the distance from the skin at this point to the retroperitoneal structures were measured vertically (0°), at 30° and at 45° from vertical toward the lower abdomen. The results were correlated with body mass index (BMI). The location of the aorta in relation to the line of insertion was also determined.

Results The abdominal wall thickness ranged from 1.1 to 5.1 cm and correlated positively with BMI. The distance from the skin to the retroperitoneal structures ranged from 7.1 to 23.6 cm and correlated positively with BMI and angle of insertion. A Veress needle or trocar inserted to its complete 11 mm length would contact retroperitoneal structures in 35% of patients if inserted vertically (0°), 23% at 30°, and 1% at 45°. In the axial plane, the insertion line perpendicular to the abdominal wall was always lateral to the aorta.

BMI (kg/m ²)	Abdominal wall Thickness (cm)	Distance from skin to retroperitoneal structures (cm)		
		Vertical (0°)	30° from vertical	45° from vertical
<25 (N=35)	2.19 (range 1.1-3.8)	10.5 (range 7.1-14.0)	12.1 (range 8.2-16.2)	14.8 (range 10.0-19.8)
25-30 (N=28)	2.9 (range 1.5-4.2)	11.8 (range 8.6-15.9)	13.6 (range 9.9-18.4)	16.6 (range 12.2-22.5)
>30 (N=15)	3.87 (range 2.0-5.1)	13.2 (range 9.5-16.7)	15.2 (range 11.0-19.3)	18.6 (range 13.4-23.6)

Conclusions Veress needles and laparoscopic trocars placed through Palmer's point should be inserted at an angle of at least 30° from vertical toward the lower abdomen to minimize the risk of injuring retroperitoneal structures. In women with a BMI <25, the angle should be increased to 45°. In the axial plane, the angle of insertion should not be shifted toward the midline, since the aorta always was medial to the instrument tip when the angle of insertion was perpendicular to the abdominal wall.

F. Andrew Rowan

EVAR vs. Open Surgery for Ruptured Abdominal Aortic Aneurysms

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Objective: With continued success of elective endovascular aneurysm repair (EVAR), the procedure has now been extended to ruptured aortas. The purpose of this study is to evaluate our results of ruptured abdominal aortic aneurysms (AAA) and to determine if EVAR is equivalent to open surgery.

Methods: We retrospectively reviewed the results of all patients presenting to our main campus who underwent repair of ruptured infrarenal AAAs between January 1990 to May 2008. Reoperative AAA surgery, juxtarenal AAA and thoraco abdominal aortic aneurysms were excluded. Co-morbidities, intra-operative details, and post operative complications were tabulated. EVAR and open repair were compared and survival analyzed by Kaplan Meier models with Cox proportional hazards used to distinguish the risk between factor levels.

Results: One hundred and sixty patients had repair of a ruptured infrarenal AAA over this time period. Thirty two of the 160 (20%) were repaired using EVAR. One hundred and twelve were considered free rupture (70%) and 48 contained rupture (30%). The average age was 72.6+9. The average APACHE score was 13.3+6.7. Intraoperative mortality was 5.6%, with no patient undergoing EVAR suffering an intraoperative death. However, 30 day/ in-hospital mortality was 31.9% with no significant difference between EVAR and open surgery ($p=.93$). There was also no difference in ICU length of stay 12+16; $p=.94$, and hospital length of stay 19+12; $p=.62$ between the two groups. Univariable analysis found that male gender ($p=.02$), and DM ($p=.01$), and tobacco use ($p=.04$) use were associated with a higher intraoperative mortality. Multivariable analysis for 30day/in-hospital mortality found that preoperative renal insufficiency OR 2.4(1.1,5.3; $p=.04$), preoperative hypotension OR 2.4(1.1,5.3; $p=.02$), and preoperative cardiac arrest 3.8 (1.1,11.6; $p=.03$) were all associated with the greatest mortality.

Conclusions: Mortality rates for ruptured infrarenal AAA remain high. The use of EVAR for these procedures now equals that for standard open repair with regard to 30day/in-hospital mortality, and is now a viable option. Nevertheless, clinical judgment on when to use this as a primary repair modality must be exercised

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Ashraf A. Sabe

THE EFFECTS OF SPLENIC ARTERY EMBOLIZATION ON NON-OPERATIVE MANAGEMENT OF BLUNT SPLENIC INJURY: A 16 YEAR EXPERIENCE

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Introduction: Non-operative management (NOM) of blunt splenic injury has become the preferred treatment for hemodynamically stable patients. The application of splenic artery embolization (SAE) in NOM has been controversial. We hypothesized that incorporation of initial use of SAE into a practice protocol for patients at high risk for NOM failure (contrast extravasation or pseudoaneurysm on CT, grade 3 injury with large hemoperitoneum, grade 4 injuries) would improve patient outcomes.

Methods: A retrospective analysis of three continuums of practice was performed: group I (January 1991 - June 1998) - SAE not part of routine non-operative management; group II (July 1998 - December 2001) - introduction and discretionary use of SAE; and group III (January 2002 - June 2007) - standardized use of initial SAE for patients considered at high risk of non-operative failure. The primary outcome measure was the success of NOM. Failure of NOM was defined as the need for abdominal operation. Secondary outcomes were mortality, length of stay, and splenic salvage.

Results: Over 16 years, 815 patients with blunt splenic injury were treated at our Level 1 trauma center. There were 222 patients in group I, 195 in group II, and 398 in group III. There was an increase in the use of SAE over time with a significant improvement in the utilization of NOM (61% in group I; 82% in group II; 88% in group III, $p < 0.05$). This was associated with an increase in successful NOM (77%, group I; 94%, group II; 97%, group III, $p < 0.0001$ group I vs. group II and III). Mortality, length of stay, and splenic salvage were similar in groups II and III but significantly improved compared to group I.

Conclusions: The increased use of initial SAE in high risk patients expanded the use of NOM, but was not associated with other incremental improvements.

Katherine Schlosser

Evaluation of Geographic Information Systems as an aid to Caribbean Basin Schistosomiasis Elimination

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Center for Global Health and Disease

Case Western Reserve University, School of Medicine

In light of recent evidence quantifying the disability associated with schistosomiasis, the World Health Organization has recently shifted its recommendations for the disease away from control and towards elimination. The purpose of this project was twofold; part one assessed the availability of geographic information systems (GIS) data to support schistosomiasis elimination in the Caribbean basin. These data were collected and compiled into maps, which were provided to PAHO as a starting point for additional mapping efforts. Part two was a pilot study evaluating the self-collection of GPS data for use with schistosomiasis elimination campaigns. Mapping efforts were focused on Antigua and Barbuda, the Dominican Republic, Guadeloupe, Martinique, Montserrat, Puerto Rico, and Saint Lucia. Data were compiled by searching the Internet for free GIS data sources. Information was collected and recorded for layers including elevation, hydrography, roads, political boundaries, land use, cities, and hospitals. Acquired data were compiled in ArcMap by country. These data were made available as PDF maps with the associated shapefiles. Part two of this project was completed in St. Lucia from June 2, 2008 through June 13, 2008. Data were collected using a Garmin eTrex Legend set to WGS84 collecting in degrees, minutes, and seconds. GPS locations were recorded for six hospitals, twenty-five health centers, and tracks were recorded for roads around the island. The main finding for the online data compilation is that high quality Caribbean data are not readily available in the public domain. The results of the pilot study were promising. These data collected in St. Lucia were more accurate and detailed than that publicly available. Smaller roads were captured by the self-collection and importantly, roads to health centers and hospitals were identified. These data are detailed enough to use to return to these sites. GIS may represent a useful tool to aid in schistosomiasis elimination. Missing information that would be most helpful to collect would include the accurate location of cities, towns, hospitals, schools, and potential sites of transmission.

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Nicholas O. Schroeder

3,4-Methylenedioxy- β -nitrostyrene (MNS) and the reduction of colony formation in osteosarcoma cells and osteoblasts.

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MNS, a known inhibitor of platelet aggregation, has been shown previously to be an inhibitor of tyrosine kinases src and syk and is being studied as a potential chemotherapeutic agent for osteosarcoma. Our lab has previously shown that MNS inhibits motility and colony formation in vitro in two families of genetically-related human osteosarcoma cell lines: non-tumorigenic/non-metastatic parental cell lines (TE85 and SAOS-2), a tumorigenic but non-metastatic cell line (MNNG), and highly tumorigenic/metastatic cell lines (143B and LM7). For this study, we examined the ability of MNS to inhibit colony formation and disrupt preformed colonies grown on collagen gels to mimic the microenvironment of the lung, the most common site for lethal micrometastases in osteosarcoma patients. MNS was shown to inhibit colony formation and quickly induce apoptosis in all five cell lines. Biochemical assays were also performed by an outside party to study the ability of MNS to inhibit a number of tyrosine kinases believed to be involved in osteosarcoma, including src and syk. Interestingly, MNS did not inhibit any tyrosine kinases, suggesting that it acts via a different mechanism

Matthew Soden

Prevalence and Predictors of Obstructive Sleep Apnea in Children with Sickle Cell Anemia

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Prevalence and Predictors of Obstructive Sleep Apnea in Children with Sickle Cell Anemia

INTRODUCTION

Obstructive sleep apnea (OSA) is a common childhood condition (prevalence 2-3%) that can play a causal role in vaso-occlusive and other adverse health outcomes like pulmonary hypertension and central nervous system (CNS) events in children with sickle cell anemia (SCA). There is a paucity of data about the prevalence of OSA in children with SCA. The specific aims of this study were to determine the prevalence and predictors of OSA in children with SCA who underwent overnight full channel polysomnography (PSG) as part of their participation in the multi-site observational cohort Sickle Asthma Cohort (SAC) study. Most of the studies linking nocturnal respiratory dysfunction to adverse outcomes in children with SCA have recorded only nocturnal oxyhemoglobin saturation without other measures of sleep, breathing or upper airway obstruction. It is unknown whether screening recommendations for OSA (ascertainment of symptoms such as snoring, observed apnea, restless sleep, or daytime sleepiness) in otherwise healthy children would be useful for children with a chronic condition like SCA. Since OSA in adults is clearly linked to hypertension, other adverse cardiovascular and CNS outcomes, and daytime dysfunction, it is possible that early detection and treatment of OSA in this vulnerable pediatric population may improve their health outcomes.

QUESTION: What is the prevalence of OSA in children with sickle cell anemia?

METHODS

A multicenter, prospective cohort study of asthma and adverse health outcomes (pain crises and acute chest syndrome) in children who were either homozygous for sickle hemoglobin or had sickle beta thalassemia 0 between the ages of 4 to 18 years were identified at three tertiary academic hematology-based care centers (London, Cleveland and St. Louis) and enrolled into the SAC study. SAC is an ongoing National Heart Lung Blood Institute-funded prospective, observational cohort study designed to evaluate the contribution of asthma and sleep abnormalities to SCA-related morbidity. This first report on prevalence includes the first 150 participants recruited into the study who underwent overnight polysomnography from the start of study in June 2006 through February 1, 2008. All sites in the study used identical sleep acquisition systems (Embla N-7000, Broomfield, CO), sensors, data collection procedures and followed current professional standards for overnight collection of sleep and breathing data in children except that carbon dioxide was not measured. Full channel overnight PSG was performed over a single recording night with studies starting at the child's usual bedtime and ending at the child's spontaneous waking or as late as 7:00 AM. Obstructive sleep apnea indices from PSG data were used to define OSA. The presence and severity of OSA was measured by the obstructive sleep apnea index (OAH13), that is, the number of obstructive apnea events plus the number of hypopnea (reduced breaths) associated with a 3% desaturation per hour. Because SCA is a chronic condition that can be associated with pre-existing oxygen desaturation or lower respiratory tract disease, the prevalence of OSA in children with SCA was evaluated using two threshold definitions: 1) OAH13 \geq 2 and 2) OAH13 \geq 5. The first definition is commonly used in clinical practice to diagnose OSA in otherwise healthy children. Definition 2 is similar, but has a more conservative threshold for hypopneas and was previously used by our group in a large community based epidemiological study of the prevalence of OSA in school-aged children. Data from standardized questionnaires regarding sleep habits, physiological and anthropometric measures (height, weight and blood pressure), and other laboratory tests (lung function, blood count) were used to examine the relationship between OSA and other risk factors or clinical findings.

RESULTS

Of the 149/150 children with successful overnight studies, we excluded 24 studies with lower quality airflow data which affected the reliability of the OAH13 measure and 3 studies because the participant was on supplemental oxygen at the time of the study which limited detection of desaturation related events. The final analytic sample was based on 122 participants.

OSA was present 26/122 (21.3%) of the children with SCA using definition 1 and 13/122 (10.6%) using definition 2. Results related to the relationships between OSA and other key covariates and potential predictors in children with SCA were not available at the time of this analysis.

Matthew Soden

CONCLUSION

At either threshold, children with SCA have a higher prevalence of OSA compared to normal children. These children may benefit from increased efforts to identify this treatable clinical problem. Future research should be directed at identifying potential predictors for OSA in children with SCA. The impact of OSA on health outcomes in children with SCA is also an important area for future research.

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Anna Strohl

Newly developed methodology to test adherence of the fetal membranes (amnion to choriodecidua)

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We have previously shown that separation of the fetal membranes (FM), amnion from choriodecidua, occurs as an integral part of the process of FM rupture. We have also reported that some spontaneous separation of FM at delivery is nearly universal and is associated with increased gestation, SROM, shorter duration of contractions, and SVD. The etiology of this spontaneous FM separation is unknown. If biochemical degradation at the amnion-choriodecidua interface is a factor, decreased adhesive force between the FM components prior to complete separation would be expected. The purpose of this project was to develop and validate machinery and procedures to measure the adhesive force between amnion and choriodecidua.

Commercial tensile testing equipment was adapted to perform a standard T-peel test (ASTM D1876) to measure FM adhesive force. FM test strip dimensions, peel speed, and orientation of test strips with respect to the placental disc were optimized for reproducibility. Test system validation was performed using Shurtape CP 60 (slow release painters masking tape) as the standard. FM were from term Caesarean or term SROM vaginal deliveries.

Equipment and procedures for a standard T-peel test on FM were developed. The adhesive force between FM ranged from 0.01 lb/in to 0.15 lb/in. A 0.5 lb load cell (sensitivity of 0.0001) was incorporated into the design and the system adjusted was so that the load cell operated in its mid-range. Reproducibility was optimal with FM test strips of 4 cm width by 6 cm length, peel speed of 10 in/min, and FM test strips oriented parallel to the placental disk. Shurtape CP 60 of decreasing widths showed reproducible, linear changes ($r^2 = 0.96$) in adhesive force over the adhesive force range of FM. FM showed greater adhesive force adjacent to the placental disc than distal from the disc ($p < .05$).

Adhesive force between the FM components can be accurately and reproducibly measured. Adherence is higher adjacent to the placental disc. We speculate that decreased adherence of the amnion to choriodecidua is a biochemically driven process contributing to FM weakening and rupture at term; this would be supported by clinical studies showing decreased FM adherence with gestation and with labor.

Michael J. Tchou

Mutations in GJA5 may lead to atrial fibrillation

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Introduction: Atrial fibrillation (AF) affects 1 in every 25 people over the age of 60 in the United States. While a large proportion of AF is caused by structural changes, 30% of cases lack a structural cause (lone AF) and may have a genetic component to their etiology. Recent research has suggested that mutations in GJA5, the gene encoding the gap junction protein Connexin 40 may be implicated in the development of AF. A case-control comparison of single nucleotide polymorphisms of the GJA5 gene may reveal significant mutations.

Methods:

Blood derived or atrial tissue derived DNA was prepared from a cohort of 200 lone AF subjects. For the detection of rare variants in the GJA5 gene, the three exons and flanking sequences were amplified and sequenced. For the detection of common single nucleotide polymorphisms (SNPs) in the GJA5 gene, the DNA was subjected to an Illumina SNP microarray and/or assay of single SNPs by the TaqMan procedure. GJA5 transcript levels were determined from atrial RNA using an Illumina gene expression microarray.

Results:

Two novel heterozygous mutations were identified among 24 lone AF samples through DNA sequence analysis. In addition a common 25 bp insertion-deletion variation in the 3' UTR that was discovered in a related project was verified in these tissues and was associated with the level of the GJA5 transcript in atrial tissue. Experience was gained in laboratory procedures including DNA extraction and preparation, gel electrophoresis, PCR, primer design, sequencing, sequence analysis, and microarray analysis.

Conclusions:

Early results indicate that variations in the GJA5 gene appear to be related to GJA5 expression levels and incidence of AF. Ongoing sample analysis will allow a more definitive association of GJA5 genetic variation and AF.

Supported by T-35

Ben Tomlinson

The impact of base excision repair activity on the sensitivity to temozolomide and the combination with methoxyamine in melanoma cell lines in vitro and in xenograft tumors

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Methoxyamine (MX) blocks the base excision repair (BER) pathway by binding to abasic sites. These sites are generated after glycosylases remove alkylated purines and pyrimidines that have been methylated by the anti-cancer drug temozolomide (TMZ) that is an effective drug used in clinics for melanoma treatment. We hypothesized that MX would sensitize melanoma cells to TMZ through its ability to specifically bind to abasic sites, resulting in the blockage of BER pathway and accumulation of DNA damage. We tested our hypothesis in two melanoma cell lines, A375 and WM164, by evaluating the expression of BER proteins and sensitivity to the TMZ alone and in combination with MX in vitro and in xenograft setting. Results showed that A375 and WM164 cell lines have normal MMR repair function and similar MGMT activity, which are well known TMZ resistant factors. However, differential levels of BER proteins were detected in these two cell lines. In comparison with A375 cells, the expression of BER proteins (including MNP, UDG, APE, and Pol β) were lower in WM164 cells. The sensitivity to TMZ alone in these two cell lines was assessed by IC90 values generated by clonogenic survival assay, showing 480 μ M in A375 and 570 μ M in WM164 cells. MX efficiently potentiated TMZ cytotoxicity in A375 but failed to enhance the killing effect of TMZ in WM164 cells. Similar results were observed in nude mice carrying human A375 or WM164 xenograft tumors that were treated with TMZ (80 mg/kg) or the combination of TMZ and MX (2 mg/kg), ip injection daily for 5 days. The results suggest that the failure of TMZ-potentialiation by MX in WM164 cells was related to the low activity of BER proteins, particularly low levels DNA glycosylases (UDG, MPG, OGG1). Deficiency of these BER proteins would decrease the formation of abasic sites, which are the targets for MX action. Thus, results indicate that BER proteins may be differentially expressed in tumor cells that impact the therapeutic effect of TMZ and the combination of TMZ and MX.

Erin Nagy Tomlinson

Patient Attitudes toward Firearm Screening in the Outpatient Clinic

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Neighborhood Family Practice

This study describes patient attitudes about guns in the home and the physician role in questioning about guns in the home. In an urban family practice clinic in Northeast Ohio, a written health habits survey was previously developed which included the question “Have a gun at home?” 387 charts were reviewed showing a 7% overall rate of guns in the home, which varied according to primary care provider. 63 subjects were interviewed via telephone to investigate their attitudes regarding the written survey and guns in the home. We found that most patients do not feel mad, uncomfortable, or judged when answering the written question “Have guns at home?” on a health habits survey. Most patients think it is an acceptable practice for a healthcare provider to ask about guns and to advise safe storage of guns, but not to recommend removal of guns from the home.

These findings support the acceptability of a written questionnaire regarding guns in the home which can assist a busy doctor or nurse practitioner in identifying patients who should be counseled regarding safe storage of firearms.

Supported by Case IRB, Neighborhood Family Practice

Jason Toy

Isolated Arthroscopic Rotator Interval Closure for Shoulder Instability

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Department of Orthopedic Surgery

Hospital for Special Surgery

Purpose: A rotator interval closure is a well described technique that can be useful as an adjunct to arthroscopic stabilizations. We have previously described an open technique with isolated closures of rotator interval defects for shoulder instability. Our hypothesis is that, in appropriately selected patients with mild glenohumeral instability, an isolated arthroscopic rotator interval closure can improve stability, relieve pain, and obviate the need for a standard arthroscopic plication or open capsular shift.

Methods: A retrospective review was performed for all patients that underwent arthroscopic shoulder surgery by the senior author at one institution over a two year period. Ten patients were identified that had an isolated arthroscopic closure of the rotator interval.

Results: Seven patients (average age, 24.4 years) were evaluated at a mean follow-up of 3.6 years range (2.1 to 4.1). All patients initially presented with pain and had grade 1 or 2 anterior and inferior instability. The average pre-operative L'Insalata score was 58.5 and post-operative was 88.6 (p-value <.05). Four out of 7 patients were very satisfied with the surgery, 2 somewhat satisfied, and 1 patient neutral. No patients were dissatisfied with the results of surgery.

Conclusions: In appropriately selected patients, with grade 1 or 2 subluxation and capsular laxity without a Bankart lesion, an arthroscopic isolated rotator interval closure can improve stability and relieve pain.

Level of Evidence: Level IV

Lauren Tuchman

Cardiac Presentations to a County Hospital in Relation to a Statewide Smoking Ban

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Rationale: Secondhand smoke has been associated with acute cardiac diseases. Passage of a public and workplace smoking ban in Ohio provided natural experimental conditions to determine whether timing of the ban was associated with reduction in acute cardiac diseases. We hypothesized that emergency department (ED) visits for acute cardiac complaints would decrease after implementation of the public smoking ban.

Methods: County hospital electronic medical records were queried. Durations queried (May 2005-May 2008) included pre-ban, post-ban, and an interim period. ED visits for acute myocardial infarction, acute ischemic heart disease, and angina were abstracted. Age, gender, race, and smoking status were noted. Locations of violations of the smoking ban were also available.

Results: Of all patients presenting with acute cardiac complaints, there was a high prevalence of smoking (45% among those reported). The average weekly number of visits increased during the time the smoking ban was in effect (mean increase = 1.44 visits per week, 95%CI 1.05 - 1.83, $p < 0.0001$). Regression analyses with interaction terms accounting for gender/age demonstrated increases in visits among men ($p = 0.03$) and in those less than 60 years old ($p = 0.01$). Percent of individuals presenting with cardiac complaints living within close proximity (1/4 mile) to establishments with violations also increased significantly from 12.7% vs. 20.5% ($p = 0.02$).

Conclusion: Surprisingly and in contrast to previous similar studies, ED admissions to our county hospital for acute cardiac complaints increased in association with timing of a public smoking ban. However, these data are limited by: 1) a possible increase in county hospital utilization in an economically challenged community, and 2) a high prevalence of primary smokers which may render irrelevant any potential effects of reducing secondhand smoke exposure. Further analyses accounting for total ED presentations are ongoing.

Meryl Twarog

Outcomes for Patients with Eating Disorders Using a Standardized Inpatient Protocol

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No prior studies have evaluated the effectiveness of a standardized protocol for inpatient medical treatment of patients with nutritional insufficiency (NI).

We hypothesize that use of an inpatient protocol at our institution will prevent refeeding syndrome and sudden cardiac death. A secondary aim of this study was to document time to resolution of medical instabilities including as bradycardia, orthostasis, hypokalemia and prolonged QTc rhythm.

A retrospective chart review was conducted from patients hospitalized for NI or medical complications of eating disorders from June 2005 to August 2008. Patients were identified by billing codes (269.9, 263.9). The data extracted included demographics, medical instabilities at admission, time to resolution of abnormalities, nutritional status at resolution and complications during refeeding. In addition, we documented change in weight, BMI and percent MBW during hospitalization for all patients and divided the data by gender for further comparison. Statistical analysis focused on determining means and medians for continuous variables and calculating percents for categorical data.

Fifty-two patients were admitted using the NI protocol (8% male, 94% female) with a median age of 17 (range 11 to 23). Mean change in percent MBW from admission to discharge was 3.35% (p value <0.001) and the average length of stay was 6.81 days. Orthostatic pulse change was the most common medical instability documented and required more days of hospitalization for correction. Comparison of admission and discharge percent MBW by gender was not statistically significant (0.756) but was limited by the small number of male subjects. Normalization of most clinical instabilities occurred when patients achieved 80% MBW, excluding hypokalemia.

This study suggests that implementation of a clinical practice guideline (CPG) results in sufficient acute weight gain for resolution of medical instabilities while preventing medical complications of refeeding. In addition, we provide evidence-based data for inpatient treatment of anorexia nervosa.

Nolan Walther

High Frequency Electrical Conduction Block for Bladder Voiding

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Case Western Reserve University, Neural Engineering Center, Functional Electrical Stimulation

Body of Abstract: (300 word max – 294 currently)

Aims: An implantable spinal sacral nerve stimulating device is currently utilized to allow controlled bladder voiding in spinal cord injured individuals with bladder dysfunction. However, this implant is frequently performed with a dorsal rhizotomy, or severing of sensory nerves. This study aimed to demonstrate (1) high frequency alternating current (HFAC) conduction block at the spinal root level, and (2) a proximal intradural HFAC reversible block of external urethral sphincter (EUS) reflexes combined with distal extradural intermittent stimulation bladder drive to improve voiding without a dorsal rhizotomy. **Methods:** In three dogs and five cats, surgical implantations of nerve cuff electrodes were placed on distal extradural roots evoking bladder and/or EUS contractions (S1, S2, or S3) and on proximal intradural ventral roots evoking bladder and EUS contractions (S1+S2 or S2+S3). Trials of proximal stimulation with distal HFAC block, and trials of distal extradural intermittent stimulation with and without proximal intradural HFAC block were conducted. EUS and bladder pressures, bladder volumes, and volume voided were recorded. **Results:** HFAC at the sacral root level partially blocked EUS and bladder contractions in proximal stimulation and distal HFAC block trials. Applying HFAC block to proximal intradural ventral sacral roots combined with distal extradural intermittent stimulation yielded no difference in voiding compared to distal extradural intermittent stimulation alone with large bladder volumes of 80 ml ($p=0.8534$) or small bladder volumes of 40 ml ($p = 0.3724$). **Conclusions:** HFAC at the sacral root level demonstrated conduction block of EUS and bladder contractions evoked from a proximal stimulus. Further experimentation is necessary to investigate the efficacy of proximal intradural ventral sacral root HFAC block combined with distal extradural intermittent stimulation to allow voiding. Proximal intradural HFAC block may provide an attractive alternative to dorsal rhizotomies accompanying implantations of extradural intermittent bladder stimulating devices.

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Arthur Wang

Mechanisms of Golf-related head injuries in the pediatric population

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As the international popularity of golf has grown over the years, so has the number of golf-associated head injuries. These head injuries frequently involve individuals being struck in the head by a swinging golf club or high velocity golf ball while observing another person playing. Many of these injuries typically require immediate neurosurgical intervention. However, even with timely treatment, some patients suffer severe intracranial injuries that lead to long term neurological deficits. Although all age groups have seen a rise in golf head injuries, children have one of the highest rates of head trauma from golfing accidents. The goals of our study were to identify potential risk factors for golf head injuries and understand the mechanism and severity behind golf club, golf cart and golf ball head injuries. A retrospective review of the patient's existing hospital and outpatient clinic charts, operative reports and radiographic images was conducted. In addition, a telephone interview and questionnaire will be administered for each patient to understand the circumstances and mechanisms behind their injury. Using this information, we will attempt to look for correlations between our variables. Between the years 1997 and 2008, 22 patients were treated at Rainbow Babies and Children's Hospital for golf related neurosurgical injuries. The average age of our patients was 7.8 years with a male:female ratio of 2:1. 14 of the 22 patients were injured after being struck by a golf club, 4 were injured by a golf ball and another 4 were injured after falling from a golf cart. Of the injuries seen, 15 patients suffered skull fractures and 7 suffered intracranial hemorrhages. An interesting finding seen was that July and August were the two months when the majority of injuries took place. Although this study is not finished, we can conclude that golf related head injuries is a novel but serious mechanism of neurological injury in children and adolescents. Children are especially susceptible because of their yet fully developed understanding of the dangers of wielding a golf club. Future injuries can be prevented with vigilant monitoring on the part of parents and a better understanding of the circumstances surrounding these injuries.

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Franklin D. Warren II

Serotonin-Dopamine Interactions in PET Knock-out Mice

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The neurotransmitter dopamine (DA) has a long association with normal functions such as motor control, cognition, and reward, as well, as a number of syndromes including drug abuse, schizophrenia, and Parkinson's disease (1). Studies have shown that serotonin, by way of several receptors located in various parts of the brain, has the ability to modulate DA neurons. There are at least fourteen serotonin (5-HT) receptor subtypes, many of which have been shown to play some role in mediating 5-HT/DA interactions (1). The effects of serotonin on DA have historically been exploited in efforts to create therapeutic drugs for drug abuse, schizophrenia, Parkinson's disease, and other disorders of DA action. We have shown previously that mild handling stress increases DA and 5-HT release in the rat prefrontal cortex (PFC). The increase in DA is blocked by administration of an antagonist at the serotonin 5-HT_{2A} receptor subtype (Pehek et al., 2006). These data indicate that 5-HT, released during stress, acts on 5-HT_{2A} receptors to augment stress-induced DA release. It is important, however, to provide alternative lines of evidence. We propose to test the hypothesis that 5-HT release is necessary for stress-induced DA release in the PFC through the use of the PET KO mouse. Brain 5-HT is severely depleted in these mice. Thus, while basal DA levels may be normal, a diminished serotonergic response to stress may result in a decreased dopaminergic response. We also wish to test the responsivity of these mice to a 5-HT_{2C} receptor antagonist. A collaborator, Dr. Bryan Roth, formerly with Case and now with the University of North Carolina, has determined that 5-HT_{2C} (but not 5-HT_{2A}) receptors are upregulated in PET KO mice. Administration of 5-HT_{2C} antagonists increases cortical DA in normal rats. We predict that, relative to wild-type (WT) controls, PET KO mice will show a heightened response to 5-HT_{2C} antagonist administration.

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David Yao

The Regulation of O-linked fucose on Notch Signaling and Notch-Ligand Interactions

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Notch receptors play an essential role in cellular differentiation, proliferation and apoptosis, thus controlling the development of multiple biological systems. In vertebrates, Notch is a family of transmembrane proteins that includes Notch1, Notch2, Notch3 and Notch4. These molecules interact with both Delta-like and Jagged ligands. The extracellular domain of Notch1 is composed of multiple tandem EGF-like repeat motifs. Each contains six highly conserved cysteine residues as well as serine/threonine residues that may be decorated with O-linked fucose. These O-linked fucose moieties can be further elongated by Fringe glycosyltransferases, which append N-acetylglucosamine molecules to fucose through a β -1,3-linkage. Although O-fucosylation of Notch receptors is clearly evident, the physiologic function of O-linked fucose in the activation of Notch signaling and Notch-ligand binding are still unclear. In this report, we first demonstrated that Fringe elongation of O-linked fucose on Notch1 enhances the binding affinity between Notch1 and Delta-like ligands including DLL1 and DLL4. Furthermore, this modification up-regulates DLL1- and DLL4-mediated Notch signaling. On the contrary, the Fringe modification does not appear to regulate the Notch1-Jagged1 (J1) interaction and thus J1-mediated Notch signaling. To further address the function of O-linked fucose, we studied the induction of Notch signaling in the presence of Lunatic Fringe after an individual O-linked fucose site was abolished. Our results suggest that multiple O-linked fucose sites on Notch1 differentially participate in DLL1- and DLL4-mediated Notch activation. Mutations at both EGF9 and EGF12 reduced Notch activity significantly. We further examined the regulation of individual O-fucose sites in Notch-ligand binding. By comparing to the wild-type control, the affinity of soluble DLL1, DLL4, and Jagged-2 ligands to Notch1 expressing cells was reduced in the presence of individual EGF9 and EGF12 mutations. These findings indicate that O-fucose sites play an essential role in Notch-ligand binding and hence the activation of Notch signaling.

David Yao

Notch-Dependent Control of Blood Lineage Development is Modified by Fucosylation

David Yao*, Quanjian Yan*, Lebing Wei, John Lowe, Lan Zhou

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Phenotypes resulting from mutations in Notch loci range from notches at the wing margin in *Drosophila* to T cell acute lymphoblastic leukemia in human. These pleiotropic effects of Notch signaling stem from the pivotal role of Notch receptors in controlling the development of multiple biological systems. Because of this critical role, Notch signaling is subject to tight regulation at multiple levels. One such regulation is the post-translational modification of Notch receptors by O-linked fucosylation, a reaction that is catalyzed by Protein O-fucosyltransferase-1 (Pofut1). To address the physiologic function of O-linked fucose moieties on Notch we have previously studied mice engineered to be conditionally deficient in O-linked fucose. These mice maintain a targeted deficiency in the FX locus which encodes an enzyme required for the de novo synthesis of GDP-fucose. Homozygosity for the FX-null allele yields a complete deficiency in O-linked fucosylation of Notch. It also produces phenotypes that include myeloproliferation and faulty T cell development, each of which is due to loss of Notch signaling in myeloid progenitor cells. In this study, the physiologic function of fucose in regulating hematopoietic lineage homeostasis was examined. We first demonstrated that FX-null multi-potential progenitors differentiated into myeloid cells while co-culturing with Notch-ligand-expressing OP9 cells. Bone marrow transplant of FX-null hematopoietic stem cells yielded an augmented myelopoietic development phenotype. Consistent with these findings, using an in vitro Notch-dependent T cell differentiation assay, we observed that mouse embryonic stem cells (ESCs) carrying targeted deficiencies in either Notch1 or Pofut1 failed to produce T lymphocytes while maintaining their ability to generate granulocytes. Moreover, in vivo hematopoietic reconstitution of CD34+ stem cells derived from Notch1-null or Pofut1-null mouse ESCs showed enhanced granulopoiesis with depressed lymphoid lineage development. Together, our results indicate that fucosylation of the Notch receptor is critical in regulating blood cell lineage homeostasis.

Karen Yeh

Characterization of Disordered Eating in Young Children versus Adolescents

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Eating disorders (EDs) have been increasingly recognized and occurring at earlier ages in recent years, with diagnosis often delayed due to the atypical presentation in younger children. This study aims to characterize the presentation of children versus adolescents with EDs to enhance clinicians' ability to detect illness. A retrospective chart review was performed on children ages 6–12 and 13–19 years diagnosed with EDs presenting to an outpatient ED clinic. Data were collected on demographics, medical and psychiatric histories, physical activity, and other factors. The groups were compared on categorical and continuous variables ($P < 0.05$). Of 77 children ages 6–12 years (mean $10.9 \pm$ SD 1.4 years) versus 127 adolescents ages 13–19 years (15.1 ± 2.0 years), a higher percentage of boys was found in the younger group (22.1% versus 10.2%, $P = 0.021$). The younger group included 14 girls (23.3%) who experienced menarche at an earlier age than in the older cohort (11.4 ± 0.6 years versus 12.3 ± 1.4 , $P < 0.001$). Compared with adolescents, young children lost less weight ($P = 0.001$), weighed less in percentage median body weight ($P = 0.019$), were diagnosed quicker ($P = 0.031$), and were less likely to binge ($P = 0.001$), count calories ($P = 0.004$), and run ($P = 0.007$). There were no differences in comorbid psychiatric diagnoses between the groups, but the young cohort was less likely to report body image disturbances ($P = 0.011$) and suicidal ideation ($P < 0.001$). With a better understanding of the ways children with EDs present differently from older patients, health care providers can provide better treatment to these young patients for improved outcomes. Care providers need to react quickly and have a high index of suspicion of EDs in children with weight loss, early puberty, and abnormal eating behaviors, without overlooking the boys. Future studies should address diverse populations of children versus adolescents to evaluate optimal interventions with younger patients.