UW Medicine

DEPARTMENT OF SURGERY

Presents

The Nineteenth Annual Research Symposium

Friday, February 22, 2013
7:00 a.m. – 2:00 p.m.

UW Tower

2700 24th Avenue East
Seattle, Washington 98112

2013 Schilling Schedule of Presentations
Moderators: Anthony Atala, M.D. and David Flum, M.D.

7:00 AM	Introduction	David Flum, M.D.
7:05 AM	Lewis	Fibrinogen Concentrate is Associated with Improved Survival in a Procine Aortic Hemorrhage Model
7:20 AM	Mulloy	Ex Vivo Reconditioning of Non-Heart-Beating Donor Lungs in a Preclinical Procine Model: Delayed Perfusion Results in Superior Lung Function
7:40 AM	McAteer	The Role of Bottle Feeding in the Etiology of Hypertrophic Pyloric Stenosis
7:55 AM	Whitney	Effect of Topical Mapk Inhibition on Hypertrophic Scarring in a Duroc Porcine Model
8:10 AM	Wallace	Survival After Ruptured AAA in the Endovascular Era: Looking Beyond the Traditional 30-Day Outcome"
8:25 AM	**Faculty Presentation: Nicole Gibran, M.D.**	
8:35 AM	Hakkarainen	Skin antiseptic agents and surgical site infection: a report from Washington State's Surgical Care and Outcomes Assessment Program
8:50 AM	Dubbins	Comparison of Outcomes in Immediate Implant-Based Breast Reconstruction Versus Mastectomy Alone
9:05 AM	Kotagal	Impact of Obamacare on 19-25 Year Olds
9:20 AM	BREAK	
9:35 AM	Plevin	The Role of LPS Structure in Monocyte Activation and Cytokine Secretion
9:50 AM	Das	Transcriptome Analysis of Recurrent Hepatocellular Carcinoma Related to Hepatitis C Virus Following Liver Transplantation: Molecular Signatures for Possible New Therapeutic Options
10:05 AM	**Faculty Presentation: Charles Mock, M.D.**	
10:15 AM	Lipira	Reconstruction of Sternal Wounds With and Without Rigid Plate Fixation
10:30 AM	Swearingen	Mismatched VCA Transplantation Without the Need for Donor Cell Chimerism: A Model to Achieve Tolerance While Eliminating Chronic Immunosuppression and the Risks of GVHD
10:45 AM	Rae	Optimizing Feedback from the Designated Level 1 Trauma/Burn Center to Referring Hospitals
11:00 AM	Fawcett	Pre-Hospital Aspiration in Trauma Patients
11:15 AM	Garland	Endoleaks After Endovascular Repair of Ruptured Abdominal Aortic Aneurysms: How Should They Be Managed?
11:30 AM	Thompson	Genetic Risk Factors for Hypertrophic Scar Development
11:45 PM	LUNCH	
12:30 PM	**Faculty Presentation: Tom Varghese, M.D.**	
12:40 PM	Morrow	Adoption of Single Incision Laparoscopy After Participation in an Industry-Sponsored Course
12:55 PM	Kansier	The ACGME Case Log: General Surgery Resident Thoracic Surgery Experience
1:10 PM	Drake	Is Appendicitis a Ticking Time Bomb? Time to Treatment and the Risk of Perforation After Patients Reach the Hospital
1:25 PM	Sham	siRNA Delivery for Gene Silencing in Hepatocellular Carcinoma
1:40 PM	Bernier	Next Generation Sequencing to Identify Inherited Mutations in All Breast Cancer Genes
1:55 PM	Closing: David Flum, M.D.	

4:00 PM **HELEN & JOHN SCHILLING LECTURE – Hogness Auditorium, UW HSB**

Regenerative Medicine: New Approaches to Healthcare

Anthony Atala, M.D.
Director, Wake Forest Institute for Regenerative Medicine
W.H. Boyce Professor and Chair, Department of Urology
Wake Forest University, Winston-Salem, NC

FIBRINOGEN CONCENTRATE IS ASSOCIATED WITH IMPROVED SURVIVAL IN A PORCINE AORTIC HEMORRHAGE MODEL

Lewis C, White N, Wang X, Bradury N, Stern S

Background: Acute coagulopathy of trauma (ACOT) is associated with significant morbidity and mortality. Fibrinolysis is one component of ACOT, and prior studies have shown that early replacement of fibrinogen improves clot formation and reduces blood loss, which may translate to improved survival. However, it is unknown whether this benefit occurs in cases of penetrating trauma with major non-compressible vascular injury as is commonly seen in the combat setting. We hypothesized that animals treated with fibrinogen concentrate (FC) as part of their resuscitation would have improved outcomes when compared to controls.

Study Design: Anesthetized pigs were subjected to a well-established arterial hemorrhage model. Hemorrhagic shock was induced by a combination of controlled catheter hemorrhage and creation of a 4 mm infrarenal aortic tear. Resuscitation then began (T_0), per Tactical Combat Casualty Care (TCCC) guidelines; control animals (n=7) received Hextend (10 ml/kg) and experimental animals (n=7) received Hextend (10 ml/kg) with FC (120 mg/kg). After 30 min all animals received a second Hextend bolus (10 ml/kg). At T_{180}, the aortic injury was repaired, and goal-directed resuscitation continued for an additional three hours. Hemodynamics were continuously monitored and recorded every five minutes. Laboratory studies including arterial blood gas (ABG), arterial lactate, Prothrombin Time (PT), Partial Thromboplastin Time (PTT), fibrinogen concentration (Fbn), and Thrombelastography (TEG) were measured at baseline, T_0, and every 60 min thereafter. Kaplan Meier survival curves were used to compare survival. Outcome variables were compared using T-tests, and serial continuous data were analyzed for significant overall effects of FC and time using two-way repeated measures ANOVA with interaction.

Results: There were no significant differences between groups in regard to baseline characteristics. Overall survival was significantly greater in the group receiving FC (86% (6/7)), compared to the group receiving hextend alone (29% (2/7), Kaplan Meier, log rank $p = 0.04$). Mean intraperitoneal blood loss was less in FC animals (12.1 ml/kg) than in control animals (21.9 ml/kg ($p = 0.09$)), and rate of blood loss was significantly less in animals that received FC (0.1 ml/kg/min, (0.04)), versus hextend alone (0.37ml/kg/min (0.11), $p = 0.02$). There was a favorable effect of FC on MAP (73 (1.2) mmHg vs 61 (1.5) mmHg, $p < 0.001$) and HR (179 (1.6) bpm vs 211 (2.0) bpm, $p < 0.001$). Laboratory studies showed no difference between groups in regard to arterial pH, arterial lactate, PT, or PTT. There was a significant interaction between FC and Fbn over time as Fbn fell significantly over time in the control group, and was preserved at baseline levels in the FC treated group (rmANOVA interaction p value = 0.03). TEG demonstrated a tendency towards preserved fibrin-specific clot strength with FC, but was not statistically significant (rmANOVA $p = 0.22$).

Conclusions: Administration of fibrinogen concentrate was associated with significantly greater survival in this large-vessel arterial hemorrhagic shock model. Improved survival may be secondary to preserved levels of circulating fibrinogen resulting in a reduced rate of internal blood loss. Additional studies will be needed to better define the underlying mechanism.

EX VIVO RECONDITIONING OF NON-HEART-BEATING DONOR LUNGS IN A PRECLINICAL PORCINE MODEL: DELAYED PERFUSION RESULTS IN SUPERIOR LUNG FUNCTION

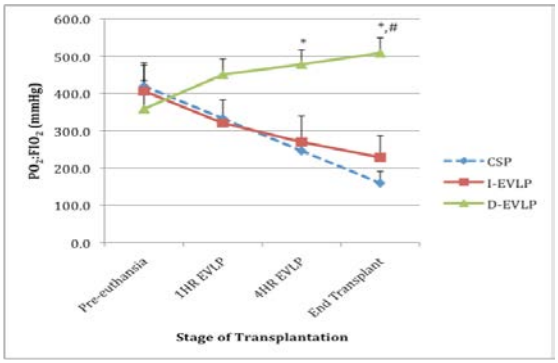
Mulloy D, Stone M, Crosby I, LaPar D, Lau C, Laubach V, Kron I

Objectives: Normothermic *Ex Vivo* Lung Perfusion (EVLP) is a promising modality for the evaluation and treatment of marginal donor lungs. The optimal timing of EVLP initiation and potential for rehabilitation of donor lungs with extended warm-ischemic times is unknown. This study compares the efficacy of different treatment strategies for non-heart-beating donor lungs.

Methods: Mature swine underwent hypoxic arrest followed by 60 minutes of no-touch warm-ischemic time. Lungs were then harvested and flushed with 4°C Perfadex®. Three groups (n=5/group) were stratified according to preservation method: standard cold-static preservation (CSP: 4 hrs storage at 4°C), immediate EVLP (I-EVLP: 4 hrs perfusion at 37°C), and delayed EVLP (D-EVLP: 4 hrs cold storage followed by 4 hrs EVLP). EVLP groups were perfused with Steen solution™ supplemented with heparin, methylprednisolone, cefazolin, and ATL-1223 (a selective adenosine 2A receptor agonist). Lungs then underwent allotransplantation followed by four hours of recipient reperfusion. Lung injury was assessed through measurement of physiologic parameters, proinflammatory cytokine expression, and histopathology.

Results: Donor blood oxygenation (PO_2:FiO_2) prior to euthanasia was not different between groups (p=0.63) (Table). Oxygenation after transplantation was significantly higher in the D-EVLP group when compared to either I-EVLP or CSP (509±40 vs. 229±59 and 159±31, respectively, p=0.002). In addition, D-EVLP treatment resulted in lower mean airway pressure decreased pulmonary artery pressure (p<0.05). Expression of the proinflammatory cytokines IL-8, IL-1β, and TNF-α was decreased in the D-EVLP group (p<0.05) and histopathologic lung injury scores were lower (p<0.05). Importantly, blood oxygenation during EVLP and after transplantation exceeded acceptable clinical levels only in the D-EVLP group.

Conclusions: Donor lungs with extended warm-ischemic times can be reconditioned for successful transplantation. Surprisingly, the combination of CSP *and* EVLP present in the D-EVLP group was necessary to reduce inflammation and restore optimal post-transplant lung function. This finding, if confirmed clinically, will allow expanded use of non-heart-beating donor lungs.



*P<0.05 vs. I-EVLP
#P<0.05 vs. CSP

THE ROLE OF BOTTLE FEEDING IN THE ETIOLOGY OF HYPERTROPHIC PYLORIC STENOSIS

McAteer J, Ledbetter D, Goldin A

Background: Hypertrophic pyloric stenosis (HPS) is the most common indication for surgery in neonates. Early exposures, including bottle feeding, have been implicated as etiologic factors. Our aim was to analyze the association between bottle feeding and HPS and determine whether that association varies according to other known risk factors.
Methods: We conducted a population-based case-control study using Washington State birth certificates linked to hospital discharge data. Cases were restricted to singleton births with codes for both HPS and pyloromyotomy. Controls were randomly chosen among all infants who did not develop HPS, and were frequency matched to cases by birth year. Bottle feeding, defined as any infant not being breastfed after birth, was determined from birth records. Logistic regression was used to adjust for covariates.
Results: We identified 714 HPS cases. Incidence decreased over the study period, from 1.4 per 1000 births in 2003 to 0.9 per 1000 births in 2009. Simultaneously, the prevalence of breastfeeding at birth discharge increased from 80% in 2003 to 94% in 2009. Compared to controls, HPS infants were more likely to be bottle feeding after birth (19.5% vs. 9.1%). After adjustment, bottle feeding was associated with an increased risk of HPS (OR=2.31, 95% CI 1.81-2.95). The HPS-breastfeeding association did not differ according to gender or maternal smoking status, but was significantly modified by maternal age (<20 years OR=0.98, 95% CI 0.51 – 1.88; 35+ years OR=6.07, 95% CI 2.81 – 13.10) and parity (nulliparous OR=1.60, 95% CI 1.07 – 2.38; multiparous OR=3.42, 95% CI 2.23 – 5.24).
Conclusion: Bottle feeding is associated with an increased risk of HPS, and this effect seems to be most important in older and multiparous women. These data suggest that bottle feeding may play a role in the etiology of HPS, and the pattern of effect modification suggests that the underlying mechanism may be hormonal in nature.

Table: Odds ratios for bottle feeding vs. breastfeeding stratified by maternal age and parity.

MATERNAL AGE	Odds Ratio	95% C.I.	p-value for effect modification
<20	0.98	0.51 - 1.88	0.005 (vs. 20-34), <0.001 (vs.35+)
20-34	2.58	1.93 - 3.44	0.050 (vs. 35+)
35+	6.07	2.81 - 13.10	
MATERNAL PARITY	Odds Ratio	95% C.I.	p-value for effect modification
Nulliparous	1.60	1.07 - 2.38	0.154 (vs. primiparous), 0.017 (vs. multiparous)
Primiparous	2.60	1.61 - 4.20	0.432 (vs. multiiparous)
Multiparous	3.42	2.23 - 5.24	
* All odds ratios adjusted for infant gender, maternal age, maternal race, maternal parity, maternal BMI, gestational age, birthweight, and smoking status.			

EFFECT OF TOPICAL MAPK INHIBITION ON HYPERTROPHIC SCARRING IN A DUROC PORCINE MODEL

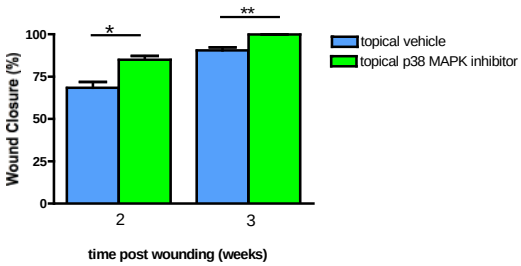
Whitney R, Warsen A, Shubin N, Hocking A, Engrav L, Arbabi S

Background: Burns are a major cause of mortality and morbidity in the world. Furthermore, severe burns may induce systemic inflammation in the form of 1) systemic inflammatory response syndrome (SIRS) 2) acute lung injury and 3) multiple organ dysfunction syndrome (MODS). It has been postulated that the local dermal inflammatory response serves as the driving force for over-stimulation of the systemic inflammatory response, ultimately leading to SIRS and MODS. We propose that inflammatory source control by local/topical administration of inflammatory signal modulators will improve outcomes by limiting the development of SIRS, MODS, and improve overall wound healing. We have previously shown that the application of p38 mitogen activated protein kinase (MAPK) inhibitor on burn wounds attenuates inflammatory signaling and improves outcomes in a murine burn model. However, to study the clinical efficacy and utility of P38 MAPK inhibition on wound healing and inflammatory source control, a model that resembles the human response is required. The Red Duroc porcine model is an established animal model that closely resembles the human wound healing phenotype. In this study, we evaluate the efficacy of topical P38 MAPK inhibition on wound healing and scarring in the Red Duroc pig model. With the previous knowledge of P38 MAPK hyperactivation being associated with a hyper-inflammatory response and hypertrophic scarring, we hypothesized that P38 MAPK inhibition will improve wound healing and decrease scar formation.

Methods: : Eight week old red Duroc pigs received 10 tangential deep partial thickness wounds (5x5 cm) on their backs using a standard electric Padget dermatome. Topical p38 MAPK inhibitor was applied over the wounds. Biopsies were taken at 2 weeks and 5 months. Periodic wound assessments with photographs were taken and image analysis was performed assessing wound closure. Histological analysis was performed. The results were compared to red Duroc pigs that had no treatment in our previous experiments.

Results: There was a significant increase in the rate of wound healing and degree of wound closure at 3 weeks ($p<0.003$) ($p<0.001$) in the animals who received topical P38 MAPK inhibitor. In addition, application of topical P38 MAPK inhibitor reduced the scar thickness when compared to control at 5 months post wounding ($p<0.0006$).

Conclusion: This study demonstrated that topical P38 MAPK inhibition resulted in decreased scar thickness, histologically decreased granulation tissue layer, decreased degree of wound contraction, and an increased rate of wound closure. Topical P38 inhibition did not increase the incidence of infection in our study nor did it cause any local changes in pigmentation or gross appearance. Our study is encouraging and suggests a role for topical P38 MAPK inhibition in wound healing in order to promote expedited wound closure with reduced hypertrophic scarring and to minimize wound contraction and subsequent loss of function. Further experiments are required to explore the utility of topical P38 MAPK inhibition in a Red Duroc burn model. This could have significant implications in burn patients to reduce mortality, end-organ dysfunction, and to improve cosmetic and functional outcome.



SURVIVAL AFTER RUPTURED AAA IN THE ENDOVASCULAR ERA: LOOKING BEYOND THE TRADITIONAL 30-DAY OUTCOME

Wallace GA, Starnes BW, Hatsukami TS, Quiroga E, Kohler T,
Tang GL, Tran NT.

Background: Emergency endovascular aneurysm repair (rEVAR) has contributed to a dramatic improvement in early patient survival after ruptured abdominal aortic aneurysm (rAAA). A contemporary series from our institution recently reported an overall 30-day mortality rate of 35% as compared to 70% in the 1980s, with favorable 30-day survival after rEVAR as compared to open surgical repair (OSR) (81% vs. 46%). Despite the international attention that the early survival after rEVAR has garnered, little is known about these patients' discharge disposition and the mid-term survival after rEVAR as compared to OSR.

Purpose: 1. To determine the discharge disposition of patients who survive rEVAR as compared to OSR of rAAA. 2. To determine the mid-term survival of patients after rEVAR as compared to OSR for rAAA.

Methods: We performed a University of Washington IRB approved, single institution, retrospective review of all patients who presented to Harborview Medical Center with rAAA July 2007 through February 2012. Primary outcomes were discharge disposition and mid-term survival (>30 days). Data were analyzed with multivariable regression and Kaplan-Meier survival curves with a log rank test.

Results: A total of 118 patients were analyzed. Eight patients received only comfort care, 10 died in the operating room, and 100 survived their index operation (39 OSR and 61 rEVAR). Average age and gender were similar (OSR 77 ± 7.8 years, 85% males; rEVAR mean 74 ± 7.4 years, 79% males). Admission lactic acidosis was similar (OSR 4.8 ± 4.0mmol/L, rEVAR 3.9 ± 3.4mmol/L, P = 0.45). Preoperative hypotension (systolic blood pressure < 90mmHG) was similar (OSR 64%, rEVAR 62%, P = .85). More patients that survived their index operation survived to discharge after rEVAR [54% OSR (21/39), 84% rEVAR (51/61), P = .001]. More patients discharged to home vs. a SNF after rEVAR [65% (33/51)] as compared to OSR [19% (4/21)] (P = .0004). Overall, the follow-up rate for determination of survival for patients who lived past 30 days was 86% (56/65, median 14 months, 25-75th interquartile 3.1-27.8). Type of procedure performed and peri-operative hypotension were predictive of discharge destination. Kaplan Meier analysis revealed a significant mid-term survival benefit for patients after rEVAR as compared to OSR (P = .01, Log Rank). Subgroup analysis of survivors past 30 days revealed similar rates of mid-term survival (P = 0.7, Log Rank). Overall mid-term relative risk reduction after rEVAR versus OSR was 35% (95% CI 0.06-0.59).

Conclusions: rEVAR saves lives and allows more patients to discharge home as compared to OSR after rAAA. The early survival benefit of rEVAR prevails at mid-term follow-up. These favorable outcomes support the utilization of rEVAR over OSR, when feasible, in centers that are able to achieve the early survival benefit. Continued durability, cost effectiveness, endoleak incidence and significance, and quality of life associated with rEVAR as compared to OSR remain to be determined in the late-term follow-up.

Skin antiseptic agents and surgical site infection: A Report from Washington State's Surgical Care and Outcomes Assessment Program (SCOAP).

Hakkarainen TW, Dellinger EP, Evans HL, Farjah F, Farrokhi E, Steele SR, Thirlby R, Flum DR; for the Surgical Care and Outcomes Assessment Program Collaborative

Background: Surgical site infections (SSI) are an important source of morbidity and mortality. Chlorhexidine in isopropyl alcohol is effective in preventing central venous-catheter associated infections, but its effectiveness in reducing SSI in clean-contaminated procedures is uncertain. Surgical studies to date have had contradictory results. We aimed to further evaluate the relationship of commonly used skin antiseptic agents and SSI, and to determine if isopropyl alcohol had a unique effect.

Methods: Data were collected prospectively for patients undergoing surgery from January 2011 through June 2012 at 47 SCOAP site hospitals. The outcome of interest was SSI during the index admission. Skin antiseptic agent was the variable of interest. Patients were stratified based upon skin antiseptic agent used, and also on the inclusion of isopropyl alcohol in the skin antiseptic agent. We used multivariate logistic regression modeling, clustered by hospital, to predict risk-adjusted rates of SSI. Observed to expected (O:E) ratios of SSI were compared across skin antiseptic agent groups using proportionality testing. Sub-group analysis of elective colorectal procedures was also performed.

Results: Among 8,014 patients the rate of SSI was 4.3%. The O:E ratios were 0.86 (p=0.22) for chlorhexidine (CHG), 1.08 (p=0.15) for chlorhexidine in isopropyl alcohol (CHG+IPA), 1.00 (p=0.96) for povidone-iodine (PVI) and 0.87 (p=0.54) for iodine-povacrylex in isopropyl alcohol (IPC+IPA). The O:E ratios were 0.91 (p=0.32) for the non-IPA group and 1.08 (p=0.25) for the IPA group. Among elective colorectal patients the O:E ratios were 0.91 (p=0.68) for CHG, 1.05 (p=0.70) for CHG+IPA, 1.12 (p=0.64) for PVI and 0.66 (p=0.34) for IPC+IPA.

Conclusions: For mostly clean-contaminated surgical cases, this large-scale regional cohort study does not demonstrate superiority of any commonly-used skin antiseptic agent in reducing the risk of SSI, nor does it find any unique effect of isopropyl alcohol. These results do not support the use of more expensive skin preparation agents, and because no single agent was found to be superior, standardizing skin antiseptic choice may not be a high value target for quality improvement.

Observed and Expected Rate of Surgical Site Infection, By Agent

Skin Antiseptic Agent	Observed Incidence SSI (% [95% CI])	Expected Incidence SSI (%[95% CI])	Observed:Expected Ratio (95% CI)	*p*-value
CHG	3.6 [2.8-4.6]	4.2[3.8-4.6]	0.86 [0.67-1.10]	0.22
CHG+IPA	4.1[3.6-4.7]	3.8 [3.5-4.0]	1.08 [0.95-1.24]	0.15
PVI	5.3[3.8-7.2]	5.3 [4.5-6.1]	1.00 [0.72-1.36]	0.96
IPC+IPA	4.6[2.8-7.0]	5.3 [4.4-6.2]	0.87 [0.53-1.32]	0.54
Sub group IPA vs non-IPA				
Non-IPA	4.1[3.4-4.9]	4.5 [4.2-4.9]	0.91 [0.76-1.09]	0.32
IPA	4.2[3.7-4.7]	3.9 [3.7-4.1]	1.08 [0.95-1.21]	0.25

Legend: CHG: chlorhexidine, CHG+IPA: chlorhexidine in isopropyl alcohol, PVI: povidone-iodine, IPC+IPA: povacrylex-iodine in isopropyl alcohol, IPA: isopropyl alcohol

COMPARISON OF OUTCOMES IN IMMEDIATE IMPLANT-BASED BREAST RECONSTRUCTION VERSUS MASTECTOMY ALONE

Dubbins J, Liu D, Feczko R, Calhoun K, Louie O, Neligan P, Said H, Mathes D

Background: Prosthetic reconstruction is common practice in breast reconstruction. Previous studies have shown an increased complication rate in the setting of immediate versus delayed reconstruction. We aim to quantify any additional risk in postoperative complications when immediate implant-based reconstruction is performed versus mastectomy alone.

Methods: We retrospectively reviewed all immediate implant-based breast reconstructions and all mastectomies without reconstruction at a tertiary medical center from 2007 to 2011 under the supervision of 4 plastic surgeons and 5 breast surgeons. Patient characteristics, operative details, and complication rates were reviewed and analyzed using univariate and multivariate analyses.

Results: Immediate implant-based reconstruction was performed in 310 consecutive women (443 breasts). Mastectomy alone was performed in 410 women (503 breasts). Patients who underwent mastectomy alone were more often unilateral, older, obese, and diabetic. Overall complications were higher in the immediate reconstruction group, largely attributable to increased rates of delayed healing and need for reoperation. Surgical site infection was similar between both groups, while seroma rates were higher in the mastectomy alone group. On multivariate analysis, independent risk factors for reoperation were smoking, nipple-sparing mastectomy, and immediate tissue expander placement. Immediate tissue expander placement was not a risk factor for delayed healing, but increased age, smoking, and nipple-sparing mastectomy were significant risk factors.

Conclusions: This study demonstrated higher complication rates in immediate implant-based breast reconstruction secondary to delayed healing and need for reoperation. Immediate breast reconstruction can be safely performed provided that patients are educated about the increased reoperation rate specific to implant complications.

IMPACT OF OBAMACARE ON 19-25 YEAR OLDS

Kotagal M, Carle AC, Kessler LG, Flum DR

Background: Young adults 19-25 years old have the lowest rates of insurance nationally. The Patient Protection and Affordable Care Act (PPACA), implemented in September 2010, mandated that insurance companies allow young adults (<26) to remain beneficiaries on their parents' insurance. Recent census data shows that the percentage of uninsured Americans decreased in 2011, attributed partly to this coverage expansion. PPACA's impact on young adults' health and health behaviors remains unknown.

Methods: To assess self-perceived health and behaviors, we evaluated publicly available surveys (Behavioral Risk Factor Surveillance System [BRFSS] and National Health Interview Survey [NHIS]) before (2009) and after (2011) PPACA implementation. BRFFS is a telephone survey of health status, behaviors, and access in non-institutionalized adults. Additionally, the NHIS examines healthcare utilization. All analyses accounted for complex survey design.

Results: Demographic characteristics of the young adult sub-population were unchanged between the two time periods, except for an increase in percentage in poverty (household income <$15,000 increased from 17.7 to 22.1%). Healthcare coverage increased significantly (68.3 to 70.3%, p=0.02). Self-perceived health status did not change significantly. There were small changes in days of poor physical and mental health, but no consistent trends indicating a changed perception of health. Respondents with a usual source of care declined (62% to 58.2%, p=<0.0001) as did those with a routine check-up in the past year (56.8 to 54.7, p=0.02). Inability to afford prescriptions and dental care declined for both insured and uninsured respondents. Vaccination rates increased for influenza (16.5 to 21.5%, p=0.001), but not for hepatitis, HPV, and tetanus. The increase in influenza vaccination was only significant for insured respondents. Sensitivity analyses by sex were similar.

Conclusions: This "first look" at nationally representative data surrounding PPACA's implementation demonstrates that healthcare coverage for young adults has increased, but that this did not affect health status, overall utilization of preventive services (except influenza vaccination), or likelihood of having a usual source of care. Our findings suggest that the link between coverage and health status is complicated, depending on both interest and ability to obtain care. The proportion of young adults reporting a recent doctor's visit, though more common among insured respondents, declined yearly since 2003; this trend continued after implementation of PPACA. Reasons for this decrease remain unclear. Self-perceived health status may not be a useful metric in this population, as young adults are generally healthy. Understanding PPACA's full impact on young adults may require a focus on those who consume more healthcare, such as those with chronic disease. Additionally, evaluating the impact of PPACA may be challenging given the recent recession. The persistent decline is a recent doctor's visits emphasize the importance of policies extending beyond coverage to improve access and quality of care.

THE ROLE OF LPS STRUCTURE IN MONOCYTE ACTIVATION AND CYTOKINE SECRETION

Plevin R, Knoll M, McKay M, Arbabi S, Cuschieri J

Background: Gram-negative sepsis is a leading cause of morbidity and mortality in the ICU through the development of organ failure. Organ failure occurs through the activation and alteration of immune cell function by being exposed to lipopolysaccharide (LPS) from the cell wall of gram-negative bacteria. Poorly regulated cytokine secretion is a key factor in the development of organ failure and progressive cellular injury. These cytokines are produced in response to LPS binding to toll-like receptor 4 (TLR4) complexes on the cell surface of monocytes. This complex is composed of the TLR4 receptor, CD14, and several supporting proteins. Binding to the TLR4 complex through interaction of CD14 activates mitogen-activated protein (MAP) kinase cascades and downstream synthesis and secretion of cytokines. However, the full contribution of LPS to the activation of this complex by LPS remains unknown. LPS has three components: a core hydrophobic lipid (Lipid A), a hydrophilic polysaccharide chain, and a hydrophilic O-antigen chain. The purpose of this study is to determine what components of LPS are needed to activate the TLR4 complex. Thus, we set out to study monocyte activation and cytokine production by various structurally modified LPS and wild-type LPS molecules.

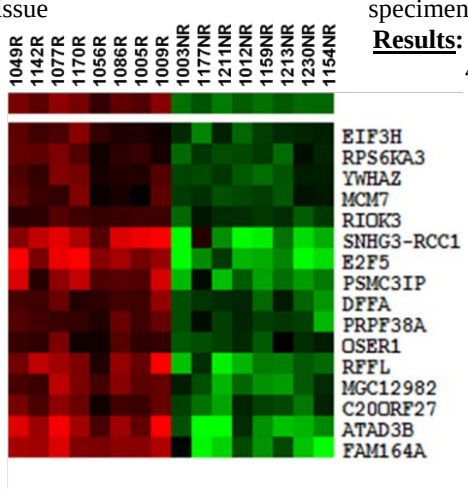
Methods: Healthy donor PBMCs were isolated and stimulated with LPS, LPS with attenuated O-antigen chain (RF5), or an LPS variant containing only Lipid A (DPL). Total cell protein was extracted and the concentration of phosphorylated and unphosphorylated p38, ERK, and JNK were assessed by Western immunoblotting. PBMCs were stimulated with LPS, DPL, or RF5 and TNF-α and IL-10 levels in cell supernatants were measured using Luminex. In order to characterize the cell-surface components involved in cytokine generation, this was repeated in PBMCs that had been pre-treated with monoclonal antibody to CD14 or TLR4. Lastly, TNF-α and IL-10 mRNA levels were measured by real-time PCR following cell stimulation.

Results: PBMC treatment with wild-type LPS activates p38, ERK, and JNK, increases de novo synthesis of both TNF-α and IL-10, and stimulates the release of both cytokines. This cytokine release appears to be CD14 and TLR4 dependent, as evidenced by decreased cytokine release from cells pre-treated with specific antibody blockers. RF5 similarly activates all three MAP kinases in a CD14 and TLR4-dependent manner and stimulates synthesis and release of TNF-α and IL-10. However, these cellular responses are attenuated compared to cells treated with wild type LPS. DPL selectively activates p38 and ERK but not JNK, and no increase in TNF-α or IL-10 synthesis is observed. However, both IL-10 and TNF-α secretion increase but to a much lower level than cells treated with either LPS or RF5.
Conclusions: Wild-type LPS has the greatest effect on monocytes by activating MAP kinase cascades, increasing TNF-α and IL-10 synthesis, and stimulating TLR4-dependent cytokine secretion. The presence of intact O-antigen appears important to this activation, as evidenced by the fact that the LPS variant with attenuated O-antigen activates the same cellular pathways as wild-type LPS, but to a much lesser degree. Isolated Lipid A selectively activates only ERK and p38 without activation of transcription of inflammatory cytokines. However, despite this attenuated transcription, isolated lipid A does result in secretion of pre-formed TNF-α and IL-10. This finding suggests that Lipid A has an alternate mechanism for stimulating cytokine release. In fact, given the hydrophobic structure of Lipid A, activation by lipid A could be due to direct plasma membrane binding which is distinct from activation by the TRL4 dependent O-antigen activation.

TRANSCRIPTOME ANALYSIS OF RECURRENT HEPATOCELLULAR CARCINOMA RELATED TO HEPATITIS C VIRUS FOLLOWING LIVER TRANSPLANTATION: MOLECULAR SIGNATURES FOR POSSIBLE NEW THERAPEUTIC OPTIONS

Das T, Diamond DL, Yeh M, Bryan JT, Hassan S, Reyes JD,
Katze MG, Perkins JD

Background: Hepatocellular Carcinoma (HCC) causes >600,000 deaths annually worldwide and is the most common primary liver cancer. Chronic hepatitis C virus (HCV) induced HCC is a primary indication for liver transplantation (LT). Unfortunately, the recurrence of HCC is a major cause of mortality in these patients. There is no standard method to identify and treat patients who are at high-risk for HCC recurrence.
Purpose: Our goal was to discover molecular signatures underlying HCC recurrence that may lead to future studies on gene regulation leading to new therapeutic options.
Methods: Following IRB approval 2 groups of liver transplant recipients with chronic HCV and HCC recurrence or non-recurrence following transplantation were selected. cDNA microarray analysis containing more than 29,000 known genes was performed on formalin-fixed paraffin embedded (FFPE) explanted liver tissue. Differentially expressed genes (based on P-value <0.05 (FDR corrected) were considered statistically significant) were determined on 8-HCC-recurrence (R) and 8-HCC non-recurrence (NR) tumor tissue specimens. Network analysis was performed using Ingenuity Pathway Analysis software.



Results: Out of 29,000 genes, 194 were differentially regulated (151 genes were up-regulated and 43 were down regulated). The main networks included: cell cycle G1/S checkpoint regulators, RAN signaling, chronic myeloid leukemia signaling, molecular mechanisms of cancer, cyclins, FXR/RXR activation, complement system and hepatic cholestasis. The highest regulated genes were 17 up-regulated genes in R group of patients (Figure: Heatmap shows differential expressions among R and NR). The microarray data for 11 genes (DFFA, RIOK3, E2F5, YWHAZ, QSER1, RPS6KA3, PRPF38A, MCM7, C20ORF27 and HDAC2) were cross-validated by quantitative real-time polymerase chain reaction (qPCR) indicating reproducible gene expression patterns. The remaining 6-genes (RFFL, SNHG3-RCC1, FAM164A, PSMC3IP, ATAD3B and MGC12982) didn't express in all patients' RNA samples simultaneously by qPCR. This non-expression is possible due to partial degradation, a drawback in utilizing FFPE samples.
Conclusions: Transcriptome evaluation using microarray analysis of FFPE tissue identified 17 highly significant genes in patients associated with HCC recurrence.

RECONSTRUCTION OF STERNAL WOUNDS, WITH AND WITHOUT RIGID PLATE FIXATION

Lipira A, Hayes A, Colohan S, Said H, Louie O, Neligan P, Mathes D

Background: Management of sternal wound complications remains a significant challenge despite advancements over the last 50 years, notably the development of muscle flap coverage. Some have advocated for sternal plating in addition to flap coverage. While there is evidence that plating high-risk patients at the time of sternotomy can reduce the incidence of sternal complications, there is little data on the use of sternal plates after the development of a sternal complication. Despite this, plating is being used in secondary sternal reconstruction in an increasing fashion. We reviewed our experience with sternal reconstruction over the past 12 years, comparing the use of flaps alone versus the use of sternal plating and flap coverage.
Purpose: To examine outcomes of plating in secondary sternal reconstruction and compare them with outcomes for traditional flap reconstruction, and to assess how plating is being used in our institution.
Methods: 70 cases of sternal reconstruction from 1997-2011 met inclusion criteria. Plates with muscle flap coverage were used in 21 patients. Flaps alone were used in the other 49. Baseline characteristics and risk factors for wound complications were obtained, as well

as indications for reconstruction and primary sternotomy. Outcome data included dehiscence, infection, reoperation, other wound complications, and length of hospital stay.

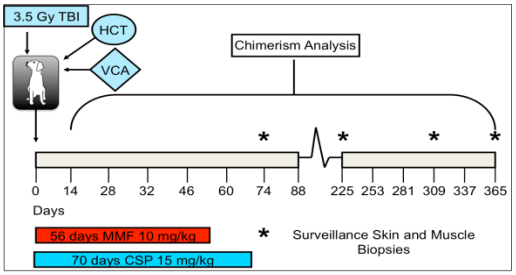
Results: Comorbidities and reason for primary sternotomy were similar between groups, but indication for reconstruction differed considerably ($p < 0.01$): 57% of plated patients had sterile dehiscence, while 69% of non-plated patients were acutely infected. Mean time from sternotomy to reconstruction was 208 days for plates, vs 60 days for non-plated. 28.6% of plates had a post-op complication, vs 44.9% of non-plated ($p = 0.3$). Reoperation was necessary in 19.1% of plates vs 28.6% of non-plated ($p = 0.6$). No plated patients dehisced, vs 20.8% of non-plated ($p = 0.03$). 3 plated patients (14%) had their hardware removed. Four patients with acute infections were plated; none had complications.

Conclusions: At our institution, plates have been used selectively, mostly for sterile dehiscence and delayed reconstructions in medically stable patients. Acutely ill and infected patients were more often treated with traditional muscle flap reconstruction without plates. None of the plated patients dehisced after reconstruction, suggesting there is a role for plating, at least in medically stable patients at high risk for wound re-dehiscence. The small number of patients with acute infections who were plated fared well, but further study is needed to evaluate the use of plates in this setting.

MISMATCHED VCA TRANSPLANTATION WITHOUT THE NEED FOR DONOR CELL CHIMERISM: A MODEL TO ACHIEVE TOLERANCE WHILE ELIMINATING CHRONIC IMMUNOSUPPRESSION AND THE RISKS OF GVHD

Swearingen B, Chang J, Butts T, Graves S, Storb R, and Mathes DW

Background: The clinical transplantation of vascularized composite allografts (VCA) to reconstruct devastating facial injuries and lost extremities offer the opportunity to truly restore both form and function. However, the clinical application of these techniques is limited by the current requirement of chronic immunosuppression. One promising method of inducing tolerance to an organ allograft is the development of mixed chimerism. We have previously demonstrated that a non-myeloablative stem cell transplant can lead to tolerance in a mismatched dog model, but the application of this protocol has been limited by graft-versus-host disease (GVHD). We have observed several animals that, after an initial period of donor cell engraftment, lost their stem cell allograft but remained tolerant to the VCA. Our hypothesis for this study was that our non-myeloablative hematopoietic stem cell transplant protocol could be used to induce tolerance to a recipient VCA without the need for persistent donor cell chimerism.



Methods: 5 Haploidentical canine recipients (median weight and age were 12.1kg and 10.9 months) received a non-myeloablative conditioning regimen of 350 cGy TBI, mobilized donor stem cells (PBMC) and VCA transplantation followed by a short course of immunosuppression (MMF for 56 days and Cyclosporine for 70 days). Peripheral blood chimerism was evaluated by PCR techniques weekly. Peripheral blood cytokine expression was evaluated by flow cytometry. VCA rejection was followed clinically and confirmed histologically after routine biopsies.

Results: All 5 animals tolerated the conditioning regimen. One dog rejected the PBMC at 35 days post transplantation and went on to reject the VCA transplant following the cessation of immunosuppression. One dog fully engrafted and converted to 100% donor chimerism and long-term tolerance to the VCA. 3 dogs demonstrated a prolonged period of transient chimerism (7 to 10 weeks post-transplant) and went on to reject their donor stem cells after the cessation of immunosuppression without acute rejection of their donor VCAs. One of these dogs was euthanized for persistent fevers at post-operative day 147 with no sign of rejection. The remaining two had long-term acceptance of their VCA (>180 days) with no evidence of acute rejection. However, more recently both of these animals have demonstrated evidence of chronic rejection. No dog developed GVHD. Early cytokine analysis in tolerant versus non-tolerant animals reveals a significantly elevated IL-4 and IL-10 compared to baseline, suggesting a Th2 profile during induction of tolerance.

Conclusions: In this study we demonstrate that our non-myeloablative protocol allows for selective rejection of donor stem cells and elimination of GVHD risks without acute rejection of the VCA transplant. Early after conditioning the establishment of aTh2 profile appears to contributing to the anti-inflammatory environment.

OPTIMIZING FEEDBACK FROM THE DESIGNATED LEVEL 1 TRAUMA/BURN CENTER TO REFERRING HOSPITALS

Rae L, Bulger E

Background: The American College of Surgeons Committee on Trauma (ACS-COT) is dedicated to improving the quality of care to severely injured patients. Many initiatives have been implemented to improve outcomes including verification and designation of trauma and burn centers and educational efforts, such as ATLS and ABLS. Along with these initiatives the ACS-COT charges designated centers to provide feedback to the referring hospital. However, there are no guidelines or recommendations as to what should be included in the feedback, and how it should be utilized. The objective of this study is to evaluate the current feedback efforts for patients transferred to Harborview Medical Center (HMC), a regional Level 1 trauma and burn center, to better understand how the feedback is utilized and to evaluate what types of feedback are most useful to the referring hospitals. HMC offers access to the transferred patient's electronic medical record (EMR) through a password protected program called U-Link. This allows trauma coordinators, managers or directors to follow up and access the chart at HMC for patients whose care was initiated at their hospital.

Methods: The current feedback methods employed by HMC were reviewed including an analysis of utilization of U-link and all other forms of feedback (ie. letters) to referring hospitals in Washington State from July 1, 2011 to July 1, 2012. In September 2012, an electronic survey was sent to all 82 Washington State hospitals that participate in the regionalized trauma system and transfer patients to HMC. Part 1 –of the survey included evaluation of U-link access and the current feedback process at HMC. Part 2 –how is feedback utilized at the referring institutions and Part 3 –what feedback and information is most needed and useful to referring hospitals.
Results: HMC admitted 5988 trauma and 763 burn patients from July 1, 2011 to July 1, 2012. Of those 3282 (54.8%) of trauma and 508 (66.5%) of burn admissions were transfers from referring hospitals. Currently 90 different hospitals have acquired a U-link account in the region to follow patients after transfer to HMC. Of those, 66 (73%) of hospitals utilized U-link over this time period. U-link was accessed 2092 times and a total of 7463 screens were viewed. The most common aspect of the chart viewed were transcripts 1973 (26%). A survey was sent to 82 hospitals in Washington State, 42 (51.2%) responded to the survey. Of those 19 (46%) use U-link 100% of the time for patients transferred to HMC, 5(12%) use it sometimes and 17 (41%) have never used U-link. Discharge summaries are the primary source of information used to generate quality assurance or case reviews (81%). The most desired feedback included; resuscitation 89.2%, admission injuries 83.8%, appropriateness of the 'decision to transfer' the patient 78.4% and deviations from ATLS/ABLS protocols 75.7%. When asked how this information would be utilized, education was indicated 100%, review for QA issues 92% and systems analysis 98.5%.
Conclusions: There is significant interest on the part of the referring hospitals to receive feedback from the designated Level 1 trauma/burn center to improve quality of care. A major goal of chart review for transferred patients is to determine areas of improvement and quality assurance. The EMR should aid trauma centers in providing feedback. A system like U-link can allowing HIPAA compliant access to review patients charts for quality improvement and feedback with little effort on the part of the designated trauma/burn center. This is a valuable tool to be utilized by tertiary referral centers to augment feedback to transferring hospitals.

PRE-HOSPITAL ASPIRATION IN TRAUMA PATIENTS

Fawcett VJ, Warner KJ, Cuschieri JC, Evans HL

Introduction: Aspiration has been associated with chemical pneumonitis and bacterial infection of the lungs, as well as the acute respiratory distress syndrome (ARDS). Rates of ventilator-associated pneumonia (VAP) are highest among patients emergently intubated following traumatic injury. We previously reported a retrospective cohort analysis demonstrating an association between subjective aspiration and pneumonia following prehospital intubation. The goal of the current study is to re-examine this relationship in a prospective manner after prehospital providers were specifically asked to note features of aspiration around the time of intubation
Methods: From May 2010 to December 2011, Seattle Medic One and King County Medics collected data regarding aspiration at the time of intubation. All trauma patients who were intubated in the field and subsequently admitted to Harborview Medical Center Trauma/Surgical ICU were included in the study. Data collection included a clinical impression of whether or not the patient aspirated, as well as explicit information regarding the presence and timing of both blood and emesis in the airway. Patient comorbidities, injury severity, physiologic variables and outcomes were drawn from the Harborview Medical Center Trauma Registry. Healthcare-associated pneumonia (HAP) was identified by medical record review of both bronchoalveolar lavage culture results and clinician discharge diagnosis. Diagnosis of ARDS was defined by the ratio of partial pressure of arterial oxygen to inspired oxygen at 48 hours, as well by clinician discharge diagnosis. Descriptive statistics and a univariate analysis of outcomes by aspiration status were performed.
Results: Two hundred and twenty-eight patients were included in the study. Eighty-nine patients (39%) were determined by medics to have aspirated blood and/or emesis. The majority (74 patients [95%] of those with blood in the airway and 17 patients [77%] of those with emesis in the airway) had aspirated prior to intubation. Increased mortality rate, hospital length of stay (LOS), ICU LOS and duration of mechanical ventilation were observed after aspiration, compared to no aspiration, but differences were not statistically significant (mortality: 21 [23.6%] vs 23 [16.6%] patients p=0.19; hospital LOS: 12.5±2.5 vs. 9.1±1.0 days, p=0.46; ICU LOS: 5.3±0.87 vs. 4.1±0.53 days, p=0.13; duration of mechanical ventilation: 5.3±1.2 vs. 3.2±0.49 days, p=0.10). Of the 89 patients who aspirated around the time of intubation, 14 (15.7%) went on to develop HAP versus only 5 (3.6%) of those who did not aspirate ($p<0.01$). Additionally, patients who aspirated in the pre-hospital setting had an increased risk of ARDS compared to those who did not aspirate (5 patients [5.8%] vs. 0 patients [0%], $p<0.01$).
Conclusion: In this prospective study of pre-hospital intubation in trauma patients, clinically diagnosed aspiration was a common event. Furthermore, the majority of patients who aspirated did so prior to intubation. Although aspiration did not convey a statistically significant increased risk of in-hospital mortality, it was associated with a significantly elevated risk of HAP as well as ARDS. Pre-hospital aspiration appears to be a clinically important event, and efforts to mitigate the negative impact of such incidents may well be warranted.

ENDOLEAKS AFTER ENDOVASCULAR REPAIR OF RUPTURED ABDOMINAL AORTIC ANEURYSMS: HOW SHOULD THEY BE MANAGED?

Garland BT, Starnes BW, Quiroga E, Tran NT

Introduction: The management of ruptured abdominal aortic aneurysms (rAAA) has undergone significant change within the last 15 years with endovascular repair now the preferred operative approach. The objective of this study is to report the incidence and characterize the behaviors of endoleaks after ruptured endovascular aneurysm repair (rEVAR).

Methods: We completed a retrospective analysis of all patients presenting to Harborview Medical Center with rAAA between July 2007 and August 2012. All patients repaired with rEVAR who underwent CT angiogram (CTA) within the first 30 days following repair

were included. CTA was read independently by attending radiologists for the presence and type of endoleak. Each patient's clinical course was reviewed including demographic data and outcomes including the need for re-intervention and length of hospital stay.

Results: Between July 2007 and August 2012, 64 patients underwent rEVAR. Of those, 47 patients had CTA performed within 30 days of the procedure. Their mean age was 74.5 years with the majority of patients being male (87.2%.) Five Type 1 or Type 3 endoleaks and nine Type 2 endoleaks were identified with an overall endoleak rate of 29.8% (14/47). Three out of five Type 1 endoleaks required urgent re-intervention due to hemodynamic instability. No type II endoleaks required further intervention. At two years, all except two endoleaks have resolved.

Conclusion: The rate of endoleak after rEVAR is higher than that reported for elective EVAR. The majority will resolve within two years and can be followed on a conservative basis. However, Type I endoleaks can quickly progress to rapid hemorrhage and require emergent intervention. We recommend 30-day follow-up with CTA for all patients with rEVAR to prevent this potentially fatal complication.

GENETIC RISK FACTORS FOR HYPERTROPHIC SCAR DEVELOPMENT

Thompson C, Hocking A, Honari S, Muffley L, Ga M, Gibran N

Introduction: Hypertrophic scars (HTS) occur in 30-72% patients following thermal injury. These red, raised, pruritic scars negatively impact quality of life with psychosocial and functional impairment. Identified risk factors for hypertrophic scarring include skin color, female gender, young age, burn site, and burn severity. Recent correlations between genetic variations and clinical conditions raise the question as to whether single nucleotide polymorphisms (SNPs) are associated with HTS formation. We hypothesized that a SNP in the $p27^{kip1}$ gene (rs36228499) that had previously been associated with decreased risk of restenosis after coronary stenting would be associated with lower Vancouver Scar Scale (VSS) measurements and decreased itching in a cohort of burn subjects.

Methods: Adult subjects (≥18 yrs old) with thermal burns were enrolled. Patient and injury characteristics were collected. Subjects were examined at two follow-up points post-injury and their VSS scores were calculated; the highest value was used for analysis. Genotyping for rs36228499 was performed using real-time PCR. Data were analyzed using Stata 12 statistical software. Categorical data were compared using χ^2 tests. Means of VSS scores and itch scores were compared using parametric and non-parametric ANOVA, respectively. Logistic regression analysis was performed to determine risk factors for hypertrophic scar as measured by a VSS score >7.

Results: 300 subjects who completed a prospective observational study to correlate SNPs with HTS formation were analyzed. Median age was 39 years (range 18-91), 69% were male & median burn size was 7% TBSA (range 0.25-80). HTS formation was associated with American Indian/Alaskan Native race (OR, 12.2; *P*=0.02), facial burns (OR, 9.4; *P*=0.04), and burn size >20% TBSA (OR, 1.99; *P*=0.03). We did not identify associations of HTS with age or gender, though age greater than 65 was associated with lower itch scores (*P*=0.04). As expected, based on published data, the $p27^{kip1}$ variant SNP occurred in 40% of the population (21% homozygous for the variant). However, no patients of Native American/Alaskan Native, African American or Pacific Islander ethnicity were homozygous for the variant. The $p27^{kip1}$ SNP was not associated with reduced HTS formation or lower itch scores in an additive, co-dominant or recessive model.

	Odds Ratio	95% CI	*P*- value
American Indian/Alaskan Native	12.2	1.5-101.9	0.02
Facial Burn	9.4	1.1-80.1	0.04
≥ 20% TBSA Burn	1.99	1.1-3.7	0.03

Conclusions: Our study suggests that American Indian/Alaskan Native ethnicity, facial burns, and higher burn surface areas are independent risk factors for hypertrophic scarring following thermal burns. These novel associations can be applied to clinical situations and potentially influence the surgical management of high risk patients. The association seen in American Indian/Alaskan Native patients may be due to yet-to-be-recognized genetic variants. Larger studies of these populations would allow for confirmation of our finding as well as genome wide investigation of associated variants. Whereas the $p27^{kip1}$ SNP may protect against vascular fibroproliferation, the effect cannot be generalized to cutaneous scar formation.

ADOPTION OF SINGLE INCISION LAPAROSCOPY AFTER PARTICIPATION IN AN INDUSTRY-SPONSORED COURSE

Morrow E, Wright AS, Figuredo E, Oelschlager BK

Objectives: We aimed to assess the rate of adoption of single incision laparoscopy among participants in industry-sponsored courses. We also aimed to learn about barriers to the adoption of single incision techniques.

Methods: We conducted 7 courses in single incision surgery from 2009 to 2012, all sponsored by one of two industry partners. Participants were General Surgeons (n=42) and Gynecologists (n=12). All courses were structured and included didactic instruction, observation of live cases, dry lab practice in a simulation center, and wet lab practice in live porcine models.
A multiple-choice survey with 12 items was constructed to assess adoption of single incision laparoscopy in this group. The survey was administered in the fall of 2013, 0-3 years from the time of course participation. Results were tabulated and descriptive statistics performed.

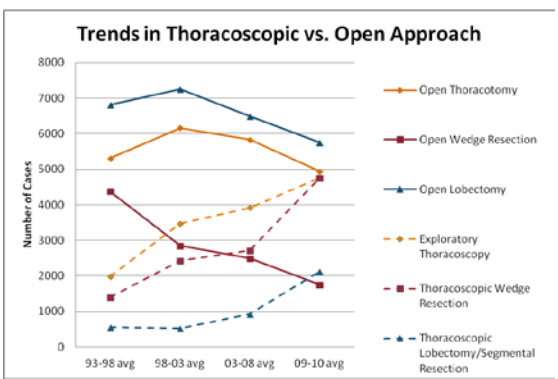
Results: Current contact information could be found for 48 participants, of whom 12 responded to the survey (25%). Prior to the course, 33% of respondents had performed at least one single incision laparoscopic procedure. Following the course, 42% of respondents performed at least one incision laparoscopic procedure. Cases that have been performed via single incision approach by respondents include appendectomy, cholecystectomy, other foregut/bariatric (excluding sleeve and band), oophorectomy, and colectomy. No respondent has performed more than 20 single incision procedures since taking the course. Only one respondent currently performs single incision laparoscopy.
Perceived barriers to single incision laparoscopy were varied; 42% said that the largest barrier was "lack of perceived benefit to patient." Other selected barriers (in order of frequency) included cost, technical challenges, and increased operative time. 67% of respondents reported that their patients are not aware of single incision laparoscopy at the time of their consultation. 84% do not discuss single incision with the patients, or do so only when asked by the patient. 17% spontaneously discuss single incision with patients. Of respondents with single incision experience, 0% felt that patient satisfaction was better after single incision laparoscopy, with 100% stating that patient satisfaction was equal between single incision and standard laparoscopy. 40% felt that complications were more common in single incision laparoscopy. Half of all respondents wrote free text responses, all of which were negative. Free text responses included comments about umbilical hernias from single incision laparoscopy, poor ergonomics, lack of traction/counter-traction, and increased cost.

Conclusions: In this group of participants in industry-sponsored single incision laparoscopy courses, adoption of single incision laparoscopy was low. The biggest barrier to use of the single incision laparoscopy was lack of perceived benefit to the patient compared to standard laparoscopy.

THE ACGME CASE LOG: GENERAL SURGERY RESIDENT THORACIC SURGERY EXPERIENCE

Kansier N, Drake F, Varghese T, Gow K

Objective: To investigate whether general surgery resident exposure to thoracic surgery has changed in quality or quantity with the advent of work hours using the National Accreditation Council for Graduate Medical Education (ACGME) case log.
Methods: The ACGME case logs for graduating general surgery residents were reviewed from academic year (AY) 1989-1990 to 2009-2010 for defined thoracic surgery cases. Data were combined into five blocks: Period I (AY1989-90 to AY1992-93), Period II (AY1993-94 to AY1997-98), Period III (AY1998-99 to AY2002-03), Period IV (AY2003-04 to AY2007-08), and Period V (AY2008-09 to AY2009-10). Periods IV and V were delineated by implementation of duty hour restrictions in 2003. Student t-test was performed to determine any difference between the time periods with significance at $p < 0.05$.
Results: A total of 19,709,505 general surgery cases were reviewed over the twenty-year period, 775,301 of which were thoracic (3.9%). When divided into time blocks, the quantity of thoracic surgery cases remained virtually unchanged, averaging 33,638 cases (3.74% of total) in Period I and 41,197 cases (4.15%) in Period V. The total number of general surgery cases also remained relatively stable over time, averaging 899,644 in Period I and 991,801 in Period V, with only a small increase in the total number of residents from 981 in 1989 to 1040 in 2010. Exploratory thoracotomy, lobectomy and wedge resection accounted for the majority of thoracic cases performed in all years, totaling 21.68%, 20.04% and 15.1% of all thoracic surgery cases, respectively. Within all three of these subcategories, however, there was a marked shift from open to thoracoscopic approaches over this time period. In Period I, virtually all exploratory procedures, lobectomies and wedge resections were performed open, while in Period V only 50.9%, 73% and 26.9% of these cases were open, respectively.



Conclusions: General surgery trainees of today have the same volume of exposure to thoracic surgery as their counterparts over the last two decades. This maintenance in caseload has occurred in spite of the onset of work-hour restrictions in 2003 and major shifts in training paradigms. However, a reflection of the evolution of the field, general surgery graduates today have a different thoracic surgery skill set at the end of

their training despite performing the same number of procedures. This is primarily due to the emergence of minimally invasive techniques. Thoracic surgery educators should take into account these differences when training future cardiothoracic surgeons.

IS APPENDICITIS A TICKING TIME BOMB?
Time to Treatment and the Risk of Perforation After Patients Reach the Hospital

Drake FT, Mottey N, and Flum DR

Background: The traditional pathophysiologic model of acute appendicitis posits a relationship between time and disease progression: luminal obstruction leads inexorably to perforation without timely intervention. Observational research has demonstrated a positive association between risk of perforation and elapsing time from symptom onset. The objective of this study was to evaluate whether there was a relationship between risk of perforation and elapsed time from presentation to surgery.

Methods: Using data from a prospective surgical registry at 52 hospitals in Washington State, we evaluated the pattern of perforation among adult patients who underwent appendectomy from January 1, 2010 to December 31, 2012. Covariates studied included patients' demographic and clinical characteristics. Wait time was measured between patients' presentation to the Emergency Department (ED), diagnostic imaging, and Operating Room (OR) start time. The relationship between in-hospital time and odds of perforation was adjusted for potential confounding using multivariate logistic regression.

Results: 9,408 adults underwent appendectomy; overall, 15.8% were perforated. Patients with perforated appendicitis were more likely to be male, older, and to have co-morbid conditions. Mean time from ED to OR was the same (8.6 hours) for perforated and non-perforated patients. In multivariate logistic regression, increasing time from ED to OR was not a significant predictor of perforation either as a continuous variable (OR 1.0 95% CI 0.99 – 0.11) or when considered as a discrete variable (patients ordered by elapsed time and divided into deciles with the first decile of patients as the reference group). In the fully adjusted multivariate model, independent predictors of perforation were male sex, increasing age, 3 or more co-morbid conditions, and lack of insurance. In this model, African Americans and Asian Americans had reduced frequency of perforation compared to whites. Patient groups who waited longer (women vs. men, younger vs. older patients, African American vs. whites) did not demonstrate increased risk of perforation.

Conclusion: Once patients have presented to the hospital there is no association between duration of ED to OR time and the risk of perforation. Lack of insurance was the only socio-economic characteristic identifiable as a risk factor for perforation. Within the typical time frame from ED presentation to OR, appendicitis does not appear likely to progress to perforation, this may be due to appropriate patient selection for urgent surgery and timely initiation of non-operative therapies such as antibiotics. These results are also consistent with the theory that perforation is most often a pre-hospital occurrence and may also reflect a more complex pathophysiologic model for appendicitis than a simple positive association between time and progression to perforation.

siRNA DELIVERY FOR GENE SILENCING IN HEPATOCELLULAR CARCINOMA

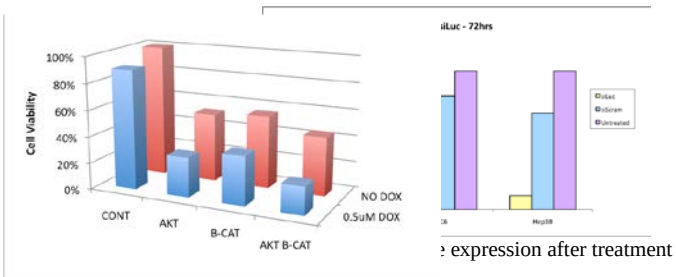
Sham J, Kievit F, Stephen Z, Wang, K, Zhang M, Park J

Background: Hepatocellular carcinoma (HCC) is the sixth most prevalent malignancy and the third leading cause of cancer related deaths worldwide. The vast majority of HCC patients present at an advanced stage when surgical therapies cannot be offered. Given the current limitations of non-surgical treatments, development of novel HCC therapies is imperative. Short interfering RNA (siRNA) provides a powerful and specific tool for gene silencing in all types of cancer, including HCC. This approach allows targeting of up-regulated pathways in the cancer and its microenvironment while limiting systemic toxicity.

Methods: For proof-of-principle experiments, RH7777 rat HCC cells stably expressing green fluorescent protein (GFP) and luciferase (Luc) were created using molecular biology techniques. GFP- and Luc-siRNA was delivered *in vitro*, and mRNA and protein knockdown were evaluated using real-time polymerase chain reaction (RT-PCR) and respective reporter assays for GFP and Luc. A syngeneic, orthotopic rat model of HCC was developed using the RH7777-Luc cell line in Buffalo rats for future *in vivo* imaging and treatment experiments, and preservation of Luc expression *in vivo* was evaluated. For preliminary siRNA treatment experiments, human HepG2 HCC cells were treated *in vitro* with siRNA directed against various pathway targets such as Akt-1 (siAKT), and beta-catenin (siCTNNB). RT-PCR, Western blot and Alamar Blue analysis was performed to evaluate mRNA, protein expression and cell viability, respectively. Combination treatments with siRNA and Doxarubicin (DOX) were also evaluated using the Alamar Blue and cell cycle arrest was examined using flow cytometry.

Results: RH7777 cells stably transfected with GFP and Luc demonstrated approximately a 400-500-fold increase in expression compared to non-transfected cells. When treated with siGFP or siLuc, expression was suppressed to 15-20%. (Figure 1 Bar graph) Tumors developed from injection of RH7777-Luc cells maintained a 400-500-fold increase in expression compared to non-transfected cells, confirming preservation of expression in an *in vivo* model of HCC. Treatment of HepG2 cells with siAKT and siCTNNB significantly decreased cell viability alone, and was able to achieve the same cytotoxic effects with a lower DOX dose. (Figure 2 Bar graph)

Conclusions: Gene-specific siRNA sequences can effectively reduce GFP and luciferase protein expression *in vitro*. RH7777-Luc cells strongly express luciferase *in vivo*, providing a



e expression after treatment

Figure 2 – *In vitro* HepG2 cell viability after treatment with siRNA and doxorubicin

reporter mRNA/protein pair for future in vivo siRNA experiments. siRNA targeted against Akt-1 and beta-catenin can independently reduce cell viability and increase sensitivity to DOX *in vitro*. The proportion of cells in G2 arrest was increased with the siRNA treatment. Further studies to evaluate the effect of *in vivo* siRNA delivery vectors are ongoing.

Next Generation Sequencing to Identify Inherited Mutations in All Breast Cancer Genes

Bernier G, Mandell J, Walsh T, Casadei S, Swisher E, King MC

Background: Breast cancer was one of the first complex diseases studied with molecular genetics. To date 18 breast cancer genes have been identified from severely affected families, most notably *BRCA* and *BRCA*. *BRCA1* and *BRCA2* account for only 10% of all breast cancers and 5% of breast cancer cases in women less than 45. Inherited mutations in *TP53, PTEN, STK11*, and *CDH1* are associated with increased risk of breast cancer as a component of their cancer syndromes. Additional mutations in the BRCA/Fanconi Anemia complex have been identified and yield a 2-fold to 4-fold increase in breast cancer risk. Most families, however, with inherited breast cancer have no known mutation identified. This is in part due to limitations of conventional genetic testing methods. We hypothesize that the remaining, as yet unidentified genes in high risk-families are due to individually rare alleles of moderate to high penetrance, many in as yet undiscovered genes. We developed a technique in our research lab using next generation sequencing and targeted genome capture to test individuals for all known breast cancer genes in one test, named BROCA. Using BROCA we can screen for single nucleotide polymorphisms (SNPs), insertions/deletions (indels) and large copy number variants (CNVs) in multiple targeted gene areas.

Objective: To determine the prevalence of deleterious mutations in known breast cancer genes in three cohorts of breast cancer patients while simultaneously evaluating for an association with candidate breast cancer genes.

Methods: We enrolled three cohorts of breast cancer patients: one cohort of average risk participants enrolled locally at Seattle Cancer Care Alliance and Providence Regional Cancer Partnership; two high risk cohorts enrolled nationally comprised of women with breast cancer diagnosed at age 40 or younger OR women with hormone receptor negative breast cancer diagnosed at any age. Extracted genomic DNA from participants was hybridized to RNA oligonucleotides of the targeted gene regions creating paired end libraries. Targeted known breast cancer genes include *BRCA1*, *BRCA2*, *ATM*, *BARD1*, *BRIP1*, *CDH1*, *CHEK2*, *FAM175A*, *MRE11A*, *NBN*, *PALB2*, *PTEN*, *RAD50*, *RAD51C*, *RAD51D*, *STK11*, *TP53* and *XRCC2*. We also targeted 14 candidate breast cancer genes selected based on their interaction with either known breast cancer genes or the BRCA/Fanconi Anemia pathway for DNA damage repair. The hybridized DNA libraries underwent massively parallel sequencing on an Illumina Hi Seq. We filtered DNA variants with high quality reads to identify rare nonsense, frameshift, and splice site mutations. For each high quality variant we confirmed with Sanger Sequencing and tested for co-inheritance of the variant in other affected members of the same family.

Results & Next Steps: To date we have enrolled over 200 participants in the average risk cohort and over 300 participants in each of the high-risk cohorts. Approximately half of the enrolled participants have been analyzed via BROCA and, thus far, for the three series combined we have identified all classes of mutations (i.e. SNPs, indels and CNVs) in multiple genes. Some mutations clearly lead to loss of function of the product. Others require additional characterization either of the splice patterns or the protein structure and function to determine potential effect on the product. We have identified clearly deleterious mutations in known breast cancer genes, such as *BRCA1/2*, *CHEK2* and *TP53*, and as well as mutations in some of the candidate breast cancer genes. We will continue to analyze samples from three cohorts and evaluate the data as it becomes available both for prevalence of mutations in known breast cancer genes and new associations with candidate breast cancer genes. For all cohorts, we will identify any patient characteristics that are associated with deleterious mutations in a particular gene. From this information we will be able to make recommendations on the patients that will benefit the most from this sequencing technique clinically. We hypothesize that the patients that will benefit from next generation sequencing techniques will be at least those that are currently recommended to undergo commercial *BRCA1* and *BRCA2* testing. Identification of non-*BRCA1*/2 mutations in these individuals will allow these patients to benefit from more aggressive detection and prevention strategies. It will allow clinicians to identify additional women at risk, allow closer surveillance, create new prevention strategies and further develop the role of genetic counseling in inherited cancer treatment.

VESICOURETERAL REFLUX AND FEBRILE URINARY TRACT INFECTIONS IN ANORECTAL MALFORMATIONS: A RETROSPECTIVE REVIEW

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Purpose: Multiple studies document a correlation between anorectal malformations (ARM) and vesicoureteral reflux (VUR), VUR and urinary tract infections (UTI), and UTI's and renal disease. We aimed to determine which characteristics in ARM patients are associated with VUR and/or UTI diagnoses to better define who would benefit from voiding cystourethrogram (VCUG) testing and/or UTI prophylactic antibiosis.

Methods: A retrospective review of ARM patients at a free-standing children's hospital from January, 1996 to December, 2011 was performed. Main patient variables included ARM classification and presence and type of co-morbid diagnoses. Simple and multivariable logistic regression was used to investigate the associations between VUR and UTI and the collected variables. Statistical significance was set at p=0.05.

Results: 190 patients were included in this study. Of them, 133 (70%) had a VCUG performed during their index admission and 41 (31%) received a diagnosis of VUR. 31 of the 190 patients had at least one febrile UTI (16%). Of these, only 14 (45%) had a diagnosis of VUR. On simple logistic regression, spina bifida (p=0.044), ectopic kidney (p=0.004), and genitourinary (GU) malformations (p=0.002) were associated with having VUR. On multivariable regression, only ectopic kidney remained associated with VUR (p=0.026). VUR (p=0.001) and a concurrent GU malformation (p=0.004) were the only variables associated with a UTI diagnosis on simple logistic regression. Controlling for VUR, the presence of GU malformations (p=0.073) remained the closest variable associated with developing a UTI.

Conclusion: In ARM patients, VUR is associated with the presence of GU and other caudal abnormalities. UTI in these patients is both related to VUR and the presence of GU malformations. Thus, VCUG testing on ARM patients should be pursued when there are other caudal and GU abnormalities, regardless of fistula location. Antibiotic prophylaxis for UTI should be considered in children with ARM and any GU malformation, not only VUR.

Unable to present