Sixty-Ninth Annual
Soma Weiss
Medical and Dental

Student Research Day

January 15, 2009

Book of Abstracts

HARVARD MEDICAL SCHOOL
Office of Enrichment Programs

The Soma Weiss Student Research Day

This day honors the memory of Soma Weiss, MD (1899-1942), an inspiring teacher and physician at HMS and an ardent supporter of student research. Soma Weiss was born January 27, 1899 in Bestercze, then a part of Hungary. He immigrated to New York in 1920 and graduated from Cornell Medical College in 1923.

Soma Weiss came to Harvard Medical School in 1925 when he was appointed assistant at the Thorndike Memorial Laboratory and Research Fellow in the Department of Medicine. He rose rapidly, demonstrating his great ability as an investigator, teacher, administrator, and clinician. Within four years, Dr. Weiss was appointed Assistant Professor of Medicine. His medical capabilities, his diplomatic handling of difficult situations, and his amicable personality led to his appointment as Director of the Second and Fourth Medical Services at Boston City Hospital in 1932. In this position, he took charge of the fourth year medical students, winning their admiration and affection. One of the important contributions he made to teaching was in his development of the Clinico-Pathological Conference at the City Hospital. His own bi-weekly Pharmacological-Therapeutic Conference gave the students unusual insight into the use of drugs.

Soma Weiss possessed all the qualifications necessary for the great clinician. He was a master of observation. His ward rounds were excellent; while conducting them, he never neglected the patients, the students, or the visiting physicians. He kept them all in proper balance while he dominated the whole. He wisely insisted that clinical work must be the basis for the study of disease.

Soma Weiss became the second Physician-in-chief of the Peter Bent Brigham Hospital in 1939. He died January 31, 1942 from the rupture of a congenital intracranial aneurysm. In the intervening years, his generous spirit, his eager and able services for the Hospital, his great abilities as a physician, investigator, and teacher, left an indelible imprint on the many students he mentored.

Harvard Medical School wishes to thank the Weiss family for their generous donation in support of the Annual Soma Weiss Student Research Day.

Soma Weiss
1899 -1942

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HARVARD MEDICAL SCHOOL

SIXTY-NINTH ANNUAL

Soma Weiss
Student Research Day
January 15, 2009

Keynote and Student Presentations
1:00 – 2:45 PM
Carl W. Walter Amphitheater, Tosteson Medical Education Center

Welcome **Eleftheria Maratos-Flier, MD**, *Associate Professor of Medicine, Beth Israel Deaconess Medical Center*

Keynote ***Industry Interactions as a Component of an Academic Career***

Moderator **Jeffrey S. Flier, MD**, *Caroline Shields Walker Professor of Medicine, Dean of the Faculty of Medicine, Harvard Medical School*

Panelists **Elliott Antman, MD**, *Professor of Medicine, Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School*
Jerome Avorn, MD, *Professor of Medicine, Harvard Medical School and Chief, Division of Pharmacoepidemiology and Pharmacoeconomics, Brigham and Women's Hospital*
Lewis Cantley, PhD, *William Bosworth Castle Professor of Medicine, Harvard Medical School; Director of the Beth Israel Deaconess Cancer Center*
George Church, PhD, *Professor of Genetics, Harvard Medical School; Wyss Institute; Broad Institute*
Laurie Glimcher, MD, *Irene Heinz Given Professor of Immunology, Harvard School of Public Health and Professor of Medicine, Harvard Medical School*

Student Speakers
Sarah Henrickson
Dynamics of T cell activation in vivo
Jonathan Shoag
PGC-1a may promote tumor growth through activation of ERRa mediated VEGF transcription
David Stark
Regional Variation in Interhemispheric Functional Connectivity
Nicholas Zwang
Identification of Phosphorylation-Dependent Binding Partners of Aquaporin-2 Using Protein Mass Spectrometry

Poster Sessions
2:45 – 4:30 PM
Atrium of the Tosteson Medical Education Center
260 Longwood Avenue, Boston, Massachusetts

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Welcome to the 69th Annual Soma Weiss Student Research Day Assembly. The first of these assemblies, held in 1940, was organized by a group of medical students to:

- **Highlight the investigative work of their colleagues in the field of medical science.**
- **Provide those students engaged in investigative work with the opportunity to present their research before an interested assembly.**
- **Demonstrate the breadth of possibilities of medical student investigative work.**

The assembly honors the memory of Soma Weiss, a Harvard Medical School teacher and physician who was noted for his inspirational support and dedication to the advancement of medical science.

Funding for student research and investigative work is provided, in part, through endowment accounts and fellowships that are managed by the Office of Enrichment Programs. The following Endowments and Fellowships are representative of those used to support our students:

Rishon M. Bialer Fund
Marshall A. Barber, PhD Memorial Fund
Edward Hickling Bradford Fellowship
Walter Bradford Cannon Fellowship in Physiology
William B. Christensen Fund for Student Research
Class of 1955 Research Fund
Doris Duke Charitable Foundation
Myer Dana and Etta Dana Fund
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Alexandra Miliotis Fellowship in Pediatric Cancer Research
Aid for Cancer Research Fellowship
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George Cheyne Shattuck Memorial
Carl W. Walter Endowment
Charles Eliot Ware Memorial
John Ware Memorial
Paul Dudley White Traveling Fellowship Fund
George Bernays Wislocki Scholarship

The abstract included in this volume are reflective of the diversity and quality of research experiences available to all Harvard medical and dental students. The presentations at today's assembly are a tribute to the memory of Soma Weiss, and a testimony to the tradition of excellence in medical and investigative research at Harvard Medical School and Harvard School of Dental Medicine.

We congratulate all of our student researchers, investigators, and their sponsors.

Predictors of Attrition and Adherence to Nicotine Patch and Exercise Intervention in an Aid-to-Cessation Trial for Women

Julie A. Adamczyk
Harvard School of Dental Medicine, William Bosworth Castle Society, Class of 2011

Taru H. Kinnunen, PhD
Department of Tobacco Dependence Treatment and Research
Harvard School of Dental Medicine

Purpose: This study sought to determine which baseline factors contribute to attrition, exercise adherence, and nicotine adherence in an aid-to-cessation clinical trial for female smokers.

Methods: Healthy females (N=300) between 18 and 55 with a desire to quit smoking were enrolled in a 52-week aid-to-cessation program combining nicotine replacement therapy with a home- or facility-based exercise regimen. Paper-and-pencil baseline surveys were administered at the initial study visit to collect demographic information, smoking history, FTND and CES-D scores, level of motivation, etc. Adherence to the nicotine patch prescription and exercise program was self-reported by participants at follow-up appointments. Baseline and follow-up data were analyzed for three major outcomes: completion of the study, adherence to the exercise prescription at the 8-week postquit follow-up, and adherence to the nicotine patch prescription for at least six weeks following quit day.

Results: Of the 300 women enrolled, 54 dropped out following the baseline visit, 115 dropped out between their quit date and the twelve-week follow-up, 72 completed through the 12-week follow-up, and 59 completed the full 52-week study program. Univariate analysis revealed significant differences in marital status ($p=0.0039$), college education ($p=0.024$), and socioeconomic status ($p=0.0153$) between those who dropped out following the baseline visit and those who completed the study. Additionally, time spent in the study correlated positively with previous quit attempts ($p=0.00793$), and negatively with number of children at home and CES-D depression scale score ($p=0.000652$ and $p=0.0218$, respectively). Those who adhered to the nicotine patch and exercise interventions were primarily white ($p=0.0231$ for patch; $p=0.0141$ for exercise), had fewer children at home ($p=0.000581$; $p=0.00187$), had made more previous quit attempts ($p=.00594$; $p=0.0268$), and had lower CES-D depression scale scores ($p=0.0107$; $p=0.00398$) than those who quit with the study but did not adhere.

Conclusion: Completion of an aid-to-cessation program and adherence to nicotine replacement and exercise intervention are most strongly predicted by having fewer children in the home and lower CES-D depression scale scores. Marital, socioeconomic, and education status may also play a role in predicting attrition. These findings call attention to the need to address depressive symptoms as they relate to smoking and exercise behaviors and may aid in designing a more effective program with lower rates of attrition and more positive outcomes.

An Assessment of Complications and Satisfaction in Post Mastectomy Radiation Therapy

Tolulope Adesiyun
Harvard Medical School, William Bosworth Castle Society, Class of 2011

Bernard T. Lee, MD
Division of Plastic and Reconstructive Surgery, Department of Surgery
Beth Israel Deaconess Medical Center

The increasing use of radiation therapy in the treatment of breast cancer has led to an increase in the number of patients presenting to surgeons with a prior history of radiation to the breast, chest wall and skin. The need to understand the effects of radiation on breast reconstruction has therefore become evident. Current literature shows an increase in the complication rates in post reconstruction radiation therapy in addition to decreased aesthetic satisfaction. The complications are higher in implant based reconstructions than with autologous flap reconstructions. In light of this, most treatment regimes today include pre-reconstruction irradiation and the use of autologous flaps when possible. This study sought to compare the satisfaction and complications of patients that underwent postmastectomy radiation therapy and autologous flap reconstruction.

We identified 70 patients that have undergone breast reconstruction and radiation therapy. A retrospective chart review was performed to collect their complication data. Patient satisfaction data and current quality of life was previously collected from the "BIDMC Breast Reconstruction Outcomes Study." A mailed survey was completed by the patients during February 2008. A 72.3 percent response rate was obtained from the survey.

A total number of 70 patients who had received postmastectomy radiation therapy were included in the study. The patients' cancers ranged from stage 0 to stage 3. Approximately 57 percent of the women received deep inferior epigastric perforator flap reconstructions, 31 percent received latissimus flaps with an implant and 11 percent of them received transverse rectus abdominis myocutaneous flaps. Eleven of the patients underwent reconstruction before receiving their radiation therapy while 59 patients had their radiation therapy prior to their reconstruction. Among the women who received radiation after reconstruction, 3 had complications that required treatment in the operating room. Among patients that received radiation before reconstruction 12 out 59 had complications requiring treatment ($p=0.61$). Comparison of satisfaction scores showed no significant difference between the two groups, however, there were trends of decreasing satisfaction among women who received radiation after reconstruction (general satisfaction 57% vs 73%, $p=0.412$; aesthetic satisfaction 57% vs 63%, $p= 0.788$).

Based on the preliminary results of the study, the complication rates from the reconstructions are identical, illustrating that it is safe to perform radiation after reconstruction and yields similar satisfaction among patients. The main limitation to the study is the small population of patients. More in-depth analysis is needed to understand the effects of post mastectomy radiation therapy.

Perceptions of Postnatal Support for Down Syndrome in Southern Israel

Jason J. Ahn*
Harvard Medical School, William Bosworth Castle Society, Class of 2011

Joav Merrick, MD, MMedSci, DMSc
Ministry of Social Affairs and Social Services, Israel

Mohammed Morad, MD
Department of Family Medicine, Ben Gurion University of the Negev

Ludwik Szymanski, MD
Children's Hospital Boston

Kerim Munir, MD, MPH, SCD
Children's Hospital Boston

Down syndrome (DS) is a common cause of intellectual disabilities in children worldwide. This is a unique biological condition that requires physicians to manage physical and mental comorbidities, providing us with a strong example for care of the intellectually disabled population as a whole.

The purpose of the research was to conduct a comparative study of perceptions of postnatal support for children with DS among Bedouin and Jewish families in Southern Israel. The clinical support and attention of a child with DS is crucial in the early postnatal years. Close monitoring of growth, sleep, thyroid function, hearing, vision, cardiovascular health, and blood tests are important in screening for congenital malformations and developmental defects in the first months of birth up to around age five. The overarching objective of this project was to enhance the understanding of DS and need for postnatal services for the improvement of the care of both Jewish and Bedouin DS patients.

The region of the Negev in the south of Israel was chosen because the institution of a national health insurance system providing universal coverage, the rise in incidence of DS in Israel when corrected for terminated pregnancies, the rise in life expectancy for DS patients, and because of cross-cultural aspects.

This was a comparative, cross-sectional, standardized survey study involving a parent questionnaire administered to a parent in person. A previous questionnaire looking at the perceptions of postnatal support for DS was modified and validated to be used in both the Bedouin and Jewish populations in the Negev, through a panel of experts. Participants were recruited from institutions, hospitals and community care centers in the Negev. A total of 38 questionnaires were collected and are being analyzed.

*This project was supported, in part, by a Mental Health/Developmental Disabilities student award funded by NIMH/NIH MH071286 at Children's Hospital Boston.

Efficacy of First-line Antiretroviral Therapy in HIV-Infected Children in Vietnam

Emily Barsky
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

Brian Luong
Harvard School of Dental Medicine, William Bosworth Castle Society, Class of 2011

Doanh Lu, MD, MPH
Vietnam CDC-HMS AIDS Partnership

Antiretroviral therapy (ART) became widely available free-of-charge for HIV-infected children in Vietnam in 2006, through the support of the President's Emergency Plan for AIDS Relief (PEPFAR). Before 2006, ART was suboptimal due to the absence of reimbursement mechanisms and suboptimal medical practice.

The efficacy and durability of the current first-line ART (as defined by the World Health Organization (WHO)) in children enrolled in the HIV outpatient clinics at Pediatric Hospital One and Two in Ho Chi Minh City, Vietnam, were evaluated. The aims were to 1) determine the effects of antiretrovirals (ARVs) on children's growth status and clinical progression, and 2) to estimate the durability of the initial ARV regimen at 48 weeks or longer as measured by time to treatment failure or death.

This retrospective chart review enrolled 256 children, ages 16 or younger, who were started on first-line ARV regimens on or before June 30th, 2007. Demographic and background data collected were as follows: age, sex, city or province, Hepatitis B and C status, previous ARV use (prior to PEPFAR/clinic opening), WHO HIV clinical stage, and mode of transmission. Quarterly data were collected on clinical outcome of treatment as measured by weight, height, CD4 count, laboratory values (Hgb, AST, ALT, Cr), incidence of opportunistic infections (OIs), deaths, treatment failure (measured by viral loads, genotyping, CD4 counts, OIs), and loss to follow-up. ARV regimens, changes, and adverse effects, as well as time to treatment failure were also abstracted. History of substandard regimens, adherence, and advanced disease were measured as potential factors for treatment failure.

The data will be analyzed for trends. The effects of ARVs on health status and clinical progression will be analyzed by measures of growth (weight and height), CD4 counts, laboratory data, and incidence of OIs. Adverse effects of ARVs such as hepatitis and drug/drug interactions will also be described. The durability of the initial ARV regimen at 48 weeks or longer will be estimated by time to treatment failure as assessed by CD4 decline, growth failure, development of a new OI or reappearance of an old one, and viral loads and genotyping when available.

Health Maintenance in Underserved Communities—A Case Study

Edo Bedzra
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2012

The area studied is the least accessible by road, the poorest by income and has some of the worst access to sanitation, water, educational and healthcare facilities in the South Tongu district of Ghana. The objective of this study was to explore what people do to obtain preventative, acute and chronic care in a rural region of low healthcare accessibility. The healthcare delivery systems implemented for communities of similar characteristics in the world were explored to answer the question, "What models of health delivery would provide better health access to the studied communities?"

A survey asking questions about what people do when they have common illnesses was administered on 118 signees over age 18 who attended an informational meeting for informed consent. The survey looked at the types of care and where people seek them and illnesses for which they seek treatment and why. Surveys were administered in eight-minute oral interviews in the local language, Ewe, with English transcription. Data was analyzed for general trends and other demographics and supplemented with data from the National Statistical Service to create demographic, health usage and disease epidemiology profiles. Literature on health care delivery models in underserved communities around the world was researched to create a matrix of health delivery models suitable for the communities studied.

118 participants (74 female, 44 male) over age 18 were enrolled. People below age 18 and those who did not attend the informational meeting were excluded for informed consent reasons. 83% of participants self-medicated with 40% obtaining drugs from unlicensed vendors. 76% of respondents attend hospitals when sick with 57% walking 6km to the nearest clinic and 53% choosing the district hospital accessible only by car. Only 30% delivered in hospitals. 33% are enrolled in the National Health Insurance Scheme while 52% can afford to buy all prescribed drugs.

Gaps were found in service delivery addressable in the short term by education of communities on health issues, adoption of a spoke and wheel model of a centralized hub of a major hospital and auxiliary rural health workforce, and in the long term by infrastructural investment in a network of health facilities to expand coverage, an incentive system that allows recruitment and retention of health personnel, and educational efforts to realign objectives in the scope of community involvement and ownership.

Risk Factors for Multiple Organ Dysfunction in Patients with Malaria in Colombia

Carly Benner
Harvard Medical School, Francis Weld Peabody Society, Class of 2011

Henry Oliveros, MD
Departments of Anesthesia and Medical Education, Hospital Militar Central de Bogotá

Over 120,000 people in Colombia contract malaria each year, many of who are soldiers stationed in rural areas of the country with limited access to healthcare. Malaria generally can be managed successfully with anti-malarial medication and appropriate supportive care. However, when left untreated, it can be life threatening or fatal. Many Colombians in rural areas and tropical regions of the country are unable to access treatment until they have severe cases of malaria, which increases their risk of complication. This study examined the risk factors associated with complicated cases of malaria, defined as cases in which the patient experiences multiple organ dysfunction (MOD). The goal of the study was to identify patients with the greatest risk of complication upon presentation to the hospital using associated risk factors to improve care and avoid complications.

I randomly selected 300 charts from the list of 687 patients who had been admitted to the Hospital Militar Central with a diagnosis of malaria between January, 2002, and May, 2008. Data on risk factors including type of malaria infection (*Plasmodium Vivax*, *Plasmodium Falciparum*, or mixed *P. Vivax* and *P. Falciparum* infection), age, gender, level of parasitemia, duration of symptoms prior to presentation, length of hospital stay, previous malaria infection, and hemoglobin level was collected from each chart. Data was also collected to determine cases of multiple organ dysfunction (MOD), which included: Pa/FIO_2, platelet count, mean arterial pressure, Glasgow score, total bilirubin, and creatinine. Multiple organ dysfunction was defined based on the Sequential Organ Failure Assessment (SOFA) score, which provides a score of 0-4 for six organ systems (pulmonary, hematological, cardiovascular, hepatic, renal, and neurological) based on indicators of the level of function for each system. A SOFA score of 8 or above was considered to be a case of multiple organ dysfunction. A statistical analysis was then performed on the data collected using STATA.

The study included all subjects age 18 years and above, and no other exclusions were made. The chart review has not yet been completed, as a sample size of 300 patients was not large enough to obtain statistically significant results. Preliminary analysis of the data revealed a strong association between hemoglobin level and multiple organ dysfunction, and a final statistical analysis will be performed upon completion of the chart review.

No final conclusions have yet been reached from the study, as the data collection is still ongoing.

Post-Operative Left Ventricular Growth in Infants with Unbalanced Complete Atrioventricular Canal (CAVC)

David D. Berg
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011
PASTEUR Summer Clinical Research Program

Sitaram Emani, MD
Department of Cardiac Surgery
Children's Hospital Boston

Complete atrioventricular canal (CAVC) is a complex congenital heart malformation involving atrial and ventricular septal defects and abnormalities of the left and right atrioventricular (AV) valves. Surgical repair of CAVC leading to a biventricular cardiac physiology is associated with excellent outcomes in infants with a "balanced" defect. In the unbalanced form of CAVC, however, the left and right components of the conjoint, malformed AV valve are not situated evenly over the ventricles. As a result, the ventricle associated with the smaller component is typically hypoplastic, which complicates a biventricular repair. The alternative surgical intervention is a staged reconstruction ultimately resulting in total cavo-pulmonary connections, also termed a Fontan operation.

The criteria for selecting the appropriate surgical intervention for unbalanced CAVC are not well defined, in part because biventricular repair changes the permanent volume loading conditions on the hypoplastic ventricle. We hypothesize that infants with unbalanced CAVC who receive a biventricular surgical repair have greater post-operative left ventricular growth rates than those who receive a univentricular procedure.

To test this hypothesis, we are conducting a retrospective cohort analysis of patients who underwent surgical repair for right-dominant unbalanced CAVC (z-score for LV end-diastolic volume indexed to body surface area between -2.0 and -5.0 at time of diagnosis) at Children's Hospital Boston from 1994 to 2008. Patients are excluded if they were born prematurely (<32 weeks gestation) or were older than one year at the time of surgical intervention. Fifty-two patients who received a biventricular repair and 33 patients who underwent univentricular palliation meet these criteria.

For each patient, LV end-diastolic and end-systolic volumes are abstracted from echocardiograms performed: 1) at initial presentation; 2) immediately post-operation; 3) 2-6 months post-operation; 4) 6-12 months post-operation; and 5) greater than 1 year post-operation. Apical four-chamber and subcostal short-axis views are used to measure LV long-axis length and short-axis area, respectively. These values are in turn used to estimate LV volumes, which are standardized to body surface area and assigned a z-score on the basis of published normative data.

An LV growth curve will be constructed for each patient and the slope of the curve will be calculated. An unpaired t-test will be used to compare mean post-operative growth rates between patients receiving biventricular and univentricular surgical repairs to evaluate the hypothesis. The results of this study will improve our understanding of postnatal ventricular plasticity, and thus improve our surgical management of the disease.

Determining the Causes of Failure to Receive Proper Treatment for Streptococcal Pharyngitis Among Children Ages Three to Fifteen in Tanzania

Regan WJ Bergmark
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011

Brian A Bergmark
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

Jeffrey Blander, PhD
Harvard Medical School

Background: Rheumatic heart disease (RHD) continues to cause significant morbidity and mortality in developing countries such as Tanzania. RHD is preventable if episodes of Group A streptococcal pharyngitis, which are most prevalent among children ages three to fifteen, are treated promptly with penicillin or another appropriate antibiotic. Despite the appearance of widespread availability of antibiotics throughout Tanzania, RHD remains a major burden. It is unknown why children are not being properly treated for GAS pharyngitis. Possibilities include not accessing medical care at all, receiving an incorrect diagnosis or inappropriate treatment, or failing to take the full course of antibiotic. Determining the major reasons for failure to properly treat streptococcal pharyngitis is critical for the development of an effective RHD program.

Objective: This research aims to identify the care access and treatment patterns common among children with RHD that prevent children from receiving adequate treatment for GAS pharyngitis.

Methods: Two types of data were collected. First, a lateral flow immunoassay rapid test for GAS was employed in the outpatient departments of public hospitals and health centers in Dar es Salaam, Tanzania to measure rates of presentation of GAS pharyngitis among children ages three to fifteen. Secondly, 50 interviews with parents of children with RHD at Muhimbili National Hospital were carried out to characterize the treatment sought by parents for children with the signs and symptoms of GAS pharyngitis as well as any trends in geographic distribution of RHD incidence.

Results: Screening of over 280 children at the outpatient sites yielded only two patients with signs and symptoms of GAS pharyngitis, both of which gave negative rapid test results. Interviews suggested that parents frequently do not consider a sore throat and fever serious enough health problems to justify accessing care in the public system for a child ages three to fifteen. Instead, a sore throat alone is treated with honey and lime or an analgesic from a local medicine store, while sore throat and a fever is treated with the above plus an anti-malarial or non-specific antibiotic from a local medicine store. Children with RHD were from varied regions (states) and tended to be from low-income families.

Conclusions: The primary barrier to treatment of GAS pharyngitis is insufficient parent knowledge that sore throat and fever require diagnosis and antibiotic treatment. This gap may best be addressed by a community education program.

Assessment of Variables Affecting Quality of Life Following Third Molar Surgery

Daniel A. Bienstock
Harvard School of Dental Medicine, William Bosworth Castle Society, Class of 2011

Sung-Kiang Chuang, DMD, MD, DMSc
Department of Oral and Maxillofacial Surgery
Massachusetts General Hospital

The purpose of this investigation was to determine the way in which certain predisposing variables relate to the patient quality of life following third molar (M3) surgery. The study illustrated this using the number of days an individual was unable to perform normal activities as the outcome variable.

This was a prospective cohort study of subjects having at least 1 M3 extracted as part of the American Association of Oral and Maxillofacial Surgeons' Age-Related Third Molar Study. The predictor variables were categorized as demographic, health status, anatomic and pathological. The outcome variable was number of days unable to perform normal daily activities. Appropriate univariate statistics were computed. A multiple logistic regression model was used to evaluate the simultaneous effects of multiple covariates.

The overall reported number of days unable to perform normal daily activities was 1.43 ± 1.75 days. Associations in unvariate analyses with a p-value that is less than or equal to 0.15 and any biologically relevant variables (age and gender) were included in a multiple regression analysis. In the multiple regression model, age, gender, inferior alveolar nerve injury, post-operative injury to the tooth adjacent to the M3, and intra-operative compromised airway were all associated with an increased number of days unable to perform normal daily activities ($p<0.01$). In addition, the lack of intra-operative complications was associated with a reduction in the number of days the patient was unable to perform normal daily activities.

The results of this study confirm that third molar surgery does result in a decreased quality of life as measured by number of days unable to perform normal daily activities. Furthermore, the results of these analyses suggest that certain predisposing factors appear to be associated with a greater decreased quality of life following M3 extractions in the form of number of days unable to perform normal daily activities.

An Investigation Into the Migratory Properties of Th17 cells in Experimental Autoimmune Encephalomyelitis (EAE)

Ian Andre Buchanan
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Ulrich von Andrian, MD, PhD
Department of Pathology
Harvard Medical School & The Immune Disease Institute

Experimental autoimmune encephalomyelitis (EAE) is one of the best animal models currently available for multiple sclerosis (MS), in that it bears striking similarity to the actual disease with regard to chronicity and degree of demyelination. Whereas the cause of MS remains unclear, EAE is a T cell-mediated autoimmune disease of the central nervous system in which myelin oligodendrocyte glycoprotein (MOG) acts as an autoantigen. While there is no paucity of data in the literature implicating the newly discovered Th17 subset in the pathogenesis of EAE, *in vivo* imaging studies of the encephalitogenic cells thought to be responsible are certainly lacking. Because studies centering on the behavior of autoaggressive effector cells are crucial to unraveling the pathogenic mechanisms underlying EAE, as well as other autoimmune disorders, we sought to elucidate the migratory properties of Th17 cells in normal and diseased settings.

Drawing on recent advances in the body of knowledge on murine Th17 differentiation, we cultured Th17 cells *in vitro* with remarkable success (purities >80%). Using naïve CD4 T cells as a control population, we then injected the Th17 subset into wildtype and MOG-immunized C57BL6 mice in order to assess predilection for, and accumulation within, the different organs. Twenty-four homing experiments revealed that Th17 cells were roughly five times more likely to accumulate in the bone marrow (BM) of WT mice and about ten times more likely to accumulate in the BM of diseased mice when using the spleen as a reference organ. Subsequent assessment of migration towards the classic BM chemokine, CXCL12, demonstrated that Th17 cells had over three-fold greater chemotactic index in comparison to their naïve CD4 precursors.

These data suggest that Th17 cells have a strong predilection for the bone marrow in normal settings, and an even stronger predilection during the diseased state. Furthermore, the significantly higher chemotactic indices exhibited by Th17 cells towards CXCL12 suggest that trafficking to the bone marrow is perhaps mediated by the surface receptor CXCR4. The preferential retention of Th17 cells in the bone marrow of EAE mice seems to be in agreement with growing evidence implicating the bone marrow as a nesting ground for autoaggressive T cells in autoimmune disorders. Two-photon microscopy studies are currently underway to visualize Th17 cells during the course of EAE.

Dental Caries Are the Primary Indication for Single Crown Placement

WaiYin Chan
Harvard School of Dental Medicine, Oliver Wendell Holmes Society, Class of 2011

Dr. Nachum Samet, DMD
Restorative Dentistry and Biomaterials Sciences
Harvard School of Dental Medicine

Although fixed partial textbooks state indications and contraindications for the placement of single crowns, there has not been a study investigating the practicing indications for single crowns. Indications to be considered include tooth decay, unstable tooth structure, after endodontic treatment, fracture and aesthetic purposes. I anticipated that severe tooth decay and unstable tooth structure would be the most common indications used in practice.

The purpose of the study is to determine which of these indications are most frequently used. This was accomplished by conducting surveys of patients and dentists in the Boston area and a chart review of single crown treatments at the Harvard School of Dental Medicine. Surveys and chart review data were analyzed and then compared to confirm that methodology did not affect the results that were obtained.

The prospective study was constructed to collect data from practitioners. However, the practitioner paper surveys yielded disappointing response rates (23 out of 148) and survey return rates at less than 13% (3 out of 23). Due to the low participation rate, the collected data could not be used for the purpose of this study.

Oral surveys of patients were conducted in the areas of Coolidge Corner (Brookline, MA), Harvard Square (Cambridge, MA), and Downtown Crossing (Boston, MA) (n= 317). "Endodontic treatment" (33.5%) was the main indication for crown treatment. The second most recalled indication was "caries beyond repair" (31.7%). It is likely that patients are unaware that endodontic treatment was necessary because of extensive caries. No other indications were statistically significant.

The chart review examined randomly selected HSDM patients receiving crown treatment between 2006 and 2008 (n=275). Results showed that caries beyond repair (31.5%) was the main indication, followed by "fracture" (24.8%) and "after endodontic treatment" (24.1%).

This study concludes that the primary indication used in single crown treatment is most likely caries and its sequelae. These findings suggest that less frequently used indications are still pertinent in single crown treatments. From a dental education perspective, the significance of this study is that dental schools need to dedicate a significant amount of time in their pre-clinical courses to better prepare students on how to treat severely broken down teeth. It is also pertinent that students have experience not only on "whole" plastic typodont teeth, but find alternatives that will better simulate clinical reality. Dental education should reflect these findings.

Multilocularity as a Defining Characteristic of the Odontogenic Keratocyst

Panasaya Charenkavanich
Harvard School of Dental Medicine, Francis Weld Peabody Society, Class of 2011

Edward B. Seldin, DMD, MD
Department of Oral and Maxillofacial Surgery
Massachusetts General Hospital

Odontogenic keratocysts (OKCs) account for approximately 3-10% of all oral cysts. OKCs have a locally aggressive clinical behavior and exhibit a high recurrence rate, thus requiring close long-term follow-up. Early diagnosis and appropriate treatment of OKCs can reduce the possibility of recurrence. Panoramic x-rays and CT scans are used to identify the presence of a possible OKC, although there is a relative lack of specific radiographic features that point to an OKC diagnosis. Final diagnosis is conducted through a combination of fine-needle aspiration biopsy and immunocytochemical testing for cytokeratin-10. Better characterization of the radiographic features of OKCs may help facilitate diagnosis of the condition as a supplement to aspiration biopsy and immunocytochemical testing.

We hypothesized that a correlation exists between multilocularity on a radiograph and the presence of an odontogenic keratocyst as well as a higher rate of recurrence. To test theses hypotheses, patients in the Massachusetts General Hospital (MGH) Department of Oral and Maxillofacial Surgery (OMFS) database were retrospectively studied from 1991-present. Data was obtained regarding the type of cyst and the radiological presentation of the cyst as either multilocular or non-multilocular. The correlation between multilocularity and the presence OKCs as well as recurrence was calculated in order to determine the extent to which multilocularity can be used as a predictor of OKCs and a higher rate of recurrence.

Analysis of study data is continuing. Currently, the results of the study are suggestive of a positive correlation between multilocularity on a radiograph and the presence of an odontogenic keratocyst. Once completed, the results of the study will provide insight as to what extent a case of OKC can be suspected if multilocularity is present on a radiograph as well as how aggressive the cyst may be in recurring. This radiographic relationship can be implemented as a predictive tool that supplements the current diagnostic procedure for OKCs.

Risk Factors for Adverse Pregnancy Outcomes Among HIV-Infected Women in Gaborone, Botswana

Jennifer Y. Chen
Harvard Medical School, Walter Bradford Cannon Society, Class of 2009

Roger L. Shapiro, MD MPH
Department of Medicine, Beth Israel Deaconess Medical Center

HIV infection has been associated with stillbirth, premature delivery, and low birthweight. It remains unknown whether these outcomes are also associated with maternal disease status or the use of highly active antiretroviral therapy (HAART) during pregnancy.

During a study period of eight months, we prospectively reviewed obstetrical records among 3741 women at Princess Marina Hospital in Gaborone, Botswana for stillbirths, premature delivery (< 37 weeks gestation), low birthweight (< 2500 g), very low birthweight (< 1500 g), and early neonatal mortality.

Of 3492 women HIV tested, 1061 (30%) were HIV-infected: 218 (20%) received no antiretrovirals, 559 (53%) received zidovudine (ZDV) alone, and 284 (27%) received HAART. HIV infection was associated with premature delivery (RR = 1.38, 95% CI: 1.23, 1.55), neonatal death (RR = 1.50, 95% CI: 1.00, 2.25), low birthweight (RR = 1.55, 95% CI: 1.34, 1.78), very low birthweight (RR = 1.42, 95% CI: 1.07, 1.88) and stillbirth (RR = 1.39, 95% CI: 0.98, 1.98). Maternal CD4 cell count was lower among HIV-infected women who received HAART ($P < 0.0001$), and CD4 $\leq$ 250 cells/mm^3 was associated with neonatal mortality (RR = 5.7, 95% CI: 1.70, 19.0) but no other adverse outcomes. Compared with ZDV alone, HAART initiated in pregnancy was associated with low birthweight (RR = 1.93, 95% CI: 1.40, 2.65) and low birthweight among term deliveries (RR = 3.32, 95% CI: 1.84, 6.01). Compared with HAART initiated in pregnancy, HAART started before pregnancy was associated with very low birthweight (RR = 2.74, 95% CI: 1.00 – 7.47), but birthweight did not differ among term deliveries.

HIV infection is associated with adverse pregnancy outcomes, and high-risk obstetrical and neonatal care services need to be supported in countries such as Botswana where HIV is prevalent. Possible associations between maternal disease status, HAART, and adverse pregnancy outcomes require further study as HAART becomes more available for treatment and for the prevention of mother-to-child transmission of HIV in the developing world.

The Cerebrospinal Fluid Controls Proliferation and Maintenance of Embryonic and Adult Neural Stem Cells

Xi Chen
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Christopher A. Walsh, MD PhD
Division of Genetics, Children's Hospital Boston

Cerebrospinal fluid (CSF), the fluid bathing the central nervous system during development and adulthood, is historically considered as the supporting fluid that maintains a stable chemical environment. Recent investigations, however, revealed abundant protein factors in the human CSF, including signaling molecules, extracellular matrix proteins, and transport proteins. Interestingly, the composition of the CSF changes during development and is different during embryonic stages and adulthood.

We hypothesized that CSF provides an inductive fluid environment for the proliferation and maintenance of the embryonic and adult neural stem cells. To test this hypothesis, primary neurospheres derived from E14 rat embryos were dissociated and cultured with embryonic CSF collected from E13, E17 or P6 rats, and adult CSF. E13, E17, P6 and adult CSF supported the generation of secondary neurospheres, composed primarily of GLAST-positive (an embryonic neural stem cell marker) cells, in the complete absence of supplemental FGF and EGF that are normally essential to maintain them, while neurospheres failed to form in the presence of artificial CSF (ACSF). These neurospheres generated in the CSF retained their responsiveness to FGF and EGF, indicating that the CSF maintains the stem cells in an uncommitted fate. In addition to the neurospheres, secondary clones generated with P6 and adult CSF included flat transitional colonies that are faintly GLAST-positive. Conditioned medium generated from embryonic choroids plexus also induced the generation of secondary neurospheres, suggesting that choroids plexus is the likely source of these proliferation signals. Cells within the neurospheres and the transitional colonies also contained differentiated neurons and glial cells. Interestingly, the GLAST positive neurospheres were maintained in culture for extended periods of time (44DIV), while the transitional colonies were not maintained. Similarly, embryonic CSF also promoted the generation of secondary neurospheres from adult brains.

Filtration analysis suggested that the CSF factors that induce generation of neurospheres range from 10kDa to 100kDa. Notably, a transient increase in Igf2 during peak neurogenesis was evident. The pattern of expression of Igf2 and its receptor Igf1R supports a role of Igf2 in neurogenesis. Indeed, supplementing basal media with Igf2 is sufficient to enhance proliferation in formation of secondary neurospheres. These data demonstrate that CSF is sufficient to maintain and stimulate proliferation of neural progenitor cells. Igf2 is a candidate molecule contributing to the proliferation and maintenance of embryonic neural stem cells. Further studies of the role of CSF during development and adulthood will be key to the understanding of central nervous system development and provide therapy possibilities for the treatment of neurological diseases.

Regulatory Mechanisms of Embryonic-like Adult Stem Cells

Yicheng Chen
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Keith D. Crawford MD PhD
Laboratory of Molecular Diagnostics
Harvard Medical School/ Brigham and Women's Hospital

Stem cells are a promising group of cells that have three key properties: the ability to self-renew, the ability to differentiate into multiple lineages and the ability to reconstitute functional tissue *in vivo*. My mentor's lab has discovered a novel source of adult stem cells in the joint synovial fluid of patients with osteoarthritis. Previous flow cytometry and gene expression data suggest that this population of adult stem cells may express genes previously thought to be unique to embryonic stem cells.

We hypothesize that these joint enriched stem cells have the ability to self renew and to differentiate into all three germ layers. The first aim of the project is to isolate and expand this population of cells. A second aim is to optimize culturing conditions to maximize the rate of expansion. The third aim is to induce the differentiation of these stem cells into all three germ layers.

Stem cells from synovial fluid were isolated and cultured in expansion, osteogenic, adopogenic, hepatogenic, neurogenic and chondrogenic media to investigate their ability to expand and differentiate into osteoblasts, adipocytes, hepatocytes, neurons and chondrocytes.

Flow cytometry analysis of synovial fluid cells revealed a population of MHC class I negative, cd235a negative cells that have a small cell volume. These are the cells we are investigating. An analysis of several different expansion media revealed that MesenPro RS™ from Invitrogen resulted in the fastest expansion of our cell population. The cells were able to continue to expand even after multiple passages. After 3 days in culture, the cells have adopted a flattened morphology and have begun to divide. The doubling time of the cells appears to be about 5-7 days. So far joint-enriched stem cells have been differentiated into adipocytes and osteoblasts. Adipocyte lineage was confirmed using oil red o staining and morphology. Osteoblast lineage was confirmed using a BCIP/NBT substrate to assay for alkaline phosphatase activity.

While there are still more cell lineages to investigate, we have confirmed the ability of joint enriched adult stem cells to differentiate into adipocytes and osteoblasts. Furthermore, these cells have the ability to self renew and appear to grow best in MesenPro RS™ media. Future differentiation experiments and genetic analysis using qPCR and microRNA arrays will be necessary to better characterize and identify this population of cells.

Characterization of the CD8 T Cell Response to MHV-68 Infection

Evelyn J. Cheung
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Hidde L. Ploegh, PhD
Whitehead Institute for Biomedical Research, Department of Biology
Massachusetts Institute of Technology

Kaposi's sarcoma-associated herpesvirus (KSHV) and Epstein Barr Virus (EBV) are two well-known gammaherpesviruses that infect humans. Because the strict species specificity of these two viruses limits detailed characterization of their pathogenesis, a closely related virus, murine gammaherpesvirus 68 (MHV-68) serves as an excellent model for understanding the nature of the in vivo infection of this class of viruses. In laboratory mice infected with MHV-68, the virus mediates a primary infection in the lung that is quickly cleared, and then manages to persist in its host by establishing life-long latency in B lymphocytes. CD8 T cells have been shown to play a critical role in clearing this initial primary infection, as well as in long-term control of the persistent infection.

Infection of mice with MHV-68 robustly activates CD8 T cells, but only six Class I MHC-restricted epitopes have been described to date for mice of the H-2b haplotype. To explore the specificity and kinetics of the CTL response in MHV-68-infected C57BL/6 mice, we sought to identify H-2Kb- and H-2Db-restricted epitopes using a MHC tetramer-based approach. Employing a consensus epitope prediction program, we selected 384 of the highest-scoring H-2Kb- and H-2Db-restricted epitopes derived from the complete MHV-68 proteome. MHC tetramer arrays presenting these predicted peptides were subsequently generated and used to screen for epitope-specific CD8 T cells.

From this screen, we identified 19 new epitopes derived from 16 different open reading frames (ORFs) comprising both early and late viral antigens. Interestingly, the CD8 T cell response is skewed toward H-2Kb-restricted epitopes. In addition, the novel epitopes can be grouped according to two distinct patterns that characterize the CD8 T cell response, with a response towards an early group of epitopes that peaks at 6 days post infection (dpi) and a later group that peaks at 10 dpi. These patterns in all likelihood reflect a continuum in the response kinetics. The respective MHC tetramer-positive CD8 T cells display an activated/effector phenotype (CD62lo and CD44hi) and produce IFN-γ upon ex vivo peptide stimulation.

MHV-68 infection in vivo elicits a response to multiple viral epitopes, thus illustrating a far broader T cell repertoire than previously known. This expanded repertoire of MHV68 epitopes not only allows for further biochemical assessment of the immune response to the virus, but also the potential development of effective vaccination strategies against MHV-68 and its related human viruses.

Pharmacogenetics of Bisphosphonate Induced Osteonecrosis of the Jaw: A pilot study

Michelle Chung
Harvard School of Dental Medicine, Oliver Wendell Holmes Society, Class of 2010

Athanasios Zavras, DMD, DMSc
Harvard School of Dental Medicine, Oral Health Policy and Epidemiology

Bisphosphonates (BPs) used to treat osteoporosis and hypercalcemia of malignancy have recently become associated with an increased risk of osteonecrosis of the jaw (ONJ). This study investigates whether the risk of osteonecrosis of the jaw is associated with genetic polymorphisms in genes controlling the function and apoptosis of the osteoclast, osteoblast, and osteocyte. A nested case-control study design is used to determine whether polymorphisms with allele frequencies of at least 5% at IGF1, IGF2, IGF1R, IGF2R and IGFBPs increase the risk of ONJ in oncologic subjects, controlling for known confounders. The study population of patients who have received intravenous BP treatments includes confirmed cases of ONJ and matched controls. Cases and controls are asked to provide DNA via a saliva sample and to complete a questionnaire, which will be used to validate the outcome and the exposures. Outcome measures of this study are unadjusted and adjusted odds ratios, which will be used as estimates of relative risk. Data collection and analysis is pending.

JCI Accreditation and Its Impact on Infection Control: A case study of Wockhardt Hospital, Bangalore, India

Meghan T. Cooper
Harvard School of Dental Medicine, William Bosworth Castle Society, Class of 2011

Mudit Saxena, MBBS, MHA
Head Administrative Services
Wockhardt Hospital Ltd., Bangalore

Over the past decade, hospital accreditation by the Joint Commission International (JCI) has become a growing trend in developing nations. Incentives for obtaining accreditation exist, such as a hospital differentiating itself from its competitors to attract both local and international patients. Accreditation status is not an assurance of good health outcomes; rather it indicates that appropriate building blocks are in place. More evidence is necessary to determine whether accreditation is a growing international trend due to economic incentives alone, or if accreditation also results in improved clinical outcomes.

A case study assessed the impact of the JCI process on the quality of patient care at Wockhardt Hospital. Infection control practices and prevalence rates of hospital acquired infections (HAIs), including ventilator acquired pneumonia (VAP), urinary tract infection (UTI), blood stream infection (BSI), and surgical site infection (SSI) were studied as a quantitative measure of quality. The infection control policies and practices prior to, during preparation for, and after JCI accreditation were examined, including the surveillance program, staff education and training to prevent and reduce infection, and HAI prevention methodology. The collection of HAI data occurred in a prospective manner from April 2007 to May 2008. There were 10,810 patient discharges in the hospital's seven units in which HAIs occurred during the study period. The data were analyzed using linear regressions to look for a dependent relationship between month of involvement in the JCI process and HAI rates. Peak rates for VAP (17/1000 Ventilator Days), BSI (19/1000 Line Days), UTI (15/1000 Catheter Days), and SSI (3.3%) all occurred during June and July of 2007. By May 2008 the rates decreased to VAP (0), BSI (0), UTI (4/1000 Catheter Days), and SSI (0.1%). A statistically significant ($p<0.05$) dependent relationship between the JCI accreditation process and rates of HAIs was found for all infection types.

The introduction of evidence-based standard operating procedures alone did not serve to institute an effective program. Education and retraining of nurses, physicians, housekeeping, and kitchen staff were essential to the creation of a functional program. Enforcement was often problematic, but staff compliance continues to increase over time. The need to meet JCI standards was the force behind the administration, allowing for the development of an effective, yet costly, infection control prevention and surveillance program.

JCI accreditation has proven itself as a framework for self-improvement. Quality of care at Wockhardt Hospital improved as infection rates declined significantly.

Molecular Circuitry of Human Regulatory T Cells

Gregory L. Cvetanovich
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

David A. Hafler, MD
Center for Neurologic Diseases, Department of Neurology
Brigham and Women's Hospital

Natural regulatory T cells (nTregs) are critical in promoting immune tolerance to self-antigens by suppressing self-reactive effector T cells through cytokine secretion and poorly understood contact-dependent mechanisms. These cells are being studied intensively because functional defects in nTregs have been identified in patients with various autoimmune diseases. In humans, $CD4^{+}CD25^{high}$ nTregs can be divided into three functionally distinct subtypes on the basis of HLA-DR and CD127 expression. We have undertaken a set of experiments aiming to expand our knowledge of the molecular circuitry underlying the normal function and pathological dysfunction of human nTregs.

In order to better understand the role of the critical nTreg transcription factor Foxp3 in human nTregs, one critical aim of this work was use genome-wide chromatin immunoprecipitation combined with sequencing (ChIP-Seq) to generate high-resolution maps of genomic loci occupied by the critical nTreg transcription factor Foxp3 in human nTregs. Initial experiments were conducted on Jurkat cells retrovirally transduced with Foxp3 and revealed that the technique has promise to work in the human nTreg system. We are in the process of analyzing data from the Jurkat cells and conducting ChIP-Seq studies using *ex vivo*, FACS-purified human nTregs of various subtypes. Initial experiments will be conducted with nTregs from healthy donors, but future work will extend to nTregs from patients with autoimmune diseases.

Another aim of this work was to conduct investigations into the epigenetic differences between the three human nTreg subsets. First, alternative splicing of Foxp3 – which produces functionally distinct isoforms containing and omitting coding exon 2 – was studied for these cell types using traditional PCR and real-time PCR. Examination of Foxp3 isoform expression patterns revealed no significant expression differences between human nTreg subsets from healthy donors. In addition, Foxp3 promoter DNA methylation patterns were examined using bisulfite sequencing and methylation-specific PCR for conserved CpG sites that have been identified previously. Initial experiments on positive-control Sssi treated cells and CD25- effector T cells revealed the expected fully methylated pattern, consistent with their low Foxp3 expression levels. Preliminary data indicates that the 7R- nTreg subset has approximately 11% methylation, consistent with published reports of $CD4^{+}CD25^{high}$ nTregs and this cell type's high Foxp3 expression.

Future work will extend on the preliminary experiments described above and will also involve determination of the gene expression profiles of human nTreg subsets from healthy donors and patients with autoimmune diseases using microarray analysis.

The Effects of FGF-2 on Bone Marrow Stromal Cells in Young and Old Mice

Christopher R. DeSesa
Harvard School of Dental Medicine, William Bosworth Castle Society, Class of 2011

Liisa T. Kuhn, PhD
Department of Reconstructive Sciences, School of Dental Medicine
University of Connecticut Health Center

There is a frequent need for bone augmentation procedures prior to dental implant placement due to the high incidence of alveolar ridge resorption that occurs following tooth loss. Evidence suggests that elderly patients often have compromised outcomes in response to bone augmentation procedures that has been linked to fewer stem mesenchymal cells; therefore a new approach is needed. A single osteogenic agent such as bone morphogenetic protein-2 (BMP-2) promotes differentiation of existing stem cells, but may be inadequate in the elderly due to their reduced number of stem cells, as well as excess bone formation at high doses. To lower the dose of BMP-2 needed, we propose to use another agent, fibroblast growth factor- 2 (FGF-2,) which will first recruit and promote proliferation of endogenous stem cells, followed by the BMP-2 treatment, which will promote the osteoblastic differentiation of that expanded cell population. The proposed method is to ultimately improve bone augmentation procedures through the use of a stem cell proliferative agent and from a scaffold that is used in conjunction with a dental implant.

Before applying this growth factor to dental implants, we set out to examine the dose-response effects of FGF-2 in vitro on primary bone marrow stromal cells. We hypothesized that FGF-2 would increase proliferation and differentiation of bone marrow stromal cells in young more than old mice due to fewer stem cells in older mice.

In order to test our hypothesis, we extracted stromal cells from the bone marrow of the tibia and femur of both young (12 weeks) and old (19 months) mice and cultured them in nutritional medium along with various concentrations of FGF-2 (0.016 ng/ml, 0.16 ng/ml, 1.6 ng/ml) and vehicle. We then measured proliferation on day 3 through an MTS assay absorbance reading on a spectrophotometer (λ 490). At day 10 and 21 we assessed the extent of osteoblastic differentiation through alkaline phosphatase and Von Kossa staining.

We found that in both young and old mice, there were no significant differences in cell proliferation of the bone marrow stromal cells between the various concentrations of FGF-2 when compared to vehicle. However, we did observe the presence of alkaline phosphatase when staining both young and old bone marrow stromal cells. Overall, it appears that FGF-2 plays a role in the differentiation of bone marrow stromal cells to osteoblastic cells, yet there still lacks significant data to support this claim.

The Unfolded Protein Response May Be Involved In Dental Fluorosis

Duy Tran Do
Harvard School of Dental Medicine, Walter Bradford Cannon Society, Class of 2011

John Bartlett, PhD
Department of Cytokine Biology
The Forsyth Institute

Background: Even though copious research has been conducted to study how fluoride causes dental fluorosis, the underlying biochemical mechanism of fluoride toxicity is still not completely understood. We have previously shown *in vitro* and *in vivo* that fluoride induces endoplasmic reticulum (ER) stress in cells responsible for enamel formation (ameloblasts). ER stress then triggers the activation of an ER-to-nucleus signaling pathway called the Unfolded Protein Response (UPR). The UPR serves to alleviate ER stress by up-regulating transcription of molecular chaperones, attenuating protein synthesis, and promoting ubiquitin-mediated proteasomal degradation of accumulated proteins. If those processes fail to relieve ER stress, the UPR will trigger apoptosis of the stressed cells. Three branches of the UPR may simultaneously be activated upon ER stress, namely the PERK, ATF6, and IRE1 pathways. We have also shown the activation of IRE1 and its downstream targets *in vitro* and *in vivo* in fluoride-treated mice. Hypothesis: Severe ER stress can activate JNK-mediated apoptosis through the IRE1 pathway. Therefore, we hypothesize that fluoride can also induce the phosphorylation and activation of JNK in ameloblasts.

Method: Mice were fed with water containing different doses of fluoride for a period of 3 to 4 weeks. Immunohistochemistry (IHC) was performed on incisors obtained from control and treated mice for the presence of phosphorylated JNK (p-JNK) and phosphorylated c-Jun (p-c-Jun).

Results and conclusions: p-c-Jun staining was detected in the nuclei of fluoride-treated ameloblasts. In addition, the intensity of staining increased with an increase in fluoride dose. However, we did not see any staining for p-JNK *in vivo*; further studies need to be performed to confirm whether JNK is activated in fluoride-induced ER stress. Future directions: To show that fluoride induces the activation of p-c-Jun, we need to perform IHC staining for c-Jun and compare the p-c-Jun/ c-Jun staining ratios of control and fluoride-treated samples. If the staining ratio of fluoride-treated mice is higher than the ratio of control mice, we can conclude that fluoride induces the phosphorylation and activation of c-Jun. In addition, we need to identify the upstream and downstream mediators of the fluoride-induced phosphorylation of c-Jun.

Oral Cancer Screening Knowledge, Practice, and Attitudes Among Massachusetts Primary Care Physicians

Adam Rubin Donnell
Harvard School of Dental Medicine, Walter Bradford Cannon Society, Class of 2011

Athanasios Zavras DMD, MS, DrMS
Office of Oral Health Policy & Epidemiology
Harvard School of Dental Medicine

Oral cancer has a high incidence rate in at risk populations and an even higher rate of mortality in those affected. Globally, oral cancer is the 5th most common form of cancer with 615,000 new cases annually. In the general population there is a 67% 5-year mortality rate, and a 90% 5-year mortality rate for those diagnosed in stages III or IV. Specific to the United States, it is the 6th most common form of cancer with 30,000 new cases annually, and it has 50% 5-year mortality rate with a lifetime prevalence of 1/72. Additionally, the oral cancer mortality rate has not changed since 1960 while mortality rates for lung, colon, rectal, prostate, and stomach cancers have all improved.

Central to decreasing the oral cancer mortality rate is decreasing the delay in diagnosis, which can be achieved by increasing the oral cancer screening rate among at risk populations. Healthcare professionals trained to perform screenings for other cancers, such as primary care physicians (PCPs), are well suited to contribute to increasing the oral cancer screening rate. This study aims to assess PCPs' knowledge of oral cancer and current screening practice, as well as their attitudes towards incorporating screening into standard care.

A 20-question survey was distributed to PCPs listed in the Massachusetts Board of Registration in Medicine database. The PCPs were recruited by mail to participate with the option of returning the survey by mail or completing it online. Two weeks following the initial mailing a reminder postcard was sent.

The PCPs demonstrated minimal oral cancer knowledge, a lack of screening practices, and a willingness to incorporate screening into their standard care. Of questions that measured oral cancer knowledge, 29% were answered correctly. Regarding practice, 75% never screened for oral cancer, and of these PCPs, 78% attributed their lack of screening practice to not being adequately trained in the examination technique. Their attitudes were favorable towards incorporating screening into standard care, with 83% interested in incorporating oral cancer examinations if trained and reimbursed.

The mortality rate of oral cancer can be decreased if oral cancer lesions are identified in the earlier stages. Increasing the rate of oral cancer screening can accomplish this, and this study has identified PCPs as healthcare professionals that are willing to add oral cancer exams to their standard care.

Polymorphisms of FAS and FAS Ligand Genes and Risk of Skin Cancer

Michael A. Dyer
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

Abrar Qureshi, MD, MPH
Channing Laboratory, Department of Medicine and Department of Dermatology
Brigham and Women's Hospital and Harvard Medical School

FAS and FAS ligand (FASLG) genes are involved in the extrinsic signaling pathway that causes cells that have been irreparably damaged by UV radiation to undergo apoptosis. Polymorphisms in these genes may contribute to the body's ability to signal pre-cancerous cells for apoptosis, and therefore, may contribute to risk of skin cancer. In this nested case-control study within the Nurses' Health Study, we assessed whether two known polymorphisms in the promoter region of the FAS gene (FAS -1377 G>A and FAS -670 A>G) and two known polymorphisms in the FASLG gene, one in the promoter region (FASLG -844 C>T) and one in the second intron (FASLG INV2nt -124 A>G), contribute to susceptibility to skin cancer. With 300 cases of basal cell carcinoma, 286 cases of squamous cell carcinoma, 219 cases of melanoma, and 874 controls, we observed that the FAS -1377 A allele and the FASLG -844 C allele were associated with increased risk of basal cell carcinoma. For FAS -1377 (AG + AA) genotypes, the adjusted odds ratio of basal cell carcinoma was 1.43 (1.07-1.92); for FASLG (CT and TT), it was 0.81 (0.65-1.00). We also found a borderline significant association between the FAS -1377 A / -670 G haplotype and basal cell carcinoma, as well as an association between the FASLG -844 / IVS2nt -124 G haplotype and melanoma. Our data suggest that polymorphisms in the FAS and FASLG genes may be associated with risk of basal cell carcinoma, but not squamous cell carcinoma or melanoma.

Characteristics of Patient Visits for Mechanical Ventilation in US Emergency Departments

Benjamin D. Easter
Harvard Medical School, Francis Weld Peabody Society, Class of 2011

Jonathan Fisher, MD, MPH
Department of Emergency Medicine
Beth Israel Deaconess Medical Center

Acute respiratory failure requiring mechanical ventilation (MV) is common in the intensive care unit, and its incidence is increasing. However, the prevalence of MV in the emergency department (ED) is unknown. Outside the ICU and the postoperative recovery area, the ED is the most common setting for the provision of critical care, including MV. While endotracheal intubation has been extensively studied in the ED, there is a paucity of studies examining MV. Only limited data offer national perspectives of ED-based MV. Information regarding the demographic and clinical characteristics of patients undergoing MV in the ED may influence their subsequent management.

This study made use of the 2002-2006 ED datasets from the National Hospital Ambulatory Medical Care Survey (NHAMCS). NHAMCS's four stage sampling design is designed to produce national estimates of the utilization of ED services. Patients were considered to have undergone a period of MV if they were intubated in the ED, and survived their ED course to either hospital admission or transfer to another facility. We sought to examine the demographic and clinical characteristics of patients undergoing MV, and to estimate their length of stay in order to understand if there is sufficient time to implement interventions aimed at reducing long-term complications of MV. We also categorized indications for MV in these patients.

The combined dataset contained 494 raw observations of intubations, representing 1,473,000 visits, and 334 raw observations for MV patients, representing approximately 994,000 visits. Approximately 58.8% were male, 75.5% Caucasian, and 66.1% above the age of 45. 9.1% of patients undergoing MV received CPR. Mean length of stay was 278.1 minutes (95% CI 234.9-321.3). Importantly, just over half (53.5%) of MV patients had ED lengths of stay greater than four hours, a sufficient amount of time to implement MV interventions. Against a backdrop of increasing ED visit times, length of stay for MV patients actually decreased over the study period ($p_{trend}<.05$). Finally, we used ICD-9 codes to sort indications for MV into one of seven categories: cardiovascular (18.1%), infection (15.5%), intoxication (10.5%), neurologic (13.0%), respiratory (19.5%), trauma (19.1%), and other (4.3%).

These data provide a picture of the epidemiology of MV in US EDs. Length of stay is sufficient for collaborating with critical care providers on implementing solutions that will reduce the complications of MV, such as ventilator-associated pneumonia, for patients ventilated in the ED.

Maternal and Child Health Outcomes in the Context of Family Violence

Huma Farid
Harvard Medical School, Francis Weld Peabody Society, Class of 2011

Anita Raj, PhD
Boston University School of Public Health

At the National Institute for Research in Reproductive Health (NIRRH), I worked on the Maternal and Child Health (MCH) Project, an Indo-US collaboration between Dr. Jay Silverman at HSPH and Dr. Saritha Nair and other researchers in India. The MCH project aimed to understand how family violence impacted maternal and child health outcomes, and the study was focused on women living in Govandi, an urban slum in Mumbai, with children who were six months of age or less. To achieve the aim of the study, researchers would conduct in-depth interviews at the Shivaji Nagar health post in Govandi with 750 women to determine the relation between domestic violence and health. The hypothesis is that family violence before, during, and after pregnancy leads to poor maternal and child health outcomes.

I worked with a team of researchers to interview women who had recently given birth and determine what questions we should incorporate on the questionnaire to better assess the connection between domestic violence and maternal and child health. These initial interviews had very open-ended questions on family violence, health, contraceptives, and demographic information and provided us with information that we could include on the questionnaire to be used during in-depth interviews.

My main task for this project was to work on the development of the questionnaire to be used during the in-depth interviews. I worked with other researchers at the NIRRH to help develop a thorough questionnaire, and also participated in the pilot testing of the questionnaire. We conducted in-depth pilot interviews at the health post in Bail Bazaar, another urban slum in Mumbai, and based on our observations from the pilot testing, we modified the questionnaire. I also helped to design a chart for the medical interns who would be working on the MCH project. This chart will be used to assess the infant's height, weight, nutritional status, and developmental status, as well as the mother's nutritional status. Both the questionnaire and the chart are now in their final form and are being implemented during the in-depth interviews, which began to be conducted on August 2, 2008.

Modification of Stem Cell Self-renewal by miR-125a

Michelle C. Fox
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011

David Scadden MD
Center for Regenerative Medicine
Massachusetts General Hospital

microRNAs have previously been shown to modify lineage fate in hematopoiesis. A loss in global microRNA processing through disruption of the small RNA processing enzyme, Dicer, ablates pluripotent stem cells in the developing mouse embryo. A conditional Dicer deletion shows multi-lineage cytopenia in the adult mouse.

Based on these previous results, we sought to identify specific microRNAs involved in the modulation of stem cell self-renewal. A cluster of three microRNAs, miR-99b, let-7e, and miR-125a, are highly conserved in long-term hematopoietic stem cells and of particular interest as potential modulators of stem cell maintenance. Constitutive expression of this cluster in bone marrow was found to enhance reconstitution in all blood lineages. Of this cluster, miR-125a transduced cells were shown to be sufficient to maintain this phenotype, but did not alter the self-renewal ability of committed progenitors.

To determine if miR-125a affected the number of reconstituting hematopoietic stem cells, we performed a limiting dilution assay using GFP+ bone marrow cells from primary transplants. We scored the ability of the transplanted stem cells to reconstitute bone marrow based on the minimum representation of test cell genotype in the blood of the recipient animals. To control for altered production of mature cells, we examined the ability of the miR-125a transduced cells to produce mature cell types and found no significant difference from control populations. By immunophenotypic histochemistry, we found that miR-125a expanded hematopoietic stem cell number by 10-fold, but did not significantly alter other progenitor populations. These results were supported in serial transplantation. We hypothesize that this increase in number reflects a decreased dependence on the stem cell niche, as this activity was maintained in culture in miR-125a expressing cells. Furthermore, inhibition of miR-125a expression in culture through an antagomir obliterated high proliferative potential and secondary colonies in assays used as surrogates for the hematopoietic stem cell compartment.

These data show that miR-125a plays a key role in the ability of stem cells to reconstitute multilineage hematopoiesis and undergo self-renewal. As miR-125a transduction did not induce leukemia or myeloproliferation in any recipient mouse, this microRNA is an attractive candidate for use in stem cell expansion. Future work is aimed at understating the molecular programs involved in stem cell self-renewal through identification of miR-125a targets.

Genetic Variants Associated with White Matter Hyperintensity

Hillary C. Frankel
Harvard Medical School, William Bosworth Castle Society, Class of 2011
PASTEUR Fellowship

Jonathan Rosand, MD, MSc
The Center for Human Genetic Research
Massachusetts General Hospital

The presence of white matter hyperintensity (WMH) in elderly patients strongly predicts risk of symptomatic stroke, cognitive decline, gait deterioration, and late-life depression. Studies have found that 95% of 60-90 year-old patients had WMH to varying degrees, with an increased prevalence and severity with age. The prevention of WMH progression could reduce age-related disability and stroke occurrence, but requires an understanding of why patients develop heterogeneous amounts of WMH volumes. WMH is thought to develop as a result of transient, moderate, repeated drops in regional blood flow leading to incomplete infarction. Through epidemiological studies, WMH has also been linked to known vascular risk factors including hypertension, atherosclerosis, homocysteine, and smoking. Sibling and family studies have demonstrated a high heritability to WMH volume, indicating that genetic variants contribute to WMH development. Despite high heritability, both linkage and association studies that have attempted to identify genetic variants that are associated with WMH have been unsuccessful.

This study seeks to use a genome-wide association to identify genetic variants associated with WMH to better understand why stroke patients present with varying WMH volumes and to eventually aid in therapeutic progress and the prevention of WMH development in future patients. We hypothesize that the volume of white matter hyperintensity in stroke patients is influenced by common genetic variation. To test our hypothesis, we used MRICro volumetric analysis to measure the WMH volume, normalized to intracranial volume, in 885 patients with first-ever symptomatic ischemic strokes and MRI within 30 days of onset. Subjects were recruited from Massachusetts General Hospital (MGH), Brigham and Women's Hospital, and through the Ischemic Stroke Genetics Study at the University of Virginia (UVA) and Mayo Clinic. Genotyping for MGH and Brigham subjects was performed on the Affymetrix 6.0 GeneChip; Mayo and UVA participants were genotyped on the Illumina610Quad platform. Following genotyping, imputation was carried out to identify likely SNPs in linkage disequilibrium and tests of association between SNPs and the log-transformed WMH were performed. The meta-analysis to combine data from the Affymetrix and Illumina platforms is ongoing.

Characterization of Kinetic and Qualitative Differences between HIV-specific CTLs Obtained from Elite Controllers and Viremic Progressors

Gaurav D Gaiha
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Bruce D Walker, MD
Partners AIDS Research Center
Massachusetts General Hospital

The cytotoxic T lymphocyte (CTL) response provides the most protective immunity in human immunodeficiency virus (HIV) infection. While the development of HIV-specific CTLs is critical to maintaining viral control, their presence alone, even in very large numbers, is not sufficient to control disease progression. Recent data from individuals who spontaneously suppress HIV to undetectable levels (i.e. 'elite' controllers) indicate that the quality of the CTL response is perhaps more important than the quantity of these cells. The goal of the study was therefore to identify qualities that define effective CTLs in order to evaluate these factors in the context of natural infection and vaccination.

Ongoing work in our laboratory has shown that distinct CTL populations can vary widely in their ability to suppress HIV. Notably, CTLs obtained from individuals with the 'protective' HLA alleles B57 and B27 appear to be more effective than those recognizing epitopes presented by other HLA. Similarly, CTLs that target *gag* protein appear to have better antiviral activity than those that target viral *env* or RT protein. Therefore, in this study we evaluated the effectiveness of a B27-restricted, gag-specific CTL clone isolated from an elite controller against a number of A3-restricted CTL clones obtained from viremic progressors through a variety of in vitro assays.

As we observed, the ability of the B27-restricted CTLs to kill both peptide-pulsed cells and HIV-infected cells was 1-2 logs higher than those obtained from viremic progressors. Inhibition by elite controller CTLs was higher despite lower peptide-HLA binding affinity and TCR affinity. Furthermore, when incubated with cells coated in anti-CD3 antibody, elite controller CTLs exhibited >10-fold greater killing suggesting differences in the coupling of TCRs and downstream signaling mechanisms.

Direct visualization of CTL clones using time-lapsed video microscopy revealed rapid killing by elite controller CTLs in comparison to clones obtained from progressors. In addition, rather than getting "hung up" on individual targets, the elite controller CTLs moved rapidly from target to target and were able to form synapses with multiple targets simultaneously.

Collectively, these data indicate that CTLs from elite controllers exhibit both kinetic and qualitative differences from clones isolated from progressors. Application of this new information could prove to be critical in the continued development and assessment of novel T cell based vaccines.

Primary Grafting with Autologous Cranial Particulate Bone Prevents Osseous Defects Following Cranial Remodeling

Lin Lin Gao
Harvard Medical School, William Bosworth Castle Society, Class of 2011
PASTEUR Fellowship

Arin Greene MD, MMSc
Craniofacial Center, Department of Plastic Surgery
Children's Hospital Boston

We previously have shown that primary autologous particulate bone grafting after frontal-orbital advancement (FOA) decreases the risk for residual osseous defects. We hypothesized that primary particulate bone grafting also would prevent bony gaps following cranial remodeling for sagittal, lambdoid or multiple suture synostosis. We studied children who underwent cranial expansion or remodeling for craniosynostosis between 1989 and 2008. In Group I, exposed dura was left open to heal spontaneously. In Group II, autologous cranial particulate bone graft was placed over dura at the time of cranial expansion. Partial thickness bone was harvested from the endo or ectocortical surface of either the sagittal, occipital or parietal segments, using a hand-driven brace and bit. Outcome variables were persistent osseous defects from incomplete ossification of exposed dura and the need for revision cranioplasty. The study included 52 children. Group I contained 15 patients: the mean age at cranial remodeling was 12.2 months (range, 1 to 36 months). Six patients (33.3 percent) had residual defects and four (26.7 percent) required corrective cranioplasty. Group II contained 37 patients: the mean age at cranial remodeling was 20.0 months (range, 3 to 79 months). Three patients (8.1 percent) had residual defects and two (5.4 percent) required revision cranioplasty. Primary autologous cranial particulate bone grafting reduces the risk of residual defects ($p = 0.033$) and decreases the need for additional cranioplasty ($p = 0.049$) following cranial remodeling for sagittal, lambdoid or multiple suture synostosis.

Dysregulated Tyrosine Phosphorylation Enhances Tau-Induced Neurodegeneration in Drosophila

Xin Gao
Harvard Medical School, Irving M. London Society of Health Sciences and Technology, Class of 2011

Mel Feany, MD, PhD
Department of Pathology
Harvard Medical School
Brigham and Women's Hospital

Alzheimer's disease is a progressive, fatal neurodegenerative disease and the most common cause of dementia. The principal histopathological features of AD brain include extracellular β-amyloid plaques and intracellular aggregates of the microtubule-associated protein tau. While amyloid aggregation is a major component of disease, tau pathology alone is sufficient to cause neurodegeneration, or a tauopathy. Tau-to-microtubule binding normally exists in a dynamic equilibrium, regulated by the actions of kinases and phosphatases. When this regulatory pathway becomes dysregulated, however, hyperphosphorylation can trigger excessive detachment of tau, leading to formation of neurofibrillary tangles. Studies on tau phosphorylation have focused on the 79 serine and threonine residues found in the longest human isoform, with at least 25 sites identified as targets of phosphorylation in Alzheimer's brains. Although comparatively little is known about the phosphorylation of the 5 tyrosine residues in tau, recent studies show such events do occur *in vitro* and may contribute to tau pathogenesis.

To further investigate the role of tyrosine phosphorylation in tauopathy, transgenic flies expressing tyrosine-to-phenylalanine mutations at all 5 tyrosine sites in human tau (tau^{YF5}) were created to mimic constitutively tyrosine dephosphorylated tau. At equivalent levels of total tau expression, tau^{YF5} flies exhibited augmented levels of neurodegeneration as compared to tau^{WT} flies. Tyrosine dephosphorylated tau triggered increased levels of neuronal apoptosis and rougher external retina, indicators of enhanced toxicity. Moreover, analysis of a series of serine-proline (SP) or threonine-proline (TP) sites whose phosphorylation is associated with tau-induced neurodegeneration showed disruption of tyrosine phosphorylation did not significantly alter SP/TP phosphorylation.

In order to identify the specific tyrosine kinases that phosphorylate tau *in vivo*, an RNAi screen involving all 31 tyrosine kinases in the Drosophila genome was performed. Our screen identified 12 enhancers of tau toxicity, including the non-receptor Src family tyrosine kinase Src42. However, knockdown of these candidates also enhanced toxicity in tau^{YF5} flies, suggesting they do not act directly in tau phosphorylation. Interestingly, over-expression of Src64, the second member of the Src family kinases in *Drosophila*, also enhanced tau toxicity. These findings are consistent with recent literature identifying the Src family tyrosine kinase Fyn as the major regulator of tau tyrosine phosphorylation *in vitro*.

Overall, our work suggests for the first time that tyrosine phosphorylation, through a mechanism separate from SP/TP phosphorylation, protects against tau-induced neurodegeneration *in vivo*. In addition, we have identified a set of tyrosine kinases, including the Src family kinases, which modulate tau toxicity independently of direct tau phosphorylation.

Assessment of Incipient Interproximal Caries Diagnostic Device Using Dye Enhanced Fluorescence

Mindy Gil
Harvard School of Dental Medicine, William Bosworth Castle Society, Class of 2011

Shigemi Nagai, DDS, MSD, PhD
Restorative Dentistry and Biomaterials Sciences
Harvard School of Dental Medicine

Background: Detecting early interproximal lesions still poses a challenge in clinical dentistry today. Invasive restorative treatment is often necessary by the time carious lesions are detectable by bitewing radiographs. Therefore, a more sensitive method for detecting early interproximal lesions would provide an opportunity for active preventive measures.

Purpose: The aim of this study was to evaluate the efficacy of a new caries detecting system using near infrared(NIR) fluorescent probes.

Methods: The overall specificity and sensitivity (Part I), and accuracy of size recognition of caries (Part II) were assessed based on histological analysis (gold standard). *Part I:* Extracted human teeth(n=33), including both intact and carious areas, were prepared for interproximal caries diagnosis by three methods. The first method was bitewing x-rays(XR). For the second and third methods, NIR fluorescent probe (OsteoSense, excitation:740nm, emission:790nm) were applied to the interproximal areas and excited with NIR light from two different angles: 1) Indirectly from occlusal surface(FO), 2) Directly to the interproximal lesions(FI). Eleven examiners assessed each lesion as "Lesion" or "No-Lesion." Consequently, sensitivity and specificity of all three methods were determined against gold standard. *Part II:* Extracted teeth with white spot lesions(n=24) were used for this assessment by the same three methods(XR, FO, FI). Six experienced dentists evaluated all three sets of images as "No lesion", "Lesion with depth <½ of Enamel width" or "Lesion with depth >½ of Enamel width". Histological analysis confirmed that all teeth had demineralization that extended to less than half the enamel width. Subsequently, every assessment was categorized into "Match", "Under-Estimate(UE)" or "Over-Estimate(OE)". One-way ANOVA and Sheff's multiple comparison test were used for a statistical analysis ($p<0.01$).

Results: Sensitivity and Specificity ranged 0-48% and 30-100% for XR, 57-78% and 70-100% for FO, 74-100% and 50-90% for FI. The mean values of MATCH percentages were 11.81%(XR), 29.17%(FO) and 64.58%(FI). UE percentages were 79.17%(XR), 53.47%(FO), 13.89%(FI) respectively. Finally, OE percentages were 9.03%(XR), 17.36%(FO), 21.53%(FI) respectively.

Conclusions: The prototype of new optical system demonstrates higher sensitivity than that of X-ray. In terms of early decay specifically, the "Match" of FI was significantly higher than that of XR and FO. This promising result suggests a potential for this new optical methodology to serve as a reliable and accurate early caries detection system.

Eligibility Criteria for Organ Transplant at US Transplant Centers: A study of practices and physician attitudes

Charity Celeste Glass
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

Lisa Lehmann, MD, PhD
Center for Bioethics, Department of Medicine
Brigham and Women's Hospital, Harvard Medical School

United Network for Organ Sharing (UNOS) is a non-profit organization that has contracted with the government to regulate the procurement and distribution of deceased donor organs. UNOS is committed to fulfilling this charge on the grounds of equity and medical utility, applying the principle of justice to both access and allocation. The guidelines put forth by UNOS are intended to uphold these principles and fairly distribute a scarce resource amongst those who need it. Each patient seeking a transplant undergoes an evaluation to determine eligibility for the waiting list. This evaluation includes medical and psychosocial components. Transplant physicians play a significant role in deciding who is eligible for an organ transplant. Whether or not patients should be allowed to simultaneously be listed at multiple centers is an example of a current eligibility criteria controversy that this study explores. Studies have shown that this strategy can sometimes lessen patient wait time because waiting times vary geographically. This raises the concern that allowing patients to be listed at multiple centers creates an access disparity between those who can afford to travel and apply at multiple centers and those who cannot. UNOS itself has switched its policy on multiple listings at least three times. The organization ruled for multiple listings in August 1987, reversed its decision in January 1988 and reversed that decision to permit multiple listings in May 1988. We are collecting empirical data to assess the prevalence of centers that allow multiple listings and the criteria centers use to list patients for transplant.. The specific aims of the study are (1) to describe the eligibility criteria transplant centers use to put patients on a waiting list (2) to determine the percentage of transplant centers that allow multiple listings (3) to assess transplant physicians' attitudes toward organ transplant practices (4) to assess transplant physicians' attitudes about who should be eligible for an organ transplant and who can be an organ donor. Transplant centers are being identified using UNOS' member list and the transplant center directors are responding to an electronic survey about transplant center policies and practices. Transplant physicians are also being surveyed regarding their attitudes toward these issues. Analysis will include descriptive statistics and multivariate regression analysis to determine associations between organ transplant eligibility criteria and the region where the transplant center is located, number of transplants performed by the transplant center and type of transplants performed by the transplant center.

Structural Characterization of Reovirus Nonstructural Protein μNS

Russell P. Goodman
Harvard Medical School, William Bosworth Castle Society, Class of 2011

Prof. Max L. Nibert , MD, PhD
Department of Microbiology and Molecular Genetics, Harvard Medical School

Family *Reoviridae* comprises a set of double-stranded ribonucleic acid (dsRNA) viruses whose genome is divided into 10 to 12 dsRNA segments, most of which encode a single protein. These viruses have no lipid envelope, possess icosahedral symmetry, and generally range in size from 60 to 85 nm in diameter. *Reoviridae* members infect a diverse range of hosts and include notable and significant human pathogens.

μNS is a "nonstructural" protein (not part of the mature virion) produced by Reovirus, the prototype member of family *Reoviridae*. When expressed in infected cells, individual μNS molecules co-associate to form very large, phase-dense structures, termed "viral factories", which are necessary for successful viral replication. Viral factories may have a number of roles in viral replication, including concentration of viral proteins for assembly, sorting of dsRNA genome segments, and packaging of viral proteins into mature virions.

No detailed structural information exists for μNS, and the protein has undergone only limited biochemical characterization. We hypothesized that the production of purified, recombinant μNS would be of great value by allowing the biochemical and structural characterization necessary to provide further insight into the roles of μNS in formation of viral factories, viral replication, and virus assembly.

To accomplish these goals, four obstacles had to be overcome. First, an expression system capable of producing sufficient quantities of μNS had to be developed. Second, μNS had to be purified to greater than 95% purity. Third, full-length μNS had to be purified away from a truncated version of μNS, μNSC, produced through translation initiation at a downstream start codon. Fourth, μNS had to be produced in sufficient concentration to allow structural techniques such as X-ray crystallography.

Each of these problems was solved in turn through experimentation with different expression systems, incorporation of polyhistidine affinity tags and ammonium sulfate precipitation for purification of the protein, and use of site-directed mutagenesis to improve protein solubility and eliminate μNSC production.

Possession of purified μNS has allowed a number of initial characterizations to be conducted. For example, gel-filtration chromatography experiments have demonstrated that μNS exists in solution as a basal oligomer, with previously identified key residues putatively involved in zinc binding required for higher-order interactions. Light scattering, proteolysis, microscopy, and crystallography experiments are ongoing.

Oral leukoplakia: Is the incidence of dysplasia and carcinoma greater than we think?

Rebecca Lindsay Grammer
Harvard School of Dental Medicine, Walter Bradford Cannon Society, Class of 2011

Mark A. Lerman, DMD
Oral Medicine, Infection, and Immunity
Harvard School of Dental Medicine

Leukoplakia is the most frequently occurring oral lesion with malignant potential. Leukoplakia is a clinical entity defined by the World Health Organization as "a white plaque that does not wipe off and cannot be characterized clinically or pathologically as any other disease and is not associated with any physical or chemical agent except tobacco". Essentially, leukoplakia is viewed as a clinical diagnosis of exclusion. Histologically, leukoplakia represents hyperorthokeratosis or parakeratosis with or without acanthosis, inflammation, and dysplasia. Hyperplasia of the squamous epithelium or a variety of other presentations, such as a non-healing ulcer or an exophytic or fungating mass may be the first step in a transformation toward a malignancy.

All cases submitted for biopsy to Pathology Services Inc., a private pathology lab in Cambridge, Massachusetts, from January 2007 to June 2008 were reviewed for inclusion in the study. Those submitted with a clinical description or diagnosis of a "white lesion", "leukoplakia", "hyperkeratosis", "lichen planus", "erythroleukoplakia", or "papilloma" were accepted for the study, totaling 1289 cases. Demographic and clinical data was collected. Two board-certified oral and maxillofacial pathologists reviewed the histopathology of all specimens from the selected cases to confirm the initial diagnoses.

Preliminary data suggest that 69.6% of the cases evaluated could be excluded from the clinical "leukoplakias" because they were assigned other specific diagnoses after histopathologic evaluation. Of the eligible cases, 69.3% revealed no evidence of epithelial dysplasia. The remaining 30.7% of lesions were described as epithelial dysplasia, carcinoma-in-situ, or squamous cell carcinoma. Dysplastic lesions accounted for 26.3% of these, while 0.6% were diagnosed as carcinoma-in-situ and 3.7% were diagnosed as squamous cell carcinoma.

Current literature suggests that 80.1% of leukoplakias reveal no histopathologic evidence of epithelial dysplasia. Historically, the term leukoplakia has included benign alveolar ridge keratosis, chronic bite injuries and probably less histologically well-defined reactive/inflammatory lesions in the category of benign leukoplakia. According to Natarajan and Woo, benign alveolar ridge keratosis is truly a separate diagnosis from leukoplakia, as the definition of leukoplakia states that the lesion must not be able to be defined as any other known condition. Therefore, clinicians and pathologists should assign it as such. Additionally, chronic bite injuries should not be included as a subtype of leukoplakia. Preliminary results suggest that after exclusion of these benign conditions, the percentage of cases of true leukoplakia that are histologically diagnosed as dysplasia, carcinoma-in-situ, and invasive squamous cell carcinoma is greater than what the current literature suggests.

Cost-Effectiveness of Prophylactic Surgery for Duodenal Cancer in Familial Adenomatous Polyposis

Wesley H. Greenblatt
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011
PASTEUR Fellowship

Chin Hur, MD, MPH
Assistant Professor of Medicine, Harvard Medical School
Director of GI Health Outcomes Research, MGH Gastrointestinal Unit

Background: Duodenal cancer is the leading cause of death in familial adenomatous polyposis (FAP) after excluding colorectal cancer. The lifetime risk of developing duodenal cancer is 4-10%. Current treatment guidelines recommend a prophylactic pancreaticoduodenectomy in advanced duodenal polyposis. Because no clinical trial has assessed this recommendation, a modeling approach was employed to evaluate the cost-effectiveness of different treatment strategies for duodenal cancer in FAP.

Methods: A Markov Cohort Model was constructed to estimate the life expectancy and cost of three different treatment options: prophylactic pancreaticoduodenectomy at Spigelman stage III duodenal polyposis, prophylactic pancreaticoduodenectomy at Spigelman stage IV polyposis, and no prophylactic surgery. A cohort of age 30 individuals with FAP who had undergone a total colectomy was simulated until age 80. The analysis was from the societal perspective. A wide sensitivity analysis was performed to assess the impact of model parameter uncertainty on the estimated cost-effectiveness.

Results: Prophylactic pancreaticoduodenectomy at stage IV had an incremental cost-effectiveness ratio of $3,200 per quality-adjusted life year gained compared to no prophylactic surgery. Surgery at stage IV was both less costly and more effective than surgery at stage III. At all stages of polyposis and all ages under 80, surgery at stage IV maximized life expectancy. The results were robust to wide variation in model parameters, but were sensitive to post-pancreaticoduodenectomy quality of life score.

Conclusions: Prophylactic pancreaticoduodenectomy at stage IV duodenal polyposis in FAP maximizes life expectancy over surgery at stage III polyposis and no prophylactic surgery, and is a cost-effective management strategy.

Autoantibody Signatures in Melanoma

Jennifer L. Greenman
Harvard Medical School, Francis Weld Peabody Society, Class of 2009

David A. Jones, MD, PhD
Department of Dermatology
Brigham and Women's Hospital

BACKGROUND: Development of improved screening tools for melanoma is an important unmet clinical need. Even though melanomas are theoretically easy to see, melanoma still kills about 10,500 Americans per year and most of these deaths could be prevented with early detection and surgical excision. Melanomas identified early are cured 97 percent of the time but if they metastasize, only 18 percent of patients live five years or more. A variety of potential biomarkers and biomarker identification strategies have been investigated for cancers, including melanoma. A very promising recent approach is to capitalize on the immune system to recognize the tumor and produce a highly amplified, easily measurable response. In particular, dysregulated protein production and dyrsregulated post-translational modifications of normal proteins in cancers leads to the production of autoantibodies against these proteins.

OBJECTIVES: This project aimed to identify a set of antigens recognized by antibodies present in melanoma plasma but not in normal control plasma.

METHODS: Using a random peptide phage-display library system and dot-blot detection assays, we analyzed serum samples from 56 patients with melanoma and 44 controls, with the samples divided into training and validation sets. A subset of 27 phage-peptides that was selected from the training set was evaluated with an independent validation set of 130 serum samples (78 from patients with melanoma and 52 from controls).

RESULTS: The subset of 27 phage-peptides had 66% specificity and 65% sensitivity in discriminating between melanoma and control serum samples.

CONCLUSIONS: Given the relatively low prevalence of melanoma, this set of particular phage-peptides may find use as a tool to screen and to follow particular at-risk populations such as those with dysplastic nevi or a family history of melanoma. Continued work is in progress to identify a phage-peptide antigen set with higher sensitivity and specificity.

A Comparison of Advertising Strategies between the Indoor Tanning and Tobacco Industries

Jennifer L. Greenman
Harvard Medical School, Francis Weld Peabody Society, Class of 2009

David A. Jones, MD, PhD
Department of Dermatology
Brigham and Women's Hospital

BACKGROUND: The indoor tanning industry is large and continues to grow, with 2007 domestic sales in excess of $5 billion. Advertising is central to shaping the consumer's perception of indoor tanning as well as driving industry demand.

OBJECTIVE: This project aimed to identify key drivers of consumer appeal by comparing tanning advertising strategies to those used by tobacco marketers. Tobacco advertising was selected as a reference framework because it is both well documented and designed to promote a product with known health hazards.

METHODS: 2000 randomly selected advertisements from four large tobacco advertisement databases were analyzed for type of advertisement strategy used, and four advertising method categories were devised to incorporate the maximum number of advertisements reviewed. Subsequently, contemporary tanning advertisements were collected from industry magazines and salon websites and evaluated relative to the identified strategy profiles.

RESULTS: Both industries have relied on similar advertising strategies, including mitigating health concerns, appealing to a sense of social acceptance, emphasizing psychotropic effects, and targeting specific population segments.

CONCLUSIONS: Given the strong parallels between tobacco and tanning advertising methodologies, further consumer education and investigation into indoor tanning's public health risks is needed. Future work will aim to analyze larger advertisement sets with more statistically rigorous methods.

Effect of Location and Restoration Type on Crestal Alveolar Bone Levels Around Single Titanium Implants

Brandon D. Grunes
Harvard School of Dental Medicine, Oliver Wendell Holmes Society, Class of 2011

German O. Gallucci, DMD
Department of Restorative Dentistry and Biomaterials Sciences
Harvard School of Dental Medicine

For the past 30 years, the use of endosseous dental implants has become a predictable and widely accepted treatment option for edentulous patients. Multiple factors exist that effect the success of implant osseointegration via their impact on crestal alveolar bone levels. Factors previously examined include the type of implant used, such as one versus two piece and submerged versus nonsubmerged designs, implant surface characteristics, and implant loading.

The objectives of this study were to examine two additional factors that could influence the integration of implants in bone. The first was whether using ceramometal versus all-ceramic restorations would have different effects on crestal bone levels. The second was how the location of the implant in relation to adjacent teeth would effect bony integration around the implant.

20 patients in need of single tooth implants were recruited and randomly assigned to receive either ceramometal or all-ceramic restorations. Four radiographs were taken of each patient, upon implant placement, upon implant loading, one year following implant loading, and two years following implant loading. Each radiograph was evaluated along three parameters for both the mesial and distal aspects of the implant: the most coronal bone to implant contact (DIB), the most coronal bone to adjacent tooth contact (BHAAT), the horizontal distance between the implant and adjacent tooth.

Objective 1: Preliminary data for the ceramometal group at the one year follow up [mean (mm)±SD(mm)]: mesial DIB (-1.61±.71), distal DIB (-2.02±.45), mesial BHAAT (1.10±.78), distal BHAAT (-.62±.49). Preliminary data for the all-ceramic group at the one year follow up: mesial DIB (-2.19±1.07), distal DIB (-2.20±.72), mesial BHAAT (1.09±1.7), distal BHAAT (-.92±.50).

Objective 2: Preliminary data for the 0-1.2mm horizontal distance group over four radiographic evaluations: mesial DIB (values decreasing), distal DIB (values decreasing), mesial BHAAT (values slightly decreasing), distal BHAAT (values decreasing). Preliminary data for the 1.2+mm horizontal distance group over four radiographic evaluations: mesial DIB (values constant), distal DIB (values constant), mesial BHAAT (values constant), distal BHAAT (values slightly decreasing).

The preliminary data suggest no significant difference in crestal bone levels around implants with ceramometal as opposed to all-ceramic restorations. For implants placed between 0 and 1.2 mm from an adjacent tooth, there appears to be a decreasing trend in crestal bone heights for both DIB and BHAAT measurements. For implants placed greater than 1.2 mm from an adjacent tooth, there appears to be no decreasing trend in crestal bone heights for DIB or BHAAT measurements.

Development of an Instrument to Assess Functional Effects of Stiffness Following Lumbar Spine Fusion

Kenneth R. Gundle
Harvard Medical School, William Bosworth Castle Society, Class of 2010

Robert A. Hart, MD
Oregon Health & Science University, Department of Orthopaedics & Rehabilitation

Introduction: One goal of spinal fusion is to eliminate motion of spinal segments in order to alleviate pain, improve deformity, and reduce disability. Independent of pain relief, however, decreased spinal mobility may impair performance of activities of daily living (ADL) as a result of stiffness of the fused segment. To assess the impact of spinal stiffness on ADL following lumbar fusion, we developed a questionnaire seeking information on these effects and assessed its reliability and external validity.

Methods: The questionnaire yields a score from 0 to 100, with higher scores indicating greater difficulty resulting from spinal stiffness in performing 10 different ADL's. The instrument was administered to 32 lumbar fusion patients a minimum of one year post-operatively. All patients completed the questionnaire twice via telephone interviews four weeks apart. Data were used to evaluate the internal consistency and retest reliability of the questionnaire. External validity of the LFFDQ was evaluated by correlating questionnaire scores with lumbar range of motion (LROM), as measured from the change in angulation between the inferior endplate of T12 to the superior endplate of S1 on standardized digital flexion-extension lateral radiographs.

Results: The instrument demonstrated high internal consistency (Cronbach's alpha = 0.89). Retest reliability was also excellent (intra-class correlation coefficient [ICC] = 0.87). External validity was demonstrated by an inverse relationship between LROM and questionnaire scores (correlation coefficient, r = -0.71, 95% confidence interval (CI): (-0.85, -0.48) $p < 0.001$), indicating a strong level of correlation.

Conclusions: This study demonstrates that the questionnaire is a reliable and valid instrument for assessing functional effects due to stiffness following lumbar spine fusion. This may represent a previously underappreciated domain of clinical outcomes following lumbar fusion surgery.

Comparison of Quality Indicators for OECD Countries in Four Categories: Acute, chronic, cancer and communicable disease care

David Guo
Harvard Medical School, Francis Weld Peabody Society, Class of 2011

Dr. Niek Klazinga, MD, PhD
Directorate for Employment, Labor and Social Affairs
Organization for Economic Cooperation and Development

Factors such as rising costs and medical errors have prompted countries to revisit the performance of their health care systems. To measure performance, nations have pursued the development of technical quality indicators for health care. In 2002, the Organization for Economic Cooperation and Development (OECD) launched the Health Care Quality Indicators (HCQI) Project, leading the effort to develop a standard set of indicators to facilitate international comparisons. The initial findings from 30 OECD countries were published in 2006, covering 17 indicators in four areas: acute conditions, cancer, chronic conditions and prevention of communicable diseases.

The objectives of my research were to evaluate the strength of OECD member countries in different categories of indicators and to explore the relation between the indicators and characteristics of health care systems. My specific aims were to develop methods of grouping OECD countries that would highlight trends in the data and to identify relationships between indicator performance and specific health system functions, including financing, resources, service delivery, and governance.

To accomplish this, I conducted a quartile analysis of each indicator, and then categorized them into the four service delivery or disease-based groups: acute, cancer, chronic, and communicable disease. To assess the strength of the countries in each category, I calculated the average of each country's quartile rankings (from 1-4) for individual indicators. Then I compared the top and bottom quartiles in terms of specific metrics in finance, resources, delivery of care, and governance.

In acute care, the top quartile countries had 97% higher health expenditure per capita, greater availability of PCI, CABG and MRI (50%, 126% and 204%, respectively), and 85% more nurses per 1000 population. In cancer care, the top quartile spent 41% more on health expenditure per capita, and had greater availability of treatment technology (51% more radiation units per million population). Conversely, in communicable disease care, the top quartile spent on average only 50% of the bottom quartile's health care expenditures and utilized slightly lower technology and staffing resources. Results from chronic care were inconclusive due to limited data availability.

The initial set of indicators suggested the importance of finances and resources in the areas of acute care and cancer care, and demonstrated the strength of the Scandinavian countries in these categories. However, the lower-income OECD countries performed better in the communicable disease category, supporting the concept of epidemiological transition between poor and wealthy countries.

SPARC-null Mice Exhibit Lower Intraocular Pressures

Ramez I. Haddadin
Harvard Medical School, Walter Bradford Cannon Society, Class of 2009

Douglas J. Rhee, MD
Howe Lab, Department of Ophthalmology
Massachusetts Eye & Ear Infirmary

The relatively elevated IOP of open-angle glaucoma is caused by impaired aqueous drainage through the trabecular meshwork (TM). The secondary pathway for aqueous drainage, the uveoscleral pathway, also demonstrates morphologic changes in the ciliary body stroma (CB) of glaucomatous eyes, similar to those found in TM. Extracellular matrix (ECM) turnover in these regions is at least one contributory factor.

SPARC, a member of the matricellular family of proteins, modulates cellular interactions with ECM in tissues undergoing remodeling. SPARC has also been implicated in conditions involving fibrosis or altered ECM deposition, such as systemic sclerosis, cirrhosis, and tumor growth. SPARC-null mice exhibit accelerated closure of dermal wounds, attenuated collagen and connective tissue, and a reduced foreign body reaction to implanted biomaterials. SPARC deficiency in these various tissues indicates that a primary function of this matricellular glycoprotein is ECM deposition.

SPARC is widely expressed in ocular tissues, and produced by both TM endothelial and CB smooth muscle cells, and present in aqueous humor. In TM cells, SPARC is one of the most highly expressed genes and, in response to mechanical stretch, is one of those most highly upregulated. With the hypothesis that SPARC contributes to IOP regulation by mediating ECM deposition in the TM and CB, we compared the IOPs of SPARC-null mice, their corresponding wild-type (WT), and heterozygous animals.

Five to eight week-old C57Bl/6x129SvJ WT, SPARC-null, and heterozygous mice were housed under identical conditions. A commercial rebound tonometer (TonoLab) was used to measure diurnal and nocturnal IOPs between 4 and 7 minutes after administration of anesthesia. Animal eyes were enucleated, and light (LM) and electron microscopy (EM) of the iridocorneal angles was performed.

During the day, the mean IOP of SPARC-null mice (n=142, 16.9±2.4 mmHg) was lower than both WT mice (n=104, 19.9±2.9 mmHg), $p<10\text{-}12$, and heterozygotes (n=38, 19.3±2.5 mmHg), $p<10^{-4}$. At night, the mean IOP of SPARC-null mice (n=66, 18.9±2.2 mmHg) was also lower than both WT mice (n=54, 23.6 ± 2.2 mmHg) and heterozygotes (n=38, 21.8±2.6 mmHg), $p<10^{-20}$ and $p<10^{-7}$, respectively. At night, the heterozygote mean IOP was lower than WT, $p<0.001$, displaying an intermediate phenotype**.** In comparison to SPARC-null and heterozygous mice, WT mice exhibited a greater proportional increase in IOP at night, $p<0.05$. LM and EM did not reveal anatomic, cellular, or quantitative extracellular matrix differences in the juxtacanalicular region.

SPARC-null mice have significantly lower IOPs than their WT counterparts. SPARC likely contributes to aqueous outflow resistance.

Determining the Relationship Between Impression Volume and Closure of Double Arch Trays

Sara Miran Hahn
Harvard School of Dental Medicine, Francis Weld Peabody Society, Class of 2011

Robert F. Wright, DDS, FACP
Director, Advanced Graduate Prosthodontics
Harvard School of Dental Medicine

The double arch impression tray is used to make simultaneous impressions of the prepared tooth, the adjacent teeth, and the opposing teeth, in an exact occlusal relationship when closed in maximum intercuspation (MI). The use of the double arch trays have been questioned for frame instability and the inability of patients to close completely into the impression material. However, the technique is still widely used because it reduces appointment time, allows the impression to be registered with the mouth closed which reportedly increases comfort and reduces the amount of impression material used.

Complete closure of teeth in maximum intercuspation is mandatory for accurately registering occlusion. The volume of impression material distributed may provide resistance and affect closure when taking an impression. We hypothesized that a larger volume of impression material compared to a smaller volume would hinder closure to MI.

Quadrant impressions were taken on articulated fracture-resistant dental casts mounted in occlusion. Three types of sideless double arch impression trays were tested along with a control, i.e. no tray: Firstbite with nylon webbing (Dentsply), Sultan with double crosshatch webbing (Sultan), and Premium with single crosshatch webbing (Clinician's Choice). Aquasil Rigid Ultra Fast Set (Dentsply) was distributed at two different volumes and impressions were made. A total of 60 tray impressions were made – ten for each group. A weight of 1.3 kg was placed on the upper arm of the articulator assuring complete closure. The impressions were allowed to set for five minutes. After set, samples were placed on a DC lightbox and a camera set at a fixed distance captured the light transparencies that were projected through the impression material. The camera transferred the information to an image analysis program, ImageJ (NIH). This system allows the different amounts of light projected through the impression to be translated into a gray scale value, which can be assigned a thickness value in a specified occlusal contact area.

Data was analyzed using a two-way ANOVA followed by a secondary test to assess significance. The volume of impression material was not significantly different. Tray type was ($p<0.05$). Sultan showed the greatest change; Premium showed the least. The difference in tray/impression light transmission occurred because of the different meshes and a dynamic interaction between the mesh, the flow of the impression material and the cusp to fossae relationship.

NKG2D Ligand Expression in Glioblastoma Following Treatment with Radiation

Brian W. Hanak
Harvard Medical School, Francis Weld Peabody Society, Class of 2011

William T. Curry, MD
Brain Tumor Research Center, Department of Neurosurgery
Massachusetts General Hospital

Virally infected and transformed tumor cells express NKG2D ligands (NKG2DL) as a means of inducing a natural killer (NK) cell and γδ T cell mediated innate immune response against these dangerous cells. It has been shown that NKG2DL expression is up-regulated by the induction of the DNA damage pathway in an ATR, ATM, and Chk1 dependent fashion. This is particularly relevant to the progression of glioblastoma given that glioblastoma stem cells, which express the protein CD133, have been shown to engage a more robust DNA damage response than their $CD133^-$ counterparts. This robust DNA damage response in $CD133^+$ stem cells appears to be the basis for their radiation resistance. With this in mind, we explored the nature of NKG2DL expression before and after treating primary glioblastoma cells with radiation.

It was our hypothesis that treatment of primary glioblastoma cells with ionizing radiation known to activate the DNA damage response would lead to the upregulation of NKG2DL expression in the $CD133^+$ population of glioblastoma stem cells, making them more susceptible to NK cell mediated lysis.

Glioblastoma tumor samples were obtained from consenting newly diagnosed patients as approved by the Institutional Review Board. The cells from these tumor samples were cultured as a single-cell monolayer following erythrocyte and non-viable cell exclusion steps. The primary glioblastoma cells were maintained in culture for 24 hours prior to being exposed to either 0 Gy, 5 Gy, or 40 Gy ionizing radiation delivered as a single dose. Twenty-four hours following the administration of ionizing radiation, flow cytometry was used to study the cell surface expression of five human NKG2DLs: MICA, MICB, ULBP1, ULBP2, and ULBP3. In addition, the cells were stained for CD133 so that expression of NKG2DLs could be studied in both the stem cell and non-stem cell populations.

Our preliminary results from the study of two primary tumor samples suggests that grade IV glioma cells (both stem and non-stem cell populations) have lost the ability to express NKG2DLs.

Primary glioblastoma cells do not upregulate expression of NKG2DLs after ionizing radiation, perhaps an immunoediting adaptation.

A Nomogram for Predicting Successful Re-Excision in Breast Cancer Patients with Close or Positive Margins

Stephanie A. Hanson
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

Michelle C. Specht, MD
Department of Surgical Oncology
Massachusetts General Hospital

Approximately 33-62% of breast cancer patients who undergo breast-conserving therapy (BCT) have a close or positive margin at lumpectomy. Currently, these patients undergo a qualitative discussion with their surgeon regarding the decision to proceed with a re-excision or a mastectomy. We sought to develop a quantitative clinical prediction tool to predict the likelihood of achieving a successful re-excision to complete BCT.

We reviewed the records of 3,737 breast cancer patients who underwent BCT at a single institution from 1997 to 2007. Of these patients, 875 had close or positive margins requiring a second procedure; 797 (91.1%) underwent a re-excision, and 78 (8.91%) a mastectomy. Pathological features of the primary tumor, surgical technique and outcome of the procedure were evaluated by univariate and multivariate logistic regression analysis.

540/797 (68%) had clear margins at re-excision and therefore achieve breast conservation with this re-excision. Number of invasive margins involved ($p = 0.025$), number of total margins involved ($p=0.009$), size of invasive cancer ($p = 0.01$) and a score to describe DCIS at the margin ($p = 0.01$) were significant in predicting probability of successful re-excision. (The DCIS score was created using a sum of DCIS involvement at each of the six margins; 0-no cancer, 1-focally close, 2-broad front close, 3-focally positive, 4-broad front positive). A nomogram to predict the probability of successful breast conservation after a re-excision was developed from the multivariate logistic regression model.

Our model permits quantification of the relative contribution of multiple clinical factors to success or failure of re-excision for breast cancer. The nomogram developed may help patients and surgeons better predict the likelihood of successful breast conservation to help avoid both futile re-excisions and unnecessary mastectomies.

Dynamics of T cell activation *in vivo*

Sarah Henrickson
Harvard Medical School, Irving M. London Society and Immunology Program, Class of 2011

Ulrich H. von Andrian, MD
Mallinkrodt Professor of Immunopathology
Department of Pathology and Immune Disease Institute
Harvard Medical School

The rules by which naive T cells decide whether to respond to antigenic stimuli are only beginning to be fully understood. T cells are activated in secondary lymph nodes (SLOs) by the recognition of signals from antigen presenting cells (APCs), usually mature dendritic cells (DCs). We showed that $CD8^+$ T cells are primed by DCs in three phases using multiphoton intravital microscopy (MP-IVM) in lymph nodes (LNs) of anesthetized mice. During phase one, T cells undergo brief, serial contacts with DCs for several hours and begin to upregulate activation markers. During phase two, which lasts approximately twelve hours, T cells engage in stable interactions with DCs, fully upregulate activation markers and secrete cytokines. The third phase is characterized by a return to serial, transient DC-T cell interactions and the initiation of T cell proliferation.

The initial phase of serial interactions was intriguing, since previous studies had suggested that T cells stop immediately upon recognition of cognate-antigen presenting APCs. We therefore examined the influence of antigen dose on the duration of phase one by varying the number of cognate peptide-MHC (pMHC) complexes per DC and the density of cognate pMHC complex-presenting DCs per LN. The duration of phase one was inversely correlated with antigen dose. Very few pMHC complexes were needed for T cell activation and there was a sharp threshold antigen dose below which T cells did not transition to phase two, migrating until they egressed from the LN. In situations of low antigen, T cells may prolong phase one and scan more DCs to determine whether to become activated.

Finally, we also investigated the importance of stable, phase two-like, DC-T cell contacts in the differentiation of effector and memory $CD8^+$ T cells. We showed that there is a concentration of antigenic peptide that does not seem to yield a population-wide transition to stable DC-$CD8^+$ T cell interactions but does yield effector and memory T cell differentiation. Overall, we provide support for an integrative mechanism for T cell activation by brief, serial encounters with DCs.

Pilot Study to Develop Clinical Case Definitions for the Diagnosis of MDRTB in Resource-Limited Settings

Alex Herrera
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2009

Douglas Wilson
Department of Medicine, Edendale Hospital, University of KwaZulu-Natal, South Africa

Diagnosing MDR-TB in HIV-infected patients is a major barrier to timely and effective MDR-TB treatment. Access to culture and sensitivity techniques are restricted in resource-limited settings, and full sensitivity testing can take up to 8 to 16 weeks. Given the high mortality associated with HIV/MDR-TB co-infection, this delay in initiating second-line TB therapy while awaiting laboratory confirmation could result in significant mortality.

Our pilot study aimed to evaluate whether clinical case definitions could accurately predict MDR-TB infection in HIV-positive patients.

HIV-positive medical inpatients who met one of 5 MDR-TB clinical case definitions were enrolled for an 8-week observation period. The definitions included: 1) Patients who remained sputum smear positive after > 2 months of treatment, 2) Sputum smear negative patients who met 'failure to thrive' criteria after ≥ 2 weeks of therapy, 3) Sputum smear positive cases who were failing re-treatment therapy after > 2 weeks, 4) Patients who completed a course of TB therapy within the last 6 months and presented with sputum positive TB or met expanded WHO smear negative TB criteria, and 5) Newly-diagnosed TB patients with a confirmed household MDR-TB contact. Sputum and other appropriate TB culture and sensitivity data were used to determine the accuracy of the definitions. 8-week mortality was also measured.

31 participants were enrolled into the study. Culture data were available for 26 participants and mortality data were available for 27 participants. The overall accuracy of the case definitions for predicting MDR-TB was 46% and the accuracy for predicting anti-TB drug resistance of any kind was 62%. Among the 17 participants with a positive TB culture, 71% had MDR-TB and 94% displayed anti-TB drug resistance of any kind. Of participants who were AFB smear positive at enrollment, 56% had MDR-TB and 81% displayed anti-TB drug resistance of any kind. The overall 8-week mortality for the study was 52%, with a 63% mortality rate among participants displaying anti-TB drug resistance of any kind and 67% mortality in participants with MDR-TB.

Preliminary results suggest that clinical case definitions may predict for drug resistance in smear-positive and culture-positive TB cases. TB smear- and culture-positive patients that meet these case definitions should be candidates for isolation wards and rapid TB drug-resistance diagnostic testing, when available. Further investigation is necessary to determine whether clinical case definitions should be used to identify candidates for empiric MDR-TB therapy.

Filum Terminale - A Novel Source of Neural Stem Cells

Thomas R. Hickey
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011

David L. Cardozo, PhD
Department of Neurobiology
Harvard Medical School

Neural stem cells (NSCs) are multipotent, self-renewing cells that proliferate as neurospheres in response to growth factors. In adult mammalian brains, neurogenesis occurs at two principal sites: the subventricular zone of the lateral ventricles and the dentate gyrus of the hippocampus. Multipotent NSCs have also been isolated from other regions including the olfactory bulb, cerebellum, spinal cord and retina. The demonstration of NSCs within the mammalian CNS presents great therapeutic potential for many CNS diseases including traumatic, degenerative (i.e. Alzheimer's, Parkinson's), and autoimmune diseases (i.e. multiple sclerosis).

The filum terminale (FT), a tissue that extends from the conus medullaris and anchors it to the coccyx, is a surgically accessible tissue that serves no essential function in humans. Recent investigations hypothesized that the FT may be a source of stem cells due to its embryonic de-differentiation into an embryonic cell type after initial neuronal differentiation. These investigations confirmed that human and rat FT tissue sections stain positive for the neural stem cell marker Nestin. Our experimental goal was to establish NSC lines from FT tissue culture in order to show that this region provides an accessible and expendable source of autologous NSCs for therapeutic use.

Rat and human models were used to test this hypothesis. FT was dissected from postnatal rats (rFT); pediatric tissue (HuFT) was obtained from children undergoing surgery for tethered cord syndrome, a procedure that involves sectioning the FT. The isolated tissue was placed in standard stem cell medium containing type II collagenase for approximately 24 hours, after which the tissue was gently triturated and returned to the incubator. Using epidermal growth factor, basic fibroblast growth factor, and leukemia inhibitory factor we isolated neurospheres from the rFT and HuFT cultures as early as 48 hours after initiation of the culture. We established approximately forty new rFT-derived and six HuFT-derived neurosphere lines over the summer.

Neurospheres were passaged every several weeks *in vitro*, and most cell lines maintained robust populations of neurospheres. Previous experiments have demonstrated that motor neurons could be generated from the neurospheres *in vitro* under specific differentiation conditions. Also, when treated with the appropriate growth factors and signaling molecules, motor neurons differentiated from human FT formed neuro-muscular junctions with rat myocytes in vitro. Our future work aims to establish the appropriate conditions for dopaminergic differentiation of the rFT- and HuFT-derived neurospheres.

Menstrual Phase Effects on Smoking Cessation in Women

Jenovie M. Hsia
Harvard School of Dental Medicine, Francis Weld Peabody Society, Class of 2011

Taru Kinnunen, MA, PhD
Assistant Professor, Department of Oral Health Policy and Epidemiology
Harvard School of Dental Medicine

Gender differences in smoking cessation has become an important area of research, particularly due to variances in rates of smoking and cessation in men and women. Differences in smoking cessation in women are thought to be caused by hormonal fluctuations due to the menstrual cycle. Women often suffer from anxiety, depression, irritability and impaired concentration during the luteal (premenstrual) phase of the menstrual cycle. These symptoms are also characteristic of nicotine withdrawal, and can therefore be alleviated with increased smoking, or conversely, intensified during smoking cessation. Therefore, it may be more likely for relapse to occur among women who try to quit during their luteal phase.

Previous studies have shown a slightly lower smoking cessation rate among women in the luteal phase, however, these studies did not have enough participants to produce statistically significant results. Furthermore, results are now mixed, with one study showing higher smoking cessation rates during the luteal phase. Nonetheless, based on results from previous studies, we hypothesize that women in their luteal phase will have slightly lower smoking cessation rates.

Participants in this study were women between ages of 18 and 65 who were part of a larger smoking cessation. Eligible participants were followed from 3 weeks before their quit dates to 52 weeks post quit date.

The number of days between onset of menstruation and quit day was determined from the self-reported menstrual history. There were 47 women in the luteal group, and 65 women in the follicular group. Their smoking logs were observed for smoking relapses. The day of relapse was the first day that started a daily smoking pattern that lasted for more than 6 days in a row or the first of three smoking episodes such as a weekend of smoking. Abstinence or relapse at the end of treatment (EOT) and end of 6 months follow up (EOF) were used as categorical variables, and days abstinent during the 6 month follow-up period was used as a continuous variable. The primary goal of this study was to compare cessation rates between the two groups.

In the luteal group, 23 women (48.9%) were abstinent at week 1, 12 women (25.5%) were abstinent at EOF, and 8 (17.0%) were abstinent at EOT. In the follicular group, 30 women (46.0%) were abstinent at week 1, 14 women (21.5%) were abstinent at EOF, and 8 (12.3%) were abstinent at EOT.

Our results suggest that women attempting to quit smoking during their luteal phase, have higher success than those in their follicular phase.

Infection Control Attitudes, Knowledge, and Practices in Settings of High Multi-Drug Resistant Tuberculosis Risk

Kristin T. L. Huang
Harvard Medical School, William Bosworth Castle Society, Class of 2011
Michael Crichton Fellowship

Krista Dong, MD
Partners AIDS Research Center, Massachusetts General Hospital
iTEACH, Edendale Hospital, Pietermaritzburg, South Africa

Background: The South African province of KwaZulu-Natal (KZN) carries a high burden of multi- and extensively-drug resistant tuberculosis (MDR-TB and XDR-TB), with 3040 cases of MDR-TB and 285 cases of XDR-TB in 2007. PubMed literature searches for health care workers' attitudes, knowledge, or fears about MDR-TB failed to retrieve any articles, indicating the dearth in awareness about health care workers' experiences with MDR-TB. Furthermore, in such a setting it is essential that infection control procedures are followed. Barriers to proper infection control practices must be identified and addressed to prevent nosocomial spread of MDR- and XDR-TB.

Aims: 1) Understand health care workers' attitudes towards working in a high-risk environment. 2) Assess staff knowledge regarding MDR-TB and infection control practices. 3) Determine current infection control practices at each hospital. 4) Identify solutions to infection control barriers.

Methods: An audit tool with 30 qualitative and quantitative questions was developed, translated into Zulu, and back-translated into English for validation. After obtaining informed consent, the questionnaire was administered to health care workers (doctors, nurses, maintenance staff, radiographers, and physiotherapists) in their preferred language. Four government hospitals in extremely high-risk environments are being surveyed: Edendale Hospital, Doris Goodwin TB Hospital, Church of Scotland Hospital, and King George V Hospital. Chi-square analyses will be conducted to determine if there are significant differences in responses among hospitals or among staff types.

Results: Preliminary results from 70 surveys at Edendale Hospital indicate a high level of concern over health risks. Sixty-nine of 70 participants (99%) reported feeling "at risk" or "very at risk" for health problems at work, and 47 out of 53 respondents (89%) selected TB as the health risk of greatest concern. Only two of 68 respondents (3%) felt that they were "very well-trained" to protect themselves from health risks like TB. Participants reported feeling "angry," "terrified," and "vulnerable" at work. Respondents requested adequate provision of protective equipment, proper isolation facilities for MDR-TB patients, and staff training. Understanding and practice of infection control measures was not high. Only 48 of 68 respondents (71%) believed it is possible to protect oneself from contracting MDR-TB, and only 41 of 69 respondents (59%) open the windows in the wards to provide natural ventilation or request that it be done. Major barriers to infection control cited by respondents include inadequate supplies and space, discomfort, patient reluctance, and lack of personal responsibility.

Conclusions: Pending completion of audit at all sites.

Evaluation of Greek General Practitioners' Knowledge, Attitudes, and Practices with Regard to Cancer Screening

Leslie Irvine
Harvard Medical School, Francis Weld Peabody Society, Class of 2011

Athanasios I. Zavras, DMD, DMSc
Department of Oral Health Policy & Epidemiology
Harvard School of Dental Medicine

Cancer is the second leading cause of death in Greece, and the World Health Organization (WHO) estimates that in 2030 over 15% of deaths will be from cancer. Screening asymptomatic populations for breast, cervical, and colorectal cancers has been shown to lead to earlier diagnosis and decreased mortality. Currently there are no population-wide screening programs in Greece, and patients often rely on the opinions and recommendations of their primary care physicians when deciding whether to undergo screening. The current study hypothesizes that general practitioners' knowledge and attitudes predict their screening practices. A validated Greek questionnaire was mailed to a random sample of physicians belonging to the Greek Association of General Practitioners (ELEGEIA). A focus group was conducted with a convenience sample of five general practitioners practicing in rural Crete in order to qualitatively assess barriers faced by providers in implementing cancer screening. Results are currently being received and descriptive and analytic statistics will be used to assess differences in knowledge, attitudes, and practices across physician populations based on region, age, and years of practice. We hope to use the results of this study to make suggestions for future studies and possible interventions to increase rates of cancer screening. The results of this project may also lead to creation of continuing education programs for physicians so that they can offer increased levels of evidence-based cancer screening. Ultimately, increased screening rates will lead to decreased mortality and a diminished burden of disease in the community.

Effect of Age-, Sex- and Location-Specific Differences in Trunk Muscle Geometry on Estimates of Loading in the Thoracic and Lumbar Spine

Sravisht Iyer
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Mary Bouxsein
Orthopedic Biomechanics Laboratory, Dept of Orthopaedic Surgery
Beth Israel Deaconess Hospital, Harvard Medical School

Vertebral fractures can occur with minimal, moderate or severe trauma and their incidence rises sharply with age. The prevalence of vertebral fractures in women rises from 6% in 50-59 year olds to 50% in 80-89 year olds. In order to understand the etiology of vertebral fractures, it is critically important to understand the loads placed on the spine during the activities of daily living. Prior approaches to estimating loads on the spine have not considered differences in muscle geometry and have exclusively used values derived from young men. We believe that there are significant age- and sex-related changes in muscle geometry and that these changes will have an impact on the loads predicted on the spine.

To investigate this hypothesis, we have developed a biomechanical model to estimate loads on both thoracic and lumbar vertebral bodies (T8 – L4) for any quasi-static activity, including bending and lifting. The model is based on a previously validated model of the trunk and has been adapted to include contributions of the rib cage and sternum. In order to quantify muscle geometry in different age groups, we used volumetric quantitative computed tomography scans from an age- and sex-stratified sample of 49 men and women, aged 35 to 83 who were among the 3529 participants in the "Framingham Heart Study Offspring and Third Generation Multidetector CT Study."

Sex-related differences ($p < 0.05$) in muscle cross-sectional area (CSA) were observed for every muscle at nearly every vertebral level studied, with CSAs in men being 13-121% higher than women. Similarly, significant sex-related differences in muscle moment arm length (MAL) were observed for all muscles at nearly every vertebral level, with the MALs in men being 3-36% higher than women. Age-related declines in muscle CSA from 5-54% and in MAL from 7-33% were also observed for all muscles at multiple vertebral levels.

Using the biomechanical model, differences in spinal compressive loads were determined based on the age- and sex-specific variation in muscle CSA and MAL. Predicted loads on the spine were higher in men vs. women for all spinal levels examined. There were also differences in predicted spinal loads between age groups for many vertebral levels. For example, predicted loads on L4 were 44% lower for old women than for young women.

These data suggest that it is important to consider subject-specific inputs to models of the spine and that age- and sex-related changes might significantly alter the distribution of load along the spine.

The International HIV Controllers Study:
A genome wide association study

Xiaoming (Sherman) Jia, M Eng
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Paul de Bakker, PhD
De Bakker Lab, Harvard-Partners Center for Genetics and Genomics
Brigham and Women's Hospital

HIV-AIDS remains one of the most important global challenges, with over 60 million individuals infected worldwide. Scientists have recently identified individuals who are able to suppress the HIV-1 virus below the limits of detection for 30 years or longer without antiretroviral therapy. With recent advancements in genetic sequencing, genotyping, and analysis methods, it is now possible to systematically examine these individuals to identify common genetic variants associated with HIV control, paving the way for novel antiretroviral therapies and vaccine candidates. A recent genome wide association study identified two factors associated with low viral load set point in caucasians: HLA-B*5701 and HLA-C. Because these factors only explain a small percentage of variation in HIV viral load within infected individuals, it is likely that many other unidentified host factors are involved.

We are performing a genome wide association study (GWAS) in 500 HIV controllers and 800 controls (HIV-infected individuals who require antiretroviral therapy). These individuals were genotyped at 650,000 SNPs in their genome using Illumina 650Y and include a combination of self-reported Whites, Blacks, Hispanics, and Asians. The primary phenotype examined was HIV controller status (viral load < 200 copies HIV-RNA/mL x 3 observations); secondary phenotypes included elite controller status (viral load < 50 copies HIV-RNA/ml x 3 observations), as well as combined chronic controller status (> 10 years) with high CD4+ count (CD4+ > 350 cells/cc). SNP imputation was performed to augment genotyping coverage. Association testing was performed within each ethnic group for each phenotype, and a meta-analysis was performed across the White, Black, and Hispanic cohorts. Stepwise logistic regression models were used to identify independently significant signals in the MHC region.

Our data shows overwhelming replication of results from a previous genome wide association study: rs2395029 (proxy for HLA-B5701) at $p = 1x10^{-10}$, rs9264942 (HLA-C) at $p = 2.5x10^{-16}$. Overall, we identified over 50 SNPs at genome-wide significance ($p < 5x10^{-8}$) in the MHC region, of which there were 4 independent associations within Whites and 2 in Blacks. Meta-analysis across Whites, Blacks, and Hispanics showed 5 independent signals in the MHC region, of which only 1 was previously identified. This is the first GWAS to 1) examine the HIV controller phenotype, and 2) study this phenotype across multiple ethnic populations. The identification of novel associations in this study brings us a step closer to understanding the host factors in HIV control and finding new pathways for drug and vaccine therapy.

A Risk Calculator to Facilitate Oral Cancer Screening, Risk Assessment, and Risk Counseling in Crete, Greece

Sharon Jin
Harvard School of Dental Medicine, Oliver Wendell Holmes Society, Class of 2011

Athanasios I. Zavras, DMD, DMSc
Department of Oral Health Policy and Epidemiology
Harvard School of Dental Medicine

Oral cancer is a significant cause of morbidity and mortality worldwide. It has a poor 5-year survival rate because most cases are diagnosed in late stages. Universal screening through a 5-minute physical exam can lead to early diagnosis and improved survival rates. Previous research in Greece suggests that physicians and dentists lack information about oral cancer and do not routinely screen or engage in risk reduction counseling with patients. Concurrently, smoking and drinking alcohol have been identified as important risk factors for men in Greece. Given these risk factors, risk reduction counseling and early detection through routine physical exams should be the primary goal in Greece.

The Oral Cancer Risk Calculator was created as a tool for patients to assess their own risk behaviors. It was implemented in various primary care settings in Crete. The objective is to encourage dialogue between patient and doctor about oral cancer, and also to serve as a reminder to perform screening. Participating doctors were recruited from those who attended the oral cancer training workshop at the University of Crete School of Medicine. They were asked to implement the form in whatever way was most suitable for their own clinic. At the end of a 4-week trial period, participants were interviewed about their experience of using the Risk Calculator.

The 6 sites where the Risk Calculator was implemented provided a good sampling of the different types of primary care settings in Crete, such as physicians vs. dentists, rural vs. urban, and hospitals vs. community clinics. Interview responses demonstrate a belief in the importance of doing screening and risk reduction counseling, but also a sense of futility for affecting a patient's behavior. Doctors felt that risk behaviors such as smoking and drinking alcohol are so integral to a patient's lifestyle and so normalized socially that patients were unlikely to change. The Risk Calculator did serve as a jumping point for discussing a patient's risk behaviors. However, the actual practice of performing screening did not change for the majority (12/15) of participants interviewed. All believed that more education and practice would improve screening and counseling. Patients must be informed as well, through public health campaigns that penetrate into the media. Overall, the Risk Calculator can be a useful tool, especially when reinforced with an educational component such as our training workshop, and if there is at least one core doctor at each site to encourage and monitor its use.

Poverty and Women's Health in Mali: Assessing a new approach to health education

Ari David Johnson
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

Arachu Castro, PhD, MPH
Harvard Medical School, Department of Social Medicine

Health education programs in Mali addressing maternal-child health focus largely on distributing information through mass media. However, the gap between health information and improved health outcomes has been repeatedly documented, particularly in settings of extreme poverty like Mali. What is necessary to improve health in communities disempowered by a lack of access to education, economic opportunities, health services, and infrastructure? The current study evaluates the impact of an integrated women's health and economic empowerment program in Mali, Project Muso Ladamunen. Using quantitative and qualitative approaches, we test whether incorporating economic empowerment with health education will create lasting maternal and child health improvements. Surveys measured 20 maternal-child health indicators in amongst women before and after their participation in an 18-month health and enterprise education program. Ethnographic life histories of 24 participants in Project Muso's education and microfinance programs explored the relationships between conditions of poverty, women's income, health care access, and maternal-child health, and assessed the role of integrated education and microfinance programs in addressing these challenges. Both qualitative and quantitative data is currently being analyzed.

A Histologic Study of Enhanced Anterior Cruciate Ligament Healing

Shilpa M. Joshi
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Martha Murray, MD
Sports Orthopedic Research Lab, Department of Orthopedics
Children's Hospital Boston

The failure of a provisional scaffold of stabilized fibrin clot to form within the knee's synovial cavity is the likely cause of poor endogenous healing of the anterior cruciate ligament (ACL). To circumvent this problem, collagen-platelet composites (CPC) have been used as a substitute scaffolding material. Collagen-platelet composites (CPC) are collagen scaffolds infiltrated with platelets obtained from the subject at surgery. Studies have shown that CPC initiates clot formation in the torn ACL and improves mechanical strength when used during repair. This study aims to investigate the hypothesis that the use of CPC to enhance suture repair of the ACL will result in significant histologic changes in the healing ACL.

Twenty-seven 30-kg female 4-month-old Yorkshire pigs underwent complete ACL transection and suture repair. In 13 knees, ACL transection was treated using suture repair alone (SUTURE group). In 14 knees, the transection was treated using suture repair augmented with a collagen-platelet composite hydrogel (CPC group). After 4 weeks, 6 weeks, or 3 months the animals were euthanized. Four intact knees were identified to serve as controls. The knees were harvested, made into histologic sections, and stained with Hemotoxylin and Eosin or alpha-smooth muscle actin antibodies. Photomicrographs were taken at 5 points along the ligament. The cellular density, vascularity, cell morphology, cell orientation, collagen uniformity, and collagen density were measured in each photomicrograph by an observer who was blinded as to the treatment groups and time points of the photomicrographs.

The greatest differences between the CPC and SUTURE group were noted at 3 months. CPC treatment caused an increase in ligament cellularity as compared to SUTURE ligament ($p = 0.0305$). CPC also induced a more fusiform cell shape than the SUTURE treated ligament ($p = 0.0003$).

Comparing CPC and SUTURE ligaments, it is evident that CPC treatment does induce changes in the ligament response after transection as compared to SUTURE treatment. These changes may be related to the increased mechanical strength seen in samples treated with CPC. The greater number of cells may result in greater deposition of collagen, creating a larger scar mass. Cells with the appropriate fusiform shape and mature fibroblast phenotype may enhance collagen production and organization. All of these changes could strengthen the ligament.

The results of this study show that the ACL is capable of endogenous repair, given the appropriate environment. Understanding the healing process may aid in engineering further improved repair in this tissue.

The Transformation of Human Fimbria Cells to Tumorgenic Serous Carcinoma Cells

Varsha Keelara
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011
Alexandra J. Miliotis Fellowship in Pediatric Oncology

Patricia K. Donahoe, MD
Professor Emeritus, Pediatric Surgery & Director, Pediatric Surgical Laboratories
Massachusetts General Hospital

Much about the development of pelvic serous carcinoma is still unknown. Association with fimbrial lesions has been shown, but a direct transformation of fimbria cells to pelvic serous carcinoma has yet to be constructed. Previously, epithelial ovarian cells have been asserted as causal of pelvic serous carcinoma by in vitro and in vivo assay of cells transformed first to non-specific tumorigenic cells and then to serous carcinoma cells. Somatic ovarian epithelial cells were transformed into non-specific tumorigenic cells by avian retroviral gene alteration of p53 deficient somatic ovarian cells. Employing this experimental model, proto-oncogenes for transformation of fimbrial cells were chosen based on presence in ovarian tumors. Previous studies have been able to recapitulate ovarian carcinoma histology by transforming ovarian surface epithelial cells in vivo with the proto-oncogenes c-myc, K-ras, Akt, and Hoxa9. Since these collective results demonstrated that epithelial ovarian cells could indeed lead to serous carcinoma in vivo, we are attempting to use this experimental model to ascertain causality between fimbria cells and pelvic serous carcinoma. Fimbrial cells were chosen because recent studies examining the fimbria of both BRCA+ and BRCA- women with pelvic serous carcinoma and salpingo-oophorectomies have shown an association via specific p53 mutations in the fimbria and associated carcinomas.

The aim of the project is to further elucidate the link between fimbrial cells and pelvic serous carcinoma by combining Hoxa9 expression with viral gene introduction of specific proto-oncogenes to create pelvic serous carcinoma cells in vivo. Fimbria cells were successfully obtained from tissue samples and p53 shRNA vectors were created. Preliminary transductions revealed feasibility for the experimental model and validation of the transductions is ongoing. Baseline data from untransformed cells is being collected and will be ready for comparison to transformed cells upon preparation. Feasibility of the experimental model was ascertained and allows for continuation of the project and successful troubleshooting allows for greater likelihood of success of future transformations.

Identification of Dental Stem Cells

Aram Kim
Harvard School of Dental Medicine, Walter Bradford Cannon Society, Class of 2011

Paul Sharpe, PhD
Department of Craniofacial Development
King's College London, United Kingdom

Identification of dental stem cells is an area of great interest for their possible applications in developing new tooth structures. However, dental stem cells have been shown to exhibit heterogeneous properties, making them difficult to isolate and manipulate. Stem cells are thought to be mobilized and proliferate to repair damaged tissues with increased blood supply. Previous studies suggested highly vascularized sites as potential stem cell niches, and the expression of perivascular markers has been shown in some populations of dental stem cells. Separate studies show that many stem cells normally proliferate infrequently and appear to be slow-cycling.

We hypothesized that dental pulp stem cells are slow-cycling cells that are derived from pericytes, and these pericytes are not only neural crest-derived but also from the circulation. To test these hypotheses, IdU (5-Iododeoxyuridine) was administered in drinking water to fifteen C57BL/6 mice at 1 mg/ml for 4 weeks, followed by 40 days in the absence of label. Mandibles and maxillas of these mice were fixed, decalcified and processed for histology. Immunohistochemistry was performed on these tissues to localize expression of IdU and three perivascular markers (αSMA, NG2, and p75-NGFR). Tooth germs of C57BL/6 mouse embryos at different stages were transplanted into a pericyte-LacZ reporter (X-LacZ4) transgenic mouse host kidney. At two weeks after the transplantation, incisors and molars were isolated from the host, and stained for β-Galactosidase.

Groups of cells that were likely to be both pericytes and slow-cycling were identified in the dental pulp of a molar and an incisor of the mice labeled with IdU nucleosides. Analysis of teeth from transplantation experiments demonstrated that pericytes migrated from the host to donor tooth germs via the circulation. The majority of migrated pericytes (LacZ+) were present in the dental papilla, near blood vessels, and near odontoblast layers. When compared to teeth of X-LacZ4 transgenic mice at a similar development stage, the teeth grown in the host kidneys had fewer LacZ+ pericytes, suggesting that local neural crest-derived pericytes were also involved in these tissues. In addition, more pericyte migration was observed in the dental pulp when the transplanted embryo tooth germs were at earlier stages of development. This is most likely due to more invasion of host vasculature in younger donor tooth germs with underdeveloped vasculature.

Targeting Metabolism in Diffuse Large B Cell Lymphoma

Amar U. Kishan
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011
Alexandra J. Miliotis Fellowship in Pediatric Oncology

Nika N. Danial, PhD
Department of Pathology
Dana-Farber Cancer Institute

Diffuse large B cell lymphomas (DLBCL)—a clinically, genetically, and morphologically heterogeneous group of tumors—comprise nearly 40% of adult non-Hodgkin's lymphoma cases, with the current regimen of CHOP chemotherapy yielding an overall survival rate of 60-70%. Recently, an unsupervised analysis of DLBCL gene expression demonstrated the existence of several subtypes of DLBCL, including an "OxPhos" cluster with increased expression of genes encoding mitochondrial proteins such as respiratory chain complexes and anti-apoptotic BCL-2 family members, and a "BCR/Proliferation" cluster demonstrating increased expression of DNA-repair and B-cell receptor signaling genes. While patients with these subtypes have similar five year survival rates upon traditional combination chemotherapy, a clearer understanding of the molecular underpinnings of these gene signatures will allow for subtype-specific targeted therapy. The large wealth of knowledge on tumor metabolism and the proven success of anti-metabolic chemotherapeutics in various model systems render the "OxPhos" cluster of DLBCL most amenable to this approach. We predict that this subclass of DLBCL is endowed with a selective bioenergetic advantage rendering them refractory to apoptosis.

To test this prediction, we have characterized various bioenergetic and metabolic properties of both the BCR and OxPhos clusters. For example, examining the steady state adenosine triphosphate (ATP) levels in the presence and absence of specific and well-characterized inhibitors of glycolysis and mitochondrial oxidative phosphorylation (OXPHOS), allowed us to deduce the relative contribution of glycolysis and OXPHOS to total cellular ATP in the OXPHOS and BCR clusters. Steady state glycolytic potential was evaluated by determining iodoacetate-sensitive lactate generation under conditions of mitochondrial inhibition. Glucose utilization was also determined by glucose tracer studies. Mitochondrial function was evaluated by two independent approaches, including fuel (glucose and fatty acid) oxidation and fuel-induced changes in mitochondrial membrane potential. These data were integrated with cell viabilility assays in which the toxicity of select anti-metabolic drugs versus standard chemotherapeutic agents was compared. Our results suggest an increased mitochondrial function in the OxPhos cluster and support the need for biochemical dissection of individual mitochondrial complex activity as well as NMR analysis of metabolitc flux to pinpoint the chief metabolic pathway in charge of OXPHOS DLBCL survival.

Biochemical Characterization of Amyloid β-protein Assemblies in the Brains of Patients with Alzheimer's Disease

Seth Kleinerman
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Dennis J. Selkoe, MD
Center for Neurologic Diseases, Department of Neurology
Brigham and Women's Hospital and Harvard Medical School

Alzheimer's disease (AD) is a progressive, uniformly fatal neurological disorder of the elderly, afflicting more than 5 million Americans and 30 million people worldwide. It is the seventh leading cause of death in the United States. The initial symptoms are memory loss and confusion; the disease inevitably continues through behavioral changes and cognitive decline towards profound dementia.

A key feature of AD histopathology is the presence of amyloid plaques, which are insoluble aggregates of amyloid β-protein (Aβ). Recently, low molecular weight (MW) oligomers of Aβ were implicated in the pathological mechanism of AD when they were observed to inhibit long-term potentiation (LTP) of hippocampal neurons; these oligomers may be the dominant synaptotoxic species.

To collect oligomers, we homogenized AD cortical tissue in Tris-buffered saline and centrifuged, retaining the buffer-soluble fraction. These extracts contain Aβ-positive species at approximately 4, 8, and 12 kDa on LDS-PAGE Western blots. Using two-dimensional Westerns, we resolved several of these spots by isoelectric point, which implies that Aβ oligmers may undergo chemical modifications modifying charge but not significantly affecting MW. To determine the biologic effects of these newly distinguished species, we extracted them from 2D gels by maceration in ammonium acetate and measured their effect on LTP of rat hippocampal neurons. Our initial data suggest that only one of the ~8 kDa dimeric species conveys a robust LTP inhibitory effect.

We also 2D-visualized Aβ species collected from APP-stably expressing cultured cells (7PA2). These produced a markedly different pattern, consistent with the theoretical MWs and isoelectric points of extensions of the Aβ peptide N-terminal to its conventional start site (Asp 1). Curiously, while the higher-MW Aβ species from the 7PA2 cells are not identical to those of AD cortex, we have observed a 12 kDa species with an isoelectric point of ~4.5 in both the cell line and certain AD brains.

Our findings suggest two avenues for further research. The first is the investigation of the nature of the charge modifications of Aβ dimers. If we confirm that one species is more synaptotoxic than another, and the two can be interconverted, there may be therapeutic implications. The second is the confirmation of an N-terminally extended Aβ species in human brain, as seems to exist in the 7PA2 medium. Given that Aβ species in the 7PA2 medium potently inhibit LTP and affect rodent cognitive behavior, this species may play a role in the pathogenesis of the human disease.

Physician Prioritization of Mentally Co-morbid Patients versus Physically Co-morbid Patients

Joseph F Konopka
Harvard Medical School, Walter Bradford Cannon Society, Class of 2010

Selwyn O. Rogers, Jr., MD, MPH
The Center for Surgery and Public Health
Brigham and Women's Hospital

Regarding the provision of general health care for the mentally ill, it is important to assess whether physicians harbor negative attitudes towards the treatment of patients with mental co morbidities that may translate to a negative impact on the general health care of the mentally ill. This survey-based study assessed physicians' desire to prioritize healthy patients over those with either a physical co-morbidity or a mental co-morbidity for life-saving treatment. 464 attending surgeons in the New England area were contacted via electronic and mailed surveys. The survey tasked respondents with completing health care dilemmas including standard gamble questions designed to elicit quality weights to nonspecific mental and physical illnesses. Additionally, respondents were asked to prioritize otherwise healthy patients versus patients with physical or mental co-morbidity for a life-saving treatment. The survey had a response rate of 13% (n = 61). Respondents assigned statistically equal quality of life measurements for both the mental and physical co-morbid condition (median physical condition = 85, median mental condition = 80, p = 0.109). However, they were almost three times more likely to prioritize normal patients over co-morbid patients for life saving surgery when the co-morbidity was mental rather than physical (58% to 21%, p =<0.01). This suggests bias on the part of physicians against patients who are suffering from a subjectively suffering from quantitatively similar burdens of disease. However, the study is limited by a low response rate that is typical of survey-based studies of physicians.

Serial Immunohistochemical Characterization of Distraction Osteogenesis in the Minipig Mandible

Matthew E. Lawler
Harvard School of Dental Medicine, Walter Bradford Cannon Society, Class of 2011

Maria J. Troulis, DDS MSc
Department of Oral And Maxillofacial Surgery
Massachusetts General Hospital

Distraction osteogenesis (DO) is the formation of new bone and soft tissue following a surgical osteotomy (bone cut) with a gradual tension provided by a device across the cut. It is a minimally invasive surgical technique for treatment of mandibular deformities requiring skeletal expansion. It has several advantages over other treatment methods because it eliminates the need for bone/soft tissue grafts, and it has the potential to minimize surgical relapse because concurrent histogenesis of surround soft tissue minimizes resistance against the newly formed bone. However, a significant limitation is the lengthy treatment protocol (2-3 months). If the underlying biological mechanism of DO were understood the system could be manipulated to decrease the overall treatment time.

It is hypothesized that early stages of mandibular distraction osteogenesis endothelial cells will be the predominant cellular component of the wound and late in distraction osteoblasts will be the major component. To test this hypothesis the Yucatan minipig was used as a model organism to determine the serial cellular events in the distraction wound with immunohistochemistry (IHC).

Seventeen female minipigs with mixed dentition (age 4-6) months were used for this study. Four of the minipigs were sacrificed on day 6 (mid-DO n=4), four were sacrificed after active distraction was ended on day 12 (end-DO n=4), four were sacrificed on day 24 (mid-fixation n=4), two were sacrificed on day 36 (end-fixation n=2), two of the animals were subjected to a 12 mm acute lengthening of the mandible (sacrificed on day 36), and one was used as a sham control. Coronal paraffin tissue sections were prepared from the center of the regenerate zone in both the anterior and posterior direction. IHC was performed using mouse anti Osteocalcin primary antibodies and a labeled streptavidin-biotin, horseradish peroxidase system.

Initial results from this study have shown inconsistent Osteocalcin staining. Slides vary from high background staining to very weak staining specimen. However, several slides have shown promising results that warrant analysis. More staining needs to be performed in order to obtain sufficient data for a quantitative comparison of the surgical groups.

Functional Analysis of Novel IL-1 Isoforms in Periapical Pathogenesis

Rayanne E. Lee
Harvard School of Dental Medicine, William Bosworth Castle Society, Class of 2011
Carl W. Walter Endowment Fellowship

Phillip Stashenko, DMD, PhD
Department of Cytokine Biology
The Forsyth Institute

The interleukin-1 (IL-1) family is known as one of the major inflammatory cytokines that exert a regulatory influence on the progression of periodontal disease involving periapical bone destruction. This study was designed to make the functional distinction of novel IL-1 isoforms –IL-1F6, IL-1F8, and IL-1F9 – in continuation of the preliminary data that had confirmed the presence of the these three novel variants in murine model of periapical inflammation. The association of the IL-1 cytokines with functional nature in regard to the inflammatory process was investigated by masking their actions through blockade with antibodies to either ligands themselves or their common receptor, IL-1Rrp2. The effects of blockade was measured by enzyme-linked immunosorbent assay (ELISA) through the quantification of regulatory cytokine proteins of inflammation. In addition the extent of periapical lesion was assessed by measuring the cross-sectional area of periapical bone destruction in mm^2 with micro-computed tomography (μ-CT).

The protein levels of osteolytic cytokines – receptor activator of NF-κB ligand (RANKL) increased significantly after the pulp exposure in all of the treatment groups compared to the negative controls. However, they were not significantly affected by functionally blocking the new IL-1 variants. However, Th2-type cytokines (IL-4 and IL10), but not Th1-type cytokine (IFN-γ), and acute inflammatory cytokines mediators (TNF-α and IL-12) reached a statistical significance in their reduction of expression only when all the IL-1 ligands were blocked simultaneously. From this we concluded that the novel IL-1 ligands have a functional redundancy and their main inflammatory influence is exerted through early immune cells and Th2 cells.

The degrees of bone loss for all the treatment groups showed reductions compared to the positive controls. In addition, anti-IL-Rrp2 and anti-IL-1F6+F8+F9 groups showed the greatest reductions although they failed to reach the statistical significance compared to the positive controls. These findings strongly indicate that the three novel IL-1 variants work by exerting proinflammatory controls although the ligands themselves may not be functionally independent.

Navigating and Circumventing a Fragmented Health System:
The patient's pathway in the Sierra Madre region of Chiapas, Mexico

Rose Leonard
Harvard Medical School, Francis Weld Peabody Society, Class of 2011

Jim Yong Kim, MD, PhD and Daniel Palazuelos, MD
Department of Social Medicine
Harvard Medical School

The purpose of this anthropological study is to gain a better understanding of where *campesinos* in the Sierra Madre region seek health care and what informs those decisions. The Sierra Madre region in southwest Chiapas is home to the "Mexicanized" Mam people. Unlike the indigenous communities in the highlands, these communities were forced to abandon their traditional customs in the 1930s. Since then, the Sierra Madre region has been largely forgotten, as it is far from the Zapatista movement and fails to attract the attention of many foreign NGOs. The *campesinos* in this area continue to live in poverty, growing only corn and beans for their families' consumption. Their sole source of income is based on the yearly coffee harvest, which depends on the variable climate and the fluctuating price of coffee. Given the unpredictable earnings coupled with their marginalized location, many *campesinos* encounter obstacles as they navigate the health system. The present study begins to fill in the dearth of information surrounding this particular region and introduces an organic community perspective about the health care system that does not exist in the literature.

The analysis reflects the decision-making processes elicited from 35 interviews in the Siltepec municipality, which serves as a lens to examine the fragmentation of the health care system in Chiapas. Participants include *campesinos*, health promoters, clinic staff, midwives, a religious leader, and an administrative director. A critique of the current health system is presented, highlighting specific points of tension among the various stakeholders: the government health system (including public clinics, hospitals, *Seguro Popular*, and vertical programs), community members, private doctors, and NGOs.

The information gathered paints a picture of rural, impoverished, and marginalized communities struggling to find health in a fragmented health system. Options for health care include: searching for a private doctor or pharmacy, traveling to Guatemala or finding a traditional healer, visiting the local public clinic or hospital, and waiting to see a foreign medical team affiliated with a NGO. Economic constraints, cultural beliefs, poor transportation infrastructure, and persistent deficiencies in the public health system shape the decisions people make in seeking treatment when they are ill.

The qualitative analysis presents the groundwork for future studies of the Mexican health care system in one of the most neglected areas of Chiapas. The data point to social networks as vehicles to disseminate health information. The data also suggest that an investment in the more efficient movement of information and of people could lead to substantial improvement in public health.

Non-Viral Transfection of Caprine Mesenchymal Stem Cells with BMP-7 Plasmid: Effects of plasmid size and type of medium

Natalie L Leong
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Myron Spector, Ph.D.
Tissue Engineering Laboratory, Boston VA Medical Center
Brigham and Women's Hospital, Department of Orthopedics

Several growth factors, including bone morphogenetic protein-7 (BMP-7), have demonstrated potential for bone and cartilage tissue engineering. Limitations in the *in vivo* use of recombinant proteins have prompted studies of methods for safely delivering their genes to sites of injury. The objective of the study was to investigate the feasibility of the non-viral delivery of a small amount of plasmid encoding BMP-7, to goat mesenchymal stem cells (MSCs) grown *in vitro* in expansion (control) and osteogenic media.

MSCs obtained from iliac crest marrow aspirates were cultured in DMEM with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS). Passage 1 MSCs were seeded in 24-well tissue culture plates at a density of 8000 cells/cm^2, and transfected with the BMP-7 gene. Two plasmids (pw24, pcBMP-7), which contained the BMP-7 gene under a CMV promoter, were employed. The sizes of the pw24 and pcBMP-7 plasmids were 10,346 bp and 6,732 bp, respectively.

Lipid transfection reagent was used to deliver the plasmid into the MSCs, according to the manufacturer's suggested protocol. Control medium was DMEM with 10% FBS and 1% PS, while osteogenic medium was additionally supplemented with dexamethasone, ascorbate-2-phosphate, and β-glycerol phosphate. Media were completely exchanged every 3 days.

At days 22 and 28, cell number was determined by measuring total DNA per sample with the Picogreen® assay. ELISA was used to evaluate the BMP-7 content of the media that were collected every three days from the cultures. Histological samples were stained with hematoxylin and eosin, and Von Kossa, and Alizarin Red S stains for mineral.

ANOVA was performed to determine the effects of the type of plasmid and type of medium on BMP-7 content of the medium. The Tukey-Kramer post-hoc test was used for all pair-wise comparisons; criterion for significance was $p<0.05$.

The results demonstrate that caprine MSCs transfected with a low load (<2 μg/well) of a particular BMP-7 plasmid using a lipid carrier can sustain production of BMP-7 for at least 28 days, with the accumulated amounts reaching therapeutic levels. The level of over-expression was affected by the size of the plasmid and the type of medium in which the cells were grown. Interestingly, after less than one week in culture, the MSC monolayers condensed spontaneously into pellet-like structures. Osteogenic medium induced mineralization, but qualitatively there was no effect of BMP-7 transfection on this process. Future applications of such non-viral transfection may be useful for select bone tissue engineering applications.

Evaluation of Electrosurgical Techniques to Remove a Simulated Ventricular Shunt Obstruction for Hydrocephalus Patients

Albert C. Leung
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011

Joseph R. Madsen, MD
Hydrocephalus Unit, Department of Neurosurgery
Children's Hospital

Hydrocephalus occurs when CSF accumulates within the brain ventricles, due to either excess CSF production by the choroid plexus or an obstruction within the CSF's drainage system. Cerebral ventricular shunts are often implanted in hydrocephalus patients to rectify the situation by siphoning excess CSF from the ventricle towards other body cavities (e.g. – peritoneum or ventricular atrium), thus reducing intracranial pressure and the symptoms associated with this pathological condition. However, the shunt failure rate is reported to be 40-50% within the first two years after implantation, with the most frequent cause of failure due to obstructive ingrowth of choroid plexus at the proximal catheter tip of the shunt. Clinically, shunt obstructions can only be managed by surgical revision. As of now, monopolar electrosurgery is the most widely used approach to ablate the shunt obstruction, but it has the following disadvantages: bystanding damage to surrounding tissue, excess heat dissipation, inefficient use of energy, and a low degree of control over the electric current's path.

The aim of this study is to use an animal model to simulate a ventricular shunt obstruction and then compare the efficacy of alternative electrosurgical techniques to monopolar electrosurgery. The alternative techniques to be tested are bipolar electrosurgery and laser photocoagulation. An important assessment will be to determine which surgical approach minimizes the risk of bleeding and tissue damage after ablation of the ventricular catheter's obstruction. Efficacy will be evaluated using the following endpoints: tissue viability, vascular coagulation, catheter patency, thermal distribution throughout the tissue, and optimal power settings of the electrosurgical device.

The jejunum tissue from the rat will be used to simulate a shunt obstruction because of its remarkable similarity to choroid plexus tissue. The shared tissue properties include a high degree of vascularity, the expansive microvilli structure, and polarized epithelial cells. The surgical protocol will place the rats under anesthesia, isolate the jejunum tissue from the abdomen, and draw the tissue into the ventricular catheter tip of the shunt to simulate an obstruction. Then the monopolar, bipolar, and laser techniques will be used to ablate the obstructed catheter tip. Afterward, the surrounding jejunum tissue will be evaluated histologically to assess for bystanding damage to surrounding tissue, the degree of bleeding near the surgical site, the thermal distribution of each device, and the post-operative patency of the catheter's tip. These parameters will used to compare the efficacy of each electrosurgical device.

This study is currently in progress and has been approved by the Children's Hospital IACUC department. The data collection is ongoing and will be used in conjunction with another animal model (sheep's brain ventricles) before final evaluation in human clinical trials.The conclusion of this study is yet to be determined, but the hypothesis anticipates the bipolar and laser photocoagulation techniques to show less bystanding damage to surrounding tissues than the monopolar electrosurgery technique. Furthermore, the extent of vascular damage and postoperative bleeding is expected to be less with the bipolar and photocoagulation techniques.

Analysis and Comparison of Hand Strains on Dentists Using Air vs. Electric Powered Drills

Keith M. Levesque
Harvard School of Dental Medicine, Francis Weld Peabody Society, Class 2011

Dr. Jack Dennerlein
Associate Professor of Ergonomics and Safety
Department of Environmental Health, Harvard School of Public Health

Dentists have an increased risk of developing upper extremity musculoskeletal disorders (MSDs) due to awkward postures, repetitive motions, and chronic pinch positions during procedures. MSDs result in lowered productivity or a career-shortening debilitation. The shape, weight, and balance of dental instruments are important in the cause of many MSDs. Pneumatic (Air-Powered) dental drills have been the most common high-speed drill for decades but are now shrinking in the market due to the quietness and high-torque of electric powered drills. The hypothesis of this study is that the muscles strains of the hand while using the heavier, larger, electric motor driven dental drills will be higher and more fatiguing than the muscles strains using the lighter, thinner air driven dental drills on dentists performing the same dental task. These high muscle strains coupled with procedure times and repetition increase the risk of debilitating musculoskeletal disorders. In addition those with small hands will have greater muscle strains from the heavier electric drills. Surface Electromyography of the flexor digitorum superficialis, flexor pollicis longus, extensor digitorum communis, and extensor carpi radialis brevis, will measure the strains of twenty dentists, performing 2 tasks with each of the drills. The mean voluntary muscle contractions of each drill for each task, the time to perform the task, and a comfort survey will determine the drill that provides the best prevention of hand musculoskeletal disorders.

Glycogen synthase kinase 3 (GSK-3) as a potential target for acute myeloid leukemia (AML) differentiation

Loretta S. Li
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2009

Kimberly Stegmaier, MD
Department of Pediatric Oncology
Dana-Farber Cancer Institute and Broad Institute of MIT and Harvard

Despite high-dose chemotherapy, the majority of patients diagnosed with acute myeloid leukemia (AML) ultimately succumb to the disease. New treatment approaches are needed. Kinases have emerged as potential therapeutic targets for AML, with inhibition leading to cell death and/or differentiation. The feasibility of differentiation therapy for AML was demonstrated with the use of all-trans retinoic acid for acute promyelocytic leukemia. However, identification of the myeloid differentiation state is not easily amenable to traditional phenotypic screening. We applied a novel approach to high-throughput screening in which gene expression signatures define the AML versus mature myeloid states. We used this signature-based approach in two parallel screens to evaluate kinase involvement in AML differentiation: small molecule library and high-throughput RNA interference (RNAi) screening.

A 32-gene signature distinguishing AML from mature myeloid cells was developed. We screened 84 kinase inhibitors, in two AML cell lines, for new agents that induce the myeloid differentiation signature. Four of the top hits from the screen have activity against glycogen synthase kinase 3 (GSK-3). Similarly, GSK-3 scored as the top hit in the RNAi screen, which sought to identify those kinases whose genetic loss induce the AML differentiation signature. These findings suggest a new role for GSK-3 as a potential therapeutic target in AML, perhaps surprising results given GSK-3's reported inhibition of sonic hedgehog and Wnt signaling. Further validation of our results is therefore critical.

We have been validating the GSK-3 inhibitor and shRNA hits using the original 32-gene differentiation signature, a cell viability assay, and morphology studies. In addition, we have tested lithium, an FDA-approved GSK-3 inhibitor used in the treatment of bipolar disease, and preliminary data suggests that it induces AML differentiation across multiple cell lines. Future studies will assess the role of GSK-3 inhibition on downstream signaling pathways in AML. Our ultimate goal is the translation of compelling preclinical findings to clinical testing. If these preclinical studies more broadly confirm anti-AML activity of lithium, we would be well positioned to rapidly bring this drug to clinical trial for patients with relapsed/refractory AML.

Evaluation of the Response of Brain Metastases from Lung Cancer to Radiosurgery

Evelyn Lilly
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011
Alexandra J. Miliotis Fellowship in Pediatric Oncology

Naren R. Ramakrishna, MD PhD
Dept. of Radiation Oncology
Dana Farber Cancer Institute

Brain metastases are a significant clinical problem. Median survival time is approximately one month without treatment and three months with treatment. Over the last decade stereotactic radiosurgery has emerged as a powerful non-invasive modality providing effective tumor control with far fewer treatment-related side effects than whole brain radiotherapy or surgery. While radiosurgery is likely to be effective in 70-80% of brain metastases, the treatment response and outcomes vary widely. At present, little is known of the factors which might contribute to the treatment response following radiosurgery. A better understanding of these factors might help to guide treatment selection for patients with brain metastases.

We hypothesize that the treatment response of tumors to radiosurgery may depend upon pretreatment characteristics. Some pretreatment characteristics which may reflect intrinsic differences in tumor biology or radiation responsiveness include the presence of cystic changes, the presence of hemorrhage, relative tumor size, and tumor location. We aggregated data from past cases of stereotactic radiosurgery of cerebral metastastic lesions from primary lung cancer, the top contributor of brain metastases and assessed volumetric changes and the incidence of hemorrhage and cystic changes over time. To allow for adequate follow-up to observe treatment-related effects, we eliminated cases without 6 months of MRI follow-up. We then used univariate and multivariate proportional hazards models to test for the influence of the tumor characteristics on local lesion control, as determined by volumetric analysis, and on the incidence of post-treatment hemorrhage or cystic changes. Models of various lesion subgroups were also tested to identify confounding factors.

The data set included 97 lesions from 40 patients (mean age 54, 12 males, 28 females). The distribution of lesions in the brain was as follows: 31 frontal, 17 parietal, 13 occipital, 6 temporal, 17 cerebellar, and 4 basal ganglia. Median duration of follow-up was 431 days. Prior to treatment, 75 percent of the lesions were cystic, and 20 percent were hemorrhagic. The median tumor volume was 0.55 cc, and the median radiosurgery dose was 1900 Gy.

We observed in our dataset, the development of new hemorrhage following radiosurgery in an additional 12.4 percent of lesions. We also observed that 12.8 percent of lesions were not locally controlled (tumor volume increased by more than 25 percent) during the follow-up period. At present, further retrospective analyses, as described in methods, are in progress to identify factors that might predict these volumetric and hemorrhagic changes.

Human Resource Crises in the Health Sector: Motivations for Maintaining a Career in Ghana

Hermioni Lokko
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

Mrs Olivia Dotse
Manna Mission Hospital, Accra, Ghana

Just like many African countries, Ghana has been severely affected by the emigration of its healthcare professionals to European and American countries. There are an estimated 600 Ghanaian doctors working in New York City. They represent about 20% of New York's physician requirement. According to the State of Ghanaian Economy Report 2002, a total of 3,157 health professionals left the country between 1993-2002, representing over 31 percent of health personnel trained in Ghana during the same period. The report said that out of 871 medical officers trained between 1993-2002, 604 (96.3 percent) left the country, leaving 267 for the entire nation. Ghana spends about 9 million each year on medical education only to lose a good percentage of its students.

It is indisputable that the brain drain problem is affecting Ghana's health sector in devastating ways. There have been many efforts by the Ghanaian government to persuade doctors to stay in Ghana. Some of the incentives have included enhanced salaries, better pensions, cars and housing allowances. Most of the efforts to solve the problem of the brain drain have been focused on encouraging people to stay. All the same, there is little understanding of what actually motivates those who chose to stay in their home countries to do so despite the inadequacies of the healthcare system. The purpose of my project was to gain an insight on why Ghanaian physicians chose to have their careers in Ghana and how those who leave, impact those who stay using qualitative research methods like one-on-one interviews. Hopefully, the findings of my study will contribute to policy-makers understanding and development of strategies to improve health workforce retention.

Overall, the study elucidated on the fact that Ghanaian physicians who chose to stay in Ghana did so for unselfish reasons like a call to serve their country and work where there is a need for physicians, be close to family and the cultural obligation to take care of older members of family and opportunities to work with indigent populations. Interesting, the study also made it clear that most Ghanaian physicians leave the country to pursue further education or postgraduate studies in medicine since such educational opportunities are very limited in Ghana and Africa as a whole. Therefore, policy makers in Ghana and other African countries must work on providing such excellent educational opportunities for physicians who train in Africa to retain them.

Un Buen Comienzo: A cluster randomized controlled trial of an early education and health intervention project in Santiago, Chile

Alice C. Lorch
Harvard Medical School, Walter Bradford Cannon Society, Class of 2010

MaryCatherine Arbour, MD
Howard Hiatt Resident in Global Health Equity and Internal Medicine
Brigham and Women's Hospital

Background. Un Buen Comienzo (UBC) is a cluster randomized controlled trial of an early education and health intervention for 4-6 year-olds attending municipal preschools in a low-income community in Santiago, Chile. The project is sponsored by Harvard University and Fundación Educacional Oportunidad with collaboration from the Chilean Ministries of Education and Health and Universidad Diego Portales. In 2008, the intervention was implemented in three intervention and three control schools, affecting 800 children. It will expand through 2010. The health intervention targets respiratory health and well-child visits for routine health screening and nutrition.

Introduction. SBOR (Sindrome bronquial obstructivo recurrente) or asthma comprises the most common chronic disease of children worldwide. In our 2007 pilot study, parents reported that 18% of the children in our study had respiratory problems, 10.34% used respiratory-related prescriptions and 16% had been hospitalized for respiratory problems at some point. The goal of the UBC respiratory intervention is to reduce overall respiratory infections among all children and improve symptom control among a subgroup of children with obstructive respiratory disease. Outcome measures include: absenteeism, number of clinic visits, number of emergency room visits, and number of hospitalizations for respiratory symptoms.

Methods. Children with respiratory problems were identified by teachers using a balanced score card and confirmed by medical record review. For each asthmatic child in the intervention group, his doctor created an individualized, written, symptom-based asthma action plan which was given to the child's parents and teachers. All children were educated about hand-washing techniques. Alcohol gel was provided to each classroom. Attendance data was collected approximately every two weeks in both intervention and control pre-Kindergarten classrooms (360 students). Medical records for asthmatic children in intervention and control schools were reviewed at the end of the year to tally number of clinic visits, emergency room visits or hospitalizations for respiratory symptoms. To strengthen our understanding of school absenteeism, we initiated an ethnographic study in one classroom which included participant observation and interviews with parents and teachers on this theme.

Results. This project is still in the process of data collection and post-test measurements will take place in November/December 2008. Preliminary results from the attendance data indicate high levels of absenteeism in both the general population (23.38% child-school days absent) and the SBOR population (26.36% child-school days absent.) Preliminary results from the ethnographic study indicate that primary reasons for absenteeism include sickness, fatigue due to late bedtimes and family events.

Proteomic Analysis of the Extracellular Matrix Components of Murine Tooth Germs

Harrison E. Mackler
Harvard School of Dental Medicine, Francis Weld Peabody Society, Class of 2011

Richard L. Maas, MD, PhD
Department of Medicine, Department of Medicine
Brigham and Women's Hospital

The restoration of missing teeth, whether from disease, trauma, or genetics, represents a substantial proportion of the daily routine of most dentists. A bioengineered tooth, made from a patient's own cells and grown in the correct location in a patient's own mouth, would in theory make the best possible replacement. Significant advances over the past 10 years have been made in the understanding of the molecular controls behind how teeth first develop. This, together with breakthroughs in stem cell biology and tissue engineering technology, make dental researchers closer than ever to the prospect of *de novo* replacement tooth formation in clinical practice.

Central to the regeneration of new dental tissue is the role of the scaffold, the substrate to which stem cells attach, proliferate, and differentiate into appropriate downstream cell types. Previous tooth tissue engineering endeavors have been met with only moderate success using man-made scaffold materials such as lactate-glycolate and silk. Here, we aimed to determine the protein composition of the endogenous extracellular matrix within which tooth precursor cells develop. We hypothesize that, in the future, tissue engineers will be able to use this "molecular blueprint" to better understand the ideal protein composition with which to design scaffolds for forthcoming research.

E16.5 murine incisor and molar tooth germs were isolated by microdissection, including both mesenchymal and epithelial cell layers. In parallel, oral tissue surrounding the tooth germs were also isolated. Crude samples were then homogenized with detergent followed by physical agitation, and the resultant proteins from the mixture were reduced, alkylated, and separated by mass on an SDS-PAGE gel. Bands from the gel were cut and subsequently analyzed by mass spectrometry to yield data on protein identity and relative concentrations within samples as well as differences between samples.

At initial analysis, 4018 proteins were identified in tooth samples, while 3861 were identified in the surrounding oral tissue. In the tooth samples, as compared to the surrounding tissues, 809 proteins were unique, 102 were upregulated, and 93 were downregulated. A more refined bioinformatic analysis is currently being performed to extract the extracellular matrix and signaling protein identities from the database. Armed with the results of this experiment, we are hopeful that tissue engineers will be able to better design scaffolds that are "biomimetic," imitating *in vivo* the natural development of a tooth within the context of clinical dental tissue regeneration.

Fragmentation Predicts Displacement in Treatment of Distal Radius Fractures

Melvin C. Makhni
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

Charles S. Day, MD
Day Research Group, Department of Orthopaedic Surgery
Beth Israel Deaconess Medical Center / Harvard Medical School

Closed reduction and plaster cast immobilization are the most common methods of treatment for closed, non-complicated distal radius fractures. However, a high incidence of secondary displacement after initial closed reduction treatment has been noticed. Further evaluation of factors leading to these poor treatment outcomes could lead to improvement in management of distal radius fractures. We hypothesized that the presence of multiple fragments would lead to both a higher rate of displacement, as well as a greater need for initial operative management.

We performed a retrospective study of 138 patients with distal radius fractures who entered the Beth Israel Deaconess Medical Center Emergency Department between April 2002 and September 2004, who satisfied the inclusion and exclusion criteria. Radiographic data was collected for these patients on presentation, after initial treatment, and at healing. Radiographic displacement was defined according to commonly used criteria. Fisher's exact and chi-square tests were used when comparing outcomes data.

At presentation, 53% of 2-part, 72% of 3-part, and 96% of >3-part fractures were radiographically displaced ($p<0.01$). After initial treatment, 81% of closed reductions secondarily displaced, compared to only 18% of operative fixations ($p<0.001$). This difference was greater for fractures with an intra-articular component: 83% were displaced after closed reduction, compared to 8% after operative treatment ($p<0.001$). Operative fixation was initially prescribed 8% and 14% of the time for 2-part and >2-part fractures. Patients with secondary displacement after casting or closed reduction underwent successful follow-up operative treatment 63% and 80% of the time, respectively. Of the initially displaced fractures, 71% of 2-part fractures secondarily displaced after closed reduction, as compared to only 33% that did so after operation. When more than two fragments were present, 88% of the fractures displaced after closed reduction, compared to only 13% after operation ($p<0.001$).

In summary: 1. More fracture parts correlates with a higher degree of radiographic displacement at presentation. 2. Operative fixation leads to the highest degree of radiographic treatment success. 3. The rate of secondary displacement after initial closed reduction correlates directly with increasing fracture fragmentation. 4. Operative fixation was similarly prescribed regardless of fragmentation. 5. Operative fixation seems equally effective regardless of the degree of fracture fragmentation or the timing of the operation.

The data suggests that displaced distal radius fractures with more than two parts may be considered for either initial or follow-up surgical management, suggesting an operative threshold based on the number of fracture parts.

Demographic and Genetic Characterization of Patients Treated with Peri-acetabular Osteotomies for Hip Dysplasia

Ana Mata-Fink
Harvard Medical School, William Bosworth Castle Society, Class of 2008
Carl W. Walter Endowment Fellowship

Matthew Warman, MD
Department of Orthopaedic Surgery, Children's Hospital Boston

Purpose: Developmental dysplasia of the hip (DDH) is a common cause of hip symptoms in the adolescent and young adult, predisposing to premature osteoarthritis. Early treatment prior to cartilage damage can delay or prevent the need for hip replacement. Demographic and genetic characterization of populations at risk may allow efficient, selective screening of adolescents and young adults. We hypothesize that there are demographic characteristics associated with adolescent and young adult DDH patients which are different from those of infantile DDH patients. In addition, we believe that there are genetic loci that predispose to DDH in a polygenic manner.

Methods: A retrospective chart review was performed to identify all patients treated with peri-acetabular osteotomies at one institution from 1991-2008. 542 patients were identified who had undergone peri-acetabular osteotomies for primary hip dysplasia, excluding those with syndromic, neuromuscular, or traumatic etiologies. These patients were sent a questionnaire regarding birth and family history.

Results: 49.9% of patients reported a family history of hip problems. 27% had a family history of hip dysplasia, hip osteoarthritis before age 45, or hip replacement before age 50 and 25.2% had a family history of hip dysplasia in infancy with long-term sequelae, hip dysplasia diagnosed in adolescence or young adulthood, hip osteoarthritis before age 45, or hip replacement before age 50. Other characteristics of this patient population include 86.8% female, 89.7% Caucasian, 34.1% diagnosed with infantile DDH, 11.7% born breech, and 40.3% first born.

Conclusions: This data suggests that there are demographic features that can be used to characterize and screen adolescents and young adults with hip dysplasia. Furthermore, there appears to be a genetic contribution to DDH diagnosed in adolescence and adulthood.

Significance: This data represents a promising first step in better understanding adolescent and young adult DDH patients. It will also serve as the basis for a genetic study to identify loci involved in adolescent- and adult-onset DDH.

Mapping the Landscape of Congenital Anomalies

Paul M. McBratney-Owen
Harvard School of Dental Medicine, William Bosworth Castle Society, Class of 2011

Rachel Badovinac Ramoni, DMD, DSc
Department of Developmental Biology
Harvard School of Dental Medicine

The ability to create a global landscape of congenital anomalies has been hampered by the fact that standard analytical approaches are limited in their abilities to consider a large set of covariates. One approach to this kind of complex data set is the Bayesian Network, a type of probabilistic graphical model. The study population will be drawn from the entire set of births with congenital anomalies contained within the Texas Birth Defects Registry (TBDR), approximately 80,000 subjects. In order to prepare the data for modeling the coding system used by TBDR, the British Pediatric Association (BPA) coding system, needed to be translated to the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). The rationale for performing this mapping was three-fold: (1) Generalizability: ICD-9-CM codes are more widely used, (2) Translation: Hospitals and clinical facilities tend to use ICD-9-CM codes, and (3) Diagnosis Reduction: BPA codes are, mostly, more precise than ICD9CM codes. For our purposes, BPA codes are too fine-grained, leaving many diagnoses with too few subjects.

Briefly, this task involved mapping each of the 1,016 individual BPA congenital anomaly codes to the appropriate ICD-9-CM codes. The BPA coding system is based on the ICD-9-CM coding system, with its aim being to add more granularity to the classifications. Given this synergy, more than half of the BPA listings were mapped by the simple removal of the final digit/s. However, there were a great number where this rule did not apply, and more sophisticated solutions were required. There were a number of instances BPA terminology variability and subcategories, and conditions that were subcategorized by anatomic site in one system and by tissue type in another. There also existed different treatments of laterality information between the two systems. These complicated issues were addressed in consultation with the senior epidemiologist and the medical geneticist from the TBDR.

After the final preparations are complete on the mapping project, the data provided by the TBDR will be translated into ICD-9-CM codes. This will then be reviewed for very low frequency events. Although the dataset is very large, some of these anomalies will be extremely rare so will need to be identified and either excluded or combined with other, similar, anomalies in consultation with a medical geneticist. This prepared data will then be modeled using Bayesian network. The results will then need to analyzed and written up, with the aim to publish results.

Developing a Monitoring and Evaluation Tool for a Midwife Training Program in Neonatal Care

Andrew C. McKown
Harvard Medical School, William Bosworth Castle Society, Class of 2011

Edward J. O'Rourke, MD
Department of Social Medicine
Harvard Medical School

The International Organization for Migration (IOM) has been working in Aceh, Indonesia since the Asian tsunami in December of 2004. After the initial disaster response, IOM focused on rebuilding and reinforcing the primary care system in Aceh that suffered from the civil war in advance of the tsunami. In an effort to specifically address maternal and child health, IOM initiated an education program for midwives on birthing emergencies that is distributed based on a "training of trainers" approach. Following the initial success with the distributed training program, IOM is working to implement another module for midwife training on neonatal home care delivered in the first 40 days of life. One component of the new module will be a monitoring and evaluation tool to encourage use of the new guidelines and assess their effectiveness.

The tool was developed through a series of steps including an evaluation of current practices, a stakeholder analysis, and a reiterative drafting process involving major project stakeholders. To begin, a series of interviews were conducted with midwives and midwife coordinators in multiple primary health care centers. These interviews elucidated current practice guidelines and general demographic data. Knowledge of everyday practice was then obtained through field visits to community health outreaches and neonatal home visits where midwife-patient interactions were observed and mothers were interviewed.

According to the information learned from the interviews, four cases were written containing different mechanisms of monitoring and evaluation, and ten midwife coordinators were invited to the IOM office for a focused discussion group surrounding the cases. The discussion resolved a consensus need for a midwife monitoring and evaluation tool and suggested a system best suited for the realities of their management incentive structure. The consensus tool was drafted, and then meetings were held with the district health offices to attain official government support and involvement, lending greater enforcement credibility to the tool. Furthermore, the district health authorities had the opportunity to suggest revisions in the system to make it most easily integrated into the official health system.

This stepwise approach to the development of the new monitoring and evaluation tool ensured that the new tool was appropriate for the system, could function in the system, and would be accepted by the system because all stakeholders took part in its development. The new tool will be taught as part of the neonatal care module in the next round of midwife training.

Effect of Two-Hour Workshop in Greece and Cyprus on Oral Cancer Awareness and Knowledge

Michelle U. Mian
Harvard School of Dental Medicine, Francis Weld Peabody Society, Class of 2011

Dr. Athanasios Zavras, DMD, DMSc
Oral Health Policy & Epidemiology
Harvard School of Dental Medicine

Over half of oral cancer diagnoses are delayed. Delayed diagnosis has been shown to reduce 5-year survival from 85% to 50%. Early signs of oral cancer frequently can be detected with cursory inspection of the oropharynx. Accordingly, the World Health Organization and US Surgeon General recommend educating primary health care workers on basic techniques for oral cancer screenings. At present, there is limited data on the efficacy of such educational programs.

A two-hour workshop on screening for oral cancer was given to 65 local health care professionals in Cyprus and Greece. The workshop consisted of a seminar on the biology and epidemiology of oral cancer, an approach to a screening on physical exam, and technologies to aid in screening. In Greece, an interactive case followed the informational session. Anonymous surveys assessing attitudes (16 statements utilizing visual analogue scale) and knowledge (20 multiple choice and true/false questions) were disseminated before, immediately after, and in Greece, 1 month after the workshop to assess the impact of the workshop on participants' perception and understanding, respectively, of oral cancer.

Analysis of participant responses revealed that the workshop increased participants' knowledge of oral cancer screening protocols ($p<0.001$). In Greece, this increase was maintained even one month after the workshop ($p<0.001$). Attitude comparison revealed only one difference in 1 of 16 items; the workshop appeared only to impact participants' perception of whether "[their] knowledge on oral cancer is current."

Our study suggests that a workshop can increase knowledge of screening techniques for oral cancer- even after one month. These data call for further exploration of educational approaches that might aid health care professionals from a variety of disciplines in helping to reduce delays in oral cancer diagnosis. In this way, education on oral cancer screening might lead to increased rates of early diagnosis, and, ultimately, improved patient outcomes.

Acoustic Neuromas: Factors Predicting the Need for Treatment

Aya Y. Michaels
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011

William Curry, MD
Neurosurgery Department
Massachusetts General Hospital

Acoustic neuromas, or vestibular schwannomas, are benign growths that arise from the vestibulo-cochlear nerve. They typically present with sensorineural hearing loss, tinnitus, facial weakness/numbness, vertigo, brainstem compression and subsequent motor dysfunction, and communicating hydrocephalus. Little is known about the natural history of acoustic neuromas, making predictions difficult and clinical decisions highly contentious among physicians. Physicians decide whether to invasively treat these rather non-threatening tumors through microsurgery, stereotactic radiosurgery, or fractionated radiotherapy and risk further cranial nerve disability, CSF leakage, infections, hydrocephalus, among other complications. On the other hand, these tumors may be conservatively managed through series of MRI studies and monitored for growth and worsening symptoms.

Many factors are considered when deciding whether or not to deliver treatment for acoustic neuromas, including the size of the tumor, demonstrated rate of growth, neurological symptoms, and the overall health of the patient. We hypothesize that patient and/or tumor-related factors can predict whether acoustic neuromas eventually require treatment after initially being managed with serial observation. We also want to understand how to manage tumors that are incidentally identified or present with minimal hearing loss and how these slowly growing tumors should be managed in the elderly.

Through a case-control study, we identified patients treated for acoustic neuromas at Massachusetts General Hospital between 1995 and 2005. We identified the subset of these patients who were not treated upon diagnosis, but who were followed for at least one year until treatment. We then selected matched controls at a 1:1 ratio. Controls came from the population of acoustic neuroma patients seen in neurosurgery and/or otolaryngology clinics for which observation was recommended and had not yet undergone treatment. By reviewing the office records of the two groups of patients, we compared them based on the following parameters: age, gender, race, indication for diagnostic image, audiogram scan, speech discrimination score, hearing loss, presence/absence of facial nerve dysfunction, presence/absence of trigeminal nerve dysfunction, location of tumor, tinnitus, vertigo, ataxia, size of tumor, and type of treatment (radiation/radiosurgery, surgery, or neither).

Preliminary analysis revealed that younger patients are typically chosen for treatment over older patients and that intracisternal tumors are more likely to grow than intracanalicular ones. Currently we have records for 106 treated patients and 59 patients who are being conservatively managed.

Clinical Equivalence of Generic and Brand-Name Drugs in Cardiovascular Disease: A systematic review and meta-analysis

Alexander S. Misono
Harvard Medical School, Walter Bradford Cannon Society, Class of 2010

William H. Shrank, MD, MSHS
Division of Pharmacoepidemiology and Pharmacoeconomics
Brigham and Women's Hospital

Generic drugs are frequently suggested as a way to reduce drug costs without affecting clinical outcomes. Small molecule generic drugs can be approved by the FDA after patent and/or market exclusivity expiry and if manufacturers show "bioequivalence," which is defined as absence of a significant difference in availability of active ingredient at the site of drug action. However, clinicians, policymakers, and patients have expressed concern that bioequivalence does not guarantee equivalence in effects on various clinical parameters. Although many studies have examined differences and/or similarities between individual drugs, to date, the literature lacks a robust review on this topic.

In an effort to fill this gap in the literature, we developed a systematic review and meta-analysis. Specifically, we sought to compare clinical outcomes of generic and brand-name small molecule drugs in cardiovascular disease, which as a group make up the largest portion of outpatient prescription drug spending. Our objective was to summarize clinical evidence and arrive at a conclusion regarding interchangeability of generic and brand-name drugs in this therapeutic area.

Systematic searches of peer-reviewed publications were conducted in MEDLINE, EMBASE, and International Pharmaceutical Abstracts from the 1984 introduction of the Hatch-Waxman Act--which created a path for generic drug approval of small molecule drugs--to current day. Studies comparing clinical efficacy and/or safety endpoints were selected. We extracted data regarding study design, clinical endpoints, and funding. A meta-analysis was conducted to determine an aggregate effect size (ES).

We identified 47 articles, covering nine subclasses of cardiovascular medications. Of these, 38 (81%) were RCTs. Clinical equivalence was noted in 7/7 (100%) RCTs of beta-blockers, 10/11 (91%) RCTs of diuretics, 5/7 (71%) RCTs of calcium-channel blockers, 3/3 (100%) RCTs of anti-platelet agents, 2/2 (100%) RCTs of statins, 1/1 (100%) RCT of ACE inhibitors, and 1/1 (100%) RCTs of alpha-blockers. Among narrow therapeutic index drugs, clinical equivalence was reported in 1/1 (100%) RCT of Class I anti-arrhythmic agents and 5/5 (100%) RCTs of warfarin. Aggregate ES was 0.03 (95% CI -0.15 – 0.22), indicating no evidence of superiority of brand-name to generic drugs.

Systematic review and meta-analysis indicates that published evidence does not support the notion that brand-name drugs used in cardiovascular disease are superior to their generic counterparts. Our results suggest that it is reasonable for physicians, policymakers, and patients to rely on generic drug approval as a proxy for clinical equivalence among a number of important cardiovascular drugs.

Dextromethorphan Fails to Improve Mechanical Allodynia in Patients with Spinal Cord Injury

Michael A. Mohan
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Christine N. Sang, MD, MPH
The Translational Pain Research Group, Department of Anesthesiology
Brigham and Women's Hospital

Chronic neuropathic pain affects up to 80% of individuals with spinal cord injury (SCI) and is often refractory to existing therapeutic options. Mechanical allodynia (pain evoked by light touch) is a common and particularly devastating aspect of neuropathic pain following SCI.

A growing body of evidence implicates altered processing of input from cutaneous Aß low-threshold mechanoreceptors – nerve fibers capable of detecting slight touch and pressure – in allodynic conditions. Namely, sensitization of the central nervous system to Aß stimulation has been proposed; in this model, increased excitatory activity in glutaminergic/N-methyl-D-aspartate (NMDA) receptor pathways in the central nervous system leads to increased neuronal excitability and the perception of pain under normally innocuous conditions. Indeed, the NMDA receptor antagonist dextromethorphan (DXM) has shown preliminary success in reducing pain and allodynia in painful diabetic neuropathy, postherpetic neuralgia, and orofacial neuralgia.

We studied the analgesic efficacy of DXM in a double-blind dose response trial (0% vs. 25% vs. 50% vs. 100% of each patient's maximally tolerated DXM dose) of adults with traumatic SCI (n=21). Allodynia was evaluated by brush mapping and computer modeling of affected area(s) as a percent of total body surface area. Additionally, we characterized central processing of Aß input by use of transcutaneous electrical stimulation. Because Aß receptors are the only nerve fibers activated at low levels of electrical current, Aß-mediated allodynia is identified when electrical current evokes pain at or near the minimum current strength necessary for detection (that is, when pain threshold:detection threshold (PT/DT) ratios are low).

Our results failed to show a reduction of allodynic body surface area with DXM administration. Mean allodynic body surface area at baseline was 41.89%. Mean change in allodynia from baseline as a percentage of body surface area was -5.11% (0% DXM), -6.32% (25% DXM), -12.70 (50% DXM), and -8.01% (100% DXM). None of the shifts from baseline reached the threshold for significance.

Similarly, DXM failed to increase PT/DT ratios observed upon transcutaneous electrical stimulation of affected skin. Mean PT/DT for 0.5 Hz single pulses was 1.50 at baseline and 1.77 (0% DXM), 1.57 (25% DXM), 1.55 (50% DXM), and 1.64 (100% DXM) following drug administration. Mean PT/DT for 100 Hz electrical trains was 1.47 at baseline and 1.86 (0% DXM), 1.59 (25% DXM), 1.42 (50% DXM), and 1.90 (100% DXM) at the respective drug dosages. Thresholds for significance were not reached, suggesting that DXM failed to alter central processing of Aß input.

Surgical Treatment of Moyamoya with Pial Synangiosis Provides Independence from Exchange Transfusions in Sickle Cell Disease

Matthew R. Naunheim
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011

Edward R. Smith, MD
Department of Neurosurgery
Children's Hospital Boston

A large percentage of children with sickle cell disease (SCD) and elevated transcranial Doppler (TCD) velocities have moyamoya disease, with approximately 11% of these children suffering a stroke by age 20. Though recent trials demonstrate that exchange transfusions may reduce the risk of stroke in some children with SCD, the potential for significant complications exists, including iron overload, hemosiderosis, transfusion-transmitted diseases, immunologic reactions, catheter-related thrombosis and infection. Any treatment capable of decreasing reliance on exchange transfusions in this population would be useful in reducing these risks. A 10 year-old boy with SCD and concomitant moyamoya experienced multiple crescendo strokes in the months prior to treatment and was dependent on exchange transfusions, which were only partially successful in reducing his symptoms. His moyamoya was subsequently treated by revascularization with pial synangiosis. Following an uncomplicated surgery, he has been free from ischemic events for 18 months despite persistent elevated TCD velocities (>300 cm/sec) and MRA findings indicating progression of his moyamoya disease. Importantly, following surgery, he has also been completely weaned from exchange transfusions. This report highlights the potential for pial synangiosis as a therapeutic technique for reducing the risk of stroke in sickle cell patients and - for the first time - documents the feasibility of surgical treatment of moyamoya to free SCD patients from transfusion therapy and its attendant risks. These findings are important to both neurosurgeons and hematologists caring for this substantial population.

The Natural History of Retinal and Visual Function in Children with Mutations in Leber Congenital Amaurosis (LCA)

Lauren Nelson
Harvard Medical School, Francis Weld Peabody Society, Class of 2011
PASTEUR Fellowship

Anne Fulton, MD
Ophthalmology Department, Children's Hospital Boston

Leber congenital amaurosis (LCA) is the earliest and most severe form of inherited retinal dystrophy. Approximately 20% of children attending schools for the blind have LCA. Overall, it accounts for approximately 5% of all inherited retinal dystrophies. LCA is an autosomal recessive disease that usually presents in children within the first year of life as severe visual impairment, nystagmus and nondetectable or severely attenuated electroretinograms (ERGs).

Mutations in ten genes have thus far been linked to the disorder (AIPL1, CRB1, CRX, GUCY2D, RDH12, LRAT, TULP1, RPE65, RPGRIP1 and CEP290). These mutations affect the photoreceptors, pigment epithelial cells, or both. Up until recently, LCA has been considered untreatable. Gene replacement trials have recently begun as an experimental treatment for RPE65 disease. It is not known whether gene replacement will be effective in children although it was in the Briard dog model of RPE65 disease. The focus of this study was to analyze the early natural course of RPE65 disease and compare it to CRB1 disease. RPE65, retinal pigment epithelium-specific 65-kDa protein, is found in pigment epithelial cells. It is believed to control the isomerase essential for converting all-trans-retynl ester to 11-cis-retinol in the visual cycle. CRB1, crumbs gene homologue 1, is currently thought to be one of the more commonly mutated genes responsible for LCA (9-13% of cases). CRB1 is located in the inner segments and is believed to play a role in organization and polarity of developing photoreceptors.

Based on the fact that the CRB1 mutation affects inner segments of photoreceptors where as RPE65 is associated with the pigment epithelium, I hypothesized that children with CRB1 mutations would show more severe early photoreceptor dysfunction (impaired DAT and thinner retinal arterioles) in comparison to children with RPE65. The dark adapted threshold (DAT) was used as the main assessment of rod photoreceptor function. In addition, image analysis software was used to measure the diameter of retinal arterioles on fundus photographs; arteriolar diameter is an indicator of overall metabolic status of the retina. Visual acuity and refraction were also analyzed.

Data indicated a rapid decline of DAT between age 4-6 in both CRB1 and RPE65 individuals. In addition, CRB1 individuals were found to have significant hyperopia, while RPE65 had mild hyperopia and in some cases myopia. No significant difference was seen between retinal arteriole diameter between CRB1 and RPE65 individuals. However, both groups had reduced retinal arteriole diameter compared to normal controls.

3T Diffusion Tensor Imaging of Mild Traumatic Brain Injury:
A prospective longitudinal study

Joshua Ng
Harvard Medical School, Francis Weld Peabody Society, Class of 2009
George Cheyne Shattuck Memorial Fellowship

Pratik Mukherjee, MD, PhD
The Neuroradiology Section, Department of Radiology and Bioengineering
University of California at San Francisco

BACKGROUND AND PURPOSE: Diffuse axonal injury (DAI) of white matter is thought to be the key mechanism of the cognitive impairment caused by traumatic brain injury (TBI). However, conventional CT and MR imaging have been unable to adequately assess DAI in mild TBI, as findings on CT and 3T MR imaging do not correlate with the patients' neurocognitive outcome. Diffusion tensor imaging (DTI) has been used to more accurately detect white matter changes in patients with moderate or severe TBI. We hypothesized that quantitative DTI tractography would be able to detect white matter changes due to DAI in patients with mild TBI, and could be used to follow the longitudinal course of axonal injury during the first year after traumatic injury.

MATERIALS AND METHODS: Thirty-one adult patients with mild TBI (25 men, 6 women; mean age, 32 +/- 9 years) and nineteen control patients (16 men, 3 women; 34 +/- 8 years) matched for age, gender, and educational level were investigated. Patients and controls underwent the California Verbal Learning Test (CVLT) to evaluate working memory. Serial 3T DTI exams were acquired acutely (<2 weeks), at one month, and at one year post-trauma for TBI patients and once only for control subjects. Quantitative three-dimensional fiber tracking was used to measure the average fractional anisotropy (FA) over whole fiber tracts bilaterally including the cingulum bundle (CB), arcuate fasciculus (AF), inferior fronto-occipital fasciculus (IFO), uncinate fasciculus (UF), corticospinal tracts (CST), and the genu and splenium of the corpus callosum.

RESULTS: Compared to controls, patients with mild TBI demonstrated reduced FA values ($p < .05$) within the IFO, UF, and the genu and splenium of the corpus callosum. No statistically significant differences were observed within the CB, AF, or the CST at any time point. No significant changes in FA were observed over the 3 time points during the first year after trauma. At all time points, the mild TBI patients had poorer working memory than controls on the CVLT ($p < .05$).

CONCLUSION: In adult mild TBI patients with neurocognitive impairment, DTI tractography reliably detected microstructural changes within several white matter tracts, most of which have frontal lobe connectivity. These tracts with acute microstructural white matter injury are the same as those most commonly affected in chronic mild TBI, as reported previously. DTI tractography did not demonstrate longitudinal changes in white matter FA over the span of a year following mild TBI.

An shRNA Screen for Regulators of Epithelial-to-Mesenchymal Transition

Lydia W. Ng
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011
Aid for Cancer Research Fellowship

Daniel A. Haber, MD, PhD
Center for Cancer Research
Massachusetts General Hospital

In cancer pathogenesis, a hallmark of malignant tumors is metastasis, the spread of a tumor to a location discontinuous to its site of origin via the lymphatics, vasculature, or direct seeding in adjacent body cavities. In fact, tumor metastases have a far higher mortality rate than primary cancers, causing 90% of human cancer fatalities. Therefore, elucidating the mechanisms by which a cancer cell can extravasate, migrate, and intravasate at a different location is critical to the development of effective cancer therapies.

Tumor seed cells are thought to undergo metastasis in a manner reminiscent of developmental epithelial-to-mesenchymal transition (EMT). As seen in developmental EMT, tumor cells that become metastatic repress expression of E-cadherin and adhesion signaling molecules, while expression of vimentin and migratory factors is upregulated. They also have increased levels of transcriptional regulators repressing E-cadherin expression, such as SNAILs, ZEB, TWIST, and an inducer recently discovered by the Haber and Brugge labs, YAP. How exactly these changes in gene expression are orchestrated remains poorly understood.

We hypothesized that multiple genes actively maintain epithelial homeostasis and that downregulation or disruption of these genes leads to EMT. We aimed to identify such genes by utilizing RNA interference, specifically a short hairpin RNA library, in a novel "whole-genome" screening methodology. In collaboration with Broad Institute's RNAi Consortium, we infected MCF10A human mammary epithelial cells with a 55,000 hairpin lentiviral library, consisting of five hairpin RNAs for each of 11,000 genes. Since one functional consequence of EMT is migratory capability, infected cells were selected for their ability to migrate through Boyden chambers. Migratory cells were pooled, and hairpins enriched in the migratory pools relative to infected growth controls were identified by microarray analysis.

We retested putative positive hairpins using the Boyden chamber migration assay. We also looked up the 23 gene targets of the putative positive hairpins and obtained two additional hairpins per gene for retesting. Our results were robust: we had a 61% retest rate for the enriched hairpins investigated and a 35% overall retest rate, with positive-retest hairpins targeting 18 genes.

For each gene with at least one positive hairpin, we used qPCR to measure knockdown levels by each of the three targeting shRNAs. After correlation of qPCR to functional results, we confirmed three genes: a transporter, a cell cycle regulator, and a cytochrome-P450. These genes represent candidate repressors of cell migration and maintainers of the epithelial state.

Characterization of Luminal Flow Effects on Drug Distribution Patterns in Stent-based Delivery

Andriana Petrova Nikolova
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Elazer Edelman, MD, PhD
Harvard-MIT Biomedical Engineering Center, MIT

Since the advent of the first FDA-approved drug-eluting stents in 2003, the procedure has evolved from an esoteric and rarely applied treatment option to one that challenges surgical revascularization in battling the number one killer in the world, namely coronary artery disease. While drug-eluting stents virtually eliminate arterial restenosis, growing evidence suggests higher incidence of late in-stent thrombosis in patients who receive DES compared to bare-metal stents. It has been hypothesized that such complications might arise from delayed arterial healing and, in particular, delayed re-endothelialization in the presence of the FDA-approved drugs. This phenomenon is manifested clinically as endothelial denudation, unresorbed fibrin deposition, and hypocellularity near the drug-eluting struts.

Our investigation seeks to examine the correlation between delayed re-endothelialization and another surface phenomenon exhibiting the same tropism, namely arterial drug distribution. Computational fluid dynamics models from our lab have established the paradigm that flow imposes recirculation zones distal and proximal to the stent strut that extend the size of the drug footprint in an asymmetrical manner. We, therefore, hypothesize that local flow patterns are responsible for creating stagnant drug pools in the vicinity of the stent strut which lead to delayed re-endothelialization and increased thrombogenicity of drug-eluting stents.

Our study model consists of Rhodamine B-coated stents deployed in arterial segments which are cannulated in a specially designed closed flow system, consisting of a reservoir, a pump and the arterial segment. Our goal is to image mural drug deposition patterns to quantify their dependence on the dynamics of luminal flow and the kinetics of drug release from the stent coating. In particular, we will examine how flow and release kinetics impact the targeting of drug to denuded interstrut regions. Our study presents a clear and quantitative analysis as to how luminal flow patterns dictate arterial drug deposition in stent-based delivery. Only by visualizing and methodically quantifying these effects would we be better able to understand the problems of re-endothelialization and thrombosis challenging these powerful technologies.

Barriers to HIV and Syphilis Screening and Treatment during Prenatal Care in Recife, PE, Brazil

Chinyere I. Obimba
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011

Dr. Arachu Castro, PhD, MPH
Department of Social Medicine
Harvard Medical School

The primary mode of infection of the HIV for children is vertical transmission, or mother-to-child transmission (MTCT). MTCT accounts for 90% of all childhood HIV infections worldwide. HIV can be transmitted from mother to infant during pregnancy, during labor and delivery, or in the course of breastfeeding. The rate of MTCT of HIV is as high as 32.5% without intervention. Congenital syphilis also poses a problem in many developing countries. Each year, maternal syphilis is responsible for 270,000 cases of congenital syphilis and 460,000 miscarriages and stillbirths worldwide.

Brazil has extensive legislation and guidelines concerning HIV and syphilis screening programs for pregnant women during prenatal care. However, Brazil continues to experience relatively high rates of vertical transmission of HIV and congenital syphilis. While the policies for both PMTCT and congenital syphilis prevention programs have been well-established in Brazil since the 1990s, there seems to be a disconnect between the national recommendations and implementation.

The purpose of this project was to characterize the barriers to the implementation of HIV and syphilis screening programs for pregnant women during prenatal care in Recife, Pernambuco, Brazil. Aims: 1) Identify the obstacles to providing HIV and syphilis screening up to the national standard of care within hospitals, clinics and maternidades in Recife through interviews. 2) Outline the challenges to providing adequate HIV and syphilis screening, prophylaxis and treatment at various levels of the health care system for the pregnant women from resource-poor areas of the region. 3) Collect ideas as to how the current standard of care could be improved.

The project was a qualitative study. Open-ended interviews were conducted. Twelve interviews were conducted in groups or individually with health care professionals (6), policy makers (3) and advocacy groups (6). The interview placed an emphasis on understanding which interventions worked and which did not, and understanding the obstacles as perceived by the interviewees. Hand-written notes from the interviews were translated, transcribed and reported into an electronic log and database for analysis.

The following barriers were identified the most during interviews: 1) lack of medications/materials available at health care centers; 2) lack of reproductive health education/advising for women; 3) ill-equipped laboratories and 4) problems of access for women from the interior of Pernambuco state to receive care. The deficiencies of the public health care system in Recife, PE are multifaceted and deserve further investigation.

Pulp Infection in Severe Early Childhood Caries

Kevin D. Oh
Harvard School of Dental Medicine, Oliver Wendell Holmes Society, Class of 2011

Anne Tanner, PhD
Department of Molecular Genetics, Forsyth Institute

Severe Early Childhood Caries (SECC) is a form of rampant caries on primary dentition that affect 10% of children in US. Affected children may develop pulp necrosis and peri-radicular lesions that require heavy treatment such as pulpotomy. It is important to recognize the causative role of oral bacteria in SECC cases. Cultures from the surface and pulp of affected teeth provided evidence that certain anaerobic bacteria are consistently present on those teeth. We hypothesize that clinical symptoms associated with SECC correlate with presence of specific virulent microorganisms in the pulp.

Previous studies have identified microorganism genus such as Propionibacterium and Peptostreptococcus using primitive culture method on necrotic pulps of primary teeth. Therefore, only cultivable bugs were identified. For this study, we extend this bacterial profile using PCR and clonal analysis in addition to culture method to include non-cultivable microorganisms. Samples from 9 different subjects were collected via sterile swipe of either vital or non-vital pulp of affected teeth. Average age of the subjects was 43.875 months including 6 males and 3 females.

Plaque samples from specific teeth were diluted serially and plated on two different blood plates (fastidious anaerobic agar and tryptic soy-broth yeast plates) and one acid plate per dilution. Samples were also plated onto Streptococcus selective plates mainly to assess the growth of Streptococcus Mutans and Sobrinus. Bacteria that grew were incubated in anaerobic chamber for 9 days for acid plates and 12 days for blood plates. Using a set of universal primer specific for oral bacteria (smaller subunit of ribosomal RNA, 16s rRNA), 30 endodontic culture isolates per sample were amplified with Touch PCR. The majority of the 30 culture isolates from each of the nine samples provided positive universal PCR results.

Plaque samples were also used to extract DNA to perform clonal analysis alongside the PCR method. Each of the nine samples generated roughly 40 clones. We were successful in cloning four of the nine samples.

The remaining portion of this study is partial sequencing of the positive PCRs which will be blasted against HOMIM (Human Microbe Identification Microarray) for identification.

Maxillofacial Injuries: Indicators of occult cervical spine injury

Jason L. Outlaw
Harvard School of Dental Medicine, Oliver Wendell Holmes Society, Class of 2011
Oral and Maxillofacial Surgery Foundation Student Research Training Award

Thomas B. Dodson, D.M.D., M.P.H.
Department of Oral and Maxillofacial Surgery
Massachusetts General Hospital

Undetected cervical spine (c-spine) injury is devastating. The presence of c-spine injury has important implications for trauma patients, influencing airway management techniques, diagnostic imaging study choices, surgical approach, and timing for repair of concomitant facial fractures. Maxillofacial injuries may be associated with and serve as a possible marker for occult c-spine injury.

The purpose of this study is to address the following question: among subjects with blunt trauma, do those with maxillofacial injuries, when compared to those without maxillofacial injuries, have an increased risk for c-spine injury? The investigators hypothesize a significant difference in the frequency estimates of c-spine injuries between subjects with and without maxillofacial injuries. The specific aims of this study are: 1) to develop and implement a retrospective cohort study composed of a sample derived from the population of subjects presenting to Massachusetts General Hospital (MGH) for evaluation and management of blunt trauma, 2) to estimate and compare the frequencies of c-spine injury between subjects with and without maxillofacial injury, and 3) to develop a multiple logistic regression model to estimate the adjusted association between maxillofacial and c-spine injuries.

The study population is derived from patients presenting to the MGH Emergency Department (ED) for a trauma evaluation between October 2002 and September 2007. The inclusion criteria include being a victim of blunt trauma, and to have a record in the MGH Trauma Database. Exclusion criteria include being a victim of penetrating trauma, being a victim of a non-blunt trauma, and incomplete data, namely inadequate documentation of injury etiology and severity.

The predictor variable is maxillofacial injury status (present or absent) and the primary outcome variable is c-spine injury (present or absent). Secondary outcome variables include c-spine injury type and severity. Other study variables can be categorized as demographic, injury, pre-hospital care, referring hospital, ED admission, ED assessment, hospital diagnosis, operations, complications, quality-assessment indicators, and hospital outcomes.

Appropriate univariate and bivariate statistics will be computed. Multiple logistic regression models will be developed to estimate the association between maxillofacial injury and cervical spine injuries adjusted for biologic (age, sex, race) and confounding variables. Confidence intervals (95%) will be computed for the estimated odds ratios. P values less than 0.05 will be considered statistically significant.

If the hypothesis is confirmed, maxillofacial injuries may serve as markers of subjects with an increased risk of c-spine injury and warrant further investigation especially in the setting of a benign clinical c-spine injury.

Plaque Assessment by 64-Slice Multidetector CT in Comparison to Intravascular Ultrasound

Milena Petranovic
Harvard Medical School, Walter Bradford Cannon Society, Class of 2009

Ricardo C. Cury, MD
Cardiac MR PET CT Program, Department of Radiology
Massachusetts General Hospital

Introduction: Determining plaque burden and plaque composition is an important step in risk stratification and prevention of acute coronary syndromes in patients with coronary artery disease (CAD). In this study, we attempt to evaluate 64-MDCT for detection, quantification, and characterization of coronary plaque.

Methods: Data from 11 patients that underwent MDCT and IVUS for clinically suspected CAD or as research protocol were collected and a total of 17 coronary segments and 122 cross-sectional slices were analyzed by MDCT and IVUS. All CT scans were performed on a 64-slice CT scanner (Sensation 64, Siemens Medical Solutions, Forchheim, Germany) using a standard Coronary CTA protocol (Detector collimation 32 x .6mm, tube voltage 120 kV, current of 800-900 mAs depending on patient size, contrast injection 5 ml/s, gantry rotation 330 ms). Coronary segments on CT were co-registered to IVUS and each obtained slice was scored by two blinded observers for presence and type of plaque. Quantitative measurements were obtained, including cross-sectional vessel area, lumen area, wall area, and plaque burden. Mean and standard deviation of Hounsfield units (HU) were recorded for plaque when present.

Results: Overall sensitivity for plaque detection was 95.0% and specificity, PPV, NPV were 88.7%, 89.1%, and 94.8%, respectively. Using IVUS as a gold standard, 64-MDCT correctly detected 12 of 14 (85.7%) slices containing calcified plaques, 37 of 41 (90.2%) slices containing non-calcified plaque, and 2 of 5 (40.0%) slices containing mixed plaques. The Cohen's kappa coefficient for the classification of plaque between MDCT and IVUS was 0.787, indicating good agreement. Spearman's correlation coefficients were 0.85, 0.75, 0.70, and 0.54 for cross sectional vessel area, lumen area, wall area, and plaque burden, respectively. In all measured parameters, MDCT overestimated the cross-sectional size and the differences between corresponding MDCT and IVUS measurements were significant ($p<0.05$). Combined non-calcified plaque had a mean CT density of 117.9 ± 94.2, which was significantly different from the mean density of calcified plaque 608.2 ± 216.9. Soft and fibrous plaques were not able to be distinguished based on their HU.

Conclusion: This study showed good diagnostic accuracy for detection of both calcified and non-calcified plaque with 64-MDCT. A sensitivity of 90.2% for non-calcified plaque detection without the use of software is in line with previous studies and confirms the added benefit of improved temporal and spatial resolution for non-calcified plaque detection with 64-MDCT. Our results, however, demonstrate the difficulty in reliably differentiating non-calcified plaque components.

A Qualitative Study of Barriers and Facilitators to Antiretroviral Adherence for HIV/AIDS Patients in Vietnam

Anh Pham
Harvard Medical School, Francis Weld Peabody Society, Class of 2011

Howard Libman, MD
Department of Medicine
Beth Israel Deaconess Medical Center

Since its introduction in the mid 1990s, highly active antiretroviral therapy (HAART) has extended the life span of individuals living with HIV/AIDS. The therapy restores daily functioning and well-being by reducing viral load and damage to the immune system. However, the effectiveness of HAART depends on excellent adherence and continuous access to treatment. Since the virus is prone to mutation, it can develop resistance to HAART and with fewer treatment options, patients may experience disease progression and even death

As little research on this important issue of adherence has been done in Vietnam, it was essential to examine adherence barriers and success strategies of HIV/AIDS patients. A qualitative study design was chosen as it allowed for a multidimensional analysis of adherence barriers and facilitators from the perspectives of the patients and healthcare providers. As such, 25 patients of the Network of Self Help Groups and 10 providers from various free HIV clinics of Ho Chi Minh City (HCMC) were interviewed in a semi-structured format about the various challenges and successes in taking HAART and their impact on the patients' quality of life.

These interviews reveal that no single variable can adequately and completely explain the level of adherence of any individual patient or group of patients. Patients and providers often point to a multitude of factors that affect adherence level. Several themes of these factors emerged through the interviews. First, the complexity of the medication regimen, e.g. number of pills, frequency, indications, etc. poses as a major challenge to adherence as it interrupted daily activities. Second, the extent of social support a patient receives predicts the success and failure of that patient's adherence. This support depends upon the trust and relationship a patient has with his healthcare provider, family and friends, surrounding community, and the Network of Self Help Groups. Positive relationship in these different social spheres greatly enhances adherence. Third, adherence education program provided at the clinics dictated the adherence level of patients. Programs that are flexible and that cater to the specific needs and challenges of patients are more likely to be successful in helping patients maintaining adherence. And finally, the concurrent use of heroin and/or alcohol represents a serious barrier for many patients. As the majority of HIV/AIDS patients in Vietnam comprise of intravenous drug users, the impact of this substance abuse on the management of HIV/AIDS in Southeast Asia cannot be ignored.

3D Volumetry of Brain and Ventricular System in Fetuses with Ventriculomegaly Correlated to Neonatal Outcomes

Danielle B. Pier
Harvard Medical School, Frances Weld Peabody Society, Class of 2010
Doris Duke Clinical Fellowship

Deborah Levine, MD
Ultrasound Medicine, Department of Radiology
Beth Israel Deaconess Medical Center

Objective: Our objective was to compare 3D volumetry of the brain parenchyma and ventricular system determined by fetal MRI, adjusted for gestational age, to neurodevelopmental outcome in fetuses diagnosed with prenatal ventriculomegaly (VM) on ultrasound or MRI.

Methods: Pregnant women were enrolled into the study after a diagnosis of fetal VM by ultrasound (lateral ventricles measuring more than 10 mm), and subsequently underwent a fetal MRI. 3D volumetry was performed on 199 fetuses. Initial comparison was live birth versus termination/demise. Subsequent outcome was obtained using standardized neurodevelopmental and psychometric testing up to age 2. Outcomes were grouped into children with either a normal or an abnormal test result. An abnormal test result was defined as an abnormal neurologic finding or 2 standard deviations below the mean on standardized tests.

Results: 164 pregnancies resulted in live deliveries, and 85 of these children had neuropsychological follow up. Parenchymal volume did not correlate with neurodevelopmental outcome ($p=0.59$), but ventricular volume did correlate with outcome ($p=0.002$). There were 75 fetuses with follow-up with a prenatal diagnosis of isolated VM with no other neurologic diagnosis. Of these, 10 were not live deliveries and 65 live deliveries with neurodevelopmental follow up. When comparing the population with isolated VM, parenchymal volume again did not correlate with neurodevelopmental outcome ($p=0.46$), but ventricular volume did correlate with outcome ($p<0.001$).

Conclusions: Ventricular volume, but not parenchymal volume, correlated with neurodevelopmental outcome in fetuses with ventriculomegaly, regardless of whether it was isolated or in combination with other CNS anomalies. This may be a useful tool in predicting outcome of fetuses with VM. Future studies will include comparing measurements of ventricular volume versus ventricular atria length in terms predicting neurodevelopmental outcome.

Procedural and Device-Related Risk Factors Leading to CSF Shunt Revisions in Hydrocephalic Patients

Angelique Pillar
Harvard Medical School, Francis Weld Peabody Society, Class of 2011
PASTEUR Fellowship

Joseph Madsen, M.D.
Department of Neurosurgery
Children's Hospital Boston

Hydrocephalus, a condition affecting 0.66 out of 1,000 live births, occurs when there is a lack of absorption, blockage of flow, or overproduction of the cerebrospinal fluid (CSF) in the ventricles or cavities of the brain. This condition is treated by placement of a shunt draining CSF from the ventricular cavities of the brain to the peritoneal, atrial, or pleural cavities. However, complication rates remain high and more than 30% of new shunts fail within 1 year. Current studies reveal that the shunt failure rate has not changed over the past decade. However it is unknown whether the increasing use of programmable shunts that may be more prone to mechanical failure may be to blame for these stagnant statistics. The purpose of this retrospective study was to determine whether the use of programmable valves, adjustable shunts, and monopolar cautery was associated with a higher risk of shunt failure and complications.

The objective was to use a Kaplan-Meier shunt survival curves and log rank analysis to compare failure rates between groups. We hypothesize that each of the three aforementioned variables are associated with a higher rate of shunt failure and shunt related complications.

The subjects of this study included patients 1 wk-18 yrs admitted to Boston Children's Hospital during calendar year 1993-2007, who during the course of their stay, were diagnosed with hydrocephalus and implanted with an original CSF shunt or revision shunt. Exclusion criteria included patients lost to follow up as evidenced by missing clinic notes. The primary outcome was defined as a need for CSF shunt revision, identified via ICD-9 procedural codes and classified by proximal catheter obstruction, distal obstruction, or valve failure in the operative note. The exposure variable was defined as programmable valve or shunt versus standard valve or shunt and monopolar cautery vs no mention of the procedure in the operative note.

This study is currently ongoing. A query of medical records revealed that overall 1833 shunt placements and revisions were carried out at CHB alone in the ten year period between Jan 1998 – Dec 2007. Of those 1883, 468 were original placements (25.5%) and 1,365 were shunt revisions (74.5%). This study may improve the understanding of the benefits or risks of monopolar cautery, programmable shunts, and adjustable valves. Information will help to prioritize device design.

A Study of Host and Pathogen Characteristics Mediating Human Immunity to *V. cholerae*

Michael Podolsky
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

Stephen Calderwood, MD
Division of Infectious Diseases
Massachusetts General Hospital

Infection with *Vibrio cholerae* leads to severe diarrhea and dehydration. Without proper treatment, death may result. In 2006, more than 230,000 cases of *V. cholerae* with 6,311 deaths were reported from 52 countries to the World Health Organization, but the true burden of disease is estimated to be ten times the number of reported cases. Among those patients who survive the acute infection, many develop protective immunity against *V. cholerae*. Unfortunately, no vaccine has yet successfully recapitulated this natural immunity. The aims of this research were (1) to identify cholera antigens important for natural protection and (2) to identify characteristics of the host that affect the development of the cholera immune response. To achieve the first aim, a protein microarray that contains the majority of the open reading frames from *V. cholerae*, transcribed and translated *in situ* from a library of DNA plasmids, was developed with the help of the Harvard Institute of Proteomics. Lymphocytes isolated from blood of cholera patients were cultured and culture supernatants containing IgG and IgA secreted into the supernatants by the lymphocytes were used to probe the protein microarrays. Lymphocytes were taken at acute and convalescent phases in order to identify those protein antigens to which cholera patients mount an antibody response over baseline. These culture supernatants were equilibrated for total IgG and IgA and then used to react with the microarrays; the data resulting is currently being analyzed. For the second aim, we examined the immune responses against specific cholera antigens among cholera patients that had or did not have concomitant parasitic infection. We found that cholera patients who were co-infected with helminths had a decreased antibody response to cholera toxin (an important and protective protein antigen) compared with non-helminth-infected cholera patients. Differences in both total IgA and IgG anti-cholera toxin responses were seen, but there were no differences seen in IgA and IgG subclasses. Analysis of cytokine production in cultured lymphocytes obtained on day 7 following infection from these two groups demonstrated that Interleukin-10 was significantly lower in lymphocyte cultures from helminth-infected cholera patients compared with non-helminth infected cholera patients. Thus parasitic worm infections appear to alter the immune response generated during acute cholera infection. We believe understanding the host and pathogen characteristics that determine immunity following natural disease will be essential for developing effective vaccination strategies against cholera as well as many other diarrheal disease pathogens which cause significant morbidity and mortality worldwide.

Reconstruction of Mandibular Defects Using Tricalcium Phosphate-Polycaprolactone Scaffolds Seeded with Autologous Mesenchymal Stem Cells

Tracy Pogal-Sussman
Harvard School of Dental Medicine, William Bosworth Castle Society, Class of 2011

Dr. Maria Troulis DDS, MSc
Department of Oral and Maxillofacial Surgery, Massachusetts General Hospital

Full thickness bone defects and congenital bone defects are difficult to treat because they heal poorly in the absence of corresponding tissue-forming cells. Innovative tissue engineering strategies are attempting to recapitulate natural tissue-formation processes by controlling the cell population, scaffold, and inductive factors involved in normal bone formation and fracture healing, in order to repair and regenerate damaged bone. Mesenchymal stem cells (MSCs) derived from bone marrow can be induced to differentiate into osteogenic cells. MSCs have great clinical utility in tissue engineering applications because they are capable of being isolated and expanded in culture. Scaffolds then serve as delivery vehicles for transplanting the cells into a bone defect site. Abukawa et al. (2004) demonstrated the successful use of biodegradable poly-DL-lactic-coglycolic acid (PLGA) scaffolds seeded with MSCs to reconstruct bony defects in a porcine mandible model. However, the practical application of this technique is limited by a lack of control over the porous architecture of the PLGA scaffolds. It is generally agreed that a highly porous microstructure with interconnected pores improves cell seeding, attachment, growth, extracellular matrix production, vascularization, and tissue ingrowth. In a more recent *in vitro* study, Sharaf et al. (2007) compared 3D-printed scaffolds that were manufactured using three-dimensional printing technology (3DPT), a procedure that allows for precise geometric control over scaffold fabrication. Sharaf et al. showed that scaffolds produced using β-Tricalcium Phosphate-Polycaprolactone (β-TCP-PCL) exhibited the greatest amount of cellular penetration into the interior of the construct following two weeks of culture. The purpose of this study was to examine the efficacy of β-TCP-PCL scaffolds for promoting cell growth and tissue formation in an *in vivo* porcine mandible model. MSCs were obtained from the iliac crest of 2 female Yucatan minipigs, isolated and expanded in cell culture, and treated with osteogenic supplements to induce differentiation into bone-forming cells. The differentiated cells were seeded onto β-TCP-PCL scaffolds, and incubated for 2 weeks. Six critical-sized mandibular defects were created in the mandible of each pig (n=12). Constructs (scaffolds and cells, n=8) and control scaffolds (scaffolds without cells, n=2) were wedged into the defect sites and the remaining defect sites (n=2) were left empty. Six weeks post-implantation, we will analyze bone formation and tissue ingrowth using radiographic and histologic methods. We anticipate that the material properties of β-TCP-PCL will enhance bone formation, and provide proof that it is a viable option for treating full thickness bone defects.

Pericardial Fat, Intra-thoracic Fat, and Left Ventricular Structure and Function: The Framingham Heart Study

Stacy A. Porter
Harvard Medical School, Francis Weld Peabody Society, Class of 2011

Caroline S. Fox, MD, MPH
Division of Endocrinology, Diabetes, and Hypertension
Brigham & Women's Hospital

Pericardial fat has been implicated in the pathogenesis of obesity-related cardiovascular disease. Whether the associations of pericardial fat and measures of cardiac structure and function are independent of the systemic effects of obesity and visceral adiposity has not been fully explored.

Participants from the Framingham Heart Study (n=1006, 54.3% women) underwent chest and abdominal CT and cardiovascular MRI between 2002 and 2005. Pericardial fat, intrathoracic fat, and visceral abdominal fat (VAT) quantified from CT, along with BMI and waist circumference, were examined in relation to CMR measures of left ventricular mass, left ventricular end diastolic volume, and left atrial dimension. In women, LV mass, LVEDV, and LA dimension were directly correlated with pericardial fat ($r=0.20$ to 0.35, $p<0.001$), intrathoracic fat ($r=0.25$ to 0.37, $p<0.001$), VAT ($r=0.24$ to 0.45, $p<0.001$), BMI ($r=0.36$ to 0.53, $p<0.001$), and WC ($r=0.30$ to 0.48, $p<0.001$). In men, LV mass and LA dimension were directly correlated with pericardial fat ($r=0.19$ to 0.37, $p<0.001$), intrathoracic fat ($r=0.17$ to 0.31, $p<0.001$), VAT ($r=0.19$ to 0.36, $p<0.001$), BMI ($r=0.32$ to 0.44, $p<0.001$), and WC ($r=0.34$ to 0.44, $p<0.001$), but LVEDV was not. Associations persisted after multivariable adjustment, but not after additional adjustments for body weight and VAT, with the exception of pericardial fat and LA dimension in men.

Pericardial fat is correlated with CMR measures, but not independent of or more strongly than other ectopic fat stores and proxy measures of visceral adiposity. An important exception is left atrial dimension in men. These results suggest that the systemic effects of obesity on cardiac structure and function may outweigh the locally pathologic effects of pericardial fat.

V173D: A True Shedding Mutation in TNF-Receptor Associated Periodic Syndrome (TRAPS)

Ali Anwaar Qureshi
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2012

Richard M. Siegel, MD, PhD
Immunoregulation Group, Autoimmunity Branch
National Institute of Arthritis and Musculoskeletal and Skin Diseases

Tumor necrosis factor-receptor type 1 (TNFR1) is a transmembrane cell-surface receptor activated by tumor necrosis factor (TNF), an important inflammatory cytokine. TNFR1 can regulate TNF-signaling via inactivation of TNFR1 by "shedding": binding of TNF to TNFR1 results in proteolytic cleavage of the extracellular domains of the TNFR1 homotrimer from the cell surface. Soluble TNFR1 (sTNFR1) acts as a competitive inhibitor, sequestering TNF extracellularly from cell-surface TNFR1 blocking downstream signaling.

Mutations in TNFR1 can cause an autosomal dominant autoinflammatory syndrome, TNFR1-associated periodic syndrome (TRAPS). Affected patients suffer from lifelong episodic fevers and inflammation with abdominal pain, urticaria, myalgia and arthralgia. Previously it was hypothesized that decreased shedding of mutant TNFR1 may lead to TRAPS, but this cannot be the major pathophysiology of the disease, since it has been shown that mutant TNFR1 has less TNF-binding capacity and is less expressed on the cell-surface. All TRAPS-associated mutations occur in the extracellular domains of TNFR1 at sites distant from the cleavage site.

Recently however, a family has been described with TRAPS caused by a novel mutation in TNFR1, V173D. This mutation occurs in the extracellular domain and in the region of TNFR1 known to be essential for shedding. We sought to examine the effect of the V173D mutation on the shedding of TNFR1 in vitro and specifically in a heterozygous setting when co-expressed with wild-type TNFR1.

Using site directed mutagenesis, we created three mutant TNFR1 constructs: C33G (a previously studied characteristic TRAPS mutation), V173D and V173P, which were singly transiently expressed in 293T-cells. Flowcytometry analysis demonstrated a similar total expression in cells and was corroborated by Western blot analysis of cell lysates. While there was reduced cell-surface expression of C33G, as previously shown, that V173D was not reduced. Analysis of the supernatant suggests that V173D is shed to a lesser extent than Wt TNFR1. Treatment of transfected cells with an inducer of TNFR1 cleavage demonstrated a five-fold increase in Wt sTNFR1 and only a slight increase in V173D sTNFR1, as detected by ELISA. Initial flowcytometry analysis seems to support these findings. Co-transfections of Wt and V173D plasmids suggest that Wt and mutant TNFR1 may be forming trimers that are expressed on the cell-surface and shed less. Cumulatively, these results suggest a possible dominant negative effect of V173D on the cleavage of TNFR1 from the cell-surface, which would elucidate a pathophysiological mechanism of TRAPS based on a true shedding defect.

Zambia's National Malaria Control Program: Scale-up for impact

Vanessa Redditt
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011

Andrew Ellner, MD, MSc
Global Health Delivery Program
Harvard Medical School, Harvard School of Public Health, and Brigham and Women's Hospital

Zambia has achieved remarkable progress in malaria control in recent years, leading to drastic reductions in malaria infection and malaria-related mortality. Through a series of semi-structured interviews with government program officials, cooperating partners, and district-level staff, this research project investigates the strategies and operations that led to this success.

Zambia's malaria control program embraces an integrated approach of prevention—using long-lasting insecticide-treated nets (LLINs), indoor residual spraying (IRS), and intermittent preventative therapy in pregnancy (IPTp)—and treatment—involving diagnostics and artemisinin-based combination therapy (ACT). Since 2006, malaria parasite prevalence in children has been reduced by 50% and moderate to severe anemia has been reduced by about 60%. More than two-thirds of Zambian households were covered by treated nets or IRS by 2008, a marked improvement from 2002.

Strong coordination of a variety of partners, led by the Ministry of Health's National Malaria Control Centre, has been key in achieving rapid scale-up and broad coverage of interventions. The joint formulation of national strategic plans and annual action plans has fostered partner commitment to core goals and enhanced has partner accountability. Regular monitoring, planning, and policy meetings with all interested partners has also improved stakeholders' involvement in the program. Increased funding from donors has also greatly enhanced the reach of interventions, through mass distribution of LLINs, higher geographical coverage of spraying, and use of improved therapies and diagnostics.

High-level political commitment to malaria control has also been crucial in the program's success. Early introduction of free ACTs as first-line malaria treatment, establishment of tax-exemption policies on antimalarial commodities, and public prioritizing of malaria control in overarching national development strategies are key examples of how political leadership has bolstered the malaria control program.

A decentralized approach to health care delivery is a cardinal feature of the Zambian health system and continuous involvement of provincial and district health teams has enhanced local ownership of malaria control interventions. Community participation in the program—from utilizing community members to conduct indoor spraying to locally-run sensitization campaigns—have also greatly contributed to increased acceptance and appropriate utilization of interventions. Furthermore, new efforts are underway to involve community health workers in malaria case management.
Key challenges the National Malaria Control Program is facing include strengthening supply chain management of ACTs and diagnostics, improving household utilization of nets, and enhancing clinician adherence to diagnosis protocols.

Outcomes Of Malignant CNS Ependymomas:
An examination of 2,408 cases through the Surveillance, Epidemiology and End Results (SEER) database (1973-2005)

Dayron Rodriguez
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011

Dr. Leonidas G. Koniaris
Division of Surgical Oncology, DeWitt Daughtry Family
Department of Surgery, University of Miami School of Medicine

Objective: Determine the role of surgery and radiation therapy for patients with malignant CNS ependymomas.

Methods: The Surveillance, Epidemiology, and End Results (SEER) database (1973-2005) was queried.

Results: Overall, a total of 2408 cases of malignant ependymomas were identified. Of these, 2132 cases (88.5%) were identified as WHO grade II ependymomas and 276 cases (11.5%) as WHO grade III (anaplastic) ependymomas. The annual incidence of ependymomas was approximately 1.97 cases per million in 2005. Overall median survival for all patients was 230 months, with a significant difference between women and men (262 months vs.196 months, respectively) ($p = 0.004$). Median age at diagnosis was 37 years among females and 34 years in males. Patients who successfully underwent surgical resection had a considerably longer median survival (237 months vs. 215 months, $p < 0.001$) as well as a significantly improved five-year survival (72.4% vs. 52.6%, $p < 0.001$). Univariate analysis demonstrated that age, gender, ethnicity, primary tumor site, WHO Grade and surgical resection were significant predictors of improved survival for ependymoma patients. Multivariate analysis identified that a WHO Grade III tumor, male gender, patient age, intracranial tumor locations and failure to undergo surgical resection were independent predictors of poorer outcomes. Multivariate analysis of partially resection cases revealed that lack of radiation was a sign of poor prognosis (HR 1.748, $p = 0.024$).

Conclusion: Surgical extirpation of ependymomas is associated with significantly improved patient survival. For partially resected tumors, radiation therapy provides significant survival benefit.

Malignant Abdominal Mesothelioma: Defining the role of surgery

Dayron Rodriguez
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011

Dr. Leonidas G. Koniaris
Division of Surgical Oncology, DeWitt Daughtry Family
Department of Surgery, University of Miami School of Medicine

Objective: Determine the role of surgery for patients with malignant abdominal mesotheliomas (MAM).

Methods: The Surveillance, Epidemiology, and End Results (SEER) database (1973-2005) was queried.

Results: Overall, 10,589 cases of malignant mesotheliomas were identified. Of these, 9211 cases were thoracic (TM) and 1112 cases were MAM (10.5%). Patients with TM presented with more localized disease than those patients with MAM ($p < 0.001$). MAM more often affected younger patients (63 years vs. 71 years) ($p < 0.001$). The annual incidence of MAM was approximately 1.00 case per 100,000 in 2005. Overall median survival for MAM patients was 8 months, with a significant difference between women and men (13 months vs. 6 months, respectively) ($p <0.001$). Patients who successfully underwent surgical resection had a considerably longer median survival (20 months vs. 4 months, $p < 0.001$) as well as a significantly higher five-year survival (28% vs. 12%, $p < 0.001$). Multivariate analysis identified that a poorly differentiated tumor grade, failure to undertake surgical resection, advanced age, and male gender were all independent predictors of poorer outcome.

Conclusion: Surgical extirpation of MAM may be associated with significantly improved survival. All patients with MAM should be evaluated for potential surgical resection.

Self-Reported Anxiety and Atherosclerotic Progression and Clinical Events Among Patients with Coronary Artery Bypass Grafts

Joshua I. Rosenbloom, MPH
Harvard Medical School, Francis Weld Peabody Society, Class of 2011

Murray A. Mittleman, MD, DrPH
Cardiovascular Epidemiology Research Unit
Beth Israel Deaconess Medical Center

Symptoms of anxiety have been associated with increased risk of coronary artery disease (CAD) and potentially poor prognosis among patients with existing CAD, but whether symptoms of anxiety specifically influence atherosclerotic progression among such patients is uncertain**.**

The Post-CABG Trial randomized patients with a history of CABG surgery to either an aggressive or moderate lipid lowering strategy and to either warfarin or placebo. Coronary angiography was conducted at enrollment and after a median follow up of 4.3 years and patients were followed up for clinical endpoints. Symptoms of anxiety were assessed at enrollment using the state portion of the Spielberger State-Trait Anxiety Inventory (STAI) in 1317 patients. In models adjusting for age, gender, race, treatment assignment and years since CABG surgery, a STAI score ≥ 40 was positively associated with risk of death or MI (odds ratio: 1.55; 95% CI: 1.01, 2.36; p=0.044). This association was attenuated slightly when depressive symptoms were included in the model. There was a statistically significant dose-response relationship between STAI score and risk of death or MI. There was no association between self-reported anxiety and atherosclerotic progression of saphenous vein grafts.

These findings suggest that anxiety symptoms are associated with increased risk of death or MI among patients with saphenous vein grafts, but that this risk does not appear to be mediated by more extensive atherosclerotic progression. More research is needed to understand the pathway of this association.

The Burden of Menthol Cigarettes: The Tobacco Intake and Dependence

Judith Rosenbloom
Harvard School of Dental Medicine, Walter Bradford Cannon Society, Class of 2011

Taru Kinnunen, MA, PhD
Tobacco Dependence Treatment and Research, Department of Oral Health Policy and Epidemiology, Harvard School of Dental Medicine

Black and female smoker sub-groups are the highest consumers of mentholated cigarettes internationally. While these subgroups report smoking fewer cigarettes per day (CPD) they tend to experience greater tobacco-related health consequences. This increased burden may be explained by higher nicotine dependence, which may in turn contribute to greater tobacco toxin exposure.

The present study examined estimated daily consumption of nicotine, tar, and carbon monoxide (CO) intake (FTC yields multiplied by CPD) and tobacco dependence among black and white menthol and non-menthol female smokers. 869 (70% white, 20% black; mean age of 41 years) potential smoking cessation research trial participants provided phone interview data regarding their CPD, time to the first cigarette (TTF), length of the longest quit attempt, and the name and style of their preferred cigarette brand.

Analysis of variance revealed significant main effects of cigarette mentholation on measures of estimated daily tar (213.0 ±1 23.5 non-menthol vs. 250.7 ± 131.8 menthol); daily nicotine (17.0 ± 10.2 non-menthol vs. 22.1 ± 23.8 menthol), and daily CO (219.1 ± 115.7 non-menthol vs. 243.4 ± 22.6 menthol). Similarly, significantly higher proportions of menthol vs. non-menthol smokers reported low TTF (50.2% vs. 43.7%; $p<.01$) and longest quit length of less than 90 days (56.6 vs. 47.2). Black menthol smokers reported significantly fewer CPD (14.7 ± 8.7) than white menthol 18.72 ± 7.4 or non-menthol smokers(19.2±8.1)(ps<.01)

Among menthol smokers, lower CPD masked higher estimated tobacco toxin intake and tobacco dependence. Because cessation is compromised by higher dependence, menthol smokers may be exposed to a greater disease burden than non-menthol smokers not only by current, but also lifetime toxin intake.

Protein Interaction Mapping in Crohn's Association Results May Have Predictive Power

Elizabeth Rossin
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Mark J. Daly, PhD
Center for Human Genetics Research, Massachusetts General Hospital

Genome-wide association studies (GWAS) have identified over 150 common variants that contribute to risk for genetic diseases such as heart disease, rheumatoid arthritis, inflammatory bowel disease, and type 2 diabetes. The identities of the underlying causal variants are still largely unknown, as are their modes of action. In aggregate, however, variants associated with a disease presumably flag causal variants that influence phenotype by perturbing genes or functional elements involved a set of biological mechanisms. Additionally, these associated variants usually explain only a portion of the risk for disease, implying that there are other loci yet to be discovered that may enhance our understanding of disease biology.

To understand whether associated loci are functionally connected, we searched for protein-protein interactions among the protein products of genes resident in the 32 loci previously associated to Crohn's disease. We were able to connect 16 of 32 loci either because resident genes' products directly bind or have a common binding partner mapping elsewhere in the genome (a common interactor). This network is significant in its mean connectedness ($p=.0013$) and appears to describe the JAK2/STAT3 signaling cascade downstream of IL23R, a pathway known to play a role in Crohn's disease. We were also surprised to find that SNPs in the common interactors' loci showed a marked excess of association signal (20.2% of common interactors were in the top 5% of all scored genes, which appears to be better than random permutation, $p=0.0090$), suggesting new candidates for replication. To test the apparent predictive power of this network, we are attempting to replicate the 7 strongest common interactor associations. In 264 affected offspring trios genotyped to date, we are able to replicate one association, in the vicinity of the *MST1R* gene ($p=0.042$ corrected for multiple testing). We are presently genotyping all 7 SNPs in a much larger sample collection which should be adequately powered to detect any other effects.

We therefore suggest that protein interaction mapping may be a powerful method for biological interpretation of GWAS results. The approach enables us to prioritize candidate genes within associated loci based on their participation in networks, through which causal variants exert their effects. In addition to ongoing replication efforts, we are also attempting similar analyses with other types of functional data (such as regulatory information) and with GWAS data from other diseases. We are confident that these approaches will greatly enhance the interpretation association results and our mechanistic understanding of disease.

Determining Unmet Needs in Malaria Treatment and Prevention in Rural India

Saurabh Saluja
Harvard Medical School, William Bosworth Castle Society, Class of 2011

Arun Mathur, MD, MBBS
Choti Sadri Community Health Center, Rajasthan, India

Despite age-old knowledge of how to treat and prevent malaria, infection with Plasmodium falciparum and vivax remain prevalent in much of rural India. In June of 2008 I traveled to rural Rajasthan, India to conduct a needs-assessment on malaria treatment and prevention for a small government-run facility in the southern part of the state. The protocol of the study involved interviewing hospital physicians, surveying ancillary staff at village subcenters, and reviewing archival data. Data were analyzed and compiled to prepare a report detailing the state of malaria treatment and prevention in the area surrounding the government-run facility. Additionally, local treatment protocols were compared to World Health Organization (WHO) guidelines and a cost analysis was performed to determine the difference between the protocols. Results from the research period are forthcoming. Preliminary data show a decreasing trend in malaria cases in the region dating back to the 1980s when data collection began. Data also suggests that the cost of implementing WHO treatment standards would vary depending on whether presumptive treatment of fever cases continue in the region or not. Even with the highest cost estimates, the benefit in terms of decreased resistance and remission may is likely to outweigh the cost of improving standards. Future studies in the region are necessary to advance the preliminary work done in this research period.

Effects of Sleep Deprivation on Blood Pressure and Vascular Cellular Adhesion Molecules

Alejandro Sanchez
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

Janet M. Mullington, PhD
Sleep and Chronobiology Unit, Department of Neurobiology
Beth Israel Deaconess Medical Center

Epidemiological studies link short sleep with an increased relative risk for developing diabetes and cardiovascular disease. Experimental sleep deprivation leads to a small elevation in inflammatory mediators and BP. The mechanisms by which these changes occur, and their overall effects on long-term health, are not fully understood. We investigated the changes in BP and soluble vascular cellular adhesion molecule-1 over the course of prolonged total sleep deprivation (TSD) and recovery sleep. We hypothesized that sleep deprivation causes an increase in vascular cellular adhesion molecules due to an increase in blood pressure.

Subjects were 22 healthy participants (7 females, 15 males, mean age 34.4 ± 1.7 yrs, no current history of any medical disorders, habitual sleep duration between 6.5 and 9.0 hrs). Systolic blood pressure (SBP) was analyzed for 21 and plasma sVCAM-1 levels analyzed for 22 participants. Starting on the 2nd baseline day, participants were equipped with recording devices to monitor blood pressure, temperature, EEG; blood and urine samples were also sampled. On the 3rd day participants were randomized to sleep or sleep deprivation conditions. Lights and posture were controlled during the 7 day in-hospital stay, and participants were closely monitored by an experimenter throughout the waking periods of the study to prevent any non-scheduled sleep episodes. Nocturnal blood pressure was measured using the Portapress System (continually measures blood pressure on a beat to beat basis). sVCAM-1 was measured in serum using Enzyme-Linked ImmunoSorbent Assay (ELISA) methodology (R&D Systems). sVCAM-1 was assayed from the 05:30 am fasting blood sample taken during sleep/sleep deprivation at baseline and through the 3 deprivation nights and during recovery sleep. Mixed models analysis of variance was used to statistically analyze the systolic blood pressure (SBP) and the sVCAM-1 data.

After our initial analysis we found that SBP increased significantly during deprivation at night for all sleep deprived subjects (on average from 112 to 135 mmHg for the sleep deprivation, compared with 111 to 110 mmHg for the sleep condition)($p<0.05$). sVCAM-1 levels significantly increased during the three nights of sleep deprivation by > 90 ng/ml over the normal sleep control ($p<0.05$) and remained elevated during the recovery sleep. sVCAM-1 increases were correlated with increased SBP levels on the second night of sleep deprivation ($r=0.04$; $p<0.05$).

In conclusion, sleep deprivation increases SBP and sVCAM-1. This elevation in blood pressure observed during sleep deprivation may lead to the increase seen in sVCAM-1.

Facial Growth in Children with Complete Clefting of the Primary Palate and Intact Secondary Palate

Elliot L. Saperstein
Harvard School of Dental Medicine, Oliver Wendell Holmes Society, Class of 2011

Bonnie L. Padwa, DMD, MD
Department of Plastic Surgery, Section of Oral and Maxillofacial Surgery
Children's Hospital Boston

Background: Patients with unoperated cleft lip and/or palate have normal facial growth. However, midfacial retrusion often occurs in patients with repaired cleft lip/palate but the etiology of abnormal facial growth is not well understood. A few studies suggest that there is abnormal intrinsic midfacial growth in patients with cleft lip and/or palate. A number of studies suggest lip repair is responsible for the deficient growth while others indicate the palate closure as the cause of the midfacial retrusion. The aim of this study is to better understand facial growth in patients with cleft lip/palate by looking at two subsets of patients; those with a unilateral or bilateral complete cleft lip/alveolus and an intact secondary palate (UCCLA, BCCLA) and those with a unilateral or bilateral complete cleft lip/palate (UCCLP, BCCLP). The only difference in treatment between these two patient groups is palatal closure. Hence comparison may provide insight into the mechanism for impaired facial growth due to surgical repair.

Methods: Retrospective, cross-sectional study of seventy-three patients (36 males and 37 females) with UCCLA, UCCLP, BCCLA, and BCCLP with a mean age of 11.12 (range 6-16) treated at Children's Hospital Boston. Inclusion criteria entail patients who had a lateral cephalogram after age 5 years and primary cleft repair by one surgeon. Lateral cephalograms were traced and angular and linear measurements of the midfacial region were made. A Student t-test was used to evaluate growth differences among the groups.

Results: Seventeen maxillary measurements and three mandibular measurements were analyzed. Patients with UCCLP and BCCLP had maxillary size and position that were significantly smaller and more retruded than patients with UCCLA and BCCLA ($p < 0.05$). There was no difference in mandibular position between groups. There were no significant differences in maxillary size and position when comparing UCCLA to BCCLA and UCCLP to BCCLP.

Conclusion: This study showed that palatal repair may be responsible for midfacial retrusion in patients with cleft lip/palate.

The Role of MicroRNAs in Vascular Inflammation

Andrew J. Schissler
Harvard Medical School, Francis Weld Peabody Society, Class of 2011
AHA Student Scholarship in Cardiovascular Disease and Stroke

Basak Icli, PhD and Mark W. Feinberg, MD
Feinberg Lab, Cardiology Division, Department of Medicine
Brigham and Women's Hospital/Harvard Medical School

Background: The vascular endothelium is critically involved in the response to inflammation. Pro-inflammatory stimuli modulate endothelial phenotype by promoting immune cell recruitment and adhesion, effects that accelerate the development of atherosclerosis. In contrast, HMG-CoA reductase inhibitors (statins) confer anti-adhesive, anti-proliferative, anti-thrombotic, and anti-oxidant properties to the endothelium. Identification of regulatory factors that mediate the effects of these opposing stimuli is of considerable interest. MicroRNAs (miRs) are endogenous 20-22-nucleotide RNAs capable of regulating complementary mRNAs. Recent studies have shown the importance of miRs in cardiovascular biology, though their function in vascular inflammation remains relatively unknown. We undertook a profiling approach to assess global patterns of miR expression and identified miR-26a as being one of the most differentially regulated under pro- and anti-inflammatory conditions. Specifically, we noted that miR-26a was induced by statins and inhibited by TNF-α. Using available bioinformatics, we identified potential miR-26a target genes, including the bone morphogenic protein (BMP) signaling effector Smad1, a critical upstream modulator of the cell cycle inhibitor $p21^{WAF1}$.

Hypothesis &Aims: Based on these findings, we hypothesize that miR-26a may play an important role in regulating vascular endothelial cell inflammatory signaling. Further, we explore the functional effects of miR-26a on endothelial cell growth and migration.

Methods & Results: We first validated miR-26a's expression in human umbilical vein endothelial cells (HUVECs) and immune cells at baseline using qPCR. We further showed that miR-26a is potently reduced by pro-inflammatory stimuli (IFN-γ and TNF-α), and increased by anti-inflammatory stimuli (statins and TGF-β). Indeed, miR-26a expression levels were markedly decreased (~-86%) in aortas from atherosclerotic-prone ApoE-/- mice in comparison to wild-type aortas by qPCR. To explore if miR-26a regulates Smad1, we transfected HUVECs with miR-26a mimics or non-specific controls (Ctrl) and examined target gene protein expression levels using Western blot analysis. In comparison to Ctrl, we found that miR-26a mimics nearly abolished Smad1 expression and increased $p21^{WAF1}$ levels. Consistently, miR-26a decreased endothelial cell growth, migration, and tube formation. Finally, preliminary data suggests that miR-26a disrupts NF- κB signaling in response to TNF-α and reduces the induction of ICAM-1 and VCAM-1.

Conclusions: These results suggest that increased miR-26a expression in endothelial cells may be protective by regulating key aspects of vascular inflammatory cell signaling and function. These studies may lead to novel therapeutic strategies for the treatment of chronic inflammatory states such as atherosclerosis.

Quality Improvement Strategies to Improve Partograph Use in a Public Hospital in Bani, Dominican Republic

Dominika L. Seidman
Harvard Medical School, William Bosworth Castle Society, Class of 2009

Kim Wilson, MD MPH
International Pediatric Center
Children's Hospital Boston

While 97% of women in the Dominican Republic (DR) give birth in a health facility, maternal mortality ratios are significantly higher than predicted given such high rates of institutionalized delivery. Infante Sano, an international non-governmental organization, hypothesized that poor quality of care leads to this paradox. Consequently, Infante Sano focused on use of the partograph to improve the quality of obstetrics services in a Dominican public health facility. The partograph is a paper chart that when used correctly, serves as an inexpensive and effective tool to monitor labor and reduce maternal and neonatal morbidity and mortality.

Infante Sano began work in Hospital Nuestra Senora de Regla, a public provincial hospital in Bani, DR where 3000 births occur annually. A team of 2 obstetricians, 1 general doctor, 1 nurse, and an Infante Sano representative formed a quality improvement team. Infante Sano trained the team in quality improvement techniques, and the team planned and led implementation of the partograph.

The team defined the problem as one of poor partograph use, and surveyed hospital staff to identify barriers to implementaiton. "Inefficient use of staff" and "material deficiencies that impeded efficient work" were identified as major obstacles. The group promoted a cooperative approach to partograph use by defining nurse/general physician/obstetrician roles, and training all staff in partograph use. The team also developed new training materials and memory aids, and reformatted the partograph to improve correct use.

Infante Sano developed a monitoring tool to assess individual parameters of the partograph according to international standards. The team reviewed 20 partographs every 3 months. Records were selected with attention to variation in time of day and provider. Accuracy of partograph completion was confirmed through review of medical records. Monitoring results were compared before and after the team's intervetions.

At Infante Sano's initial site assessment, no partographs were completed at Hospital Nuestra Senora de Regla. At 3 month follow-up, 75% of records had partographs, of which 30% were adequately completed. At 6 month follow-up, 95% of records had partographs, of which 40% were adequately completed.

Partograph use can be improved through the formation of quality improvement teams in public hospitals in the Dominican Republic. More research must be done to determine whether improved partograph use correlates with improved quality of care and reduced maternal mortality in Dominican public health facilities.

International Influences on Stem Cell Scientists in Singapore

Diane Shao
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Michael M.J. Fischer, PhD
Professor of Anthropology and Science and Technology Studies
Massachusetts Instutite of Technology

Stem cell science in Singapore epitomizes the ideals of global scientific collaboration. Prominent investigators from across the world have been drawn into its ranks, bringing cutting edge technology and ties to their previous institutions. The backgrounds of principal investigators influence the collaborations and opportunities of trainees working in their laboratories. The global nature of science fosters great work, but also has drawbacks for scientific training. Through participant observation and informal interviews, I address the dynamics of a prominent stem cell laboratory in Singapore in which a foreign-trained principal investigator divides his time between two laboratories across the world – one in Singapore and one in Harvard Medical School.

Patient Satisfaction in DIEP Flap Breast Reconstruction: A comparative evaluation with TRAM flap, latissimus flap, and implant techniques

Shira S. Simon
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2012

Bernard T. Lee, MD
Division of Plastic and Reconstructive Surgery, Department of Surgery
Beth Israel Deaconess Medical Center

Breast reconstruction is an essential component of the overall treatment plan for patients considering mastectomy. As perforator flap techniques, such as the deep inferior epigastric perforator (DIEP) flap, continue to gain in popularity there is still no consensus on the optimal method of reconstruction. The purpose of this study is to compare patient satisfaction with the DIEP flap to the more commonly practiced techniques in breast reconstruction and identify factors influencing satisfaction.

A retrospective chart review was performed on all women who had undergone postmastectomy breast reconstruction at an academic teaching hospital. A total of 615 patients who had primary reconstruction with tissue expander/implant, latissimus dorsi flap, pedicled or free transverse rectus abdominis myocutaneous (TRAM) flap, and DIEP flap were identified between the years of 1999 and 2006. A questionnaire was designed to collect the following information from patients after reconstruction: (1) demographic information, (2) general and aesthetic satisfaction developed by the Michigan Breast Reconstruction Outcome Study[1], and (3) health related quality of life provided by the SF-12v2 (QualityMetric, Lincoln, RI).

Patient response to the questionnaire was 75%, with 462 completed questionnaires returned. This group included 87 tissue expander/implant patients, 115 latissimus dorsi flap patients, 143 TRAM flap patients, and 117 DIEP flap patients. There was no significant difference in the quality of life scores among the four groups. In comparison to all the other reconstructive procedures, the DIEP flap patients had the highest level of general and aesthetic satisfaction ($p<0.001$ and $p=0.001$)(Table 1). Autologous reconstruction had significantly higher general and aesthetic satisfaction rates in comparison to implant-based reconstruction ($p=0.026$ and $p<0.001$). Reconstruction using an abdominal donor site had significantly higher general and aesthetic satisfaction rates than latissimus flap reconstruction ($p=0.002$ and $p=0.033$). When compared directly with the TRAM flap group, the DIEP flap group had a higher general satisfaction rate ($p=0.031$); however, the aesthetic satisfaction rate was similar. Besides the type of reconstruction chosen, other significant covariates on patient satisfaction included quality of life and length of time since surgery.

Breast reconstruction with the DIEP flap technique has the highest patient satisfaction rates among the four types of reconstructions evaluated. Although reconstruction based on an abdominal donor site revealed higher overall levels of satisfaction, the DIEP flap patients had a higher general satisfaction level when compared to a traditional TRAM flap. Discussing satisfaction outcomes with patients will help in making educated decisions about breast reconstruction.

PGC-1α May Promote Tumor Growth through Activation of ERRα Mediated VEGF Transcription

Jonathan Evan Shoag
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Zoltan Arany, MD PhD
Cardiology Division, Department of Medicine
Beth Israel Deaconess Medical Center

Angiogenesis, the creation of new blood vessels, has emerged as a central process in the growth of solid tumors. Tumors recruit a blood supply via the release of pro-angiogenic proteins, notably vascular endothelial growth factor (VEGF). Our group has recently reported a novel pathway for VEGF transcription regulated by the transcriptional coactivator PGC-1α. It was found that in skeletal muscle hypoxia induces PGC-1α, which is then able to induce VEGF expression. It was also demonstrated that PGC-1 acted independently of the canonical Hypoxia-Inducible Factor (HIF-1) pathway via binding to the transcription factor Estrogen-Related Receptor-α (ERRα).

We sought to examine the role of PGC-1α in tumor angiogenesis. We found that a mouse melanoma cell line (B16F10) upregulated both PGC-1α and VEGF in response to nutrient deprivation. Furthermore, retroviral expression of PGC-1α in this cell line was sufficient to induce VEGF transcription.

In order to investigate this pathway in human tumors, a human colon cancer cell line, Colo-205, was identified via an in-silico based approach. Using a lentiviral expression system, we found that short-hairpins directed against PGC-1α, PGC-1β (a protein closely related to PGC-1α), and ERRα were able to substantially inhibit baseline VEGF expression.

We also found that exposure of this cell line to nutrient deprivation dramatically induced the expression of both PGC-1α and VEGF; while cells expressing short-hairpins against PGC-1α showed a markedly diminished VEGF response. We then investigated the mechanism by which PGC-1α is induced in response to nutrient deprivation, via treatment with various cell-signaling inhibitors. Notably, inhibitors of Mitogen-Activated Protein Kinase (MAPK) completely abrogated both the PGC-1α and VEGF responses to nutrient deprivation.

The host microenvironment has also been shown be an important source of pro-angiogenic factors. We therefore explored the role of PGC-1α in the host by injecting PGC-1α deficient mice with B16F10 and Lewis-Lung Carcinoma (LLC) cells. We found that PGC-1α deficient mice develop substantially smaller tumors than wild type mice in this context.

These data therefore indicate that PGC-1α plays a substantial role in tumor angiogenesis. Both mouse and human tumor cell lines utilize the PGC-1α pro-angiogenic pathway. This pathway appears to be crucial for VEGF induction in response to some physiologic stimuli, and we have begun to elucidate the molecular mechanism of PGC-1α induction. This pathway could therefore be an attractive target for cancer therapy as it may contribute to both tumor and stromal angiogenic responses.

Dopaminergic Differentiation of Filum Terminale Derived Neurospheres

Peter A. Soden
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

David L. Cardozo, Ph.D.
Department of Neurobiology
Harvard Medical School

Background: The *filum terminale* (*FT*) has recently been shown to be a novel source of neural progenitor cells (neurospheres) in both rats and humans. It is also an accessible vestigial structure at the caudal end of the spinal cord that plays no role in the human post-natal nervous system. Both of these statements make the multipotent neurosphere's, derived from FT tissue, of great clinical interest due to their potential use in neurodegenerative disorders. For example, replacement of the degenerated dopamine (DA) secreting neurons of the substantia nigra in the midbrain could help resolve the symptoms of Parkinson's disease.

Purpose/Hypothesis: This project is testing whether a standard stem cell medium with Fetal Bovine Serum can be used to differentiate FT derived neurospheres into dopamine secreting neurons. We hypothesized that this medium would cause neuronal differentiation but would not lead to formation of dopamine secreting neurons.

Methods: FT derived neurospheres were grown from resected FT tissue from P5 rats over the course of 9 days in a standard stem cell medium containing growth factors basic Fibroblast Growth Factor (bFGF), Leukemia Inhibitory Factor (LIF), and Epidermal Growth Factor (EGF), as well as Amphotericin B to control for contamination. At the end of 9 days four neuropsheres were siphoned out and plated on four Poly-L-Lysine/Laminin coated cover slips. A minimal amount of extracellular matrix and standard stem cell medium (same as mentioned above) was added to each cover slip to prevent dessication. The following day each plated neurosphere was immersed in the same standard stem cell medium with the addition of 10% Fetal Bovine Serum. After 13 days of incubation the plated cells were fixed and immunocytochemistry was used to stain for β-Tubulin III and Tyrosine Hydroxylase.

Results: All four plated neurospheres showed high levels of β-Tubulin III staining illustrating that neuronal differentiation did occur. However, none of the four cover slips stained positive for Tyrosine Hydroxylase.

Conclusions: The presence of β-Tubulin III showed that the differentiated neurospheres had neuronal qualities and the absence of Tyrosine Hydroxylase demonstrated that they did not have the required trait to be DA neurons. Further research using dopamine differentiation specific media needs to be conducted on this novel source of neural stem cells to continue assessing its multipotent properties.

Mechanical and Mesenchymal Mechanisms of Secondary Cartilage Induction

R. Christian Solem
Harvard School of Dental Medicine, Oliver Wendell Holmes Society, Class of 2010

Richard Schneider, Ph.D.
Department of Orthopaedic Surgery
University of California at San Francisco

Strategies for engineering tissue replacements or for devising molecular and cell-based therapies to treat articular cartilage damaged by injury or degenerative joint disease can benefit from understanding mechanical and developmental mechanisms that control the induction and maintenance of secondary cartilage. Secondary cartilage arises subsequent to osteogenesis and after the formation of the primary cartilaginous skeleton, which is typically replaced by bone. Secondary cartilages are found within joints, ligaments, and tendons, which points to their importance for proper kinetic movement of the skeleton. Much evidence indicates that initiation of secondary chondrogenesis relies on mechanical force and accordingly, the evolutionary presence or absence of secondary cartilage reflects species-specific variations in functional anatomy.

To investigate molecular, cellular, and biomechanical mechanisms that regulate secondary cartilage formation, we conducted a series of experiments using two species of quail and duck, which exhibit considerably different musculoskeletal morphologies. In conjunction with their distinct mode of feeding via levered straining, duck develop a pronounced secondary cartilage at the insertion (or enthesis) of the jaw adductor muscle on the mandible. An equivalent cartilage is absent in quail, which peck at their food. We hypothesized that species-specific differences in jaw morphology and a concomitant dissimilarity in the local mechanical environment promote formation of secondary cartilage in duck versus quail.

To test our hypothesis we employed two experimental approaches. First, we altered the mechanical environment in duck by paralyzing the skeletal musculature and by blocking mechanotransduction through stretch activated channels. Second, we transformed the duck jaw to resemble that found in quail. We accomplished this by transplanting from quail to duck the mesenchymal precursor cells destined to form the skeletal and connective tissues of the jaw. Such mesenchyme is known to generate species-specific pattern in chimeric "quck". Both experimental approaches resulted in a loss of secondary cartilage in duck and chimeric quck as evidenced by anatomical, histological, and immunohistochemical data.

To understand the molecular basis for such alterations, we analyzed the expression of genes associated with the development of cartilage, bone, muscle, and tendon in control quail and duck, paralyzed duck, and chimeric quck embryos. Secondary chondrogenesis was mediated by the expression of *sox9*, *fgfr2*, and *bmp4* only under mechanical load. This response correlates with local mechanical forces that vary according to the species-specific anatomy of the musculoskeletal complex. Our experiments demonstrate that mesenchyme-dependent changes in gene expression, musculoskeletal pattern, and ultimately mechanical forces, control the induction of secondary cartilage.

The SUCCEED Trial:
A collaborative care program to improve depression treatment in cardiac patients

Gillian Sowden
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

Jeff Huffman, MD
Psychiatry Department, Massachusetts General Hospital

Background: Major depression is common in patients with acute cardiac disease, occurring in approximately 15-20% of hospitalized cardiac patients. Depression has serious effects on a patient's prognosis after an acute cardiac event, adversely affecting quality of life, as well as significantly increasing the risk of mortality. Despite its high prevalence and serious consequences, however, depression in cardiac patients commonly goes undiagnosed and untreated, even though treatment of depression in cardiac patients has been shown to be both safe and effective. Studies have shown that a 2-item screening tool is effective in identifying depressed individuals and that collaborative care models, which coordinate care between psychiatrists and medical treatment teams, can improve rates of depression treatment in outpatient settings.

Objectives: The goal of the SUCCEED trial is to determine the efficacy of using a 2-item depression screening tool to diagnose depression in hospitalized cardiac patients, and whether a collaborative care depression management program improves outcomes at follow-up.

Methods: Patients admitted to MGH with acute cardiac disease undergo a two-item depression screen (PHQ-2) as part of the initial nursing interview. When positive, a study social work care manager meets with the patient and performs a formal standardized interview for depression (PHQ-9). Patients diagnosed with clinical depression are randomized to either usual care or collaborative, and depression levels and medical outcomes are measured at 2,6,12 and 24 weeks post-enrollment.

Results: Nurses are screening approximately 75% of hospitalized cardiac patients, of whom approximately 7% are diagnosed with a clinical depression. Approximately 80% of patients in the collaborative care group receive adequate depression treatment upon discharge, compared with only 10% of patients in the usual care group. Six weeks after enrollment, 70% of collaborative care patients are in recovery and only 11% depressed, compared with only 35% of usual care patients in recovery and 35% still depressed.

Conclusions: A 2-item depression screen as part of the nursing interview is an efficient way to detect depression in cardiac patients, although this tool may need to be refined to improve its sensitivity. Furthermore, a collaborative care model appears effective at improving depression levels in depressed cardiac patients at 6-week follow-up, although more work is needed to determine whether this is effective and feasible in the long-term.

The 'Critical Thinking' Concept: What is it in medicine?

Jared Sprague
Harvard School of Dental Medicine, Walter Bradford Cannon Society, Class of 2011

Edward Krupat, PhD
Director, Center for Evaluation
Harvard Medical School

Medical educators strive to instill the knowledge, skills and attitudes necessary for professionals to thrive in the modern era of medicine. Rather than focusing curricula on facts, educators are emphasizing the *process* of learning to develop in students the most important outcomes of an education. One of these outcomes is to develop life-long 'critical thinkers', who will practice evidence-based medicine through more and more complex issues and situations. It is clear that 'critical thinking' is important, however, it is not clear what 'critical thinking' actually is. Professional descriptions of 'critical thinking', obtained through a survey to clinicians, shows that there is still considerable variety as to what 'critical thinking' means.

The survey that was used for this research was relatively simple. It asked what the responder defines 'critical thinking' to be. It also had the responder define a clinical scenario, where 'critical thinking' was important. The responder needed to delineate differences in actions and words of someone who engaged in 'critical thinking' versus someone who did not. Then, they were asked to provide the respective outcomes.

The aim of the study was to allow a wide path of perceptions and possibilities to be incorporated into the answers. The results of the survey show many classic skills of 'critical thinking', including: analyzing, synthesizing, utilizing, reasoning, prioritizing, implementing, integrating, and judging. 'Critical thinking' is additionally described as: creative, systematic, rigorous, practical, reflective, rational, analytical, accurate, deliberate, and clear. The results also link attitudes with 'critical thinking,' such as: courage, careful attention, vigilance, and flexibility. And more than what 'critical thinking' is, some hinted that 'critical thinking' could be anything, solely depending on what is needed and respective circumstances.

While many respondents described a process closely linked with wisdom, the overall and general form of 'critical thinking' focused on informational processing. The generic model of response required the clinician to be able to collect, understand, and manipulate information in such a way that results in desirable outcomes.

Fast Responses Without False Starts: Dorsal anterior cingulate cortex in simple reactive behavior

Lakshminarayan Srinivasan, PhD
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2009

Emad N. Eskandar, MD
Center for Nervous System Repair
Department of Neurosurgery
Massachusetts General Hospital

Survival in life and success in sports demand fast reaction times to simple cues without false starts. However, the precise mechanism by which the human brain achieves rapid and reliable reactive behavior remains unknown. The anterior cingulate cortex (ACC) is ideally positioned to play a prime role in action timing, because it directly connects with cognitive, motor, and arousal systems.

Here we demonstrate that bilateral surgical ablation of the dorsal anterior cingulate cortex (dACC) in human subjects immediately and dramatically disrupts the control of movement onset timing in reaction to a simple cue, increasing false starts and slowing reaction times in apparent violation of the speed-accuracy tradeoff. This paradox is resolved by a drift-diffusion model of dACC as a switch that permits movement execution only when detection, decision making, and motor planning processes are complete. These findings demonstrate a causal role for human dACC in action timing for simple reactive behavior.

Center of Pressure Dynamics in Parkinson's Disease Patients with Freezing of Gait: Failed postural adjustments?

Jennifer M. Srygley
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2009
Sinclair Kennedy Traveling Fellowship

Jeffrey M. Hausdorff, PhD
Harvard Medical School and Tel Aviv University
Movement Disorders Unit, Sourasky Medical Center

Parkinson's disease (PD) is a neurodegenerative disease with myriad symptoms, the most prominent of which affect gait and movement. Freezing of gait (FOG) is a symptom of PD in which the patient is unable to initiate or continue normal forward motion and feels as if the feet are glued to the ground. FOG can be a debilitating symptom and correlates with adverse measures of quality of life, increased risk of falls, and higher rates of nursing home admission. While several aspects of FOG have been studied, the underlying pathophysiology of FOG remains unclear. The purpose of this study was to obtain simultaneous recordings of force magnitude and center of pressure (COP) dynamics during freezing episodes in order to better understand the possible pathophysiologic mechanisms involved in FOG.

Thirty subjects with PD performed three 2-min. walks back and forth along a 20m corridor, with a 180º turn at each end while "on" anti-parkinsonian medicaitons. All participants wore piezoelectric force-sensitive insoles that use an array of sensors to measure the forces under the feet.

Twenty-seven FOG events were observed and all occurred while subjects were turning. During freezing episodes, several features of the force and COP dynamics were consistently noted. FOG episodes were characterized by a rapid, alternating pattern of weight shifts from foot to foot at a frequency of 2-4 Hz. Whereas the occurrence of FOG was accompanied by a shift of the COP to the anterior part of the foot, the termination of the episode was marked by a posterior weight shift. Thus, freezing ended only when all of bodyweight was borne by one foot, with the COP in a relatively posterior position. This gradual backward COP shift, superimposed on the back-and-forth weight shifts gave freezing episodes a characteristic signature. Freezing episodes clinically characterized by shuffling gait also displayed the same pattern of progressive backshift of the COP as that seen in those without foot lifting. In contrast, a pattern of oscillatory weight-shifts was never observed during turns that were executed without freezing.

Prior to this study, data on COP dynamics had not been recorded during a freezing episode. We observed a characteristic pattern of force and COP dynamics during FOG episodes that was consistent between subjects and events. This pattern closely resembles multiple failed anticipatory postural adjustments (APAs). While a single APA is required to initiate a step from quiet standing, this pattern of multiple, failed APAs during freezing suggests that impairments in motor planning may contribute to FOG episodes. The act of turning seems to especially challenge to patients with PD, and to trigger FOG episodes, in part because of deteriorations in these patients' motor planning systems. Further study of this phenomenon may help illuminate the pathophysiologic mechanisms of FOG and, ultimately, lead to the development of new strategies for preventing or shortening FOG episodes.

Degree of Right Hand Dominance Predicts Differential Patterns of Intrinsic Functional Connectivity during Rest

David E. Stark
Harvard Medical School, Francis Weld Peabody Society, Class of 2009

F. Xavier Castellanos
Phyllis Green and Randolph Cōwen Institute for Pediatric Neuroscience at the NYU Child Study Center

Given functional and anatomical differences associated with handedness, neuropsychological and neuroimaging studies of psychiatric illness commonly limit population heterogeneity by excluding left-hand dominant and ambidextrous participants. However, as demonstrated by handedness inventories, right-hand dominance is more complete in some individuals than others. Here, we examine the extent to which the functional connectivity of motor, language, somatosensory and visual attention regions varies among right-handers, depending upon degree of hand dominance.

For each of 39 healthy right-handed participants, a 6.5 minute functional magnetic resonance imaging (fMRI) scan obtained during rest was acquired. Participants also completed a 22-item modified Edinburgh Handedness Inventory, providing a laterality quotient for each individual. Functional connectivity analyses utilized each of the following *a priori* regions of interest (Harvard-Oxford Structural Atlas): precentral and postcentral gyrus, supplementary motor areas, superior parietal lobule, supramarginal gyrus, inferior frontal gyrus, and planum temporale. For each region of interest, we examined the relationship between laterality quotient and voxelwise measures of functional connectivity using mixed-effects analyses (FLAME; clusterwise correction: $Z > 2.3$; $p < 0.05$, corrected).

Stronger right-hand dominance was associated with greater functional connectivity between each of the following regions and the lingual gyrus: precentral gyrus, postcentral gyrus, supramarginal gyrus, superior parietal lobule, right supplementary motor area, and right planum temporale.

Our results suggest that motor, somatosensory and visual attention regions all exhibit greater functional connectivity with the lingual gyrus among individuals with greater right-hand dominance. This pattern verifies the potential impact of heterogeneity among right-handers on functional connectivity and demonstrates its detectability during rest.

Regional Variation in Interhemispheric Coordination of Intrinsic Hemodynamic Fluctuations

David E. Stark
Harvard Medical School, Francis Weld Peabody Society, Class of 2009

F. Xavier Castellanos
Phyllis Green and Randolph Cowen Institute for Pediatric Neuroscience at the NYU Child Study Center

Electrophysiological studies have long demonstrated a high degree of correlated activity between the left and right hemispheres, however little is known about regional variation in this interhemispheric coordination. While cognitive models and neuroanatomical evidence suggest differences in coordination across primary sensory-motor cortices versus higher-order association areas, these have not been characterized.

Here, we used resting-state functional magnetic resonance imaging data acquired from 62 healthy volunteers to examine interregional correlation in spontaneous low-frequency hemodynamic fluctuations. Using a probabilistic atlas, we cross-correlated probability-weighted time series from 112 regions comprising the entire cerebrum. We then examined regional variation in correlated activity between homotopic regions (corresponding anatomical regions in opposite hemispheres). We specifically contrasted correlated activity across primary sensory-motor cortices, unimodal association areas, and heteromodal association areas.

Consistent with previous studies, robustly correlated spontaneous activity was noted between all homotopic regions, which was significantly higher than that between nonhomotopic (heterotopic and intrahemispheric) regions. We further demonstrated substantial regional variation in homotopic interhemispheric correlations that was highly consistent across subjects. Specifically, there was a gradient of interhemispheric correlation, with highest correlations across primary sensory-motor cortices, significantly lower correlations across unimodal association areas, and still lower correlations across heteromodal association areas.

These results demonstrate functional differences in interhemispheric coordination related to the brain's hierarchical subdivisions. Synchrony across primary cortices may reflect networks engaged in bilateral sensory integration and motor coordination while lower coordination across heteromodal association areas is consistent with functional lateralization of these regions. This novel method of examining interhemispheric coordination may yield insights regarding diverse disease processes as well as healthy development.

Costs and Patterns of Cardiovascular and Anti-hypertensive Drug Use Among PhilHealth Beneficiaries

Jordan B. Strom
Harvard Medical School, William Bosworth Castle Society, Class of 2011

Dennis Ross-Degnan, ScD
Department of Ambulatory Care and Prevention
Harvard Medical School

An estimated 7.76 million Filipinos above 20 years of age have diagnosed hypertension, responsible for considerable morbidity and mortality and roughly US$16 million in costs every year to the national health insurance, Philippine Health Insurance Corporation (PhilHealth). The largest part of this cost (34%) is spent on medicines, although no formal analysis has been performed to study prescription patterns. In anticipation of a planned outpatient benefit for anti-hypertensive medications, we conducted a cost identification analysis to collect information on cardiovascular drug usage (including hospital prescribed, generic name, brand name, therapeutic class, formulation, quantity, unit price, and payment) from 1,200 randomly selected claims to PhilHealth for individuals 40 and older with a discharge diagnosis of essential hypertension, defined as having an ICD-10 code of I10.x or I15.x; with or without other diagnoses, discharged within the period July to December, 2007. Subsequently, the database containing this prescription drug information was merged with a PhilHealth electronic database containing PhilHealth member information and analyzed with STATA statistical software nationally and by region for minimum, 25th percentile, median, mean, mode, 75th percentile, standard deviation, maximum for all variables, price per defined daily dose (DDD), most commonly prescribed medications (brand names and generic names), lowest and highest cost medications, charges and medication frequency and type per hospital, and total charges. A sensitivity analysis was performed to estimate cost savings from establishing the anti-hypertensive medication benefit with a restricted formulary. RESULTS: A total of 1,167 claims were located (33 missing), and 955 were valid (defined as containing information on cardiovascular drug pricing) with 212 invalid. Of the 1,167 claims analyzed, 590 (469 valid, 121 invalid) were from the NCR, 278 (221 valid, 57 invalid) were from Region VII, and 299 (265 valid, 34 invalid) were from Region X. The following represents the most frequent entry in each category with respect to charges and frequency respectively: hospital (Capitol Medical Center/Chong Hua Hospital), generic name (Nicardipine/Captopril), brand name (Norvasc/Norvasc), therapeutic class (Calcium Channel Blockers/Calcium Channel Blockers), formulation (tablet/tablet). CONCLUSIONS: Usage of Nicardipine is responsible for 29.6% of total costs to PhilHealth and hydrochlorothiazide usage, recommended as starting therapy for hypertension by the Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, was responsible for 0.002% of total costs, underlining the need for rational drug use.

The Role of HNF4α in the Regulation of Fgl2 Expression in Macrophages

Bethany Strong
Harvard Medical School, William Bosworth Castle Society, Class of 2011

Jorge Plutzky, MD and Gabriela Orasanu, MD
Department of Cardiology, Brigham and Women's Hospital,
Harvard Medical School

Studies of atherosclerotic plaques have shown that ruptured lesions are rich in macrophages, implicating the macrophage as a regulator of plaque stability. Activated macrophages express prothrombinase/fibrinogen-like protein 2 (fgl2), which is a procoagulant factor that can induce intravascular thrombosis. Whereas our previous studies showed that hepatocyte nuclear factor 4 alpha (HNF4α) is a transcription factor expressed in mouse macrophages (RAW 264.7 cells), the regulation of IFNγ-induced fgl2 mRNA expression by HNF4α in RAW 264.7 cells is unknown. To study this, the mRNA expression levels of IFNγ-induced fgl2 were assayed by quantitative real-time PCR. It was determined that IFNγ (100U/mL) induced in a time-dependent manner fgl2 mRNA expression (at 6, 12, and 24 hour time points the fold induction was 871.6, 2721.1, and 435.03, respectively). Also, IFNγ induced in a dose-dependent manner fgl2 mRNA expression (50, 100, and 200 U/ml were accompanied by fold inductions of 34.4, 66.3, and 90.5, respectively). To assess the role of HNF4α in regulation of fgl2 mRNA expression we used an activator of AMP-activated protein kinase - 5-aminoimidazole-4-carboxamide-1-ß-D-ribofuranoside (AICAR) which is known to diminish HNF4α protein levels and target gene expression. Raw 264.7 cells were pretreated with increasing concentrations of AICAR (0.01, 0.025, 0.05, and 0.1 mM) for 24h and IFNγ (100U/mL) after 12h. AICAR significantly decreased IFNγ-induced fgl2 mRNA expression in a dose dependent manner (fold inductions 38.9, 30.5, 4.0, and 6.9 at 0.01, 0.025, and 0.05 mM, respectively). These results show that HNF4α may regulate IFNγ-induced fgl2 mRNA expression in mouse macrophages. Future experiments include siRNA to knockdown HNF4α in Raw 264.7 cells and investigation of fgl2 responses in a mouse atheroma.

Structurally Linking HIV/AIDS and Family Planning Services in Kwazulu-Natal, South Africa

Kimberly L. Sue
Harvard Medical School, William Bosworth Castle Society, Class of 2008

Jenni Smit, PhD
Reproductive Health & HIV Research Unit, Durban Branch
University of the Witwatersrand

Despite the clear link between unsafe sex and the risk of both unintended pregnancies and the acquisition of HIV and sexually transmitted infection (STI), few reproductive health programs address the fundamental interconnection between family planning (FP) and HIV/AIDS prevention. The linking of FP and HIV services is especially relevant to the sub-Saharan region in light of the high HIV prevalence in these countries. The prevalence of HIV in South Africa is amongst the highest in the world, with women disproportionately affected. Yet there is a pattern of low condom use, high hormonal injectable contraceptive use, and almost non-existent intrauterine device (IUD) availability. In addition, even though South Africa follows an integrated primary health care (PHC) model, health services related to HIV/AIDS provided in a largely vertical fashion. As a result, services such as FP, Prevention of Mother to Child Transmission (PMTCT), HIV, Antiretroviral (ARV), and other STI services are poorly integrated into primary care.

With this information in mind, the aim of this three-year demonstration project was to create an innovative model of integrated RH service delivery that links family planning and barrier method services with HIV/AIDS services at the district level in KwaZulu-Natal, South Africa. This model includes integrating and expanding several existing programs in order to create a more comprehensive approach to reproductive health by enhancing contraceptive choices as well as HIV prevention through standardized approach to dual protection and barrier method promotion.

We hypothesized that such a model can contribute to the reduction in the number of unnecessary clinic visits, a reduction in the number of unwanted pregnancies and also a reduction in the number of new HIV infections, as well as to raise awareness about the reproductive rights of HIV-positive and HIV-negative patients alike. Evaluation and data capture of the current state of health care integration was necessary in order to design a state of the art integrated reproductive model that is attentive to the resources and capacity of the local clinics. Part of the data capture for the baseline assessment included development of three tools: a facility inventory, a provider interview and a client interview. Over the next several years, we will use this data to inform an integrated approach to HIV and family planning that can both support ongoing integration efforts as well as address barriers to integration at the primary health care level in the eThekwini district.

Brain Drain in Croatia: An observational study of experiences and incentives for higher science education in Split, Croatia

Shyam K. Tanguturi
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011

Dimitri Krainc, MD, PhD
MassGeneral Institute for Neurodegenerative Disease
Massachusetts General Hospital

Brain drain is an active and pervasive phenomenon in several Central and Eastern European countries particularly Croatia. Despite major strides in Croatian development, improvements in medical research and education have been significantly limited by a dearth of intellectual and human capital. Studies indicate that brain drain is particularly active within students, an alarming fact for the future of medical science in Croatia.

The Mediterranean Institute for Life Sciences (MedILS) is an international cohort of scientists with the aim of creating a 'hotbed' of original intellects to train Croatia's "best young talents" in leading the future Croatia's life sciences and reversing brain drain. Since its conception in 2003, however, no formal study has been made on its impact on brain drain.

This case study consisted of interviews with principle investigators and students from both MedILS and the local public institution at the University of Split. Interviews were used to assess the respective 1) incentives to study and pursue careers in science, 2) obstacles to science education, and 3) overall satisfaction with education and mentorship. 45 interviews were conducted: 17 were conducted at MedILS (3 MS students, 5 PhD students, 1 Postdoctoral Student, 8 mentors), and 28 were conducted at the University of Split (16 MD students, 5 PhD Students, 7 Mentors).

Interview data suggested that MedILS has indeed shaped incentives and obstacles to science. Mentors and students at the University of Split regularly met obstacles like poor structural resources, misallocation of university funds, discrimination, administrative obligations, poor collaboration, and unmotivated students and colleagues; members of MedILS were contrarily pleased with the caliber of students and colleagues, group funding, opportunities for collaboration, flexibility, and independence. Students at MedILS were uniquely in impressed by the location of MedILS, opportunities for outreach, collaboration, and international externships to foreign labs. Nonetheless, MedILS appears to have fallen short in the areas of mentorship, quality of life, and administration: problems included isolation, poor management and hierarchy, lack of institutional standards, impediments for foreign scientists, undefined positions of students, and general problems with Croatian science (customs regulations).

This study demonstrates that in two years of functionality, MedILS has greatly changed the experience of Croatian science by mentors and students. By continuing its progress with group funding, collaboration, student externships, etc. and focusing its attention to improving mentorship, and administration, MedILS may indeed provide an innovative approach to reversing Croatian brain drain.

A Qualitative Study of Barriers and Facilitators to Antiretroviral Adherence for HIV/AIDS Patients in Vietnam

Sonya Trinh
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

Howard Libman, MD
Department of Medicine
Beth Israel Deaconess Medical Center

Since its introduction in the mid 1990s, highly active antiretroviral therapy (HAART) has extended the life span of individuals living with HIV/AIDS. The therapy restores daily functioning and well-being by reducing viral load and damage to the immune system. However, the effectiveness of HAART depends on excellent adherence and continuous access to treatment. Since the virus is prone to mutation, it can develop resistance to HAART and with fewer treatment options, patients may experience disease progression and even death

As little research on this important issue of adherence has been done in Vietnam, it was essential to examine adherence barriers and success strategies of HIV/AIDS patients. A qualitative study design was chosen as it allowed for a multidimensional analysis of adherence barriers and facilitators from the perspectives of the patients and healthcare providers. As such, 25 patients of the Network of Self Help Groups and 10 providers from various free HIV clinics of Ho Chi Minh City (HCMC) were interviewed in a semi-structured format about the various challenges and successes in taking HAART and their impact on the patients' quality of life.

These interviews reveal that no single variable can adequately and completely explain the level of adherence of any individual patient or group of patients. Patients and providers often point to a multitude of factors that affect adherence level. Several themes of these factors emerged through the interviews. First, the complexity of the medication regimen, e.g. number of pills, frequency, indications, etc. poses as a major challenge to adherence as it interrupted daily activities. Second, the extent of social support a patient receives predicts the success and failure of that patient's adherence. This support depends upon the trust and relationship a patient has with his healthcare provider, family and friends, surrounding community, and the Network of Self Help Groups. Positive relationship in these different social spheres greatly enhances adherence. Third, adherence education program provided at the clinics dictated the adherence level of patients. Programs that are flexible and that cater to the specific needs and challenges of patients are more likely to be successful in helping patients maintaining adherence. And finally, the concurrent use of heroin and/or alcohol represents a serious barrier for many patients. As the majority of HIV/AIDS patients in Vietnam comprise of intravenous drug users, the impact of this substance abuse on the management of HIV/AIDS in Southeast Asia cannot be ignored.

Evaluation of ARV Decentralization Program in Pietermaritzburg, South Africa: Phase I

Ailis M. Tweed-Kent
Harvard Medical School, Francis Weld Peabody Society, Class of 2011

Krista Dong, M.D.
iTEACH, Edendale Hospital
Pietermaritzburg, South Africa

Introduction*:* ARV services that provide safe and effective HIV/AIDS treatment are in great demand. Edendale Hospital in Pietermaritzburg, South Africa (PMB) serves a catchment area of approximately 900,000 individuals with an adult HIV/AIDS prevalence of over 40%. As of 2004, the South African government initiated a country-wide roll out of ARVs to large municipal hospitals. However, in 2007 only 10% of those in need of ARVs were receiving the life-saving treatment due to barriers such as distance to the hospital, lack of doctors, and long wait times for initiation. In order to alleviate the bottleneck for treatment, the government decided to decentralize ARV initiation and treatment from large hospitals to smaller community clinics. In PMB, ARVs are now initiated at both the Communicable Diseases Clinic (CDC), a large ARV clinic at Edendale Hospital, and Richmond Clinic, a community healthcare center. Before scaling up the decentralization process to additional clinics, it is important to assess the current services at both Richmond and the CDC. Identifying the challenges is crucial to meeting the present and future demand for ARVs.

Aims: To perform an audit of the ARV services being provided at the CDC and Richmond clinic in order to better understand the current status of and existing challenges surrounding the roll-out and decentralization processes. To provide feedback to Integration of TB in Education and Care for HIV/AIDS (iTEACH) to enable implementation of immediate interventions to address the identified challenges. To create a document summarizing the auditing process and methodology employed for other clinics to use to evaluate their ARV programs.

Methods*:* Both quantitative and qualitative measures will be used to evaluate the current services at CDC and Richmond. *Qualitative:* Comprehensive questionnaire tools were developed to identify existing gaps and challenges in providing ARV services. Separate tools were created for CDC and Richmond. Tools will be administered to ARV-associated staff by members of the assessment team. Data will be collected and analyzed in a Microsoft Access database. *Quantitative:* Quantitative data from a cohort of 200 patients each at CDC and Richmond will be collected from patient files and captured in a Microsoft Excel database. Data will be analyzed using chosen quantitative indicators.

Results: Pending collection of both qualitative and quantitative data.

Discussion: Pending collection of both qualitative and quantitative data.

Lateralized Motor Evoked Potentials via Transcranial Magnetic Stimulation

Andrew M. Vahabzadeh-Hagh
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011

Alexander Rotenberg, MD, PhD
Neurology, Children's Hospital, Harvard Medical School

Alvaro Pascual-Leone, MD, PhD
Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School

Transcranial Magnetic Stimulation (TMS) is two-decade-old method for non-invasive focal brain activation where small electrical intracranial currents are generated by a powerful fluctuating extracranial magnetic field. TMS provides a safe means of measuring corticospinal excitability and integrity of motor pathways in conditions such as epilepsy, stroke and spinal cord trauma. However, work with TMS in animal disease models has been limited, mostly due to lack of downscaling of TMS coils from humans to rats. Large human TMS coils, which stimulate with a resolution of about $1cm^3$, activate many regions of the cortex and more distal parts of the corticospinal tract (CST) in rats. As such, TMS elicited motor evoked potentials (MEPs) in rats may be composed of signals from various locations along the CST.

The purpose of this study is to determine if conventional TMS coils can be used in rats to elicit cortically derived MEPs, which could provide quantifiable metrics for cortical excitability, expanding TMS application to animal models of neurologic disease. In so doing we will also determine whether TMS or focal epicranial electrical stimulation (EES) in animal models is a better approximation for TMS in humans.

We hypothesized that with focal brain activation by TMS, the cortical component of the MEP, as in humans, should be lateralized to contralateral limbs. To test this hypothesis we delivered unilateral TMS and compared MEPs recorded via electromyography (EMG) from the brachioradialis muscle in both forelimbs to test for signal lateralization. Subsequently, we stimulated the motor cortex unilaterally by EES, and compared the EES- and TMS-elicited signals..

Unilateral TMS produced two populations of signals with latencies of 6 and 11ms. The later signal (11ms) lateralized to the contralateral limb and therefore was the predicted cortical signal. EES confirmed TMS results. It is therefore likely that this signal is cortically derived as it matches latency and morphology of EES MEPs. It was also found that TMS and EES evoked this cortical signal 32 and 96% of the time respectively. This is likely due to diffuse TMS activation, suggesting the best approximation of TMS in humans may not be TMS in rats.

Healthcare Management in the World Health Organization's Office of the Director-General

Nina Vasan
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011

Ian Smith, MD, MPH
Office of the Director-General
World Health Organization

During my time working as an intern in the Office of the Director-General at the World Health Organization, I completed 6 healthcare management projects for the Director-General:

(1) I co-authored the *WHO Headquarters Influenza Pandemic Business Continuity Plan.*

(2) Working with leaders of the H8 global health organizations, I managed the development of *Global Health Issues: A Roadmap for the UN Secretary General* on strategically advancing progress towards the Millennium Development Goals. The priority issues identified were broadcast in Secretary General Ban Ki-Moon's address at the 2008 G8 Summit in Japan.

(3) I helped write Director-General Margaret Chan's speech on "The Feminization of the HIV/AIDS Epidemic" for the XVII International AIDS Conference in Mexico City, Mexico.

(4) I created a framework and algorithm for selecting, monitoring, and evaluating corporate travel.

(5) I edited *State-Centered Approaches to Global Health Governance* for the United States Institute of Medicine.

(6) I identified priority island nations to target regarding vulnerability to climate change.

Effects of N-Acetylcysteine on Clinical Outcomes in Acute Liver Failure

Chetan Vedvyas
Harvard Medical School, Walter Bradford Cannon Society, Class of 2011

Raymond T. Chung, MD
Basic Research, Gastrointestinal Unit
Massachusetts General Hospital

Acute liver failure (ALF) is a potentially lethal condition in which precipitous decline in hepatocyte function leads to significant morbidity and mortality. Chief among the defining features of ALF is widespread hepatic necrosis, which may lead to coma and death; other common sequelae include coagulopathy, renal failure, and metabolic derangements. Viral hepatitis and drug-related hepatotoxicity are the most common ALF etiologies.

Intravenous N-acetylcysteine (NAC) has been established for decades as an effective antidote for acetaminophen hepatotoxicity. Importantly, recent data from the US Acute Liver Failure Study Group has shown that intravenous NAC can also increase the likelihood of spontaneous survival in early stage non-acetaminophen-related ALF. This development is particularly notable given that orthotopic liver transplant is indicated for ALF patients who are unlikely to be spontaneous survivors. The mechanism by which intravenous NAC exerts its clinical benefit in non-acetaminophen-related ALF is poorly understood.

Along with hepatocyte necrosis, apoptosis factors prominently into ALF. Recently, serum M-30 antigen has been demonstrated to be a sensitive indicator of hepatocyte-specific apoptosis in chronic HCV, as well as in all-cause ALF. In this regard, we hypothesized that intravenous NAC exerts its beneficial effect on clinical outcome in ALF by means of attenuating hepatocyte apoptosis and that serum M-30 antigen correlates with intravenous NAC's beneficial effects in ALF.

In this study, we used ELISA to examine serum M-30 antigen levels in 73 non-acetaminophen-related ALF patients given intravenous NAC and a separate group of 73 given placebo. We attempted to correlate a change in serum M-30 antigen levels with patient outcomes to provide evidence that it contributes to the benefit observed with NAC.

Additionally, recent studies have provided some support for serum M-30 antigen level assay as a potential indicator for clinical outcomes in acute liver failure. The current larger study sought to bolster the use of M-30 antigen level as a clinical outcome prognosticator in ALF.

Analysis has not yet been completed. Preliminary results indicated that mean M-30 antigen level was higher in ALF patients who were transplanted and/or died compared to those who spontaneously survived. Though this supports the use of M-30 antigen level as a clinical outcome prognosticator in ALF, further analysis needs to be completed to provide statistical significance to this assertion.

Tumescent Mastectomy Technique as a Risk Factor for Skin Flap Necrosis Following Immediate Breast Reconstruction

Kapil D. Verma
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

Yoon S. Chun, MD
Department of Plastic Surgery
Brigham and Women's Hospital

The use of the tumescent mastectomy technique allows sharp dissection with minimal blood loss, however it remains unclear whether the use of the tumescent technique adversely affects native skin flap viability post-operatively. The authors assessed whether use of the tumescent technique serves as a risk factor for post-operative skin flap necrosis.

The authors conducted a retrospective review of all mastectomies followed by immediate breast reconstructions performed over a 6 year period at Faulkner Hospital. Mastectomy technique, patient characteristics, and post-operative native skin flap necrosis rates were reviewed. Logistic regression analysis adjusted for clustering was performed.

A total of 275 patients underwent 170 unilateral and 105 bilateral mastectomies with immediate reconstruction, totaling 380 reconstructions. 100 mastectomies were performed using the tumescent mastectomy technique and 280 were performed without using the tumescent mastectomy technique. Logistic regression analysis adjusted for clustering showed that patient age, BMI, and the use of the tumescent mastectomy technique were statistically significant risk factors for developing post-operative major native skin flap necrosis. For each 10-year increase in patient age, the odds of major necrosis increased by 1.57 times ($p = 0.010$). For each 1 unit increase in BMI, the odds of major necrosis increased by 1.10 times ($p= 0.006$). Radiation received before reconstruction increased the odds of major necrosis by 3.10 times ($p = 0.014$). Finally, patients that underwent tumescent technique during the mastectomy had 3.64 times the odds of major necrosis than patients who did not undergo tumescent technique during mastectomy ($p < 0.001$).

Based on a retrospective medical charts analysis with logistic regression adjusted for clustering, use of the tumescent mastectomy technique is shown to increase the risk of post-operative major skin flap necrosis in an immediate breast reconstruction setting.

Restoring BCR regulation in SLE and RA Patients with Mutations in Sialic Acid Acetyl Esterase

Erin Xiaolu Wei
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011
Arthritis Foundation Summer Fellowship

Shiv Pillai, MD, PhD
Department of Medicine-Cancer Center
Massachusetts General Hospital

B cell functional defects have been implicated in many systemic autoimmune diseases, including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Sequencing data from our laboratory has shown that some anti-nuclear antibody positive patients with SLE and RA carry point substitutions in the sialyl acetyl esterase (*SIAE*) gene that result in loss of activity of the enzyme. Such substitutions can cause the increase of B cell receptor (BCR) induced calcium flux, reflecting an augmentation in BCR activity. Similarly, *Siae-/-* mice show both BCR over-activity and the generation of autoimmune antibodies. These findings are suggestive of an active role for *SIAE* mutations in the progression of SLE and RA.

BCR signaling is a highly regulated process. One key negative regulator of BCR signaling, CD22, is a 140 kD membrane protein belonging to the SIGLEC family of lectins. CD22 has seven extracellular immunoglobulin-like domains, and of these, the two N-terminal Ig domains bind alpha-2,6-linked (a2,6) sialic acid-bearing ligands. Covalent modifications of alpha-2,6 sialic acid have been found to alter its affinity for CD22 binding sites. One such modification is acetylation of the 9-OH position, whereby the outermost hydroxyl group is modified by a sialyl 9-O-acetyl transferase. Removal of the acetyl group restores ligand recognition by CD22. SIAE removes acetyl group from the 9-hydroxyl position of sialic acid and is a potential regulator of substrate binding to CD22, thus providing fine-tuning of BCR signaling.

Our recent data show that SIAE undergoes an unusual time course in its post-translational modification. There exists a consistent size difference between the pre-secretory and membrane-bound forms of wildtype SIAE. Pulse-chase analysis has not detected membrane-bound SIAE inside cells, suggesting the modifications occur immediately prior to localization to the membrane. The difference in migration of membrane-bound SIAE and pre-secreted SIAE disappeared following treatment with PNGase F, further suggesting that the final modification of SIAE is due to the addition of a sugar moiety.

For functional analysis of human SIAE, wild-type *h*SIAE has been cloned in two pET vector systems for prokaryotic expression. Stable mammalian lines of SIAE have also been created and are currently being tested for hSIAE secretion. We plan to purify SIAE in large quantities and incubate the wildtype protein with mutant B cell lines. This may represent an important first step towards restoring BCR regulation in B cells with mutations in *SIAE*.

Remodeling Characteristics in Mouse Midpalatal Suture Osteochondroprogenitor Cells in Response to Mechanical Force

Kimberly G. Whippy
Harvard School of Dental Medicine, Oliver Wendell Holmes Society, Class of 2011

Bjorn R. Olsen, MD, PhD
Department of Developmental Biology
Harvard School of Dental Medicine

The skeleton is a metabolically active organ that undergoes continuous post-developmental remodeling and differentiation, processes regulated by complex interactions between genetic and epigenetic factors. Many current orthodontic and orthopedic procedures depend on the ability of bone and cartilage cells to respond to mechanical stress. Furthermore, cellular alterations that result from stress are major contributors to pathological conditions like osteoarthritis and osteoporosis. Unfortunately, the molecular signaling pathways that modulate gene expression to control bone remodeling and differentiation remain ambiguous. Our aim was to explore how mechanical force application influences skeletal structure and function to elucidate bone biology and signaling pathways to ultimately improve strategies for treating skeletal diseases, injuries and malocclusion.

The midpalatal suture cartilage contains mesenchyme-like cells, osteochondroprogenitor cells, which are highly proliferative and have a unique ability to differentiate into either chondrocytes or osteoblasts. It was previously found that application of expansive force to this suture in mice promoted bone resorption via osteoclast activation and bone formation via increased osteochondroprogenitor cell proliferation, migration and differentiation into osteoblasts in the midpalatal suture within the first 7 days. The purpose of this study was to reveal the molecular signals involved in the intitial osteochondroprogenitor cell proliferation in response to expansive force application.

A previously characterized expansion procedure or sham operation were performed on wild-type 6 week male mice and differential midpalatal suture mRNA expression was analyzed in these two groups of mice after sacrifice, palatal dissection and RNA purification and amplification using RT-PCR and microarray molecular technology.

RT-PCR analysis suggested that the proliferating osteochondroprogenitor cells respond to stress by upregulating expression of bone specific markers as compared to cartilage specific markers significantly more than wild type mice. Microarray data has not been obtained at this time. The project should be completed in the next month.

Preferences and Attitudes of Women Undergoing Second Trimester Abortion

Monica H. Wojcik
Harvard Medical School, Francis Weld Peabody Society, Class of 2011

Lisa Lehmann, MD
Center for Bioethics, Department of Medicine
Brigham and Women's Hospital, Harvard Medical School

Abortions after the first trimester have long been the subject of much controversy, as the fetus reaches later stages of development and approaches viability. It has been observed that while the abortion debate may be without resolution, most Americans support the right to abortion so long as there is some degree of limitation. In the past few years, determination of the extent of these limitations, particularly regarding procedures performed at later gestational age, has been borne out not by physicians and medical experts, but by lawyers and lawmakers. The 2003 Partial-Birth Abortion Ban Act was intended to proscribe a particular method of ending fetal life in the later stages of pregnancy ("intact dilation and evacuation") by specifying anatomical landmarks beyond which the fetus may not be removed alive. The constitutionality of this Act, that does not include a maternal health exemption, was challenged in the Supreme Court case *Gonzales v Carhart* In April, 2007, the Court decided that these objections were invalidd. Therefore, after *Gonzales v Carhart*, physicians who perform second trimester abortions are presented with a mandate to demonstrate intent to cause fetal demise prior to uterine evacuation in order to avoid legal prosecution. As a result, many physicians have begun using a feticidal injection to cause fetal demise prior to the surgical abortion procedure. These injections, typically intraamniotic digoxin or intrafetal potassium chloride, have been received with much controversy among practitioners. However, while the safety, efficacy, and necessity of adopting feticidal injections into second trimester abortion protocols has been debated in the medical community, patient preference for or against feticidal injection, and the attitudes behind these preferences, remains unclear. With a lack of data available regarding the preferences of patients who may be affected by the *Gonzales v Carhart* ruling, we investigated whether these patients have a preference for abortion with or without feticidal injection, and which factors were major determinants of these preferences, in a survey-based cohort study with an enrollment goal of 150-200 patients at multiple facilities. We also investigated whether the implementation of second-trimester abortion protocols using feticidal injection would affect the patient's decision to have an abortion. It is hoped that the results of this survey study will motivate further dialogue between physicians who perform second trimester abortions and their patients, and will contribute to the ongoing debate within the medical community.

Protective Effects of Prior Obesity on Bone Mass in Anorexia Nervosa

Monica H. Wojcik
Harvard Medical School, Francis Weld Peabody Society, Class of 2011

Karen K. Miller, MD
Neuroendocrine Unit
Massachusetts General Hospital, Harvard Medical School

Anorexia nervosa (AN), characterized by extreme low-weight and amenorrhea, is complicated by severe bone loss. In contrast, obesity is protective against bone loss, including that seen in postmenopausal estrogen deficiency. However, it is not known whether a history of being overweight or obese protects against low bone mass in AN and whether this could be in part mediated by an earlier age of skeletal estrogen exposure. Therefore, we studied 309 women with AN, mean age 24.7 ± 6.2 (SD) years, mean % IBW 76.3 ± 6.0 (SD), and mean BMI 17.0 ± 1.4 (SD) kg/m^2, of whom 31 were previously overweight or obese (PO) ($\geq$ 25 kg/m^2). Mean current weight, duration of AN, duration of amenorrhea and age of AN onset were similar between the PO group and the not previously overweight or obese group (non-PO). Age of menarche was lower in PO than non-PO (12.7 ± 0.2 vs. 13.6 ± 0.1 [SEM], p = 0.003). Mean PA spine (-1.4 ± 0.1 vs. -1.0 ± 0.2 [SEM], p = 0.036) and lateral spine (-1.7 ± 0.1 vs. -1.1 ± 0.2 [SEM], p = 0.035) Z-scores were lower in non-PO than PO. The differences in BMD between groups remained significant after controlling for age of menarche. There was no difference between groups in BMD at the hip or radius. BMD Z-score was positively associated with previous highest weight at all skeletal sites (PA spine: R=0.32, lateral spine: R=0.30, hip: R=0.28; radius: R=0.29; $p \leq 0.0001$ at all skeletal sites). Our data demonstrate that a prior history of being overweight or obese predicts a higher spine BMD despite subsequent extreme low weight. One mechanism may relate to earlier age at menarche and resultant estrogen exposure establishing a higher pre-morbid trabecular bone BMD. Studies are warranted to investigate this hypothesis further.

Analysis of Electroencephalogram Activity and Anesthetic Drug Administration in Carotid Endarterectomy Patients

Arthur W. Wood
Harvard Medical School, Francis Weld Peabody Society, Class of 2009
Doris Duke Clinical Research Fellowship

Emery N. Brown, MD, PhD
The Department of Anesthesia and Critical Care
Massachusetts General Hospital

Despite improvements in anesthetic drugs, EEG recording technology, and signal processing algorithms, there has been limited study to date of EEG changes across the stages of general anesthesia using full-montage EEG. The objective of this study is to develop more precise EEG-based neurophysiological correlates of arousal states under general anesthesia.

From the intraoperative monitoring database at Massachusetts General Hospital we identified 8 subjects who underwent carotid endarterectomy (CEA) surgery using a 10-20 system of EEG recordings to monitor for cerebral ischemia. The EEG recordings used were limited to those from subjects without a history of neurological or vascular problems (stroke, previous CEA) and whose CEA procedure was executed without anesthetic or surgical complication.

We used multitaper spectral methods to compute the time-varying power spectral density and coherence continuously throughout the entire operation. Average power spectral density and coherence were then calculated for shorter representative time segments corresponding to the following clinical states: baseline, induction, maintenance and recovery. Values were calculated in the delta (0-3.5Hz), theta (3.5-7.5Hz), alpha (7.5-12.5Hz), beta (12.5-25Hz) and gamma (25-100Hz) frequency bands.

From a qualitative perspective, there were several observable differences in the spectral results between arousal states. During the baseline state, the EEGs displayed a uniform level of power in the theta through gamma band with a slightly higher level in the delta band. The induction and maintenance stages showed a drop in power across the gamma band and an increase in power in the alpha and beta bands. Upon recovery, the power spectrum returned to the baseline pattern. Inter-hemispheric and anterior-posterior coherence were high in the delta through alpha range and low in the beta through gamma range at baseline. During induction and maintenance, the coherence in the delta through alpha range was lost and there was an increase in beta through gamma coherence.

Preliminary statistical analysis of the coherence shows that the mean coherences among all subjects differ significantly between baseline and post-induction states in all frequency bands. These results suggest that, in addition to power spectral density, coherence changes appreciably between arousal states and could be used to improve the efficacy of EEG-based anesthesia monitors.

The Roles of Thrombin and Gα12 Signaling in Neuronal Hypoxia

Vijay Yanamadala
Harvard Medical School, Irving M. London Society of Health Sciences & Technology, Class of 2011
Huntington's Disease Society of America Donald A. King Fellowship

Robert M. Friedlander, MD
Department of Neurosurgery
Brigham and Women's Hospital

Acute neurodegeneration resulting from ischemic stroke, brain trauma, ischemia-reperfusion injury, and spinal cord injury is associated with high morbidity and mortality with few effective treatments. We are most limited by the central nervous system's limited capacity to regenerate. Effective treatments should be able to limit the extent of damage and degeneration in patients immediately after injury, and the development of such treatments is dependent upon the elucidation of the pathways and mechanisms of neuronal apoptosis. Further elucidation of pathways that can control these central apoptotic processes will be of undoubted therapeutic benefit. In this regard, thrombin and its protease activated receptors (PARs) have been sparsely examined, even though thrombin levels are acutely elevated in various models of central nervous system (CNS) injury. Like all G protein coupled receptors, these PARs are highly cell type specific and easily targeted extracellularly, thus presenting an ideal approach for therapy.

We have recently shown that thrombin induces apoptosis in several immortalized cell lines by activating the heterotrimeric G protein Gα12 and inducing downstream JNK1 activation, IκBα upregulation, and Bcl-2 degradation. Herein we demonstrate that both hypoxia and thrombin activate Gα12 in primary cortical neurons. These pathways are associated with induction of JNK1 phosphorylation, upregulation of IκBα expression, and modulation of Bcl-2. However, the pathways also show a high degree of divergence. Hypoxia induces the activation of Caspase 3 and initiates an intrinsic apoptosis cascade leading to neuronal death. Conversely, thrombin does not activate Caspase 3. Additionally, thrombin protects neurons from this hypoxia induced death, as demonstrated morphologically and through quantitative cell death assays. This is associated with the preservation of IκBα and Bcl-2 expression and inhibition of Caspase 3 activation.

Further elucidation of these pathways will reveal the precise mechanism through which thrombin protects cortical neurons from hypoxia induced apoptosis, with the potential to develop neuron specific thrombin receptor agonists for the treatment ischemic neurodegenerative diseases.

The Role of Regional Referral Centers in Breast Reconstruction: The New England Perforator Flap Experience

Janet H. Yueh
Harvard Medical School, Francis Weld Peabody Society, Class of 2009
Doris Duke Clinical Research Fellowship

Bernard T. Lee, MD
Division of Plastic and Reconstructive Surgery, Department of Surgery
Beth Israel Deaconess Medical Center

The deep inferior epigastric perforator flap has become an increasingly popular option for autologous breast reconstruction after mastectomy. Despite its reported advantages, perforator flap breast reconstruction has been slow to integrate as considerable technical expertise is necessary. Due to the lack of availability, many patients may go to great effort to find surgeons that offer this operation. In February 2004, the Beth Israel Deaconess Medical Center developed a microsurgery program for perforator flap breast reconstruction. By providing the full spectrum of reconstructive techniques, our institution became a referral center for the New England region. The purpose of this study is to analyze the impact of this program before and after initiation with attention to reconstruction rates, patient satisfaction, and referral patterns.

A retrospective chart review was performed on all women who had undergone mastectomy or breast reconstruction at our accredited ACS Commission-on-Cancer hospital between 1999 and 2006. 1073 women underwent 1341 complete mastectomies at our center for treatment of breast cancer or prophylactic removal of the breast. 101 women had 118 complete mastectomies at an outside institution but chose to have delayed reconstruction at our center. Patients who had breast reconstruction received a validated questionnaire on satisfaction, health-related quality of life, and sociodemographic data. A 75.6 percent response rate was obtained.

Since the inception of the perforator flap program in 2004, there has been a significant increase in the immediate reconstruction rate from 51.5 percent to 63.9 percent ($p < 0.001$). While the percentage of prosthetic-based reconstructions have remained constant, the proportion of latissimus dorsi and transverse rectus abdominis myocutaneous flaps have significantly decreased as the proportion of microsurgical flaps have significantly increased. Between the two time periods, general patient satisfaction after breast reconstruction increased from 58.5 percent to 74.4 percent ($p < 0.001$), while aesthetic satisfaction increased from 58.5 percent to 69.9 percent ($p = 0.010$). Furthermore, we have seen at 4.1 fold increase in the number of patients per year coming from outside institutions seeking to have delayed or secondary breast reconstruction.

The addition of a perforator flap breast reconstruction program to accredited cancer centers can increase both patient satisfaction and reconstruction rates. The availability of microsurgical breast reconstruction at an institution will not only dramatically shift the proportion of reconstructive techniques performed, but also attract patients from outside the institution. This shift in referral patterns further emphasizes the role of breast reconstruction within a regional referral center.

The Prevalence and Associated Risk Factors of Pentavalent Antimonial Resistant Leishmaniasis in Colombia

Josue Zapata
Harvard Medical School, Oliver Wendell Holmes Society, Class of 2011

Henry Oliveros, MD
Departments of Anesthesia and Medical Education
Hospital Militar Central

Each year over two million people around the world are affected by clinical leishmaniasis at a cost of around sixty thousand lives and 2,357,000 disability-adjusted life years. Newly acquired resistance to pentavalent antimonials, the primary treatment option in Colombia, as well as an increase in prevalence of the disease adds to the importance of providing every individual with appropriate treatment and prevention methods. Based on previous studies, we hypothesize that there are certain risk factors that are associated with resistance to pentavalent antimonial drugs that, if elucidated, can help mitigate the problems associated with resistance.

To test this hypothesis, we conducted a retrospective case-control chart review of 200 patients from the Hospital Militar Central or a Military Dispensary in Bogotá, Colombia in which 50 patients had documented cases of resistance to pentavalent antimonials, while the other 150 had no documentation of resistance. Resistance was defined as patients who were referred to the Hospital Militar Central due to lack of response to treatment, patients whose lesions fail to close at least 50% after treatment, or patients who require a "second line" drug aside from the traditional therapy of Pentavalent Antimonials. Potential risk factors for resistance including nutritional information (BMI), time of presentation, past history of leishmaniasis treatment, age, geographic location, location of bite, number of lesions, and description of the lesions were also collected from the patient records. Finally, this data was subjected to statistical analysis to examine associations between the hypothesized risk factors and the outcome of resistance.

The results are pending.

Identification of Phosphorylation-Dependent Binding Partners of Aquaporin-2 Using Protein Mass Spectrometry

Nicholas A. Zwang
Harvard Medical School, Francis Weld Peabody Society, Class of 2009
Howard Hughes Medical Institute-National Institutes of Health Research Scholars Program

Mark A. Knepper, MD, PhD
Chief, Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute
National Institutes of Health, Bethesda, MD

Vasopressin-mediated control of water permeability in the renal collecting duct occurs in part through regulation of the distribution of AQP2 between the apical plasma membrane and intracellular membrane compartments. Phosphorylation of Ser-256 at AQP2's cytoplasmic COOH-terminus is well-accepted as a critical step for translocation. The aim of this study was to identify binding partners to phosphorylated versus nonphosphorylated forms of the AQP2 COOH-terminus using mass spectrometry. Cytosol from inner medullary collecting ducts isolated from rat kidneys was incubated with "bait" peptides, representing the COOH-terminal AQP2 tail in its nonphosphorylated and phosphorylated forms, to capture differentially-bound proteins prior to LC-MS/MS analysis. Mass spectrometric results were confirmed by immunoblotting. Immunoprecipitation, performed using an AQP2 COOH-terminal antibody combined with immunblotting against the proposed binding partners, was performed to demonstrate protein-protein interactions with native AQP2. Our studies confirmed previously identified interactions between AQP2 and hsc70, hsp70-1 and -2, and annexin II. These proteins were found to bind less to the Ser-256 phosphorylated AQP2 than to the non-phosphorylated form. In contrast, another heat shock protein bound to phosphorylated AQP2 more avidly than to non-phosphorylated AQP2, namely hsp70-5 (BiP). Immunogold EM studies demonstrated that BiP is present, not only in the ER but in the cytoplasm and apical plasma membrane of rat collecting duct cells. Furthermore, confocal immunofluorescence studies showed partial colocalization with AQP2 in non-ER compartments. Finally, three other proteins were found that bound more avidly to non-phospho-AQP2 than to the phosphorylated form: ras-related nuclear protein, GDP dissociation inhibitor 2, and protein phosphatase 1 catalytic subunit.