

26<sup>th</sup> Annual Medical Student Research Symposium  
Stanford Hospital Atrium  
Thursday, May 7, 2009  
3:00pm-6:00pm



3:00pm Opening Remarks – Philip A. Pizzo, MD, Dean of the School of Medicine  
3:00-5:45pm Poster Presentations  
5:45pm Closing Remarks – Charles G. Prober, MD, Sr. Associate Dean, Medical Education  
5:50pm Announcement of Awards – William Rhine, MD, SUMCAA President

Abstracts for these projects are located online at: [http://med.stanford.edu/student\\_research/events.html#symposium](http://med.stanford.edu/student_research/events.html#symposium)

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Agbo, Chioma	TROPONIN I: TIME-TO-POSITIVITY IN EMERGENCY DEPARTMENT PATIENTS WITH ACUTE MYOCARDIAL INFARCTION	Schreiber, Donald
Baras, Jacqueline	THE RELATIONSHIP BETWEEN MRI AVAILABILITY AND LOW BACK PAIN CARE UTILIZATION	Baker, Laurence
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**Thank you to the 2009 Symposium Committee, who helped plan the event, read all abstracts, and judged poster presentations.** Dr. Laurence Baker, Dr. Patricia Cross, Chris Cueva, Alana Frost, Matt Goldstein, Gene Ma, Elise Min, Sarah Nelson, Adeoti Oshinowo, Wendy Pang, Sarah Pickard, Mara Violanti, Judy Yeh

**Thank you to the additional 2009 Symposium Judges, who volunteered their time and effort!** Marissa Aillaud, James Berbee, Tiffany Castillo, Patricia Foo, Dr. Neil Gesundheit, Joshua Goldner, Mariko Howe, Dr. Susan Knox, Andrew Lee, Abed Meer, Laura Prolo, Jeremiah Ray, Dr. Oscar Salvatierra, Jessica Tsai, Angela Venegas, Jane Whitney

**And a HUGE thank you to the Stanford University Medical Center Alumni Association for their continued, generous support of this year's Medical Student Research Symposium.**



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# THE EVALUATION OF PRE AND POST PROCESSING SEMEN ANALYSIS PARAMETERS AT THE TIME OF INTRA-UTERINE INSEMINATION IN COUPLES DIAGNOSED WITH MALE FACTOR INFERTILITY

**Chioma Agbo** and Michael H Dahan M.D., Department of Obstetrics & Gynecology, Stanford Reproductive Endocrinology and Infertility (REI) Center

Male infertility is generally defined as an inability to conceive by the male partner that is attributable to several causes such as low sperm production, abnormal sperm morphology or poor sperm motility.

Intrauterine insemination (IUI) remains a popular initial treatment option for infertile couples due to its lower costs and greater religious acceptability, especially for couples with male factor infertility. IUI involves placing processed donor sperm directly into the uterine cavity.

Semen analysis is the most common method of evaluating male reproductive potential and is often performed prior to an IUI. Unfortunately, there is very limited literature available evaluating the potential semen analysis parameters in predicting pregnancy outcome, particularly in severe male infertility.

In this study we performed a retrospective case-control of three hundred and eighty six couples undergoing one thousand IUI cycles in which the male partner had a semen analysis done at Stanford Reproductive Endocrinology and Infertility (REI) Center to determine whether the couple conceived with the intrauterine insemination. Statistical analysis revealed that couples with extreme male infertility, defined as 0.5 cc or less volume of semen specimen post-processing is strongly correlated with failure to achieve pregnancy.

*Funding provided by the Stanford Medical Scholars Fellowship Program.*

## TROPONIN I: TIME-TO-POSITIVITY IN EMERGENCY DEPARTMENT PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

**Chioma Agbo** and Donald Schreiber M.D., Department of Surgery – Emergency Medicine, Stanford University School of Medicine

**Background:** The American College of Cardiology (ACC)/European Society of Cardiology (ESC) criteria for the diagnosis of acute myocardial infarction are based on a rise and fall of cardiac markers such as troponin I (TnI) and CKMB associated with clinical symptoms compatible with myocardial ischemia or infarction and specific ECG changes. Several studies have shown that serial measurements of serum cardiac troponins and/or CKMB over an 8 to 12 hour period of observation can reliably identify or exclude an AMI. Unfortunately, there is no established serial serum testing regimen recommended for exclusion of AMI using the current ACC/ESC criteria for AMI to guide physician medical decision process. **Objectives:** Our objective is to determine optimum serial serum testing time points for TnI after ED arrival time that can be used to rule in or rule out AMI with a high sensitivity. The aim of this study is to improve the clinical decision algorithm for patients with possible AMI in the ED. **Methods:** A retrospective observational study was conducted on patients presenting to a university center ED with an AMI. Patients with AMI during hospitalization or STEMI were excluded. We retrospectively determined the blood sample collection times for cardiac markers compared to time of initial ED presentation. Time to positivity of TnI was calculated using a statistical analysis software program, SAS version 9.1.3. **Results:** A chart review was conducted on 638 patients. 120 patients had a final diagnosis of NSTEMI. 50% were positive within 1 hour from baseline. 95% of patients were positive within 8 hrs. **Conclusions:** Our findings indicate that 95% of patients diagnosed with NSTEMI have a positive result within 8 hours from ED arrival to blood draw. Results from this study suggest a prospective study needs to be done to evaluate if time intervals of 0, 4 and 8 hours is suitable for serial measurements of TnI.

## THE RELATIONSHIP BETWEEN MRI AVAILABILITY AND LOW BACK PAIN CARE UTILIZATION

**Jacqueline Baras** and Laurence Baker, Department of Health Research and Policy, Stanford University.

The rapid expansion in the number of magnetic resonance imaging (MRI) scanners in the United States has enabled more patients to receive cutting-edge imaging that can produce valuable diagnostic information. However, for patients with low back pain, the use of MRI is controversial. Spinal abnormalities detected by MRI often do not correlate with symptoms and can lead to additional, unnecessary interventions, including surgery, which in many patients is of uncertain efficacy. This paper investigates the relationship between the expanding MRI supply and the diagnosis and treatment of low back pain in approximately 800,000 Medicare beneficiaries from 1998-2005, providing new information about the broader effects of increased imaging availability in a relatively large group of patients.

We use logistic regression models in which the key independent variable is a measure of MRI availability (distribution of MRI units per million population); the dependent variables are indicators of receipt of low back MRI or surgery within 30, 90, 180, or 365 days of an “index visit” for low back pain; and controls include patient demographic and health characteristics as well as fixed effects for areas and years. We find that patients in areas in the highest quartile of MRI availability, compared to patients in the lowest quartile, were 15.2% more likely to receive MRI within 30 days of their index visit, and 9.6% more likely within 365 days (both  $p < .001$ ). Increases in MRI availability are also associated with higher probabilities of low back surgery receipt.

Diffusion of MRI equipment is associated with increased early use of low back MRI and subsequent surgery, both of which are discouraged for the majority of patients with new onset low back pain. These results raise concerns that the widespread expansion of MRI may adversely impact quality of care for low back pain patients. Policy efforts that assess the value of technology diffusion should consider both the monetary and non-monetary costs that increased MRI availability may have for particular patient groups.

*Funding provided by the Stanford Medical Scholars Fellowship Program.*

## IDENTIFICATION OF PERIPHERAL AND CNS SIGNALING PROTEINS ASSOCIATED WITH ALZHEIMER'S DISEASE

**Sarah L. Bauer Huang**, Markus Britschgi, Tony Wyss-Coray. Department of Neurology & Neurological Sciences, Stanford School of Medicine

Alzheimer's disease (AD) is a neurodegenerative disease that is clinically characterized by development of initial mild loss of episodic memory that progresses into a severe dementia with additional psychomotor symptoms at later stages of the disease. Pathophysiological hallmarks of AD include:  $\beta$ -amyloid ( $A\beta$ ) plaques, tau-hyperphosphorylation, neurodegeneration, and neuroinflammation. A number of cytokines and growth factors have also been shown to be transported across the blood brain barrier in both directions (reviewed in [1]), and changes of levels of several of these factors in the brain and in the blood have been associated with AD. Signaling proteins characterized in this research, as well as in previous research in our lab [2], could be used as pre-dementia biomarkers of AD, which would have implications both in diagnosis as well as in treatment. Indeed, a set of 18 out of 120 simultaneously detected plasma signaling proteins has been recently identified to classify blinded plasma samples from patients with early AD and non-demented controls with high accuracy [2]. For this current study, we hypothesize that peripheral and CSF levels of signaling proteins are associated with AD-related psychopathological changes and molecular biomarkers.

Using Luminex technology, we measured concentrations of 74 soluble intercellular signaling proteins in plasma samples of 51 AD patients and 87 healthy normal controls, as well as 45 CSF samples. Using the statistical tool SAM (Significance Analysis of Microarrays) we did multivariate and linear regression analyses to find relationships between signaling proteins and molecular biomarkers of AD (CSF levels of Tau, pTau, or  $A\beta_{42}$ ) or clinical scores. For the plasma analysis, IgE and ENA-78 were negatively correlated with clinical diagnosis. EN-RAGE was positively associated with Tau, pTau and negatively associated with the  $A\beta_{42}/p\text{Tau}$  ratio. No factors were associated with  $A\beta_{42}$  alone. For the CSF analysis, Fatty Acid Binding Protein (FABP) was positively associated with pTau and negatively associated with  $A\beta_{42}/p\text{Tau}$ . FABP, MMP-3, Stem Cell factor, Complement 3, TNF-RII,  $\beta$ -2 microglobulin,  $\alpha$ -2 macroglobulin, IL-16, Tissue factor, VCAM and PAI-1 were all positively associated with Tau. No factors in the CSF were associated with the clinical diagnosis or pTau.

In our study, a number of factors were associated with clinical diagnosis or molecular AD biomarkers. Surprisingly, those factors found to correlate to disease in plasma did not overlap with those found in the CSF, suggesting the signaling proteins associated with AD may compartmentalize to the CNS or periphery. Future studies will examine the biological role of these proteins in AD.

1. Britschgi and Wyss-Coray (2007)
2. Ray et al (2007)

*Funding provided by the Stanford Medical Scholars Fellowship Program*



## A BAYESIAN NETWORK TO ASSIST MAMMOGRAPHY INTERPRETATION

**Stephanie W. Chan**, Ryan W. Woods, Ross D. Shachter, Elizabeth S. Burnside, Daniel L. Rubin. Department of Diagnostic Radiology, Department of Medical Informatics.

Breast cancer is the most frequently diagnosed malignancy among American women and the second leading cause of cancer death among women of all ages. Improving mammography interpretation is critical to promote early diagnosis, the most effective means of decreasing the death rate from this disease. At the same time, there is variation in practice among mammography practitioners, and methods to improve their accuracy are needed to ensure high quality practice. One challenge that radiologists face is to evaluate whether negative biopsies in suspicious cases result from sampling error. Our goal is to develop a computer application to aid radiologists with this challenging diagnostic process.

We are working with a Bayesian network previously developed to represent the probabilistic relationships among key predictor variables, which appears useful in assessing the likelihood of malignancy given the abnormalities seen on mammography. By applying a probabilistic framework to relate a negative result of pathology to the degree of suspicion of malignancy on mammography of the lesion biopsied, we calculate a probability that malignancy is present in the patient. Preliminary studies show that this probability of malignancy is increased in cases that were found by clinical follow-up to be malignant, after initial biopsies were benign.

These preliminary results suggest that the Bayesian Network can be used to assist radiologists with evaluating how suspicious a lesion seen on mammography is for malignancy. This information can assist a radiologist in deciding which lesions seen on mammography should undergo biopsy. In addition, this information can assist a radiologist with deciding whether a negative biopsy is discordant with the lesion seen on mammography and may require repeat biopsies to overcome initial sampling error. Our ultimate goal is an application that can help radiologists identify those cases that are most suspicious of being discordant and that would benefit most from additional sampling to ensure cancer is not missed.

*Funding provided by the Stanford Medical Scholars Fellowship Program.*

## **ROLE OF MHC CLASS I MOLECULES IN THE PATHOGENESIS OF AUTOIMMUNE DIABETES**

**Pearl Chang**, Remi J. Creusot, and C. Garrison Fathman. Stanford University, Department of Medicine, Division of Immunology and Rheumatology

Major histocompatibility complex (MHC) proteins have been implicated in autoimmune diseases such as type 1 diabetes (T1D) but their exact role in disease pathogenesis and progression remains unclear. Recent microarray data from Kodama et al. (Clin. Immunol. 2008) suggests tissue-specific age-dependent differences in expression of certain classical MHC class I (MHC Ia) and nonclassical nonpolymorphic MHC class Ib genes in the non-obese diabetic (NOD) mice, a T1D mouse model, compared to the disease-free congenic NOD.B10 mice that differs in one segment of the MHC gene cluster. At 4 weeks of age, around the initial onset of insulinitis, there was lower expression of the MHC Ia gene H2-D1 and seven of the MHC Ib genes in the pancreatic lymph nodes (PLN) of NOD mice, but not in the peripheral blood cells (PBC) or spleen. In contrast, at 12 weeks of age, when islet destruction begins, MHC expression was lower in the PBC but not PLN or spleen.

Based on these observations, we examined gene and/or protein expression by quantitative PCR or FACS, respectively, of H2-D1 and the class Ib molecules Qa1 and Qa2 in PLN and PBC of 4- and 12-week-old NOD and NOD.B10 female mice. We chose to focus on Qa1 and Qa2 because of their potential roles in immune suppression (Lu et al., Immunol. Rev. 2006; Hogarth et al., J. Immunol. 1985). We found significant differences in total Qa2 gene and protein expression in NOD mice relative to age-matched NOD.B10 mice, as well as differences in Qa1 gene expression and H2-D1 protein expression.

These preliminary results indicate that differences in both MHC Ia and Ib expression may play a role in T1D disease pathogenesis and suggests the possibility of certain MHC genes serving as potential biomarkers of disease susceptibility or progression. Further studies are needed to determine the exact role of these MHC molecules in the immune-mediated destruction of pancreatic islets and autoimmunity.

# ONCOLOGIC AND FUNCTIONAL RESULTS OF GRADE 1 CHONDROSARCOMAS AND ENCHONDROMAS AFTER CURETTAGE AND CRYOSURGICAL TREATMENT

Richard Chiu, David McCall, David G. Mohler

Departments of Orthopaedic Surgery and Epidemiology, Stanford Medical School

Chondrosarcomas are malignant cartilage tumors that are traditionally treated by radical resection. This procedure involves amputation or removal of the entire bone segment on which the tumor resides, which results in lifelong disability and reduced quality of life. Grade 1 chondrosarcomas are often histologically and clinically indistinguishable from benign enchondromas, and are less aggressive than chondrosarcomas of higher grades (grades 2 and 3). Given their lower metastatic potential, grade 1 chondrosarcomas can potentially be treated by curettage with cryosurgery, an intralesional procedure that has been used to treat benign tumors. Curettage with cryosurgery avoids the post-operative morbidities resulting from radical resections, but may be associated with a higher risk of recurrence due to the incomplete removal of tumor cells. This study was performed to assess the efficacy of curettage and cryosurgery in treating grade 1 chondrosarcomas and in preserving the functional performance of patients.

We retrospectively reviewed the medical records of 51 patients treated by curettage and cryosurgery for grade 1 chondrosarcomas and enchondromas between 1995 and 2009 with a minimum follow-up of 12 months in clinic or by phone. Primary outcomes were tumor recurrence and functional score measured by the MSTS (Musculoskeletal Tumor Society) scoring scale. Patients were also noted for secondary outcomes of tumor site, preoperative pain, bone scan results, and radiographic signs of endosteal scalloping. A summary of results is provided in the Table below.

	Patient	Clinic	Phone	MSTS		Contact	Tumor	Pre-Op.	Bone	Endo.	Tumor
	Age	Followup	Followup	Score	Gender	Mode	Recur	Pain	Scan	Scallop	Site
Median	46	29	54	29	17 M	36 clinic	1 yes	46 yes	19 yes	32 yes	46 long bone
Mean	49	38	55.4	26.9	34 F	15 phone	50 no	5 no	32 no/NA	19 no/NA	5 other *
Range	20-79	13-134	17-135	11-30	*other refers to sacrum, pelvic ilium, metatarsal, or phalanx						

Followups given in months; MSTS score out of 30

There were 16 cases of grade 1 chondrosarcomas, 16 cases of enchondromas, and 19 cases of indistinguishable tumors (grade 1 chondrosarcoma or enchondroma), most of which were tumors of the long bones. Only 1 case of recurrence occurred out of the 51 patients. Median and mean  $\pm$  SD of MSTS functional score (maximum 30) were 29 and  $26.9 \pm 4.7$  respectively. No significant differences between categories of gender, contact mode, or diagnosis (grade 1 chondrosarcoma, enchondroma, indistinguishable) were observed for recurrence or functional score. Curettage/cryosurgery yielded a negligible recurrence rate (2%), and the functional outcomes were superior to those of radical resection (data from literature). With appropriate follow-up, curettage and cryosurgery may serve as a good alternative to radical resection as the mainstay therapy of grade 1 chondrosarcomas, as this intralesional procedure yields negligible recurrence rates but superior functional results.

*This study was funded by the Stanford Medical Scholars Research Fellowship.*

## **POLYMETHYLMETHACRYLATE PARTICLES INHIBIT THE EXPRESSION OF OSTEOGENIC TRANSCRIPTION FACTORS RUNX2, OSTERIX, AND DLX5 IN OSTEOPROGENITOR CELLS**

**Richard Chiu**, Kierann Smith, Gene Ma, R. Lane Smith, Stuart B. Goodman  
Departments of Orthopaedic Surgery and Bioengineering, Stanford Medical School

Wear debris particles generated from total joint replacements have been implicated as a significant inhibitory factor of osteoprogenitor differentiation. Previously, our study has shown that particles of polymethylmethacrylate (PMMA) cement inhibit the osteogenesis of osteoprogenitors with respect to mineralization and alkaline phosphatase expression. However, whether this inhibition results from direct effects of particles on transcription factors or signaling pathways that regulate osteogenesis is unknown. Runx2, osterix, and Dlx5 are the primary transcription factors that regulate osteoblast differentiation and bone formation.  $\beta$ -catenin is a transcriptional activator of the canonical Wnt signaling pathway, which activates Runx2 gene transcription. Msx2 is a reciprocal antagonist of Dlx5-mediated osteogenesis. In this study, we examined the effects of PMMA particles on the expression of Runx2, osterix, Dlx5, Msx2, and  $\beta$ -catenin in osteoprogenitor cells.

Confluent cultures of MC3T3-E1 osteoprogenitor cells (ATCC) were treated with PMMA particles (1-10  $\mu$ m, Polysciences) at concentrations of 0.00, 0.15, 0.30, and 0.60% v/v on their first day of differentiation in osteogenic medium containing 50  $\mu$ g/mL ascorbic acid and 100 mM  $\beta$ -glycerophosphate. Cells were treated with PMMA particles during the first six days of differentiation. RNA was extracted from cell samples by the TRizol method each day throughout this six-day period, reverse transcribed into cDNA, and quantified by real time-PCR using primers for mouse Runx2, osterix, Dlx5, Msx2, and  $\beta$ -catenin, with normalization to 18S expression.

MC3T3-E1 cells challenged with PMMA particles showed a significant dose-dependent decrease in the expression of Runx2, osterix, and Dlx5 throughout the 6-day period, and through days 1-4 for  $\beta$ -catenin. The expression of Msx2, a reciprocal antagonist of Dlx5-mediated osteogenesis, was not significantly reduced except at the highest particle concentration (0.60% v/v) and only during days 1-4. This study has shown that PMMA particles inhibit the differentiation of osteoprogenitor cells by suppressing the expression of Runx2, osterix, Dlx5, and  $\beta$ -catenin. The inhibition of osteoprogenitor differentiation by implant wear debris is therefore mediated by direct inhibitory effects of particles on the expression of osteogenic transcription factors.

*This study was funded by the Stanford Medical Scholars Research Fellowship.*

## **POLYMETHYLMETHACRYLATE PARTICLES IMPAIR OSTEOPROGENITOR VIABILITY BY CELL NECROSIS NOT APOPTOSIS**

**Richard Chiu**, Gene Ma, Kierann Smith, R. Lane Smith, Stuart B. Goodman  
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Osteolysis in total joint replacement is mediated in part by the biologic reactions of bone cells and progenitors to implant wear debris. Recent studies have shown that particles of titanium implants induce cell death by apoptosis in rat calvarial osteoblasts, murine osteoclasts, and human mesenchymal stem cells. Previously, our study has shown that particles of polymethylmethacrylate (PMMA) cement inhibit the osteogenesis of murine osteoprogenitor cells, but the effects of these particles on the viability of these cells has not been studied. The purpose of this study was to determine whether PMMA particles impair the viability of murine osteoprogenitor cells, and whether this cell death occurs by apoptosis or necrosis.

Confluent cultures of murine MC3T3-E1 osteoprogenitor cells (ATCC) were treated with PMMA particles (1-10  $\mu\text{m}$ , Polysciences) at doses of 0.000, 0.038, 0.075, 0.150, 0.300, and 0.600% v/v for 72 hrs. Culture supernatant levels of lactate dehydrogenase (LDH), an intracellular enzyme released from dead cells, were measured at 24-hr intervals. Cell number was determined at similar time intervals by hemocytometer cell count with trypan blue staining. Particle effects on proliferation were assessed by incubating cells in BrdU nucleoside for 24 hrs following particle challenge for 24, 48, and 72 hrs, with subsequent spectrophotometric measurement of BrdU uptake. A flow cytometry-based TUNEL assay was performed to detect cells with fragmented DNA, a hallmark of apoptosis, in cultures challenged with particles at doses of 0.075 and 0.300% v/v for 48 and 72 hrs. Cells were observed for morphology and evidence of particle phagocytosis.

MC3T3-E1 cells challenged with PMMA particles showed a significant dose- and time-dependent increase in LDH release, a dose-dependent decrease in cell number, and a dose-dependent decrease in BrdU uptake for cells challenged with particles for  $\geq 48$  hrs. MC3T3-E1 cells showed evidence of particle phagocytosis, swelling, and lysis as observed under the microscope. TUNEL assay revealed no apoptotic cells in particle-treated cultures at all days and particle doses tested. This study has shown that PMMA particles induce osteoprogenitor cell death, as evidenced by the increase in LDH release and the decreases in cell number and BrdU uptake. Evidence of cell swelling and lysis, a characteristic of necrosis, and the absence of apoptotic cells as determined by the TUNEL assay, indicate that cell death occurs by necrosis rather than apoptosis. The mechanism of cell death may depend on the chemical composition of the particle involved (e.g., metal vs. polymeric, metal ion effect). Bone loss induced by wear debris particles therefore involves reduction of osteoblast numbers due to cytotoxic effects on osteoprogenitor cells.

*This study was funded by the Stanford Medical Scholars Research Fellowship.*

## **Longitudinal investigation of cancer biomarker expression levels pre- and post-chemoradiotherapy treatment using multiplexed proximity ligation assays (PLA).**

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**Background:** Pancreatic cancer is the 4<sup>th</sup> leading cause of death in the United States with an overall 5-year survival of less than 5%. Although there have been significant improvements in the overall survival rates for many cancers in the past 25 years, there has been little progress for pancreatic cancer. In a prospective Phase II study, we investigated the efficacy of integrating stereotactic body radiotherapy (SBRT) with gemcitabine chemotherapy. Our primary endpoint was overall survival and local progression-free survival. In addition, we analyzed a panel of 21 biomarkers from the plasma of these patients before and after SBRT to determine if there were any biomarkers that were predictive of response to therapy or overall survival.

**Methods:** We analyzed the expression levels of 21 cancer biomarkers via multiplexed proximity ligation assays (PLA<sup>1,2</sup>) in 32 pancreatic cancer patients and 19 age-matched controls. These patient samples were assessed for these biomarkers prior to initiating therapy. In a subset of patients, we also compared changes in this biomarker profile with the goal of identifying a plasma biomarker profile predictive of outcome or response to treatment.

**Results:** In the clinical trial<sup>3</sup> were treated with SBRT and a median of 5 cycles of gemcitabine chemotherapy resulted in a median survival of 11.8 months, a 1 year survival of 50% and a local control rate of 94%. Results of biomarker studies corroborate prior studies showing significant differences in biomarker expression levels between controls and patients prior to treatment. Three biomarkers, EpCAM, MESO, and CA125 were significantly predictive of pancreatic patient survival. In addition, decreasing levels of EpCAM after therapy was an excellent predictor of patient survival. Future work will use a larger patient pool to verify these changes in biomarker profile before and after chemoradiotherapy treatment.

**Conclusions:** This study demonstrated that SBRT and gemcitabine chemotherapy was a promising treatment strategy. Furthermore, PLA was able to identify several prognostic markers for this disease and may be used in the future to select the patients that would be most appropriate for this therapy.

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## IDENTIFICATION OF POTENTIAL VIRULENCE GENES IN *FRANCISELLA TULARENSIS* BY INFECTIVITY ASSAYS PERFORMED IN *DROSOPHILA MELANOGASTER*

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Tularemia is a serious zoonotic disease in humans that is caused by the gram-negative coccobacillus *Francisella tularensis*. To date, our understanding of *Francisella* virulence mechanisms remains rudimentary, and this has prevented the development of improved interventions or preventive vaccines for *F. tularensis* infection. Recent work using genome-wide screens has begun to change this, but such studies have identified dozens of candidate virulence genes, and it will be increasingly difficult to test all of them efficiently in traditional mouse models. To address this, recent work has established the fruit fly *Drosophila melanogaster* as a viable model host for the study of *F. tularensis* infection.

In the present research, we made use of *D. melanogaster* to perform an efficient small-scale analysis of potential *F. tularensis* virulence genes and their impacts on infectivity. A “competition assay” approach was used to test each candidate gene. In this type of experiment, the infectivity of wildtype *Francisella* is directly compared to the infectivity of a mutant *Francisella* strain that has a putative loss-of-function mutation in a candidate virulence gene. Through these assays, we identified four candidate genes that do not appear grossly necessary for the *in vitro* growth of *Francisella* but whose absence reduces the bacterium’s *in vivo* growth by at least ten-fold. The predicted products of these genes include an MDR-type secretion protein, a two-component response regulator, and a Cro/CI-family transcriptional regulator.

Future work will aim to characterize these candidate *Francisella* virulence genes. Specifically, the mutant *Francisella* strains will be tested in mice to determine the relevance of the identified virulence genes to mammalian infection. These mutant strains will also be tested in *Drosophila* that possess defective immune pathways. If the mutant *Francisella* regains infectivity in an immunocompromised host, this may provide insight into the function of host immunity in *Francisella* infection.

## **SUBTHALAMIC NUCLEUS AND MOTOR CORTEX LOCAL FIELD POTENTIALS IN PATIENTS WITH DYSTONIA AND PARKINSON'S DISEASE**

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The pathophysiology of dystonia is poorly understood. A recent hypothesis of the pathophysiology of Parkinson's disease (PD) implicates excessive oscillatory activity in an "antikinetic" frequency range in the subthalamic nucleus (STN). However, oscillatory frequencies characteristic of dystonia in STN have not been studied. Here we compare power spectral density from local field potentials from the STN in PD and dystonia. Additionally, to assess the role of cortical input in determining characteristic oscillation frequencies, we compare activity from the STN to motor cortex activity using electrocorticography, which has not previously been done in movement disorders patients.

Local field potentials (LFPs) were recorded simultaneously from the STN and the primary motor cortex (M1) in patients undergoing deep brain stimulation surgery for either PD or dystonia. These signals were recorded during alternating periods of rest and movement. The distribution of power in physiologically relevant frequency bands was analyzed, comparing PD vs dystonia, rest vs movement, and STN vs M1. Results show that dystonia patients have increased power in the low gamma (31-55Hz) range compared to PD patients, but much less power in the low beta (13-21 Hz) range. This is true under both resting and movement conditions, although both groups exhibit a decrease in power in the low beta range during movement compared to rest periods. Recordings from the STN and M1 were very similar.

These data support the hypothesis that Parkinson's disease is associated with beta band oscillations in the STN, and suggest that increased oscillations in the gamma frequency range in the STN may be associated with dystonia. These data further suggest that motor cortex activity in patients with PD or dystonia may reflect pathophysiologic activity in the STN. Increased gamma activity in dystonia provides a mechanism for the known increase in cortical plasticity in a variety of dystonic conditions.

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## **NATIONAL TRENDS OF COMPLICATION RATES DURING HOSPITALIZATION IN THE U.S. FROM 1998-2005**

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**Context:** Although patient safety and quality of care are adversely affected by complications (preventable adverse events), little is known about their rates of incidence. **Objective:** To establish national benchmark trends for preventable adverse events during inpatient hospitalization in the United States from 1998-2005. **Design and Setting:** Using Patient Safety Indicator (PSI) software from the Agency for Healthcare Research and Quality (AHRQ), preventable adverse events (PAE) were identified in the Nationwide Inpatient Sample (NIS) between 1998 and 2005, which encompasses nearly 300 million discharges from 1,054 acute-care hospitals across 37 states in the U.S. **Main Outcome Measure:** Risk-adjusted incidence rates and joinpoint trends for the 20 PSIs.

**Results:** During the 8 years analyzed 6.7 million PSI events occurred. Out of the 20 PSIs, 15 showed statistically significant trends ( $p < 0.05$ ). Eight PSIs were increasing: 1, complications of anesthesia; 3, decubitus ulcer; 7, infections due to medical care; 10, postoperative physiological or metabolic derangement; 11, postoperative respiratory failure; 12, postoperative pulmonary embolism or deep vein thrombosis; 13, postoperative sepsis; and 15, accidental puncture or laceration. Seven PSIs showed decreasing trends: 4, failure to rescue; 6, iatrogenic pneumothorax; 8, postoperative hip fracture; 17, birth trauma injury to neonate; 18, obstetric trauma- vaginal with instrument; 19, obstetric trauma- vaginal without instrument; and 20, obstetric trauma- cesarean delivery. The remaining five PSIs showed no statistically significant trend: 2, death in low-mortality DRGs; 5, foreign body left during procedure; 9, postoperative hemorrhage or hematoma; 14, postoperative wound dehiscence; and 16, transfusion reaction.

**Conclusion:** This is the first study to establish national trends of PAEs during the last decade using a nationally representative database. Statistically significant decreasing trends indicate PAEs where progress has been made and increasing trends identify PAEs where further investigation, monitoring and intervention should be directed.

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## **SEROTONIN TRANSPORTER SHORT ALLELE IS ASSOCIATED WITH PRO-INFLAMMATORY BIAS AT BASELINE AND AFTER PSYCHOSOCIAL STRESS**

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Individuals who carry two short alleles (SS) for the promoter for the serotonin transporter (5HTT) gene, SLC6A4, carry an increased risk of depression after stressful life events. Since pro-inflammatory cytokines are known to mediate aspects of depression, and since psychological stress has been shown to increase circulating concentrations of pro-inflammatory cytokines, we hypothesized that an exaggerated pro-inflammatory cytokine response to stress may partially mediate this genetic vulnerability.

Fifteen individuals with two copies of the long allele (LL) and eleven individuals with two copies of the SLC6A4 short allele participated in a standardized psychosocial stress task. The pro-inflammatory cytokine IL-6 and the anti-inflammatory cytokine IL-10 were measured in serum collected at baseline and at 30, 60, 90, and 120 minute intervals after the completion of the 20 minute stress task. Compared to LL individuals, individuals with the SS genotype showed an elevated IL-6/IL-10 ratio across all time points.

These findings suggest that SS individuals may have a pro-inflammatory bias that leaves them vulnerable to the depressogenic effects of life stressors, and point to the need for further clarification of how genetic vulnerability might be transduced into psychiatric symptomatology by inflammation.

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## PULLULAN DELIVERY FILM FOR TARGETED ISCHEMIC PRECONDITIONING

**Michael G. Galvez BA**, Jayakumar Rajadas PhD, Geoffrey C. Gurtner MD. Department of Surgery, Division of Plastic and Reconstructive Surgery

Ischemic preconditioning (IPC) is a phenomenon whereby a brief episode of ischemia-reperfusion (I/R) triggers potent mechanisms to protect tissue from subsequent sustained I/R. The intentional creation of IPC before flap transposition, results in increased ischemic tolerance and improved flap survival. However, partial or complete flap necrosis continues to be a challenging problem, due inadequate neovascularization and I/R-induced cell necrosis and apoptosis. Deferoxamine (DFO) a small molecule that increases hypoxia inducible factor-1 (HIF-1), has been shown to decrease tissue necrosis, and can be effective for pharmacological IPC. Covalently conjugating DFO to pullulan, a biodegradable carbohydrate, was investigated for enhanced IPC in flap tissue before transposition.

Delivery of DFO from pullulan films was measured and compared to subcutaneous DFO injections. A skin flap was elevated dorsally on C57Bl/6 mice, then a silicone sheet was implanted between the flap and wound bed, and before flap transposition a pullulan film was placed directly above this sheet. Three groups were assessed: DFO film, control film, and no film. Flap survival was quantified, photographed, and harvested for histology. Neovascularization, ROS, and HIF stabilization were assessed. A novel drug delivery system was successfully created through the conjugation of DFO to pullulan. The DFO film proved to have consistent release of active drug *in vivo*. Flaps had decreased necrosis, and increased viability and perfusion. A prolonged period of protection was evident compared with controls, which correlated strongly with HIF stabilization.

IPC may help to increase the success rate of flap transposition and decrease the complications associated with I/R injury. Delivery of targeted DFO conjugated to pullulan is a practical method to enhance IPC to protect against tissue injury, prevent flap necrosis, increase neovascularization, and provide enhanced cellular tolerance to hypoxic stress.

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## **TRAINING TRAINERS IN HEALTH AND HUMAN RIGHTS: IMPLEMENTING CURRICULA REFORM IN SOUTH AFRICAN HEALTH SCIENCES INSTITUTIONS**

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Health sector complicity in apartheid era abuses prompted calls to integrate human rights into the educational curricula of health professionals in South Africa. To address this aim, a Train the Trainers course in Health and Human Rights was established to train faculty members in teaching human rights to students. This study sought to follow-up trainees to determine their experiences in implementing human rights teaching since attending the course.

A survey was distributed to past participants of Train the Trainer courses held from 1998-2006. Quantitative and qualitative data were collected and analyzed. Of 162 past participants, 46 completed the survey. Half of these respondents (23) reported implementing a total of 33 human rights educational activities that were part of the formal curriculum at their institutions. Additionally, 72 extracurricular activities were offered by 21 respondents, not all of whom had successfully implemented formal coursework. Co-workers were most commonly identified as allies for human rights educational initiatives. Barriers to implementation were budget and time constraints and perceived apathy of co-workers or students. Overall, respondents reported personal growth since taking the course and positively perceived their work in human rights.

Training courses in health and human rights provide faculty members with the historical context, educational competencies, and collective volition to incorporate human rights in formal health sciences curricula. Despite institutional barriers, co-workers are vital allies in supporting human rights teaching. Thus, training of fellow staff and those in key leadership roles is perceived as vital to future curriculum reform efforts. There is room for future research in the form of comparison studies between faculty formally trained in health and human rights with those who are not. A further longitudinal study of students educated at institutions with and without human rights curricula would reiterate the need for formal training in this area to ensure the protection of patients in the years beyond the medical classroom.

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## CHIPPING AWAY AT CANCER DETECTION WITH MAGNETIC NANOTECHNOLOGY

**Richard S. Gaster**, Drew A. Hall, and Shan X. Wang, Department of Bioengineering, Electrical Engineering and Materials Science Engineering, Stanford University

Medical decision-making is increasingly based on molecular testing; quantitative detection of disease-specific proteins in serum and other bodily fluids forms the basis of many diagnostic tests to direct therapy in diverse areas of clinical medicine. Current methods of protein detection, however, are limited by their sensitivity, multiplexing capacity, or, most importantly, robustness to the composition of complex biological samples. Detection across varied samples is crucial: for instance, a urologist may provide urine, a neurologist cerebrospinal fluid, a cardiologist blood, or an oncologist cell lysates. The diversity of such matrices (such as autofluorescence, pH, ionic strength, and temperature) has hindered the generalizability and sensitivity of the majority of protein detection platforms, thus greatly reducing their clinical utility. Here, we present a magnetic nanosensing protein detection technology to address this pressing need.

Fortunately, even the most complex biological samples lack a magnetic background signal and do not interfere with the magnetic transduction mechanism. Therefore, a magnetic field-based detection platform would appear to be ideally suited for exceptionally sensitive protein detection in diverse clinical samples. With our technology we show the ability to measure an entire panel of proteins down to low femtomolar ( $10^{-15}$ ) concentrations with equally quantifiable detection in phosphate buffers, serum, cell lysates, urine, and saliva samples. In addition, we show application of our technology to multiplex cancer diagnostics and sensitive tumor marker profiling that is orders of magnitude more sensitive than competing technologies with exceptionally broad linear dynamic range.

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## **OVERSEAS RESIDENCY TRAINING: TO WHAT EXTENT DO NON-UNIVERSITY AFFILIATED INTERNATIONAL ROTATIONS PROVIDE TRAINING WITHIN ACGME CORE COMPETENCIES?**

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The Accreditation Council for Graduate Medical Education (ACGME) Outcomes Project calls for greater emphasis on education highlighting competency in professionalism and interpersonal communication through cross-cultural experience, however there is little known about the structure and outcomes of overseas rotations. We conducted a web-based census to identify educational practices of non-university affiliated U.S. volunteer organizations in low-income nations. Organizations were identified via internet search and electronic surveys were sent to each organization with data analysis using SPSS.

We identified 172 organizations, and excluded 70 for the following reasons: residents not accepted (31), contact information not valid (22), non-medical work (15), not based in the U.S. (1), or University- affiliated (1). We achieved a 54% response rate. Licensed anesthesiologists volunteer for 80% of organizations and 53% invite anesthesia residents. While 55% consider resident education part of their mission, only 27% are familiar with ACGME Core Competencies. Over 79% do not have stated goals and expectations, and <50% provide formal learning activities on compassion and/or ethics (35%), cultural competence (46%), interpersonal and/or communication skills (47%), teaching skills (27%), application of medical knowledge (27%) or morbidity & mortality conferences (11%). However 85% include volunteers with teaching experience, 80% state that residents are supervised and 78% provide feedback. Regarding core competencies, 67% stated that residents frequently or always achieve goals related to patient care, medical knowledge (73%), interpersonal and communication skills (93%), professionalism (94%), practiced-based learning (40%), and systems practice (75%). Eighty percent agreed that volunteer experience makes residents better physicians.

While a majority of organizations are not familiar with ACGME core competencies, their programs often include opportunities which fulfill competencies and basic curriculum. Our future work will include a case-study of the Anesthesia Department, Kilimanjaro Christian Medical Center, Tanzania to determine the applicability of U.S. competency-based educational goals to overseas rotations.

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## **SLEEP AND THE CORTISOL AWAKENING RESPONSE IN WOMEN WITH METASTATIC OR RECURRENT BREAST CANCER**

**Cheryl L. Green**, Sandra E. Sephton, Bita Nouriani, Helena C. Kraemer, Laura C. Lazzeroni, Janine E. Giese-Davis, Robert M. Sapolsky, and David Spiegel.

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Flattened diurnal cortisol slopes are associated with decreased longevity in women with metastatic or recurrent breast cancer. The larger of the two components determining the diurnal slope is the value of the morning rise in cortisol, which is in turn highly dependent upon sleep. Prior studies of the relationship between sleep and the cortisol awakening response (CAR) have resulted in seemingly contradictory findings. The purpose of our study was to examine several variables as to sleep quality and quantity simultaneously in the hope of clarifying the relationship between sleep and the rate of rise (slope) in the CAR. Our hypothesis was that poorer sleep quality and shorter sleep duration would be associated with greater CAR slopes. Our method was to track sleep data in 19 women with breast cancer using the Nightcap electronic sleep system. In addition, we measured salivary cortisol levels at awakening and at four successive 10-minute intervals post-awakening.

Results were that sleep quantity, though not sleep quality (measured by sleep efficiency, sleep latency, and number of nocturnal awakenings), was associated with log CAR slope. Both shorter sleep duration and earlier awakening time were individually correlated with CAR slope ( $r = 0.61$ ,  $p < 0.0001$ ;  $r = .82$ ,  $p < 0.0001$ ), and they were not correlated with one another. These two variables also interacted with one another in influencing CAR slope such that shorter sleep duration is associated with increased CAR slope when a breast cancer patient awakens earlier, but not when she awakens later in the day, and earlier awakening time is associated with increased CAR slope when a patient sleeps for an insufficient length of time, but not otherwise ( $F = 8.7$ ,  $p = 0.01$ ).

Thus, decrements in sleep quantity, though not in sleep quality, were associated with greater CAR slope in metastatic breast cancer patients. Given prior findings that greater CAR slope is responsible for the flattened diurnal cortisol slopes found in breast cancer patients with reduced longevity, this study was significant in that it: clarified which of many sleep variables are associated with the steeper CAR slopes, suggested that the steeper morning slopes might represent a stress response to awakening, and potentially elucidated one mechanism whereby host resistance to cancer, and thereby longevity, was diminished. Future studies might include developing a mouse model whereby links between breast cancer, diminished sleep, spiking morning cortisol, and longevity might be examined prospectively.

## NOTCH SIGNALING MEDIATES COCHLEAR PROGENITOR CELL DIFFERENTIATION

**Gary M. Green**, Stefan Heller, Alan G. Cheng. Stanford University Department of Otolaryngology

Jagged 1, a member of the DSL class of Notch ligands, is selectively expressed in supporting cells of postnatal cochlea. Following hair cell injury with neomycin, supporting cells proliferate and transdifferentiate into hair cells. Pharmacological inhibition of the Notch receptor results in increased numbers of hair cells in the rodent cochlea. Using *in vitro* cochlear culture and novel sphere-based assays, we have explored the role of Jagged1-Notch interactions on progenitor cell fate in early postnatal murine cochlea.

Our research confirmed the localization of Jagged1 to specific supporting cell populations. Notch inhibitor increased the number of hair cells, reversing some neomycin-induced loss. In sphere-based progenitor cell assays, we discovered that organs of Corti cultured in the presence of neomycin yield fewer sphere-forming cells than those in control media, suggesting a contracting progenitor cell population due to transdifferentiation or cell loss in acute injury. In a limited trial, we did not detect a difference with addition of Notch inhibitor. *In vitro*, organ of Corti spheres differentiate into Myosin VIIa+/Math+ hair cell-like cells at the periphery of Jagged1 cell islands, recapitulating the *in vivo* relationship. Math1+ cells appear to be more strongly associated with Jagged1 regions than Myosin VII+ cells. This underscores the association of Jagged1-Notch interactions with the hair cell maturation process. Finally, we have begun to use FACS to select Jagged1+ cell populations. Jagged1 antibody labels 2-3% of cells from the dissected Organ of Corti.

Future research includes performing RT-PCR on FACS sorted cells and culturing these cells in isolation and with Notch+ cells. We will also look at Notch inhibitor effects on progenitor cell fate at different time points to better understand the temporal control of this pathway. We will also analyze the effects of Notch agonists on spheres in suspension culture and in differentiation protocols. A greater understanding of the Jagged-Notch pathway may help develop pharmacologic treatments to regenerate hair cells and to create *in vitro* derived cells for transplant applications in sensorineural hearing loss.



## **AMYLOID PRECURSOR PROTEIN C-TERMINAL FRAGMENTS FORM LARGER AGGREGATES AND ARE TRAFFICKED MORE SLOWLY THAN FULL-LENGTH COUNTERPARTS**

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Memory loss in Alzheimer's disease (AD) patients is largely due to degeneration of basal forebrain cholinergic neurons (BFCN). Interestingly, individuals with Down Syndrome (DS) who have a triplication of the gene encoding amyloid precursor protein (APP) all develop AD-like neuropathological symptoms. Studies have suggested that accumulation of APP or its cleaved carboxyl-terminal fragment(s) (CTFs) may disrupt retrograde axonal transport of nerve growth factor (NGF), a vital factor for neuronal maintenance of BFCN. However, the cellular and molecular mechanism(s) for such disruption remain unknown. This study targeted elucidating the intracellular localization of APP and its 99 amino acid CTF (C99) in PC12M cells. It is not clear where these cleavage events occur and how the CTFs can mediate trafficking deficits.

APP and C99 fragments with C-terminal GFP tags were transfected into PC12 cells, a rat adrenal tumor cell line that can be induced to differentiate into neurons by NGF. Cells were then stained with antibodies recognizing various compartments along the endocytic pathway to determine localization. APP and C99 are contained in morphologically distinct vesicles. This could be caused by the higher levels of C99, a smaller protein. C99 tends to form large aggregated structures that colocalize with the early endosomal markers Rab5 and EEA1. There is some colocalization of both APP and C99 with the TrkA receptor for NGF, indicating that they could be transported together. Additionally, live imaging of transfected cells allows for some quantification of the rate of transport of APP and C99 containing vesicles. C99 is not trafficked as readily as APP, showing slower movement and more accumulated, bright punctae on live imaging.

These results together suggest that the pathology of APP overexpression might be mediated by the interaction of C99 and Rab5 on the signaling endosome. Further investigation is underway to rigorously quantify the rate of NGF trafficking under APP and C99 overexpression conditions. Also, it will be necessary to purify the vesicles containing APP and C99 and use proteomic analysis to determine what other modulating proteins can be implicated. All this will shed light on the relationship between APP overexpression and the neuronal degeneration seen in AD.

## **STRATEGIES FOR MANAGING THE CONSEQUENCES OF A MANUFACTURING DEFECT AND RECALL OF A DRUG PRODUCT PRODUCED IN A CONTRACT MANUFACTURING FACILITY**

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Drug product defect and recall is an increasingly frequent occurrence that can harm patients, physicians, products, and companies. In March 2008, generic pharmaceutical giant Actavis voluntarily recalled all lots of its fentanyl transdermal patch sold in the United States. The recall, involving nearly 3 million patches, was due to a fold-over defect in the manufacturing process that could cause leakage of the fentanyl from the patch; direct exposure to high concentrations of fentanyl can lead to serious and potentially fatal adverse events.

The recall of this generic drug product, contract manufactured by Corium and marketed and distributed by Actavis, can serve as an illustration of the challenges and strategies for preventing, recognizing, and managing a recall based on a manufacturing defect. Actavis and Corium must work together to recall the patch from the public, identify and correct the manufacturing defect, and preserve the value of the drug product and of the company itself during the recall. This report describes some of the lessons learned in past drug recalls to illustrate the challenges facing Corium and Actavis as they handle the recall of the fentanyl patch.

## **SECONDARY SCHOOL STUDENTS AS COMMUNITY EDUCATORS ABOUT MALARIA: ADULT AND STUDENT PERSPECTIVES FROM THE GAMBIA**

**Jocelyn James**, Brian Blackburn, Kalifa Bojang. Department of Internal Medicine, Stanford University, and Medical Research Council, The Gambia.

Malaria contributes substantially to morbidity and mortality in Sub-Saharan Africa, particularly through its effects on pregnant women and children under 5. Uptake of prevention measures such as insecticide-treated nets poses an important challenge to malaria control. Arguments have been made for engaging schoolchildren in malaria education and prevention in Africa. However, traditional constructs of age-related knowledge and authority may limit the efficacy and acceptability of using students as community educators, and this social context has been only minimally explored. In The Gambia, secondary school students have helped to conduct community education about malaria. We set out to explore the practice and acceptability of this practice in The Gambia through focus group interviews with secondary school students and adults. In February and March 2008, we conducted individual oral questionnaires and focus group interviews in 3 schools and 3 communities of the Basse area. Thirty-one adults and thirty-six students participated in discussions separated by gender.

Comments from students and adults revealed that secondary school students are well respected and assume important roles in the community. Students perceived that malaria is important to their communities and that they gain knowledge, confidence, and practical skills by participating in malaria education programs. They distinguished between teaching women and men and between teaching about malaria and HIV/AIDS. They were aware and respectful of behavioral norms and values. Adults spoke positively of student-conducted activities on malaria. However, interviews also revealed the profound importance of traditional role relationships and values toward age and knowledge.

We conclude that using secondary school students as community educators is a promising component of malaria education, but that value to students and potential contributions to prevention practices must be weighed against the risk of alienating elders and altering role relationships. The design and implementation of such activities should actively and substantially involve elders and traditional leaders.

## **CHARACTERIZATION OF KAPOSI'S SARCOMA-ASSOCIATED IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME IN SUB-SAHARAN AFRICA**

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Background: Immune reconstitution inflammatory syndrome (IRIS) can occur in HIV-infected patients upon initiation of antiretroviral therapy (ART). IRIS, which is believed to result from a newly reconstituted immune system aggressively responding to residual opportunistic pathogens, can vary in severity from minor signs and symptoms to death. In sub-Saharan Africa, in addition to the AIDS epidemic, there is also a high prevalence of infection with the virus that causes Kaposi's sarcoma (KS). This has resulted in KS becoming the most common cancer in the region. Now that ART is becoming available in Africa, it is important to investigate the frequency and severity of IRIS in the context of treating patients with AIDS-related KS.

Objective: To estimate the frequency and severity of IRIS in patients with AIDS-related KS who initiate ART.

Methods: We investigated the occurrence of IRIS in the context of a clinical trial comparing two ART regimens given to patients with AIDS-related KS. Participants were examined prior to therapy and then every 4 weeks for 48 weeks. Given limited prior information regarding KS-IRIS, we used a pilot phase to develop a protocol to capture events suspected to represent KS-IRIS. A questionnaire was designed to record relevant signs and symptoms and digital photography was used to document cutaneous lesion changes.

Results: Of 23 participants with at least 8 weeks of observation, the median age was 33 years, 43 percent were women, median pre-ART CD4+ T cell count was 74, and 13 (57 percent) developed a sign or symptom compatible with KS-IRIS. Clinical findings were varied and, in cutaneous lesions, included swelling, increased pigmentation, erythema, tenderness, warmth, and abnormal sensations. There were visceral manifestations in at least one participant, in the form of a refractory symptomatic pleural effusion. While death occurred in one participant, the majority experienced improvement without additional interventions.

Discussion: We found that KS-IRIS occurs at a clinically relevant frequency and has heterogeneous manifestations that are difficult to distinguish from natural disease progression, thereby complicating patient management. While KS-IRIS is often self-limiting, sometimes it is not, underscoring the importance of developing real-time diagnostic tests to differentiate inflammatory-based disease from natural progression of KS.

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## IDENTIFICATION OF CANCER STEM CELLS AND CHARACTERIZATION OF THEIR ROLE IN CHEMOTHERAPY RESISTANCE IN OSTEOSARCOMA

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The most common primary bone malignancy in children is osteosarcoma (OS). This cancer is exceedingly rare, with approximately 400 pediatric cases occurring yearly in the U.S. Due to the rarity of this disease, it has been difficult to perform large-scale, systematic analyses for a variety of markers, both diagnostic and prognostic. We have established a multi-institutional collaboration to obtain tumor samples from patients with OS to evaluate these samples for cell surface markers that may be either utilized diagnostically or characteristic of a poor prognosis. In particular, we are interested in examining OS for the presence of tumor-initiating cells (TICs), also referred to as “cancer stem cells”. It has been shown that TICs from other tumor types are more resistant to chemo- and radiotherapy, and as such, an abundance of these cells within a tumor may correlate with a negative overall outcome. Since no data exists as to whether this subpopulation of cells is present within OS tumors, our studies will provide great insight into the biology of this cancer. We have screened multiple osteosarcoma samples and have defined several markers which show heterogeneous expression among tumor cell populations, and have preliminary data suggesting that the abundance of one cell surface protein, CD146, may distinguish TICs from the bulk population of tumor cells. We are currently in the process of confirming that this marker does in fact serve to distinguish a cells tumor initiating capacity.

Further analysis will determine if in fact putative TICs in osteosarcoma are therapy-resistant and whether they serve as useful predictors of outcome. The development of novel, and reliable, prognostic and diagnostic markers for osteosarcoma will have significant impact upon the clinical management of patients with this disease.

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## ADVENTITIAL VEGF SIGNALING IS CRITICAL FOR RESTENOSIS AFTER VASCULAR INJURY

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Coronary heart disease (CHD) is the leading cause of death in the developed world. Percutaneous transluminal coronary angioplasty (PTCA) with concomitant mechanical stent deployment, is the dominant procedure used to achieve revascularization for CHD. In the United States alone nearly 1.3 million PTCA procedures were performed in 2004. The major hurdle limiting PTCA is restenosis of the vessel lumen, occurring in 10-50% of cases, and resulting from neointimal hyperplasia, a process characterized by vascular smooth muscle cell invasion into the tunica intima, proliferation, and secretion of extracellular matrix. Drug eluting coronary stents (DES) which elute inhibitors of cell cycle progression reduce restenosis; however, considerable concern has emerged regarding the safety of DES due to an increased risk of late stent thrombosis, probably due to delayed endothelial repair after injury. Although this complication is rare, it is frequently lethal, resulting in death or myocardial infarction in 85% of cases. The safety concerns over DES have created an urgent need define the cellular and molecular mechanisms underlying the biology of restenosis. Vascular endothelial growth factor (VEGF) is an important modulator of vasculogenesis and angiogenesis, and as such, has been the target of intense scrutiny with regard to restenosis. However, previous investigations of the role for VEGF signaling in restenosis and in post-injury reendothelialization have reached conflicting conclusions.

In order to examine the role for VEGF signaling in restenosis and in post-injury reendothelialization, we used a genetic approach to achieve temporal and cell type-specific inhibition of VEGFR-2 signaling in a validated mouse model of vascular injury. We found that global inhibition of VEGFR-2 signaling suppressed restenosis, but inhibiting smooth muscle, endothelium, and bone marrow alone did not. Using differential tissue-restricted inhibition of VEGFR-2, we were able to isolate a population of adventitial resident progenitor cells responsible for mediating the pro-hyperplastic effect of VEGFR-2. In order to determine the mechanism for stem cell mediated neointimal hyperplasia, we used periadventitial dye and adenovirus labeling to demonstrate activation, migration, and differentiation of adventitial stem cells which contribute to luminal restenosis. We also assessed reendothelialization of vessels after VEGFR2 inhibition by analyzing entire vessels for reendothelialization and found that reendothelialization was not reduced, and may be accelerated.

Together, these results suggest a new model for restenosis after vascular injury in which adventitial progenitor cells, activated by VEGF, are key players in formation of neointimal hyperplasia after vascular injury. Inhibition of VEGF signaling via VEGFR-2 reduces restenosis *in vivo* and reduces *in vitro* migration and differentiation of adventitial progenitor cells in response to VEGF. Furthermore, VEGFR-2 inhibition *in vivo* does not cause delayed endothelial repair. Our results suggest a novel role for VEGF in restenosis and could form the basis for an improved therapeutic strategy.

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## THE ROLE OF THE RETINOBLASTOMA/E2F1 TUMOR SUPPRESSOR PATHWAY IN THE DNA LESION RECOGNITION STEP OF NUCLEOTIDE EXCISION REPAIR

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The retinoblastoma Rb/E2F tumor suppressor pathway plays a major role in the regulation of mammalian cell cycle progression. The pRb protein, along with closely related proteins p107 and p130, exerts its anti-proliferative effects by binding to the E2F family of transcription factors known to regulate essential genes throughout the cell cycle. We sought to investigate the role of the Rb/E2F1 pathway in the lesion recognition step of nucleotide excision repair (NER) in mouse embryonic fibroblasts (MEFs). Rb<sup>-/-</sup>;p107<sup>-/-</sup>;p130<sup>-/-</sup> MEFs repaired both cyclobutane pyrimidine dimers (CPD) and 6-4 photoproducts (6-4PPs) at higher efficiency than did wildtype cells following UV-C irradiation. The expression of damaged DNA binding gene DDB2 involved in the DNA lesion recognition step was elevated in the Rb family-deficient MEFs. To determine if the enhanced DNA repair in the absence of the Rb gene family is due to the derepression of E2F1, we assayed the ability of E2F1-deficient cells to repair damaged DNA and demonstrated that E2F1<sup>-/-</sup> MEFs are impaired for the removal of both CPDs and 6-4PPs. Furthermore, wildtype cells induced a higher expression of DDB2 and xeroderma pigmentosum gene XPC transcript levels than did E2F1<sup>-/-</sup> cells following UV-C irradiation. Using an E2F SiteScan algorithm, we uncovered a putative E2F-responsive element in the XPC promoter upstream of the transcription start site. We showed with chromatin immunoprecipitation assays the binding of E2F1 to the XPC promoter in a UV-dependent manner, suggesting that E2F1 is a transcriptional regulator of XPC. Our study identifies a novel E2F1 gene target and further supports the growing body of evidence that the Rb/E2F1 tumor suppressor pathway is involved in the regulation of the DNA lesion recognition step of NER. Future insights into the mechanism of E2F1-mediated DNA repair may provide crucial links for understanding cellular homeostasis and the development of cancer.

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## **STOPPING A SILENT KILLER IN THE UNDERSERVED ASIAN COMMUNITY: A NOVEL LIVER CANCER PREVENTION CLINIC**

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Asian and Pacific Islanders bear a disproportionate burden of liver disease caused by chronic hepatitis B virus (HBV) infection, which is associated with a 25% risk of death from cirrhosis or liver cancer. However, few community based programs exist to tackle this serious public health problem among low income, uninsured Asian and Pacific Islander immigrants. The Hep B Free Clinic is a student-run grassroots organization that focuses on HBV and liver cancer prevention in one of the nation's largest immigrant communities: San Jose, California. From 2007-2008, we provided free HBV serological screening to 510 patients. For chronically infected patients who elected to undergo follow up monitoring, a series of blood tests were given to evaluate for liver damage (alanine transaminase, ALT), a liver cancer marker (alpha-fetoprotein, AFP), and HBV replication (HBV DNA levels).

Of those screened, 17% were chronically infected. Remarkably, one-third (33%) of infected patients were unaware that they were infected. Of those chronically infected, 100% showed signs of active liver damage as measured by elevated ALT, and 9% had elevated AFP tumor markers for hepatocellular carcinoma. Nearly one-quarter (24%) of those chronically infected carried HBV DNA levels that met the criteria for treatment. Patients who were candidates for antiviral therapy were signed up for free drug assistance programs, and those requiring triphasic CT scans for possible liver cancer were referred. Uninfected patients lacking protective HBV antibodies were provided free vaccinations. Our striking findings call for more aggressive liver cancer prevention in this community, including universal screening for HBV.

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## **IFOX PHARMACOGENOMICS: A PRACTICALITY TEST FOR A QRT-PCR FFPE TUMOR ANALYSIS METHOD**

Brian Francisco, **James T. Link**, Meghan Ramsey, Erich Schwarz, Branimir I. Sikic, Department of Medicine, Oncology Division

The objective of this study is to correlate the genomic characteristics of tumors from 72 colorectal cancer patients with patient clinical outcomes after treatment with the IFOX chemotherapeutic regimen. The regimen consists of an EGFR inhibitor (gefitinib) added to a standard treatment (5-fluorouracil, oxaliplatin, and leucovorin). The remission rates of 74% for treatment naive patients and 36% for previously treated patients are promising. A correlation between the cancer genomics and the treatment outcome could allow for more effective treatment selections and accurate prognoses.

The primary experiment is to determine the expression of 20 genes that are implicated in the drug mechanisms. The Paradise<sup>TM</sup> system was selected to evaluate the mRNA expression profiles of the genes from formalin fixed paraffin embedded (FFPE) samples. The first step is designed to screen out highly degraded samples. The method compares the qRT-PCR derived levels of two regions of  $\beta$ -actin mRNA: a 5' region and a 3' region. Degradation occurs from the 5' end so those with high 3'/5' ratios are not recommended for testing. Of the 63 available samples, 42 passed the QC test (67%, 58% of trial samples). The 42 samples underwent laser capture microdissection to isolate the tumor from the stroma, RNA isolation, and an amplification protocol. Primers that fall within the 3' UTR region of 5 of the genes (EGFR, TYMS, DPYD, TYMP, and ERCC1 plus  $\beta$ -actin) were then employed in successful qRT-PCR experiments. The identity of the  $\beta$ -actin PCR products was examined by gel electrophoresis. Unfortunately, 20% of the products did not have the correct sequence excluding those samples. At this stage, 35 of the 72 trial patients had samples suitable for further study potentially precluding statistical significance.

The results for EGFR mRNA levels were compared with EGFR protein levels detected by immunohistochemistry and found to correlate 73% (22 out of 30) of the time. Contrary to earlier single drug studies, initial analyses of the qRT-PCR data and correlation with survival surprisingly suggest that higher EGFR and ERCC1 and lower DPYD and TYMP mRNA levels correlate with longer survival. Efforts are underway to determine if the results are caused by confounding KRAS mutations. Overall, the reagent system allowed for quantification of mRNA samples from FFPE samples, but its ability to screen out low quality samples and its precision with moderately degraded samples are questionable.

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## THE ROLE OF WINGLESS PROTEIN (WNT) IN SCARLESS VS SCARRING WOUND REPAIR

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Unlike postnatal mammals, fetal mammals can heal scarlessly post-insult. The mechanisms for this ability are not fully understood but have implications for treating fibrosis and scarring in numerous systems. The wnt protein family is highly conserved in evolution and plays a crucial role in organ system morphogenesis. Wnt has been shown to promote self-renewal of stem cells and expansion of dividing pluripotent progenitor cells. Our goal was to understand the differential role of the wnt signaling in scarless and scarring wound healing.

In vivo methods involved a mouse model that expresses  $\beta$ -galactosidase when wnt is activated (BAT gal mouse) which allowed X-gal staining to visualize wnt activity after cutaneous injury. Postnatal and embryonic mice were injured. Wnt activity was assessed at several timepoints post-injury. Wnt expression did not change in embryonic scarless healing, but increased in postnatal, scarring wounds at all timepoints. Next, in vitro fetal and postnatal fibroblasts were treated with recombinant Wnt3a or recombinant TGF- $\beta$ 1. After treatment, expression of genes involved in wound healing and scar formation were assayed with quantitative RT-PCR. TGF- $\beta$ 1 is known to be involved in driving inflammation and scarring. Wnt3a was observed to increase expression of genes which are considered pro-fibrotic, including Type I Collagen, HAS1 and Hyal2 in postnatal cells and in a manner similar to TGF-beta1.

We conclude that Wnt3a increases markers of scar formation in postnatal dermal fibroblasts in a manner similar to TGF-beta1. Wnt3a effects embryonic and postnatal cells differentially: two genes that were transcriptionally increased in fetal cells but not in postnatal cells were HAS2 and 3. In order to further understand the role of Wnt signaling in scarless compared to scarring wound healing, future studies will explore the relationship of hypoxia, wnt expression, fibroblast migration and proliferation.

## **INTERFERON- $\beta$ THERAPY IN TH1 AND TH17 MODELS OF CENTRAL NERVOUS SYSTEM AUTOIMMUNITY**

**Robert Mair**, Robert Axtell, Lawrence Steinman, Stanford University

Although Interferon- $\beta$  (IFN- $\beta$ ) is one of the major treatments for relapsing remitting multiple sclerosis (MS), its mechanism of action is not fully understood. Recent work in the Steinman Lab using the mouse model of MS, experimental autoimmune encephalomyelitis (EAE), demonstrates that IFN- $\beta$  requires IFN- $\gamma$  signaling to suppress disease. This research suggests that IFN- $\beta$  treatment in the presence of IFN- $\gamma$  signaling inhibits the differentiation of TH17 cells and promotes the expression of the anti-inflammatory cytokine IL-10 in CD4 T-cells. To test the roles of IFN- $\gamma$  signaling and IL-17 inhibition in IFN- $\beta$  therapy, we assessed IFN- $\beta$  treatment in EAE induced by adoptive transfer of either TH1 or TH17 polarized CD4 T-cells. We hypothesized that the therapy would be most effective against the TH1 model of disease since it involves a high level of IFN- $\gamma$  expression and similarly effective against the TH17 model due to IFN- $\beta$  mediated inhibition of IL-17.

We found that IFN- $\beta$  is effective in treating TH1 EAE but not TH17 induced EAE. IFN- $\beta$  increases IL-10 production in the periphery in TH1 induced EAE, which correlates with the therapeutic effect. The IFN- $\beta$  therapy inhibits peripheral IL-17 production but is not sufficient to reduce disease in TH17 induced EAE. These findings further demonstrate that IFN- $\beta$  requires high levels of IFN- $\gamma$  to effectively inhibit EAE by up-regulating IL-10. They suggest that baseline levels of IFN- $\gamma$  and other TH1 cytokines could be predictive markers for therapeutic response to IFN- $\beta$  in multiple sclerosis.

## DETECTION OF NOVEL CYTOGENETIC ABNORMALITIES IN LOW-GRADE B-CELL LYMPHOMAS

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**Purpose:** In non-Hodgkin lymphomas, cytogenetic abnormalities not only define certain types of lymphomas, but they also predict prognosis. Our aim was to identify novel cytogenetic abnormalities in a subset of low grade B-cell lymphomas using fluorescence *in situ* hybridization (FISH) and polymerase chain reaction (PCR).

**Patient Samples and Methods:** Samples were formalin-fixed paraffin-embedded tissue from patients with biopsies of either lymphoplasmacytic lymphoma (LP) or splenic marginal zone lymphoma (SMZL). Samples were subjected to FISH using a commercially available probe for the immunoglobulin heavy chain (*IGH*) locus and a protocol previously used by George *et al* (*J Mol Diagn* 2005, 7:346-351). Hybridized slides were analyzed by a single microscopist and intact non-overlapping nuclei were scored for split signals and fusion signals. Two sets of 100 consecutive interphase nuclei were examined for the number of green (G), red (R), and fusion (F) signals, for a total of 200 nuclei for each sample. A nucleus was considered negative for the *IGH* translocation if it contained two fusion signals (2F), and positive if it contained two red signals and two green signals (2R2G) or one fusion signal pattern (1R1G1F). Background levels of signal pattern were determined by scoring 1000 negative control tonsil nuclei. Samples found to be positive for the translocation involving the *IGH* locus were screened for known partner genes by FISH using commercially available probes. Samples which were negative by secondary screening were then subjected to Pan-Handle PCR using standard techniques.

**Results:** 13 samples were evaluated using FISH for a translocation involving *IGH*. The diagnosis was LP for 5 cases and SMZL for 8 cases. The background level of positive signal patterns was 5.4%. 3 cases (cases 23, 24, and 26) were positive for an *IGH* containing translocation with 30%, 76%, and 18% positive signal patterns respectively. All 3 cases had the diagnosis of SMZL. Of those 3 cases, case 24 was found by FISH to be positive for a known translocation involving *IGH* and *BCL2* and was eliminated from further study. The remaining cases were negative for all known translocations by FISH. When subjected to Pan-Handle PCR, case 23 was found to have a known translocation involving *IGH* and *BCL2*, despite being negative for this translocation on previous FISH screening. Pan-Handle PCR was unable to identify a partner gene for case 26.

**Conclusion:** Of all 13 cases screened in this study, 3 cases (23%) had an *IGH* containing translocation. On secondary screening, all 3 cases were eliminated because they had a previously described translocation (*IGH/BCL2*) or because Pan-Handle PCR did not identify a partner gene (case 26). Our goal was to identify novel cytogenetic abnormalities in order to provide further information on their role in lymphoma pathophysiology. While our study did not find a novel cytogenetic abnormality, it did highlight some areas of difficulty that can be adjusted in further studies. Our results may have been limited by our small sample size and future studies should include more cases for the initial screening stage. Since all of the *IGH* containing translocations were found in SMZL cases, we will focus on this type of lymphoma in future studies. It is well known that chromosomal translocations play an important role in the pathophysiology of many hematologic and non-hematologic malignancies. Identification and characterization of these cytogenetic abnormalities may provide information useful for diagnostic and/or prognostic purposes and further investigation is warranted.

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## ELITE GOLF SWING BIOMECHANICS DRIVE BENCHMARKS FOR AMATEURS

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The essential elements of golf swing power generation have yet to be determined. We examined the relationship between golf swing biomechanics and clubhead speed at impact (CSI) in ten elite professional golfers. Three amateurs were then compared to these benchmark curves. Three-dimensional kinematics and kinetics including upper torso rotation, pelvic rotation, X-factor, O-factor, S-factor, and stance torque/kg were assessed in relation to CSI. Results revealed that peak torque/kg and peak X-factor were highly consistent among golfers; the coefficients-of-variation of hard swings were 6.8% and 7.4%, respectively. Downswing was initiated by a reversal of pelvic rotation, followed by upper torso rotation. Peak torque occurred in initial downswing and was preceded by peak X-factor in all swings. Peak torque/kg ( $\rho=0.858$ ), peak X-factor ( $\rho=0.886$ ), X-factor at impact ( $\rho=0.886$ ) and peak S-factor ( $\rho=0.829$ ) had very strong within-subject correlations to CSI. Peak X-factor was a stronger predictor of CSI than either peak pelvic or peak upper torso rotation independently. In the amateur golfers, the number of biomechanical factors which fell outside both one and two standard deviations of the professional mean increased with handicap. The data indicate a consistent pattern of elite rotational biomechanics in relation to power generation and may offer a basis for strategic training and injury prevention.

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## PRE-HOSPITAL CARE FOR OBSTETRIC EMERGENCIES IN INDIA

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A centralized EMS-model was introduced in Andhra Pradesh, India, by the Emergency Management and Research Institute (EMRI) in August 2005 and currently provides prehospital care to all 80 million residents. Given the paucity of data on prehospital pregnancy-related emergencies, shortage of skilled attendants during deliveries (48%) and high maternal mortality in India, this study seeks to describe the epidemiology and patient outcomes of prehospital obstetric emergencies following the introduction of EMRI's services. The cross-sectional study utilized a standardized questionnaire for real-time data collection from Emergency Medical Technicians responding to pregnancy-related emergency calls. All obstetric patients calling EMRI for emergency medical care within a predetermined 2-hour sampling interval were enrolled in the study. Over 12 days, every 2-hour interval of the 24-hour day was sampled. The EMRI call center computer database was used to determine call times and transport distances for each patient. Mortality data was determined by phone follow-up at 48-72 hours following prehospital care and at 42 days following completion of pregnancy (for women delivering within 42-72 hours of EMS transport).

During the study period, EMRI ambulances responded to 719 pregnancy-related calls, accounting for 18% of all emergency medical calls. The average patient age was 22.6 years, and 98.7% of patients were in their 3<sup>rd</sup> trimester. Five percent of calls were from urban areas and 95% from rural/tribal areas. Rural/tribal patients had longer call to scene times, were transported further distances and had lengthier transport times than urban patients (one-way between-subjects ANOVA,  $p < .002$ ). Nineteen percent of obstetric emergency calls were complicated, and 20% of these patients had more than one complication. Of the pregnancy-related complications, the most frequent were premature birth (28%), hypertension (22%), ambulance delivery (10%), and hemorrhage (9%). During the first 48-72 hours, 86% of pregnant women delivered and 5% of newborns died. Newborn mortality was significantly correlated with the number of pregnancy-related complications ( $p < 0.05$ ). Only one maternal death was identified by 48-72 hour ( $n=700$ ) and 42-day follow-up calls ( $n=492$ ). There was no difference in the number of complications, maternal mortality, or newborn mortality between urban versus rural/tribal groups (T-Test,  $p > .05$ ).

This is the first study to describe the epidemiology of prehospital obstetric emergencies in India. The maternal mortality ratio (MMR) for women receiving prehospital care was 204 compared to an adjusted MMR for India of 450. Further investigation is needed to determine the utility and efficacy of prehospital care models and their impact on maternal and newborn mortality in developing nations.

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## **ACCEPTANCE AND ACCESS HOME-BASED HIV COUNSELING AND TESTING AND BARRIERS TO CARE IN RURAL WESTERN KENYA**

**Jolene H. Nakao**, Daniel R. Feikin, N. Grant Miller. Stanford University

The HIV prevalence rate in Kenya is 7.4% among adults age 15-64, one of the highest national prevalence rates in the world. Although centers for HIV Voluntary Counseling and Testing (VCT) have been made numerous throughout Kenya and care/treatment for people HIV positive has been made available to patients free of charge, the proportion of the population who has been tested for HIV and who knows his/her HIV serostatus is surprisingly low, at 36% of adults ages 15-64. Furthermore, the proportion of HIV positive people in need of treatment who are actually receiving treatment is also surprisingly low, at 35%. Home-based HIV counseling and testing (HBCT) is a way to provide confidential HIV testing in a person's home. As HBCT has not yet been evaluated on a wide scale in Kenya among adult individuals, this project is designed to assess in rural Kenya 1) overall acceptance rates and variables that predict differential acceptance rates of HBCT, 2) reasons for refusal, and 3) barriers to seeking treatment for people who are HIV positive. A one-time door-to-door individual survey was performed among 4675 adults and minors over age 15 in the rural community of Asembo in western Kenya from January through August 2008; a follow-up survey was offered to those who tested positive. Trained HBCT counselors visited households in 14 villages to offer home-based HIV testing, counseling, survey participation, and a referral for free care as needed.

Overall, the HBCT acceptance rate was 76.9%, and HIV prevalence was 13.8% among those tested during HBCT. Men were 18% less likely to accept HBCT as compared to women. Participants did not directly report stigma to be the main reason for refusal of HBCT or the main barrier to seeking treatment for those testing HIV positive. Reasons for refusing HBCT included wanting to test at a later date, being afraid to know the test results, and thinking he/she was not at risk of getting HIV. 46.8% of those who tested HIV positive during HBCT who received a follow-up visit presented for care between the time of HBCT and the follow-up visit. Reasons given for not presenting for care included "planning to go later" and being "too busy."

The high acceptance of HBCT suggests it is an acceptable alternative to VCT that would likely increase rates of testing. The next step would be to consider the feasibility of implementing HBCT, weighing its benefits against its costs. Future studies may further elucidate the reasons for HBCT refusal and non-presentation for care, and may be used to augment efforts to increase HIV testing and access to treatment and care for those individuals most in need.

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## **SYNTHETICALLY LETHAL COMPOUND INHIBITS RENAL CELL CANCER THROUGH INHIBITING ANAEROBIC METABOLISM**

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Renal cell cancer (RCC) is one of the deadliest urologic cancers. Therapies for the advanced form of the cancer are limited by the fact that it is resistant to both conventional chemotherapy and radiation therapy. This contributes to the cancer's high mortality rate. We took a different approach to find anti-RCC compounds by utilizing the concept of synthetic lethality. 70-80% of RCC exhibit loss of the tumor suppressor gene VHL. We are identifying small compounds that inhibit parallel, essential pathways to VHL, thus killing RCC cells through its synthetic lethality with the loss of VHL. We screened 64,000 compounds and found several families of compounds that are synthetically lethal to VHL-deficient RCC cells. Our goal was to identify and characterize one of these compounds as a potential drug against RCC.

The 3-series compound (30408) family was independently confirmed to decrease VHL-deficient RCC cell survival in vitro. 30408 was also found to inhibit RCC growth in vivo in an orthotopic mouse model. Mechanistically, we found that 30408 inhibits glucose utilization by VHL-deficient RCC cells. There is decreased glucose retention in VHL-deficient RCC cells, likely secondary to decreased hexokinase ability to phosphorylate and therefore retain glucose inside cells. The decrease in intracellular glucose leads to a cell cycle arrest at the S phase, and a decrease ATP production, leading to increased cell death. 30408 therefore inhibits glycolysis. Because VHL-deficiency causes cells to switch from an aerobic metabolism to anaerobic metabolism, VHL-deficient RCC cells are selectively affected as they are more dependent on anaerobic metabolism than their RCC counterpart. Because of the selective toxicity of 30408 against renal cancer cells with a loss of VHL but not against renal cells with intact VHL, 30408 serves as a potential drug to selectively inhibit renal cell cancer, sparing normal renal tissues. Future work will focus on the exact mechanism of 30408 toxicities.

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## PPAR-DELTA SENSES AND ORCHESTRATES THE CLEARANCE OF APOPTOTIC CELLS TO PROMOTE TOLERANCE

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Macrophages rapidly engulf apoptotic cells to limit the release of noxious cellular contents and to restrict autoimmune responses against self antigens. Although factors participating in recognition and engulfment of apoptotic cells have been identified, the transcriptional basis for the sensing and silently disposing of apoptotic cells is unknown. Here we show that peroxisome proliferator activated receptor delta (PPARd) functions as a transcriptional sensor of dying cells. Genetic deletion of PPARd dramatically decreases expression of opsonins, such as C1qb, resulting in impairment of apoptotic cell clearance and reduction in anti-inflammatory cytokine production. This increases autoantibody production and predisposes PPARd-deficient mice to autoimmune kidney disease, a phenotype resembling the human disease systemic lupus erythematosus. Thus, PPARd orchestrating the timely disposal of apoptotic cells by macrophages, ensuring that tolerance to self is maintained.

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## PREVALENCE AND NATURAL HISTORY OF PEDIATRIC DELIRIUM IN THE INTENSIVE CARE SETTING

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**Objectives:** To determine the prevalence of delirium in a pediatric post-surgical intensive care unit (ICU) population, and explore the relationship between delirium and age, type of surgery, and length of stay in the ICU.

**Study Design:** In this cross-sectional study, we recruited over a 3 month period all children one month to 18 years old admitted to the Pediatric and Cardiovascular Pediatric Intensive Care Units following surgery. A total of 56 patients were enrolled. Subjects underwent daily assessment for the development of delirium by a multidisciplinary team, and vital signs, pain scores, sedative medications administered, and nursing assessments were recorded daily. The prevalence of delirium and its correlation with age, surgery type, length of stay and intubation duration were analyzed.

**Results:** Out of the 56 patients enrolled, 20 (35.7%) were diagnosed with delirium. Patients who developed delirium were younger (mean age, 3.1 years vs 5.7 years,  $p < 0.046$ ), had longer duration of tracheal intubation and treatment with sedative medications (4.2 days vs 0.7 days,  $p < 0.001$ ), and had longer post-extubation ICU stay (4.5 days vs 2.08 days,  $p < 0.01$ ). They were also more likely to have undergone cardiovascular surgery than patients who did not develop delirium ( $p < 0.016$ ).

**Conclusions:** Delirium is a frequent complication in post-operative pediatric intensive care unit patients. Patients most at risk may be young children, patients undergoing cardiovascular surgery, and those with prolonged intubation and length of stay in the ICU. Further studies are needed to determine the scope and severity of this problem, and to develop effective assessment and treatment tools.

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## **COMPARING UTILIZATION PATTERNS OF PRIVATE HEALTH INSURANCE ENROLLEES WITHIN A PRIVATE HEALTH INSTITUTION IN SOUTHWESTERN NIGERIA**

**Olufisayo O. Ositelu**, Corinna Haberland, and Kate Bundorf. Center for Health Policy, Department of Health Research and Policy.

Inadequate and low quality health services provided in public facilities coupled with the high proportion of out-of-pocket medical spending has greatly contributed to the emergence of powerful non-governmental players such as private medical institutions and managed care health maintenance organizations (HMOs) in Nigeria. Using frequency of hospital visits and admission rates as indicators, we compared the utilization pattern of health care among individuals with different levels of managed care insurance coverage in a single private medical facility in Lagos, Nigeria.

The HMO offers three tiers of health coverage- gold, silver and standard, with gold offering the highest level of coverage and standard the least. In 2008, a total of 1037 individuals (standard- 844, silver-147 and gold- 46) were enrolled in the HMO. The number of patients that had at least one visit to the medical facility is 128 (15%), 12 (8%) and 5 (11%) for standard, silver and gold respectively. We examined the patient charts for retrospective data on number of visits, admissions and diagnosis. The average number of visits for standard, silver and gold is 4.78, 4.92 and 8.6 respectively. However, unpaired t test results show the differences are not statistically significant. Differences in admission rates were also not significant. Malaria accounted for the most number of hospital visits for both standard and silver enrollees. On the other hand, gold enrollees presented mostly for hypertension management. It is unclear whether this pattern of disease incidence is related to the difference in mean ages between the insurance subgroups or the socio-economic status of the enrollees (gold patients tend to be older and more well-off than other patients). Further research will address these findings.

## THE EFFECT OF GASTRIC BYPASS SURGERY ON COGNITION

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The increasing prevalence of morbid obesity and its comorbidities such as diabetes makes studying the effects vital. Higher glucose levels are associated with cognitive impairment and dementia. Currently, gastric bypass (GB) surgery is one of the few effective methods for long-term weight loss and glycemic control.

Previous studies have demonstrated that obesity and poor glycemic control are associated with neuronal loss, cognitive decline and dementia. A study of eight diabetic bariatric patients showed increases in cognition with weight loss. This suggests that improving glycemic markers would improve cognitive function and reverse impairment. We aim to improve previous studies by increasing sample size and heterogeneity. We hypothesize GB will increase glycemic control and cognition scores for both diabetic and non-diabetic patients.

Cognition scores and glycemic markers of a cohort of 30 obese patients undergoing GB were assessed preoperatively and at three and six months postoperatively. The battery of tests covered six broad areas of cognition: verbal memory and learning, attention, visuospatial performance, processing speed and executive function.

Preliminary results suggest GB is associated with significant improvement in: attention and memory ( $p$ -value  $< 0.002$ ), processing speed ( $p < 0.03$ ) and verbal learning ( $p < 0.04$ ) after 6 months in both patient populations. In addition, other tests involving executive functioning and verbal fluency show trends toward significance and will be interesting to follow.

These results demonstrate an additional benefit of GB and enhance our knowledge of the relationship between obesity, diabetes and cognition and may provide insight into other cognitive diseases such as dementia.

## THE STUDY OF NEUROBLASTOMA FROM A STEM CELL PERSPECTIVE

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Neuroblastoma is an embryonal tumor of the developing autonomic nervous system and the most common extracranial solid tumor of childhood. Cancer stem cells (CSCs) are defined as the subpopulation of tumorigenic cells that are uniquely responsible for the maintenance of tumors, and are hypothesized to be the mechanism for the widespread recurrence and resistance to therapy exhibited by various cancers. If CSCs exist in neuroblastoma, their prospective identification and isolation may be the first step in being able to unlock the key mechanisms active in neuroblastoma growth and subsequent identification of targets for therapeutic intervention.

We were able to engraft human tumors into non-obese diabetic (NOD)/severe combined immunodeficiency (NOD-SCID) mice. In order to evaluate the tumors by fluorescence-activated cell sorting (FACS), we also developed a method by which the xenografts were made into single cell suspensions while preserving viability of the tumor cells. Two xenograft lines of neuroblastoma tumors were screened for surface markers that define the tumorigenic subpopulation of cells. Significant surface marker heterogeneity within the tumors were observed. A number of colony forming assays which consisted of sorting and plating isolated neuroblastoma cells in culture on irradiated mouse fibroblasts demonstrated differential capacity of certain cell surface markers to enrich for cells with increased colony forming capacity. Cells that expressed high levels of SSEA-1, CD9, CD166, CD164, formed greater numbers of colonies than cells with low expression of these surface markers. Other markers of interest include CXCR4, CD49f, CD71, CD271, and CD171. In limiting dilution xenograft assays, we found that a high percentage of the viable cells within a tumor were capable of forming new tumors.

Further studies are necessary to define the cancer stem cell compartment of neuroblastoma. The high percentage of cells that are capable of forming tumors suggest that neuroblastoma may be a defect in differentiation that allows for primitive neural crest cells to retain the capacity for self-renewal, migration, and metastasis underlying the pathogenesis of neuroblastoma. Melanoma, another tumor thought to be derived from cells of neural crest origin that may exhibit a similar biology, demonstrates the remarkable ability to generate new tumors from transplantation of a single cell. The surface markers identified will facilitate further study of neuroblastoma with the hope of identifying the genetic or biochemical pathways necessary for the pathogenesis of neuroblastoma.

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## **PROTEOMICS: LOW MOLECULAR WEIGHT MARKERS IN SERUM OF GLIOBLASTOMA PATIENTS**

**Ashley Plant**, Harvey Cohen, John Whitin, Tom Yu. Stanford University

Background: Glioblastoma(GBM) is the most common and most aggressive adult brain tumor. Median survival for patients diagnosed with glioblastoma is 6 to 12 months with only 5% survival at 5 years. Prognosis has only changed by 3% in the last 30 years. Studies have identified a multitude of potential biomarkers for diagnosis and prognosis. These potential biomarkers include VEGF, Cox2 expression, heat shock proteins, MMP9, GFAP, PGD2S, etc. Early results from mice trials have also demonstrated the ability to change long term survival with early identification and treatment. A easily identified biomarker with measurable levels in the serum or urine of at risk patients may prove to be of large consequence in diagnosis and prognosis of glioblastoma patients.

Methods: 20 serum samples from glioblastoma patients as well as 20 controls consisting of 12 meningioma and 8 arterio-vascular malformation samples were extracted from the Stanford brain bank. All samples were collected between 2005 and 2007 and taken at initial preoperative time points. Samples were fractionated and ran on Protein Chip SELDI system on both CM10 and H50 chips under low, medium, and high laser. Data analysis, thus far, has included cluster formation, p value statistics, local and global false discovery rates (FDR), and technical clustering.

Results: 2 peaks with p values  $<.01$  and local false discovery rate  $<.05$  were consistently identified when comparing GBM vs. controls or GBM vs. AVM or GBM vs. MEN alone. These peaks were significantly lower in the GBM population than in either the AVM or MEN populations. These peaks were at m/z of 8961 and 8951 with p values of  $2.12E-7$  and  $4.07E-7$  with local FDR's of .000243 and .000243 respectively. Technical clustering revealed both were distinct peaks.

Conclusions: Potential biomarkers for glioblastoma exist in the serum of patients with GBM when compared to other malignant and non-malignant brain lesions. These biomarkers are decreased in GBM when compared to controls suggesting higher rates of proteolysis or lower rates of production of these proteins in GBM patients. Further research will be required to study the significance of these biomarkers when compared to normal patients and the usefulness of these markers in identifying GBM early on.

## **TOTAL HIP ARTHROPLASTY USING AN ANTERIOR APPROACH AND A FRACTURE TABLE: A COMMUNITY HOSPITAL EXPERIENCE**

Steven T. Woolson, **Michael A. Pouliot**, James I. Huddleston. Stanford Department of Orthopaedic Surgery.

There is no data regarding the safety and efficacy of minimally-invasive total hip arthroplasty (THA) performed by community practice general orthopaedic surgeons. Increasingly, community orthopaedists are adopting the minimally invasive anterior approach, often with minimal or no prior training with the technique. The procedure is reported by its innovator to improve patient recovery time and reduce the risk of post-operative dislocation. Additional incentives for adopting this procedure include improved marketing and patient demand for “minimally invasive” techniques.

The early clinical and radiographic results of primary THA using a minimally invasive anterior approach to the hip performed on a fracture table were studied. 231 consecutive patients (247 hip replacements) of 5 community practice surgeons were studied. These surgeons had trained in and used the standard posterior approach for over ten years prior to converting exclusively to the anterior approach in October of 2004. For comparison, clinical and radiographic results from two years prior to adoption of the anterior approach were collected.

The average surgical time (164 minutes) and estimated blood loss (858 ml) were more than double and the major complication rate (9%) was 6 times that reported by an innovator of the anterior approach. However, no postoperative dislocations occurred. These findings lead us to conclude that adequate training is critical to reduce the risk of complications during the learning experience of minimally invasive hip arthroplasty procedures by community practice surgeons. Our data questions the “minimally invasiveness” of this procedure in the hands of low volume, non fellowship-trained orthopaedic surgeons.

## **OPTICAL TRACKING FOR DEVICE DESIGN AND SURGICAL EDUCATION IN SINGLE INCISION LAPAROSCOPIC SURGERY**

**Brian C. Pridgen**, Pablo Garcia, and Sanjeev Dutta. Stanford Research Institute (SRI) International & Department of Surgery.

Laparoscopic surgery through a single incision within the umbilicus has been successfully performed at a number of institutions in recent years. This technique promises to deliver greater benefits to patients than those benefits realized already with traditional laparoscopic surgery. However, this nascent field is limited both by the use of rigid straight instruments designed for traditional laparoscopic surgery and by the lack of a suitable simulation training platform. The goal of this project was to develop a system that would allow real-time spatial tracking of instruments used in single incision laparoscopic surgery (SILS) procedures. This information could be used to aid in the design of instruments purpose-built for SILS and to provide a platform for SILS training.

Optical tracking systems have been successfully employed for accurate spatial tracking of instruments used for traditional laparoscopic surgery training. However, commercially available systems are not capable of tracking instruments used in SILS due to the close proximity of the instruments and the inability of the system to discriminate between them. Using commercially available software, infrared cameras, and reflective markers, we have developed a system capable of tracking multiple instruments in close proximity. Additionally, this system is capable of tracking movement of intracorporeal joints controlled by extracorporeal mechanical linkages, which are popular design considerations for SILS instruments.

This novel system allows for real-time spatial tracking of instruments used in SILS procedures. This data will permit quantitative assessment of instrument prototypes to inform the design process. Additionally, this system can be used to aid in surgical education for SILS procedures.

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## **AGE ADJUSTMENT: A POOR STRATEGY FOR PREDICTING OUTCOME IN CHILDREN BORN PREMATURELY**

**Kristin S Raj**, Trenna L Sutcliffe, Anne M Debattista, Cheryl Goldfarb-Greenwood, Lynne C Huffman. Pediatrics, Stanford University School of Medicine and Lucile Packard Children's Hospital.

Children born prematurely have greater neurodevelopmental difficulties compared with children born at term, though early interventions (EI) can improve their outcomes. Testing, such as the Bayley Scales of Infant Development–2nd edition (BSID-II), determines eligibility for EI. The scores are calculated based on adjusted age for prematurity or on chronologic age. Age-adjusted scores may suggest that a child is developing typically, while her chronologic scores may suggest delay and need for EI. State programs frequently recognize age-adjusted rather than chronologic scores until 24 months of age when determining eligibility. Research is needed, however, to determine whether adjusted versus chronologic scores best reflect a child's abilities and need for EI services.

To investigate this issue, seventy subjects born before 37 weeks gestation were identified from a database of premature infants at Lucile Packard Children's Hospital. The BSID-II was administered at 18-24 months of age. The Wechsler Preschool and Primary Scale of Intelligence-3rd edition (WPPSI-III) was administered at 31-38 months of age. Scores based on adjusted and chronologic ages were calculated for the BSID-II. The sensitivity and specificity of adjusted and chronologic BSID-II scores for predicting delay (score <85) on the WPPSI-III were measured. Receiver operating characteristic (ROC) curves were generated to assess predictive ability and estimate appropriate cutoff scores, using adjusted and chronologic BSID-II scores. When predicting preschool WPPSI-III delay, the chronologic toddler MDI scores showed a sensitivity and specificity of 1.00 and 0.27, respectively. In contrast, the adjusted toddler MDI scores showed a sensitivity and specificity of 0.71 and 0.97, respectively. Chronologic MDI scores of 77 were highly sensitive indicators (95% sensitive) of later delay with an area under the ROC curve (AUC) of 0.795. However, adjusted MDI scores up to 96.5 were needed to maintain the same sensitivity.

The use of chronologic scores provides greater sensitivity when identifying preterm children who will likely be delayed at preschool age and therefore benefit from EI. High sensitivity ensures that all children at risk for delay will be identified. Our analysis suggests that adjusting for prematurity when measuring developmental level may be grossly insensitive in picking up delay. If using adjusted scores, a score of 96.5 should be used as the threshold for predicting delay to preserve sensitivity. These results may have significant impact on current early intervention policies for children born prematurely.

## **CORRELATION BETWEEN SYMPTOM SCORES, NASAL ENDOSCOPY AND IN-OFFICE COMPUTED TOMOGRAPHY IN CHRONIC RHINOSINUSITIS PATIENTS AFTER FUNCTIONAL ENDOSCOPIC SINUS SURGERY**

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The diagnosis and treatment of patients with chronic rhinosinusitis (CRS) is based on a comprehensive evaluation of symptoms, nasal endoscopy and radiographic examination. However, it is a well-established clinical conundrum that there is poor correlation between objective and subjective findings in pre-surgical patients. The post-surgical patient population is a cohort of interest since patients often require medical care even after ESS. Correlations between subjective and objective findings in this population have not been studied, but are certainly important to understand in the context of medical treatment decision-making. Furthermore, the use of an in-office CT scanner might allow for a more appropriate comparison of simultaneous subjective and objective findings.

We recruited a cohort of 50 sequential CRS patients who had undergone endoscopic sinus surgery in the previous 3 years. During one office visit, they completed a validated SNOT-20 symptom questionnaire, underwent nasal endoscopy and received an in-office sinus CT scan. CT scans and nasal endoscopy were scored using the Lund-MacKay and Lund-Kennedy scoring systems, respectively. Seventy-five percent of participants presented with disease on nasal endoscopy and 84 percent had positive findings on sinus CT. There was a positive correlation between total right- and left-sided CT scan and nasal endoscopy scores ( $r = .684$  and  $r = .759$ , respectively). A positive correlation also existed for total Lund-MacKay and Lund-Kennedy scores ( $r = .753$ ). Nasal drainage of pus and nasal congestion were most positively correlated with nasal endoscopy and CT scan scores.

Our findings demonstrate a strong correlation between findings on sinus CT scan and nasal endoscopy performed during the same clinic visit. However, as seen in the pre-operative population, our study also shows that symptoms as measured by the SNOT-20 as well as a visual analogue scale, do not correlate well with objective findings in postoperative patients. Additional studies should evaluate the effect of medical therapy on symptom score, nasal endoscopy and CT findings in post-ESS patients with symptomatic CRS.

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## **EFFECT OF THE MEDIA ON AN INDIVIDUAL'S LEVEL OF CONFIDENCE IN HIS OR HER HEALTHCARE SYSTEM**

**Chandler D. Robinson** and Mary K. Bundorf, Department of Health Research and Policy, Stanford University.

Confidence in healthcare systems and health care professionals is on the decline. This has coincided with increasing attention paid by the media to issues surrounding medical errors and physician fallibility. The objective of this research was to examine the impact of the media on an individual's level of confidence in his or her healthcare system. Our hypothesis was that the current heightened attention paid to medical errors by the media has caused individuals to have less confidence in their healthcare system. To address this, the 2006 Eurobarometer 64.1 survey was employed and the study undertakes multivariate analysis. The results provide evidence of individuals who read or hear about medical errors often being more likely to have less confidence in their healthcare system than those who read or hear about errors less often. An additional interesting finding was that those who are likely to have used the system the most, elderly patients, tend to have the most confidence in the healthcare system. This suggests that those with fewer personal experiences are relying on external information sources such as media coverage to formulate an opinion. Thus, this heightened media coverage might be causing individuals to think their healthcare system is more likely to fail them than is actually the case.

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## **INTELLECTUAL PROPERTY PROTECTIONS FOR PHARMACEUTICALS AND ACCESS TO MEDICINES IN DEVELOPING COUNTRIES: A CASE STUDY ANALYSIS IN GUATEMALA**

**Joshua A. Rolnick, Corinna Haberland**, Department of Health Research and Policy

In the last fifteen years, international treaties have transformed intellectual property for pharmaceuticals. Many developing countries have drug patents for the first time, leading health advocates to argue that these rules restrict drug availability and affect health. Yet empirical study is scarce. In 2000, Guatemala implemented patents on pharmaceuticals and a 5-year prohibition on the use of patent holders' clinical data by generic companies (data exclusivity). This study uses key informant questionnaires/interviews and analysis of data on drug exclusivity and patents to assess the impact.

Key informants were physicians, generic company executives, and drug purchasers. A short questionnaire on drugs and IP was written and translated to Spanish by the primary investigator. 29 surveys were distributed via email (8 physicians, 12 purchasers, 9 generics) and 17 (58%, 6 physicians, 8 purchasers, 3 generics) were returned. Follow up interviews took place with 15 (52%). Interviews were also performed with representatives of the Ministry of Health, the patent office, and the WHO. Data was obtained on patents and data-protected drugs. Results showed a perceived impact of both patents and drug protection. 100% of drug purchasers stated that they had noticed an impact of drug patents, 88% said that prices had increased since 2000, and 75% had decreased the quantity of drugs they purchased. All three generic manufacturers stated that certain drugs produced in 2000 are no longer available, but specific evidence existed only for omeprazole and sildenafil. Interviews revealed that vaccines, ARVs, and many essential drugs are purchased via international programs unaffected by patents.

Guatemala has successfully implemented an intellectual property regime and key informants perceive the rules to have affected drug prices and availability. Domestic production of a small set of drugs has been curtailed. However, the impact on health is unclear. Further analysis will focus on characterizing trends in patents/data-protected drugs and comparing prices of patented/protected drugs to clinical substitutes. Future studies should attempt to obtain longitudinal data on prices, availability, and health outcomes.

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## FLEXOR TENDON TISSUE ENGINEERING: BIOREACTOR CYCLIC STRAIN INCREASES CONSTRUCT STRENGTH

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Mutilating injuries of the hand and upper extremity result in tendon losses too great to be replaced by autologous grafts. The goal of this study was to use tissue engineering techniques to produce additional tendon material. We used a custom bioreactor to apply cyclic mechanical loading onto tissue engineered tendon constructs to study ultimate tensile stress (UTS), elastic modulus (E), construct framework, and cell orientation. Constructs used were acellularized rabbit hindpaw FDP equivalents reseeded with tenocytes or left unseeded. Tendon constructs were subjected to a stretch force of 1.25N over a 5-day course.

Seeded tendon constructs that were exposed to bioreactor loading had a significantly increased UTS ( $71.17 \pm 14.15$  N) compared to non-loaded controls ( $35.69 \pm 5.62$  N); ( $p = 0.001$ ). Similarly, seeded constructs exposed to bioreactor loading also had a significantly higher E ( $1091 \pm 169$  MPa) compared to non-loaded controls ( $632 \pm 86$  MPa); ( $p = 0.001$ ). Histologically, bioreactor-treated tendons showed a more homogeneous collagen framework. Cyclic strain further caused the cells to reorient parallel to the direction of strain. This alignment was in stark contrast to the random cell orientation of constructs not exposed to bioreactor treatment.

This study shows that cyclic loading of tendon constructs increases the ultimate tensile stress and elastic modulus of seeded constructs and changes the constructs' framework and cell orientation. The use of the bioreactor may therefore accelerate the *in vitro* production of strong, non-immunogenic tendon material that can potentially be used clinically to reconstruct significant tendon losses.

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## **SYSTEMATIC REVIEW: THE HEALTH BENEFITS AND HARMS OF GARDENING**

**Sarah Jane Selig, MS**, Dena Bravata, MD, MS, Jennifer Schneider, MD, Investigators; Ingram Olkin, Mentor Stanford University, Department of Health Policy and Research

This study will be the first to synthesize quantitatively the literature on the effectiveness of gardening interventions to increase physical activity and improve dietary choices among the elderly. The results of this meta-analysis could be used to inform the physical activity recommendations that clinicians and other health professionals make to their patients. If gardening interventions are associated with increases in physical activity among the elderly, the results of this analysis will be used to inform a proposal to seek additional funding for a clinical trial using of a gardening intervention at a local community health center to increase physical activity among elderly outpatients.

So far, we have performed searches in eight databases, including: PubMed, PsychInfo, AgeLine, ToxLine, Ovid, ERIC, CAB, and Cinhal. From these searches, we have identified 7,040 articles, of which, roughly 400 met criteria for level 2 abstraction. Of these 400 articles that underwent level 2 abstraction, roughly 50 have met inclusion criteria.

Preliminary results suggest that gardening interventions do increases people's consumption of fruits and vegetables, both in the elderly and in children.

We are actively searching the EMBASE database and re-running all databases above in order not to miss any recently published articles. In addition, we continue to analyze data re: harms and other health outcomes.

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## 3D-FSE-CUBE FOR DETECTION OF HIP PATHOLOGY WITH MR ARTHROGRAPHY

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Magnetic resonance imaging (MRI) can be used to evaluate hip joint pathology, but conventional MRI is suboptimal for assessment of the acetabular labrum and articular cartilage. Magnetic resonance arthrography (MRA) is sensitive for labral pathology, but not for articular cartilage lesions. Currently, MRI and MRA of the hip are performed using two-dimensional sequences in multiple planes, but these sequences suffer from partial volume artifacts and do not lend themselves to reformations. Using three-dimensional fast spin-echo (3D-FSE) techniques, we overcome these limitations by allowing isotropic voxels that permit image reconstruction in multiple planes.

We compared 3D-FSE-Cube XETA, an isotropic fast spin-echo acquisition with an extended echo train, to two-dimensional coronal proton density and sagittal T1 sequences at 1.5T. A musculoskeletal radiologist, who was blinded to clinical and surgical findings, evaluated articular cartilage, labral, and ligamentum teres pathology. For sensitivity and specificity calculations, the gold standard was the pathology documented during hip arthroscopy. During arthroscopy, a total of 37 lesions were discovered in the articular cartilage (n = 8), acetabular labrum (n = 17), and ligamentum teres (n = 12). The results are summarized in Table 1.

**TABLE 1:** Comparison of 3D-FSE-CUBE XETA with Coronal Proton Density and Sagittal T1 with Fat Saturation

<b>Imaging sequence</b>	<b>Cartilage</b>	<b>Labrum</b>	<b>Ligamentum Teres</b>
<i>3D-FSE-CUBE XETA</i>			
Sensitivity % (95% CI)	63 (26, 90)	82 (56, 95)	42 (16, 71)
Specificity % (95% CI)	67 (31, 91)	N/A	60 (17, 93)
<i>Coronal Proton Density</i>			
Sensitivity % (95% CI)	75 (36, 96)	65 (39, 85)	50 (22, 78)
Specificity % (95% CI)	44 (15, 77)	N/A	60 (17, 93)
<i>Sagittal T1 with Fat Saturation</i>			
Sensitivity % (95% CI)	100 (60, 100)	76 (50, 92)	0 (0, 30)
Specificity % (95% CI)	67 (31, 91)	N/A	100 (46, 100)

3D-FSE-Cube enables reformatting in multiple planes and appears more sensitive for assessment of labral pathology than conventional imaging techniques. Cartilage lesions were not better seen, perhaps due to blurring from the long echo train. Further studies will compare 3D-FSE-Cube sequences using higher magnetic fields to conventional techniques.

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## EXPLORING TRIGGERS OF ATRIAL FIBRILLATION

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Atrial fibrillation (AF) is a supraventricular tachyarrhythmia caused by disorganized atrial depolarizations. AF, which occurs in 2.3 million Americans, leads to greater risk for stroke and other embolic events from atrial thrombi. In addition, AF causes symptoms of palpitations, fatigue, shortness of breath, and lightheadedness, which severely impair quality of life. Although many risk factors have been identified, no explanation has been made regarding why some patients with similar risk factors are more susceptible to develop AF than others. It is possible that specific events/activities may trigger AF in combination with specific genetic markers that increase the tendency to develop AF. We hypothesize that the interplay among the triggers, an individual's relevant underlying diseases, and the individual's inherent biologic susceptibility toward AF is the major determinant of outcome. Therefore, the specific aim of this study is to identify significant events/activities or their combinatorial effects with various underlying diseases that predispose individuals to develop atrial fibrillation (AF).

In order to address this specific aim, we are conducting an extensive survey to AF patients with loop monitors. The survey is a prospective, cohort study with a nest case-control design that is initiated to record events or activities preceding an AF episode. We are currently still in the process of patient recruitment and have 31 patients at this time. In the next six months, however, we hope to have at least 50 patients enrolled in the study, allowing us to analyze the data and identify potential triggers for atrial fibrillation.



## REGROWING THE BRAIN: THE EFFECT OF TRANSPLANTING HUMAN NEURAL PROGENITOR CELLS AFTER STROKE

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Stem cell transplantation has been shown to enhance functional recovery after stroke in rats, but their mechanism of action is not well understood. In this study, we test the hypothesis that grafted human neural progenitor cells (hNPCs) enhance endogenous repair mechanisms that occur naturally after stroke, such as dendritic plasticity.

hNPCs or buffer were transplanted into the ischemic cortex of 8 week old adult male Nude rats 7 days after a permanent distal middle cerebral artery occlusion (dMCAO). Animals were sacrificed at 2 weeks and 4 weeks after transplantation. Dendrites were stained using the Golgi-Cox method and impregnated brains were sliced into 150 $\mu$ m sections using a cryostat. The brains were then visualized using a stereotactic microscope and Stereoinvestigator software at 40X. Dendritic branching, total dendritic length, and branch order were measured in layer V of the ipsilesional and contralesional cortex.

hNPCs were shown to enhance dendritic branching and length after stroke, in both the ipsilesional and contralesional hemispheres at both 2 and 4 weeks after transplantation. At 2 weeks post transplantation, the number of dendritic branches was significantly increased in the hNPC group as compared to the buffer group in the contralesional hemisphere ( $p=0.029$ ) and the ipsilesional hemisphere ( $p=0.0006$ ). In addition, the total dendritic length was also significantly increased in the hNPC groups at 2 weeks in both the contralesional ( $p=0.0032$ ) and ipsilesional ( $p=0.0136$ ) hemispheres. Similar increases ( $p<0.05$ ) in dendritic branching and dendritic length were seen between the hNPC group and buffer group in the ipsilesional hemisphere at the 4 week time point. Branch order was also shown to be enhanced by hNPC transplantation, with an increase in the 2<sup>nd</sup> and 3<sup>rd</sup> order branches in particular. hNPCs significantly enhance endogenous repair mechanisms that occur after stroke resulting in increased dendritic branching and dendritic length on both the contralesional and ipsilesional hemispheres.

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## PROTEIN AND GENE PROFILES OF OSTEOPROGENITOR CELLS ON ALLOGRAFT BONE

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Autografts possess the three components necessary for bone production: viable osteoblasts, scaffold and growth factors, yet autograft is limited in supply and harvesting is often associated with residual morbidity. Allograft bone is an alternative, but incorporation proceeds more slowly. Osteoinductive growth factors may improve allograft integration, but delivering the necessary growth factors at biologically critical times and doses during osteoprogenitor cell proliferation and differentiation is challenging. We hypothesized that the addition of exogenous osteoprogenitor cells would improve bone formation and that the cells would express a specific, ordered sequence of growth factors when cultured on corticocancellous allograft bone. We tested this hypothesis over a 4-week period using a novel murine model. 1.5 mm thick corticocancellous allograft bone discs were made from the distal femurs of euthanized 10-week old C57 male mice. Osteoprogenitor cells were harvested from the long bones of other mice, expanded, and plated on the allograft discs.  $1 \times 10^6$  cells were seeded on each allograft for 72 hours, at which time osteogenic media was added. The seeded allografts were maintained under osteogenic culture conditions for 4 weeks. The culture media was assayed at 0, 2 and 4 days, and every 4 days subsequent for BMP-2, IGF-1, Osteocalcin, TGF- $\beta$ , and VEGF-a protein using ELISA. RT-PCR was used to assay 18S, BMP-2, BMP-7, Ctnn-b1, FGF-b, IGF-1, PDGF-a, PDGF-b, Runx-2, TGF- $\beta$ , and VEGF-a mRNA levels. SPSS 14.0 was utilized to perform a one-way ANOVA with a Tukey test for each assay ( $p < 0.05$ ). New bone formation of cell-seeded grafts was measured with  $\mu$ CT at 0 and 4 weeks.

The concentration of osteocalcin peaked at 4 days at 13 pg/ml, a significant increase over all other time points ( $p < 0.05$ ). IGF-I protein decreased significantly to 575 pg/ml ( $p < 0.05$ ) at 2 days compared to 8-day values. VEGF protein increased significantly to 164 pg/ml ( $p < 0.05$ ) at 8 days compared to 0, 24, and 28-day time points. TGF- $\beta$  protein increased significantly to 69 pg/ml ( $p < 0.05$ ) at 2 days compared to time points from 8 days to 28 days [Fig. 4]. mRNA patterns did not precede protein expression.  $\mu$ CT showed a 7.7% increase in bone volume.

Our experiments demonstrate specific time dependent expression patterns for key bone-related proteins when osteoprogenitor cells are cultured on cortico-cancellous allograft bone. Although these expression patterns generally parallel those found when osteoprogenitor cells are cultured alone, differences in the profiles suggest that the allograft bone scaffold is modulating osteoprogenitor cellular activity. Furthermore, the finding that the protein often increases earlier in the time course than mRNA suggests that pre-formed protein is released, or that there is a decoupling between the mRNA and protein expression in these circumstances. Allograft incorporation may potentially be enhanced by adding osteoprogenitor cells, choosing scaffolds with specific physico-chemical properties, or adding specific exogenous growth factors at critical times during osteoprogenitor cell proliferation and differentiation.

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## IDENTIFYING MODIFIABLE RISK FACTORS FOR STRESS FRACTURES IN HIGH SCHOOL FEMALE DISTANCE RUNNERS

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Stress fracture injuries account for up to one-third of all injuries in female runners. Previous research reported that participation in ball sports during adolescence was associated with lower prevalence of stress fractures in older, elite distance runners. Here we seek to further evaluate this relationship in a younger population of high school female distance runners. We hypothesized that previous participation in ball sports would be protective against development of stress fracture injuries, and risk factors to developing stress fracture injury include previous injuries and menstrual irregularities.

We used a cross-sectional design. An online questionnaire was administered to 153 subjects (age and SD: 15.4±1.1 years) who currently participate in long distance running in high school. The online questionnaire included questions on previous participation in ball sports (basketball and soccer), menstrual history, previous running injuries and stress fractures. Of respondents, 78% reported participation in soccer or basketball, 72% reported a previous running injury and eight subjects reported a previous stress fracture injury. Stress fractures were diagnosed by a medical doctor and confirmed using diagnostic imaging. Subjects in the stress fracture group trended towards being older at onset of ball sports participation (8.6±2.5 versus 7.0±2.2 years old,  $P=0.06$ ) and had a higher prevalence of menstrual irregularities (OR and 95% CI = 12.90(CI=9.43-17.64)) and reported shin splint injuries (1.99(CI=0.67-5.89)).

Findings suggest participation in ball sports may protect against developing stress fractures in a dose-dependent manner, as subjects with stress fractures participated at an older age and for shorter duration. Soccer and basketball require sprinting, jumping, accelerating, and decelerating, and place transverse and torsion loads on the skeleton. Our findings are consistent with previous studies that have shown long-term exposure to these ball sports improves bone mineral density, bone stiffness, and bone symmetrical properties. Menstrual irregularities and shin splints are associated with developing stress fractures.

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# THE USE OF METFORMIN AS A CARDIOPROTECTIVE AGENT IN HEART TRANSPLANTATION DECREASES ISCHEMIA-REPERFUSION INJURY AND INCREASES GRAFT FUNCTION AND SURVIVAL

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**Background:** While pharmacological advancements in immunomodulation have dramatically improved the short-term outcome of cardiac transplantation, chronic rejection, or graft coronary artery disease (GCAD) remains the major obstacle to the long-term survival of cardiac transplant recipients. Ischemia-reperfusion (I-R) injury has been shown to be the strongest alloantigen-independent factor in the development of GCAD. Recently much attention has been given to metformin and its potential cardioprotective properties after a large prospective clinical study suggested that metformin offers cardiac benefits independent of its glucose controlling properties. In this study we examined the use of metformin as a cardioprotective agent in limiting the initial I-R injury and subsequent development of GCAD.

**Methods:** To determine the effects of metformin therapy on I-R injury we performed murine heterotopic heart transplantations using a total mismatch model and twenty-four hours later compared levels of apoptosis and cell death between the control group and metformin-treated donor hearts. Briefly, the metformin-treated group had the donor treated by IP injection of metformin one hour prior to surgery and an additional IVC injection of metformin into the heart 2 minutes before harvesting. During the preparation of the recipient mouse the cold-ischemic time was approximately 20 minutes and during this time the metformin-treated hearts were placed in a PBS/metformin solution. Caspase-3 activity, cytoplasmic histone-associated-DNA fragments, and TUNEL staining were used as indicators of apoptosis and cell death. To assess GCAD development we performed heterotopic heart transplantations using a 52-day MHC Class II mismatch model. Graft viability was assessed by direct abdominal palpation daily. Allografts were harvested at 52 days. Morphometric analysis was performed to assess luminal narrowing (intimal proliferation).

**Results:** In our acute model, animals treated with metformin have a 1.8 fold decrease in caspase-3 activity ( $p < 0.001$ ), a 3.1 fold decrease in cytoplasmic histone-associated-DNA fragments ( $p < 0.01$ ), and a 38% decrease in %TUNEL-positive cells ( $p < 0.01$ ). In the chronic rejection model animals treated with metformin had a significant increase in their cardiac graft beating score and had a significant decrease in GCAD development as measured by luminal narrowing ( $46.77 \pm 3.63\%$  vs.  $67.95 \pm 4.42\%$ , respectively, with a p-value of  $< 0.001$ ).

**Conclusions:** The results of our study show that preconditioning donor hearts with metformin is a novel approach to reduce GCAD development. Importantly, the beneficial effects seen with metformin therapy may be due to a reduction in apoptosis.

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## **RACIAL/ETHNIC DISPARITIES IN CHILD MORTALITY RATES IN CALIFORNIA, 1989-2004**

**Marie E. Wang**, Vandana Sudaram, Paul H. Wise. Department of Pediatrics and Center for Health Policy/Primary Care Outcomes Research. Stanford University, Stanford, CA.

Reducing health inequalities has been identified by the Department of Health and Human Services as one of the nation's most important health priorities. Child mortality rates are considered to be a standard measure of health status in children and have seen significant declines over the last few decades due to decreases in unintentional injuries, congenital anomalies, and certain diseases. However, racial/ethnic disparities have persisted despite overall improvements in child mortality.

Our objective was to identify the primary contributors to disparities in child mortality rates in California from 1989—2004. Data were obtained through the National Vital Statistics System and California Department of Finance and analyzed by race/ethnicity, age, cause of death, and urban/rural place of residence. Many racial/ethnic disparities were noted, primarily among African-Americans, Hispanics and Native Americans when compared to whites and in the urban population. For example, among children ages 1-4, African-Americans and Native Americans had higher risk ratios of child mortality from accidents compared to whites (1.44 and 3.54, respectively). African-Americans had the greatest number of excess deaths among the urban population (2879 excess deaths over 15 years), with the main contributors being the mortality rate from homicide in the age 15-19 group, followed by accidents among children age 1-9 and homicide in children ages 1-4. Hispanics had 1260 excess deaths in the urban population, with the main contributor also being the mortality rate from homicide in the age 15-19 population. Native Americans had 984 excess deaths.

Racial/ethnic disparities in child mortality rates continue to persist in California among African-Americans, Hispanics and Native Americans and within the urban population. While accidents remain the most common cause of child mortality, the disparities resulting in the greatest excess mortality come from homicides in children age 15-19. Further investigation is necessary to clarify the drivers of these disparities and inform future prevention efforts.

## PREFERENCE FOR TRADITIONAL BIRTH ATTENDANTS AMONG MIGRANT WOMEN IN BAJA CALIFORNIA

**Emilee Wilhelm-Leen** and Paul Wise. Health Research and Policy, Department of Pediatrics, Stanford University

This study was designed to explore the factors that influence preference for traditional birth attendant care (TBA) care among women living in a rural agricultural community near Vicente Guerrero, Baja California, Mexico. The study employed semi-structured, narrative interviews. Seventeen interviews were conducted, eight with women who had migrated to Baja California within five years (“recent migrants”) and nine with women who had migrated more than five years ago (“distant migrants”).

No study subjects reported using TBA care in Baja California, but many recent migrants reported indirect familiarity with local TBA care; fewer distant migrants knew of women who used local TBA care. Many recent migrants reported preference for TBA care, in contrast to few distant migrants who reported such preference. Among recent migrants who preferred TBA care, women most often cited the special skills TBAs possess, such as *sobado* (prenatal massage) as the reason for their preference. Distant migrants employed a similar strategy to explain their preference for physician care by citing examples of special skills unique to physicians, such as ultrasonography. Most distant migrants shared a common narrative to explain the preference of recent migrants for TBA care which bore little resemblance to recent migrants’ own rationale for their preference. Distant migrants asserted that recent migrants prefer TBA care, not because TBAs possess special skills, but because recent migrants are insufficiently acculturated to normative practices for pregnancy and birth in Baja California.

Results from this and subsequent studies may be used to inform outreach efforts by service and governmental organizations operating in Baja California with the ultimate goal of improving maternity care in rural and transitional communities in Mexico.

## **GASTRIC BYPASS IMPROVES MUSCULOSKELETAL FUNCTION**

**Gavitt A Woodard**, BS; Betsy Encarnacion, BS, BS; Gaurav Banka, BS; Stephanie Bravo; Tina Hernandez-Boussard, PhD, MPH; John M Morton, MD, MPH

**Background:** The leading public health crisis of the industrialized world is morbid obesity which results in a wide range of musculoskeletal disability and is associated with dysmotility, joint pain, and osteoarthritis. We hypothesize that bariatric surgery improves musculoskeletal function and that these improvements correlate with weight loss, improvements in cholesterol levels, and decreases in hs-CRP.

**Methods:** At a single academic institution between 2006-2007, 45 Roux-en-Y gastric bypass (RNYGB) completed the Short Musculoskeletal Function Assessment questionnaire (SMFA) pre-operatively and at 3, 6, and 12 months post-operatively. SMFA scores are broken down into a total score, a dysfunction index, a bother index, and scores for daily function, emotional impact, arm/hand function, and mobility. SMFA measures, percent excess weight loss (%EWL), cholesterol levels, and hs-CRP were compared between pre and post-op and to established overall population normal values with paired two-tailed t-tests and Pearson correlations.

**Results:** The demographic distribution and post-op weight loss of the study participants was representative of RNYGB demographics and outcomes reported in the literature. Pre-op SMFA scores showed significantly worse musculoskeletal function than population norms in every category. Post-op all categories significantly improved from pre-op and were statistically similar to population norms. Pre and post-op SMFA scores correlated with pre-op and post-op hs-CRP. Impaired pre-op mobility scores were correlated with less weight loss post-RNYGB.

**Conclusions:** In this study, patients undergoing RNYGB have significantly impaired musculoskeletal function by every measure. RNYGB improves musculoskeletal function to population norms. Higher hs-CRP values correlate with worse SMFA scores. SMFA can be given to patients pre-operatively as one measure of motility that can predict some degree of weight loss success. Morbidly obese patients should consider RNYGB to improve musculoskeletal measures such as their ability to perform daily activities, the emotional impact of musculoskeletal dysfunction, and their mobility.

## LONG-TERM IMPROVEMENT IN CARDIAC RISK FACTORS FOLLOWING RNYGB

**Gavitt A Woodard**, BS; Loren Toplosky, BA; Karen Chong; Stephanie Bravo; Tina Hernandez-Boussard, PhD, MPH; John M Morton, MD, MPH

**Background:** Coronary artery disease (CAD) is the leading cause of death in the industrialized world with obesity as a leading preventable risk factor for CAD. Certain biochemical cardiac risk markers (BCRF) have demonstrated strong prediction for cardiovascular events, and we have previously shown that Roux-en-Y gastric bypass (RNYGB) improves BCRF at 1 year post-op. We hypothesize that in addition to the weight reduction benefit, Roux-en-Y gastric bypass (RYGB) will also induce a salutary effect upon biochemical markers for cardiac risk.

**Methods:** At a single academic institution (2004-2008), we measured BCRF in 456 consecutive gastric bypass patients preoperatively and at 3, 6, 12, 18, 24, 30, 36, and 48 months postoperatively. All surgeries were performed by a single surgeon with a 30cc pouch and a 100cm Roux limb. BCRF included total cholesterol (TC), triglycerides (Trig), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, high sensitivity C-reactive protein (hs-CRP), and fasting insulin (FI). Time points were compared by two-sample paired t-test of equal variance with  $p < .05$  as significant.

**Results:** There was significant improvement in weight and all BCRF from pre-operative to all post-op time points. Beyond its increase from pre-op (42.8 mg/dL) to 1 year (53.8) HDL continued to significantly improve at 2 years (59.8) and 4 years (65.0) post-op. Weight loss at 1 year was 78.3% and patients maintained this weight loss at 2 years (77.3%), 3 years (80.0%), and 4 years (77.6%) post-op. Trig, LDL, hs-CRP, and FI levels continued to decrease after 1 year but these further reductions were not significantly reduced from 1 year values, though they were significantly improved from pre-op.

**Conclusion:** In this study, the improvements in BCRF seen at 1 year post-RNYGB remained or further improved at 2, 3, and 4 years post-operatively. These improvements in BCRF are not only durable beyond 1 year post-op, but in some markers, such as HDL and hs-CRP, undergo significantly further improvement at 2 years, 3 years, and 4 years post-op. Patients in our study maintained their excess weight loss through 4 years post-operatively.

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## PROBIOTICS IMPROVE OUTCOMES AFTER ROUX-EN-Y GASTRIC BYPASS SURGERY: A PROSPECTIVE RANDOMIZED TRIAL

Gavitt A. Woodard BS, Betsy Encarnacion BS, John R. Downey BA, Karen Chong, Tina Hernandez-Boussard, PhD, MPH, John M. Morton, MD, MPH, FACS

**Introduction:** Roux-en-Y gastric bypass surgery (RNYGB) offers an effective and enduring treatment for morbid obesity but it is not without potential risks. Gastric bypass may alter gastrointestinal (GI) flora possibly resulting in bacterial overgrowth (BO) and dysmotility. Previous data support that use of probiotics may effect BO and GI motility. Our hypothesis was that daily use of probiotics would improve GI outcomes after RNYGB.

**Methods:** 44 patients undergoing RNYGB were randomized to either a probiotic or control group. 2.4 billion colonies of Lactobacillus were administered daily post-op to the probiotic group. The outcomes of H<sub>2</sub> levels indicative of bacterial overgrowth, GI related quality of life (GIQoL), and weight loss were measured pre-operatively and at 3 and 6 months post-operatively. Categorical variables were analyzed by  $\chi^2$  test and continuous variables were analyzed by t-test with a  $p < 0.05$  for significance.

**Results:** At 6 months a statistically significant reduction in bacterial overgrowth was achieved in the probiotic group with a pre to post-op change of sum H<sub>2</sub> ppm (probiotics = -32.13, controls = 0.80). Surprisingly, the probiotic group attained significantly greater percent excess weight loss than that control group at 6 weeks (controls = 25.5%, probiotic = 29.9%) and 3 months (38.55%, 47.68%). This also continued but was not significant at 6 months (60.78%, 67.15%). Both probiotic and control groups significantly improved their GIQoL.

**Conclusion:** In this novel study, probiotic administration improves bacterial overgrowth and weight loss after RNYGB. These data may provide further evidence that altering the GI microbiota can influence weight loss.

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## **RNYGB ULCER OR STRICTURE COMPLICATION ASSOCIATED WITH INCREASED WEIGHT LOSS**

**Gavitt A Woodard**, BS; Joe Peraza, BS; Karen Chong; Gaurav Banka BS; Tina Hernandez-Boussard, PhD, MPH; John M Morton, MD, MPH

**Introduction:** The leading public health crisis of the industrialized world is morbid obesity with bariatric surgery being the only effective and enduring treatment. Marginal ulcers and strictures at the gastrojejunal (GJ) anastomosis are two of the most commonly reported complications following Roux-en-Y gastric bypass surgery (RNYGB). The long term effects of these complications on weight loss and cardiac risk improvement is unknown.

**Methods:** A prospective database was maintained on 706 consecutive RNYGB patients at a single academic institution from 2004-2008. Data collected included weight, BMI, age, sex, race, type of insurance, pre-operative weight loss, comorbidities, and post-operative complications including strictures and ulcers. Weight and serologic cardiac risk factors were collected pre-operatively and at 3, 6, and 12 months post-operatively. Weight loss was measured by BMI and % excess weight loss (%EWL). Statistical analysis was done with a student's t-test and a backwards elimination logistical regression.

**Results:** The rate of ulcers and strictures was 3.7% in pts post RNYGB. There were no significant differences in age, pre-op BMI, pre-op weight loss, sex, race, insurance, or pre-op comorbidities of hypertension, depression, and diabetes. Patients who had an ulcer or stricture complication had significantly greater weight loss at 12 months post-op (BMI of 27.6 kg/m<sup>2</sup> vs. 31.3 kg/m<sup>2</sup>, %EWL of 95.8% vs. 78.3%). There were no significant differences in any serologic lab values pre-op or post-op except 12 month post-op hs-CRP was significantly higher in pts who had had an ulcer or stricture (5.57 mg/L vs. 2.56 mg/L). Logistic regression showed that an ulcer/stricture complication lead to a 17% increase in 12 month %EWL, followed by pre-op diabetes which decreased 12 month %EWL by 9%, and being female which lead do 8% greater 12 month %EWL.

**Conclusions:** Patients who have an ulcer or stricture post RNYGB have greater weight loss at 12 months post-op. These patients have higher hs-CRP values but otherwise undergo the same post-op improvements in serologic cardiac risk factors. No demographic or pre-op data is predictive of who will experience an ulcer or stricture. Post-op ulcers or strictures have a greater impact on 12 month weight loss than pre-op DM, sex, and all other measured variables. There were no adverse long-term survival, weight, or lab outcomes from an ulcer or stricture complication.

## **IMPAIRED ALCOHOL METABOLISM AFTER GASTRIC BYPASS SURGERY: A CASE-CROSSOVER TRIAL**

**Gavitt A. Woodard, BS**; Betsy Encarnacion, BS; John Downey, MPH; Joe Peraza, BS; Tina Hernandez-Boussard, PhD, MPH, John M. Morton, MD, MPH, FACS

**Introduction:** Morbid obesity remains the leading public health crisis of the industrialized world with the only effective and enduring treatment being bariatric surgery. Roux-en-Y gastric bypass (RNYGB) results in a dramatic mortality reduction from disease but leads to increases in mortality from accidents and suicide. Poor psychological adjustment has been reported, including addition transfer and new onset alcohol addiction post-operatively. Evidence suggests that patients may metabolize alcohol differently following gastric bypass.

**Methods:** Pre-operatively and at 3 and 6 months post-operatively, 19 RNYGB patients breath alcohol content (BAC) were measured every 5 minutes after drinking 5 oz. of red wine to determine peak BAC and time until sober. Patients reported symptoms experienced while intoxicated and answered a questionnaire of drinking habits.

**Results:** The peak BAC in patients following RNYGB was significantly higher at 3 months (0.059%) and 6 months (0.088%) post-operatively than matched pre-op levels (0.024%). Patients also took significantly more time to return to sober at 3 months (61 minutes) and 6 months (88 minutes) than at pre-op (49 minutes). Post-op intoxication was associated with lower levels of diaphoresis, flushing, and hyperactivity and higher levels of dizziness, warmth, and double vision. Post-op patients reported drinking significantly less alcohol, fewer preferred beer, and more preferred wine than prior to surgery.

**Conclusions:** This is the first study to match pre-op to post-op alcohol metabolism in gastric bypass patients. Post-RNYGB patients have much higher peak BAC and require more time to become sober. Patients also experience different symptoms of intoxication and therefore may not recognize when they have had too much to drink. Patients who drink alcohol following gastric bypass surgery must exercise extreme caution.