

UW Medicine

DEPARTMENT OF SURGERY

Presents

**The Seventeenth Annual
Resident & Fellow
Research Symposium**

Friday, February 18, 2011
7:30 a.m. – 1:00 p.m.

Museum of History and Industry
McEachern Auditorium
2700 24th Avenue East
Seattle, Washington 98112

SCHEDULE OF PRESENTATIONS

Moderators: Julie Freischlag, M.D. and Alexander Clowes, M.D.

7:30am	Introduction	Alexander Clowes, M.D.
7:45am	Shalhub	Blunt Abdominal Aortic Injury: A Single Institution's Experience
8:00am	Davidson	Long Term Survival of Adult Trauma Patients
8:15am	Jayaraj	A Comparison of the Villalta-Prandoni and Venous Clinical Severity Scoring Instruments in the Assessment of Post Thrombotic Syndrome
8:30am	Wassenaar	Pepsin Detection in Patients with Laryngopharyngeal Reflux Before and After Fundoplication
8:45am	Petersen	Comprehensive Evaluation of an Endoscopic Fundoplication Using the EsophyX™ Device
9:00am	Swanson	Trajectories to Death: An Analysis of Adult Non-Survivors in the National Burn Repository
9:15am	Brown	Propranolol Dosing Practices in Burn Patients: Implications for Safety & Efficacy
9:30am	Chung	Role of Mitogen Kinase Phosphatase-1 (MKP-1) in the Mechanism of Adrenergic Influence of Innate Immunity
9:45am	Kohler	TNF-Alpha and IL1-Beta Production are Differentially Modulated by Zinc Deficiency in Murine Macrophages
10:00am	Smith	Unsaturated Fatty Acids Affect Mesenchymal Stem Cell Secretion of Angiogenic and Inflammatory Mediators
10:15am	BREAK	
10:30am	Moreno	Preventing Vein Graft Stenosis in Peripheral Vascular Surgery
10:45am	Gao	Syndecan-1 is Induced by Arterial Injury and is Regulated by a NF-KB Dependent Pathway in Smooth Muscle Cells
11:00am	De Wispelaere	Sphingosine 1-Phosphate Induced Expression of Smooth Muscle Alpha Actin Requires Myocardin Related Transcription Factor-B and Calmodulin Kinase II γ
11:15am	Lao, V	Methylated TIMP3 is a Potential Predictive Marker for EGFR Inhibitor Therapy in Colorectal Cancers
11:30am	Massarweh	Comparative Assessment of the Safety and Effectiveness of Radio Frequency Ablation Among Elderly Medicare Beneficiaries with Hepatocellular Carcinoma
11:45pm	Kwon, S	The Use, Safety, and Cost of Bariatric Surgery Before and After Medicare's National Coverage Decision
12:00pm	Mulloy	Intraoperative Cryoablation During LVAD Implantation Reduces the Incidence of Postoperative Ventricular Tachyarrhythmia
12:15pm	LaRiviere	Closing the Loop: Factors Associated with Children Discharged and Re-Presenting with Appendicitis
12:30pm	Liu	Patterns of Antibiotic Use and Surgical Site Infection in Autologous Breast Reconstruction
12:45pm	Chang	Immunological Tolerance to Composite Tissue Allotransplantation in a DLA-Identical Canine Model
4:00pm	HELEN & JOHN SCHILLING LECTURE – Hogness Auditorium, UW HSB <i>Clinical & Personal Comparative Effectiveness</i> Julie A. Freischlag, M.D. The William Stewart Halsted Professor Chair, Department of Surgery Surgeon-in-Chief Johns Hopkins Hospital; Baltimore, Maryland	

BLUNT ABDOMINAL AORTIC INJURY: A SINGLE INSTITUTION'S EXPERIENCE

Shalhub S, Tran N, Lundgren R, Quade S, Gunn M, Starnes B

Background: Blunt Abdominal Aortic Injury (BAAI) is rare. It is often seen in high speed motor vehicle collisions, and associated with major blunt intra-abdominal injury and thoraco-lumbar fractures. This is a review of our institutional experience over the last fifteen years.

Methods: This is a retrospective review of patients who presented with blunt traumatic injury to the abdominal aorta between 1996 and 2010. Data collected included demographics, mechanism of injury, associated injuries, ISS, type of intervention, procedural complications, and subsequent CT imaging.

Results: Over the study period, 28 cases of BAAI were identified (68% male, median age 28.5 years old, range 6-61). The leading cause of injury was motor vehicle collisions (57%). The median ISS was 45 (range 16-86), and 39% were hypotensive upon presentation. Associated small bowel and colon injury were seen in 39% of the cases and 50% had associated spine injuries. Aortic injury presented as free rupture (29%), pseudoaneurysm (PSA) (11%), large intimal flap (LIF) (39%), or intimal tears (21%). The most common location was infra-renal and management was dependent on hemodynamics and type of injury: non-operative (32%), open aortic repair (36%), endovascular repair (21%), and multimodality (10%). Overall mortality was 32%, with the majority occurring during the initial operative exploration. Free aortic rupture mortality was 100%. Intimal tears resolved or remained stable. Patients who underwent repair of their aortic injury had no vascular complications except for one patient who underwent a hybrid repair and died from SMA thrombosis and bowel ischemia.

Conclusions: This is the largest series of Blunt Abdominal Aortic Injury described in the literature at a single institution. BAAIs range from intimal tears to free rupture with outcomes and management modalities correlating with type of injury. Non operative management is successful in the cases of intimal tears with minimal thrombus formation. Free rupture remains a devastating injury with 100% mortality. For all other categories of aortic injury, successful repair correlates with a favorable prognosis.

LONG TERM SURVIVAL OF ADULT TRAUMA PATIENTS

Davidson G, Hamlat C, Rivara F, Koepsell T, Jurkovich G, Arbabi S

Context: Inpatient case fatality rates for trauma may not be comprehensive for measuring survival after trauma care. To date there have been few large studies evaluating long term mortality in trauma patients and identifying predictors that increase risk for death following discharge.

Objective: To determine the long-term mortality of patients following trauma admission and to evaluate survivorship in relation to discharge disposition.

Methods: Cohort study of 128,024 injured adult patients during 1995–2008 using the Washington State Trauma Registry linked to Death Certificate data. Kaplan-Meier and Cox proportional hazards models were used to evaluate long term mortality following admission for trauma.

Results: Of the 128,024 trauma patients, 7,479 died before discharge and 21,364 died following discharge. Cumulative mortality at three years post injury was 16% compared to the expected population cumulative mortality of 5.9%. In-hospital mortality improved during the 14 year study period whereas long-term mortality increased. After adjustments for confounders, patients who were older and those who were discharged to a skilled nursing facility had the highest risk of death. The hazard ratio for death after discharge to a skilled nursing facility compared to that after discharge home was 1.50 (0.76-2.93) for ages 18-30, 1.92 (1.36-2.73) for ages 30-45, 2.14 (1.48-3.09) for ages 46-55, 2.07 (1.52-2.82) for ages 56-65, 1.54 (1.19-2.01) for ages 66-75, 1.55 (1.28- 1.87) for ages 76-80, and 1.36 (1.07-1.71) for those over 80. Other significant predictors of mortality after discharge included maximum head Abbreviated Injury Scale Score, Injury Severity Score, Functional Independence Measure, mechanism of injury, length of stay and insurance status.

Conclusion: While there has been a decline in in-hospital death, long-term mortality continues to be high in trauma patients. Discharge to a skilled nursing facility at any age following trauma admission signaled high risk of subsequent mortality.

A COMPARISON OF THE VILLALTA-PRANDONI AND VENOUS CLINICAL SEVERITY SCORING INSTRUMENTS IN THE ASSESSMENT OF POST THROMBOTIC SYNDROME

Jayaraj A, Meissner M

Background: Post-thrombotic syndrome is a common chronic complication of acute deep venous thrombosis (DVT), with as many as two-thirds of patients developing symptoms of pain, edema, hyperpigmentation, or ulceration. There exist multiple instruments to assess post thrombotic syndrome including the commonly used scoring systems put forth by Villalta et al and the American Venous Forum's Venous Clinical Severity Score (VCSS). At present, studies comparing the two in their ability to identify and grade the severity of post thrombotic syndrome (PTS) do not exist. This is important to enable comparison of studies that have used different instruments to assess PTS. The purpose of this study is to compare the two instruments as part of a larger randomized controlled study that assessed the impact of graduated compressive stockings in the prevention of post thrombotic syndrome.

Methods: 138 extremities in 69 consecutive patients with an acute deep venous thrombosis documented by duplex ultrasonography were randomized to treatment with graduated compressive stockings (GCS) that provided compression of 30-40mm Hg or no stockings to assess impact of GCS on the prevention of PTS. As part of this study, these patients were sequentially followed at months 1, 3, 6, 12, 18 and 24 following diagnosis of DVT. Post-thrombotic syndrome scores as defined by Villalta et al (PTSV) and the Venous Clinical Severity Score (VCSS) were assessed at these follow up visits. The PTSV was scored as Absent (Score <3 or =3 without objective criteria), Mild to Moderate (score ≥ 3 with one objective criteria) or Severe (score ≥ 4) while the VCSS score was assessed as Absent (score ≤ 3), Mild to Moderate (score 4-7) and Severe (score ≥ 8) based on performance characteristics of the venous clinical severity score. Each extremity was considered separately for analysis. The two instruments were compared using Pearson's Chi square analysis at the various time points mentioned above. Additionally correlational statistics including Spearman correlation and Gamma statistic were computed.

Results: A significant difference was not detected in the ability of PTSV and VCSS instruments to detect mild to moderate disease. (Spearman correlation: 0.41 to 0.73, Gamma statistic: 0.71 to 0.98, $p < 0.05$). For severe disease, the Chi square test suggests a difference in the ability of the two instruments to detect disease although there exists good correlation (Spearman correlation: 0.20 to 0.59, Gamma statistic: 0.71 to 1.0, $p < 0.05$) between the two instruments.

Conclusion: Both PTSV and the VCSS scoring systems are important tools in the identification and follow up of post thrombotic syndrome. There exists agreement between the two instruments for detecting both mild to moderate and severe disease.

PEPSIN DETECTION IN PATIENTS WITH LARYNGOPHARYNGEAL REFLUX BEFORE AND AFTER FUNDOPLICATION

Wassenaar E, Johnston N, Merati A, Montenovolo M, Petersen R,
Tatum R, Pellegrini C, Oelschlager B

Background: Laryngopharyngeal reflux (LPR) is an extreme manifestation of gastroesophageal reflux disease that can lead to substantial airway damage. No single diagnostic test can accurately determine its presence. We hypothesized that the presence of pepsin (which originates in the stomach) in the epithelium of the larynx and potentially in sputum may provide the diagnostic accuracy that is needed to guide therapy.

Methods: Ten patients with clinical LPR, undergoing fundoplication were enrolled in this pilot study. Pre-operative laryngoscopy, laryngeal epithelial biopsy and/or sputum analysis, 24-hr pH monitoring, and a standardized questionnaire about symptoms were completed. The same testing was performed 6 months post-fundoplication. Pepsin content was measured by Western blot analysis.

Results: The primary presenting LPR symptom was hoarseness in 7, cough in 2 and globus in 1 patient. Pepsin was detected in 8 of the 10 patients pre-operatively. There was correlation between biopsy and sputum (+/+ or -/-) in 4 of 5 patients who had both analyzed pre-operatively. Nine patients were available for follow-up. Post-operative pH monitoring improved in all patients and normalized in 5 of 8 patients studied. Eight of 9 patients had improvement of their primary LPR symptom (6 good and 2 mild). Only one patient (who had negative pre-operative pepsin) reported no response to treatment of her primary LPR symptom. Post-operatively pepsin was detected in only 1 patient, though it was substantially decreased compared with the pre-operative value.

Conclusion: Our study shows that pepsin is found consistently in the laryngeal epithelial biopsy and sputum of patients with pH-proven GERD and symptoms of LPR. In such patients, a fundoplication results in the clearance of pepsin from the upper airway and corresponds to clinical improvement. Detection of pepsin may have value in the diagnostic armamentarium of LPR. A larger clinical trial is needed to further delineate its predictive value.

COMPREHENSIVE EVALUATION OF AN ENDOSCOPIC FUNDOPLICATION USING THE ESOPHYX™ DEVICE

Petersen R, Fillipa L, Wassenaar E, Martin A, Tatum R, Oelschlager B

Introduction: There are limited studies that evaluate the efficacy of an endoscopic fundoplication (EF) for GERD with EsophyX™ device, especially with the most recent procedural iteration (TIF-2). This study is a prospective evaluation of our early experience with this device and procedure.

Methods: Data were collected prospectively on 23 consecutive patients undergoing EF between March 2009 and August 2010. All patients completed a symptom questionnaire assessing frequency and severity of GI & respiratory symptoms, 24 hr pH and manometry studies preoperatively and were encouraged to repeat these at 6 months.

Results: All patients had an abnormal pH study and were on PPI therapy prior to EF. The mean age was 47 ± 11 and 6 (27%) were male. Nine (41%) patients had a BMI > 30, and three had a small hiatal hernia (≤ 2 cm) detected on endoscopy. The procedure was aborted in 2 patients for retained food, though subsequently successful in one. EF was incomplete in 2 patients due to difficulty with maintenance of insufflation. The median LOS was 1 day and there were no major perioperative complications. To date, 6 month follow-up is complete in 9 patients (Table 1). One patient, who had an incomplete EF, has undergone a subsequent Nissen fundoplication secondary to persistent GERD.

Conclusion: EF with EsophyX™ is associated with a significant reduction in heartburn and abnormal acid exposure at 6 months, although many patients ultimately resume PPI therapy. The procedure has an acceptable safety profile, but further study is necessary to determine its place in the treatment of GERD.

6 Month Outcome (n=9)	Preop	Postop: 6mos	p value
Heartburn*	7.2 ± 3.2	4.3 ± 3.0	0.01
Regurgitation*	5.1 ± 3.6	3.3 ± 3.7	0.18
PPI use	9 (100%)	7 (78%)	0.47
LESP (mmHg)	28 ± 30	26 ± 25	0.90
Total % contact time pH<4	10.9 ± 4.6	4.2 ± 1.5	0.01

*Symptom Severity Scale: 0 (never) to 10 (worst)

TRAJECTORIES TO DEATH: AN ANALYSIS OF ADULT NON-SURVIVORS IN THE NATIONAL BURN REPOSITORY

Swanson J, Klein M, Gibran N, Kramer C, Young S, Pham T

Introduction: Survival following burn injury has reached unprecedented levels in young adults, yet mortality remains a significant problem for older adults. In contrast to trauma, where a trimodal distribution of deaths has been observed, the temporal distribution of deaths has not yet been well characterized following burns. In this study, we analyzed adult deaths in the National Burn Repository (NBR) by age group, and specifically focused on different time trajectories to death in younger and older adults.

Methods: We reviewed the NBR records of all patients aged 18 and older who did not survive hospitalization from 1995 to 2007. In order to better understand age effects on time to death, patients older than 55 years were compared to 18-54 year olds. We analyzed patient and injury characteristics, comorbidities and complications associated with death. We also compared patients who died early (≤ 72 hours) with those who died later in their hospitalization.

Results: During the study period, 5,975 patients died during acute hospitalization (table 1). Early death was associated with higher mean percentage total body surface area (TBSA) burns in both age groups ($p < 0.001$). Inhalation injury was strongly associated with late deaths among younger adults, and early deaths among older adults ($p < 0.001$). A histogram of deaths indicates a consistent asymptotic distribution as a function of time post-burn in both age groups (figure 1).

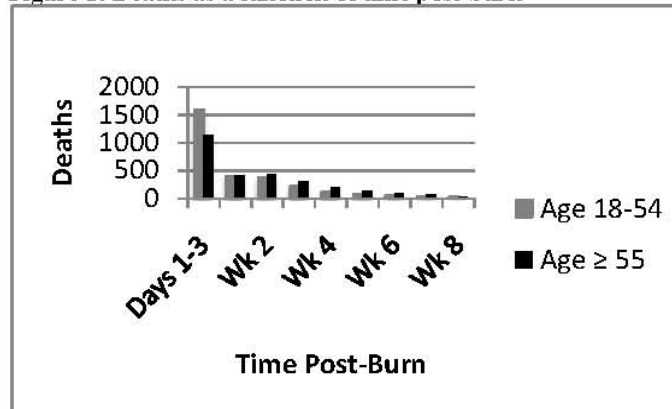
Conclusions: Death predominantly occurred within 72 hours of injury in both younger and older adults. However, early deaths may be due not only to failure of resuscitation but early withdrawal of care, especially in the older adults. There was no discrete mortality peak following 72 hours in either age group, and the death distribution curve does not correlate to that described for non-burn trauma deaths. Our findings suggest that to improve overall burn survival, emphasis should be directed to the resuscitation phase, where mortality burden is concentrated.

Table 1: Characteristics of Death by Age and Timing

	Early Death (<72 hours)	Late Death (>72 hours)	p-value
18-54 years old (n=2,984)			
N (%)	1593 (53%)	1391 (47%)	
Percent male	78	73	
Avg TBSA (SD)	68 (28)	50 (24)	<0.001
Inhalation injury, n (%)	382 (24%)	598 (43%)	<0.001
55+ years old (n=2,997)			
N (%)	1136 (38%)	1861 (62%)	
Percent male	56	60	
Avg TBSA (SD)	52 (27)	27 (19)	<0.001
Inhalation injury, n (%)	648 (57%)	689 (37%)	<0.001

TBSA = total body surface area
SD = standard deviation

Figure 1: Deaths as a function of time post-burn



PROPRANOLOL DOSING PRACTICES IN BURN PATIENTS: IMPLICATIONS FOR SAFETY & EFFICACY

Brown D, Gibbons J, Honari S, Klein M, Pham T, Gibran N

Introduction: Propranolol is commonly used for the treatment of hypermetabolism associated with burn injury. Studies in children have demonstrated a reduction in muscle protein wasting with propranolol administration; results and dosing in adults are less well established. Experience at our institution suggests that modest dosing of propranolol results in frequent episodes of hypotension or bradycardia.

Methods: Medical records were reviewed for burn-injured patients receiving propranolol during 2008-2010. Patients were included if they were between 18 and 65 years-old and had at least 20% total body surface area (TBSA) burn.

Results: Sixty-five patients met the criteria with a mean age of 39 years, mean burn size of 38% TBSA, and mean length of hospital stay of 46 days. Patients received propranolol dosages ranging from 0.08 to 3.8 mg/kg/day, with a mean maximum dosage of 0.6 mg/kg/day. Propranolol was administered on an average of 64% of hospital days. Heart rate decreased by a mean of 19% after 1 week and 24% after 3 weeks, while systolic blood pressures decreased by a mean of 7% and 8%, respectively. Fifty-eight percent of patients experienced at least one episode of hypotension (defined as systolic blood pressure < 90 mm Hg) and 12% experienced bradycardia (heart rate < 60 bpm) following propranolol administration. Doses of propranolol were most frequently held for low blood pressure; in 45% of patients at least one dose was held for hypotension, with a mean lowest systolic blood pressure of 87 mm Hg. The risk of hypotension requiring at least one dose to be held was 4.1% per day of propranolol therapy.

Conclusions: In this retrospective analysis, we observed many instances of hypotension attributable to propranolol administration. Our data suggest that adults do not tolerate the high doses reported in pediatric patients. Despite the potential beneficial anabolic effects of propranolol, burn care providers and researchers must recognize the potential iatrogenic hemodynamic effects of this intervention. Our data support the need for future prospective multi-center studies to delineate the safety and efficacy of propranolol in adult burn-injured patients.

ROLE OF MITOGEN KINASE PHOSPHATASE-1 (MKP-1) IN THE MECHANISM OF ADRENERGIC INFLUENCE OF INNATE IMMUNITY

Chung C, Pham T, Hassan M, Knoll M, O'Keefe G

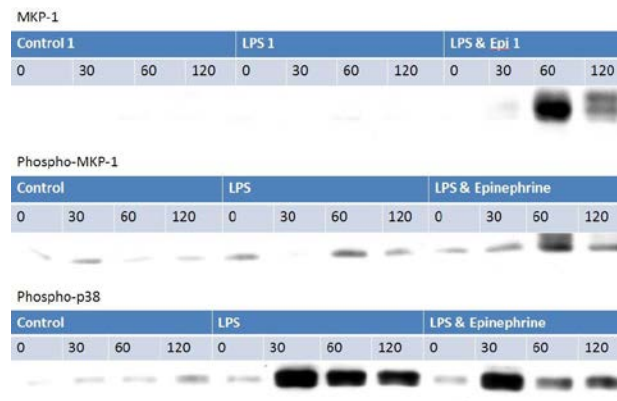
Background: Activation through the Mitogen-Activated Protein Kinase (MAPK) pathway is a key component of inflammatory signaling in monocytes and macrophages. It has been shown that beta-adrenergic receptor stimulation suppresses inflammatory (eg. TNF-alpha) signaling, but the mechanism is uncertain. The intracellular inhibitor, MAPK Phosphatase-1 (MKP-1) deactivates MAPK proteins and is one mechanism of feedback inhibition of inflammatory signaling and cytokine production.

Purpose: We sought to determine whether MKP-1 might be involved in epinephrine-induced suppression of inflammatory signaling. We tested the hypothesis that epinephrine stimulation will result in early MKP-1 phosphorylation and MAPK (JNK and p38) protein dephosphorylation after LPS exposure in human monocytes.

Methods: Vitamin D₃ treated THP-1 cells were stimulated by lipopolysaccharide (LPS), epinephrine, combined LPS and epinephrine, or vehicle followed by cell harvest and protein extraction procedures after 0, 30, 60 or 90 minutes. Protein expression for MKP-1, phosphorylated MKP-1, and the phosphorylated MAPK proteins (p-JNK, and p-p38) were measured by western blot analysis.

Results: A sample western blot of our experiments is shown below. When compared to LPS stimulation alone, combined LPS and epinephrine stimulation resulted in (1) increased total MKP-1 protein peaking at 60 minutes, (2) increased MKP-1 phosphorylation beginning at 30 minutes and peaking at 60 minutes, and (3) accelerated reduction of phosphorylated MAPK proteins at 60 and 120 minutes. It appears that the increase in pMKP-1 precedes the accelerated reduction in phosphorylated MAPK proteins.

Conclusions: Combined epinephrine and LPS stimulation induces both total MKP-1 and pMKP-1 and accelerates dephosphorylation of p-p38 and pJNK in comparison to LPS exposure alone. These findings suggest that phosphorylation of existing MKP-1 following beta-adrenergic receptor stimulation is a key mediator in the early suppression of inflammatory signaling. Increased total MKP-1 protein may be involved in subsequent and ongoing feedback inhibition of MAPK activation.



TNF-ALPHA AND IL1-BETA PRODUCTION ARE DIFFERENTIALLY MODULATED BY ZINC DEFICIENCY IN MURINE MACROPHAGES

Kohler J, Blass A, Williams M, Lederer J, Kelly E, Soybel D

Introduction: Circulating levels of Zn^{2+} are acutely depleted during systemic surgical stress and infection. Zinc deficiency is associated with impaired resistance to localized infection and increased mortality during sepsis, while repletion of Zn^{2+} improves immune functions and improves survival. The physiology of zinc in the response to critical illness is poorly understood. However, a critical component of the response to systemic stress is the release of inflammatory modulators from macrophages. In this study, we utilized an *in vitro* model of murine macrophage function to determine whether expression and secretion of key effector cytokines (TNF-alpha, IL1-beta) is changed in response to Zn^{2+} depletion.

Methods: Activated primary macrophages were harvested from mouse peritoneal exudates 4 days following instillation of thioglycollate. Assays included ELISAs of media to measure secretion and RT-qPCR to monitor generation of mRNAs for TNF-alpha and IL1-beta. Cell groups (n=8 each) were exposed to standard culture media under baseline conditions and then maximally stimulated with 4 hr exposures to purified lipopolysaccharide (LPS, 100ng/ml). Cell groups were also exposed to TPEN (~2 μ M) or DTPA (~2 μ M) which bind and chelate intracellular and extracellular Zn^{2+} , respectively. Results are expressed as means \pm SE, *p<0.05 compared to baseline and +p<0.05 compared to LPS alone.

Results: At baseline, primary macrophages secrete significant levels of TNF-alpha and IL1-beta, which are only modestly enhanced by exposure to pathologically relevant concentrations of LPS. Depletion of intracellular Zn^{2+} with TPEN significantly decreases baseline and LPS-stimulated secretions of TNF-alpha, and markedly increases secretions of IL1-beta. Release of IL1-beta, but not TNF-alpha, was amplified in response to chelation of extracellular Zn^{2+} with DTPA. In both primary macrophages and RAW cells, exposure to TPEN had minimal to moderate effects on transcription of mRNA for TNFalpha and IL1-beta, confirming that observed effects are not due to *de novo* transcription.

Conclusions: In freshly harvested mouse macrophages, decreases in intracellular free Zn^{2+} suppress secretion of TNF-alpha while up-regulating that of IL1-beta. These responses to changes in extracellular Zn^{2+} levels are not associated with marked alterations in levels of their encoding mRNAs. Secretion of TNFalpha is dependent on adequate intracellular levels of Zn^{2+} . The sensitivity of IL1-beta to depletion of intra- and extracellular Zn^{2+} offers the novel perspective that availability of circulating Zn^{2+} may acutely regulate the cytokine profile released in response to sepsis or systemic surgical stress.

		baseline	DTPA	TPEN	LPS	LPS/DTPA	LPS/TPEN
TNFα	mRNA fold-incr	1.0 \pm 0.0	1.1 \pm 0.3	4.4 \pm 2.7	2.1 \pm 0.7	2.2 \pm 0.9	4.2 \pm 2.7
	Secretion pg/ml	330 \pm 35	326 \pm 29	258 \pm 27*	395 \pm 29	383 \pm 28	272 \pm 28+
IL1B	mRNA fold-incr	1.0 \pm 0.0	1.1 \pm 0.1	0.8 \pm 0.2	1.8 \pm 0.4	2.3 \pm 0.7	0.8 \pm 0.2
	Secretion pg/ml	54 \pm 7	184 \pm 48*	374 \pm 57*	73 \pm 13	127 \pm 24*+	378 \pm 59*+

UNSATURATED FATTY ACIDS AFFECT MESENCHYMAL STEM CELL SECRETION OF ANGIOGENIC AND INFLAMMATORY MEDIATORS

Smith A, Muffley L, Bell A, Numhom S, Hocking A

Background: The promise of mesenchymal stem cell (MSC) therapy to treat diabetes mellitus and diabetic complications has generated significant scientific and clinical interest. Ongoing clinical trials in progress are primarily focused on the ability of these multipotent mesenchymal stromal cells to ameliorate tissue damage by secreting cytokines and growth factors that promote angiogenesis while reducing inflammation and fibrosis. However, critical to the development of MSC-based therapies for patients with type 2 diabetes is an understanding of how their metabolic environment, which consists of high levels of glucose and fatty acids, impacts MSC biology. To date, most studies have investigated the effect of hyperglycemia on MSC; in contrast little is known about the impact of elevated plasma fatty acid levels. It remains to be determined whether chronic exposure to elevated levels of fatty acids affects MSC ability to release soluble factors that regulate cellular responses to injury.

Purpose: To determine whether unsaturated fatty acids alter MSC secretion of angiogenic and inflammatory mediators

Methods: Primary human MSC were exposed to elevated levels of either omega-6 polyunsaturated fatty acids (linoleic acid and arachidonic acid) or monounsaturated fatty acids (oleic acid) for seven days in the presence of either normal (5.38 mM) or high (20 mM) glucose. Control MSC were cultured without the addition of unsaturated fatty acids. Outcomes measured included MSC proliferation, differentiation, gene expression and protein secretion. MSC proliferation was measured by both counting viable cells and quantifying BrdU incorporation during DNA synthesis. MSC differentiation potential was determined using *in vitro* differentiation assays for bone and fat. MSC gene expression was assessed using relative quantitative real time PCR and protein secretion was assessed using ELISAs.

Results: Exposure to unsaturated fatty acids inhibited human MSC proliferation but had no significant effect on MSC differentiation into bone or fat. MSC expression and secretion of growth factors and cytokines was also altered after exposure to unsaturated fatty acids. Linoleic acid up-regulated MSC expression and secretion of VEGF, IL-1 β , IL-6 and IL-8; arachidonic acid elicited similar effects. In contrast, oleic acid had no significant effect on VEGF and IL-8 mRNA and secreted protein levels.

Conclusion: Collectively, these data suggest that perturbations in the metabolic environment may influence MSC regulation of cellular responses to injury. Furthermore, these data support the need for *in vitro* and *in vivo* studies to define the functional consequences of an altered MSC secretome. This work is directly relevant for the development of MSC therapy targeting the pancreas and diabetic complications including cardiovascular disease, nephropathy and chronic non-healing wounds.

PREVENTING VEIN GRAFT STENOSIS IN PERIPHERAL VASCULAR SURGERY

Moreno K, Yagi M, Murray-Wijelath J, Sobel M

Background: Infrainguinal autogenous vein grafts are especially prone to narrowing and failure with both inflammatory and thrombotic pathways implicated. Platelets and monocytes are key thrombo-inflammatory cells that arrive first at sites of vascular injury. These cells have potent interactions that recruit and activate one another, propagating thrombotic and inflammatory responses within the vessel wall. Excessive platelet-monocyte interactions are associated with myocardial infarction, unstable coronary syndrome, and cerebral vascular accidents. We hypothesized that elevated levels of platelet-monocyte aggregates may be associated with graft stenosis and failure. We also wished to identify markers that predicted a patient's susceptibility to developing vein graft stenosis.

Methods: We first conducted a retrospective study designed to compare patients with established stenoses of their autogenous leg bypass grafts to those with widely patent grafts. Twenty-nine patients were studied in a stable period after surgery. Levels of platelet-monocyte aggregates (PMA) in whole blood was quantified by flow cytometry. Three measures of aggregates were developed: Basal, or in vivo aggregates were estimated by immediate analysis; Spontaneous PMA were measured after ex-vivo incubation. Finally, responsiveness to thrombin stimulation of the blood was quantified by the in vitro dose response to an exogenous thrombin receptor activating peptide (sflrn). Encouraged by data from the retrospective study, we proceeded to conduct a longitudinal prospective study of patients undergoing autogenous vein infrainguinal bypass grafts. Blood samples are obtained pre- and postoperatively at day 1, 3-5, 4-6 weeks, 3 months, and 6 months. PMA are measured as above, and other soluble markers of inflammation and thrombosis are measured. To date, 38 vascular surgery patients have been studied. Primary clinical endpoints are hemodynamic stenosis >50% within the graft, or any graft revision to maintain patency.

Results: In the retrospective study, 18 patients had hemodynamically significant graft stenoses, and 11 were free of stenosis. Baseline PMA levels were no different in patients with vein graft stenosis vs. non-stenosis (16.3% + 2.8 versus 14.5% + 2.7 respectively, mean + sem). However, patients with stenosis showed higher Spontaneous Generation of PMA (58.5% +/- 4.5 vs. 42 % +/- 3.7, p<.05), and higher responses to thrombin agonist (p<.001, ANOVA). Covariables of smoking, diabetes, statin, or antithrombotic therapy could not account for these differences. In the prospective study, 12 patients have developed a stenosis, and twenty-six are free from stenosis. The results from this prospective study so far confirm the trends of the retrospective study: baseline PMA levels are similar, and while spontaneous generation of PMA is higher in those developing stenosis.

Conclusions: Platelet-monocyte reactivity may play a role in the development of vein graft stenosis. Those with/without stenosis differed primarily in their threshold, or predilection to form aggregates, while their basal circulating levels of PMA were similar. Provocative measurements may unmask pathologic differences in thrombo-inflammatory responsiveness that are not apparent in basal measurements. Understanding the causes and mechanisms leading to abnormal platelet-monocyte responses may improve approaches to predicting or preventing vein graft stenosis.

SYNDECAN-1 IS INDUCED BY ARTERIAL INJURY AND IS REGULATED BY A NF-KB DEPENDENT PATHWAY IN SMOOTH MUSCLE CELLS

Gao L, Fukai N, Kinsella M, Chen L, Kenagy R, Daum G, Clowes A

Background: Arterial injury induces smooth muscle cell (SMC) proliferation, migration, and intimal accumulation of cells and extracellular matrix, which contribute to stenosis following reconstruction. These processes can be suppressed by the administration of the glycosaminoglycans heparin and heparan sulfate. We have previously shown that an endogenous heparan sulfate proteoglycan, syndecan-1, inhibits SMC proliferation and migration and intimal formation after murine arterial injury.

Purpose: To determine whether expression of syndecan-1 is regulated after arterial injury in vivo and molecular mechanisms of syndecan-1 regulation in SMCs in vitro.

Methods: Total common carotid artery ligation was performed in male C57Bl/6 mice. After 0, 2, 4, 7, and 14 days, arteries were removed for RNA isolation. Aortic SMCs were growth arrested in serum-free medium and then stimulated with various growth factors for up to 8 hours before extraction of RNA and analysis of syndecan-1 expression by quantitative RT-PCR. A panel of inhibitors was used to determine signaling pathways involved in the regulation of syndecan-1 expression.

Results: After arterial injury, syndecan-1 expression was first induced at day 7 (5.03 ± 1.1 fold of control; mean \pm SEM; $p < 0.01$, $n=5$) and remained high at day 14 (2.59 ± 0.57 ; $p < 0.05$, $n=3$). In addition, both medial and intimal SMC proliferation peak at day 7 and fall dramatically thereafter. Levels of syndecan-1 mRNA in SMCs were increased by 10% serum (3.79 ± 0.45 fold of control; $p < 0.0001$, $n=10$) and 10 ng/ml PDGF-BB (4.12 ± 1.07 ; $p < 0.05$, $n=5$) but not by PDGF-AA, PDGF-CC, FGF2 (all 10ng/ml), EGF(20ng/ml), thrombin(10nM), sphingosine-1-phosphate (S1P, 1 μ M), or TGF- β 1 (2 ng/ml). Inhibition of phospholipase C (PLC; 10 μ M U73212), Src kinases (10 μ M PP2), Ca⁺⁺ (10 μ M BABTA or 3 μ M thapsigargin), and PKCs (10 μ M GFX 109203) attenuated syndecan-1 induction by 70-100% ($n=2-3$). Blockade of the NF-kB pathway using a proteasome inhibitor (10 μ M MG132) or two IKK inhibitors (20 μ M IKK II and 10 μ M IKKI-2) suppressed syndecan-1 induction by 60-100%. Finally, induction of syndecan-1 by serum or PDGF-BB was completely blocked by pre-treatment of SMCs with either actinomycin-D ($106.6 \pm 0.67\%$ or $105.3 \pm 0.11\%$ inhibition, respectively) or cycloheximide ($93.5 \pm 9.4\%$ or $101.7 \pm 0.24\%$ inhibition, respectively).

Conclusions: These data show that syndecan-1 is induced by arterial injury in parallel with intimal and medial SMC proliferation, thus moderating the hyperplastic response to injury. In addition, the induction of syndecan-1 in vitro is transcriptionally regulated through a molecular pathway involving Ca⁺⁺ release, activation of PLC, Src kinase, IKK, and NF-kB.

SPHINGOSINE 1-PHOSPHATE INDUCED EXPRESSION OF SMOOTH MUSCLE ALPHA ACTIN REQUIRES MYOCARDIN RELATED TRANSCRIPTION FACTOR-B AND CALMODULIN KINASE II γ

De Wispelaere A, Mahoney W, Reidy M, Clowes A, Daum G

In response to vascular injury, smooth muscle cells (SMCs) become activated and undergo a process termed phenotypic modulation. This event is characterized by increased proliferation, migration and decreased expression of SMC genes, including smooth muscle α -actin (SMA). We recently demonstrated that re-expression of SMA after injury depends on the type 2 receptor for sphingosine-1-phosphate (S1P2R) and in cultured primary SMCs, sphingosine-1-phosphate (S1P) induces SMA in an S1P2R-dependent manner. It is known that SMC genes, including SMA, are transcriptionally regulated by serum response factor (SRF) and its co-factors myocardin, myocardin-related transcription factor A and B (MRTF-A, MRTF-B). We have previously reported that S1P-stimulated SMA expression requires activation of Rho and release of calcium from intracellular stores.

Here, we demonstrate that the nature of the SRF co-factors required for S1P-stimulated SMA expression depends upon the vascular bed from which the SMCs are isolated. In addition, we identify calmodulin kinase II γ (CaMKII γ) as the calcium-dependent target in regulating SMA expression induced by S1P. Using siRNA technology to suppress expression of SRF, myocardin, MRTF-A and MRTF-B in carotid and aortic SMCs, we found that both cell types require SRF for S1P-induced SMA expression.

Surprisingly, only carotid SMCs utilized MRTF-B, whereas aortic SMCs utilized MRTF-A and myocardin instead. A selective usage of SRF-cofactors between SMCs isolated from vessels of different embryonic origin has not been noted previously and may reflect alternate processes in development. Recently, MRTF-B was shown to be critical for the development of neural-crest-derived vessels, but dispensable for other arteries. In the presence of jasplakinolide (Jas), which stabilizes F-actin, inhibition of Rho by C3 exotoxin no longer inhibits S1P induced SMA expression. This suggests that the sole requirement for Rho in SMA expression by S1P is to promote actin polymerization and the release of MRTFs from G-actin. Consistent with this observation is that Jas treatment alone induces SMA expression. Importantly, S1P further stimulates SMA expression even in the presence of Jas and this process is calcium-dependent.

To investigate the nature of the calcium requirement in S1P-induced SMA expression, we utilized pharmacologic inhibitors and found that KN-93, a calmodulin kinase inhibitor, prevented the additional increase in SMA expression by S1P. Using siRNA against two CaMKII isoforms, gamma (γ) and delta (δ), we identified that CaMKII γ is the CaMKII isoform involved. This finding is interesting in the light of a previous observation that expression of CaMKII γ decreases after vascular injury which now indicates that loss of CaMKII γ may contribute to decreased SMA expression after injury.

METHYLATED TIMP3 IS A POTENTIAL PREDICTIVE MARKER FOR EGFR INHIBITOR THERAPY IN COLORECTAL CANCERS

Lao V, Grady W

Background: Colorectal cancer is the third most common cancer world wide and is a leading cause of cancer deaths in the United States. The current strategy using tumor stage for determining patient response to treatment is suboptimal. There is an underlying heterogeneity among the clinical behavior of colorectal cancer that likely reflects the molecular heterogeneity that is not well assessed using histological methods. Thus, there is a critical need for biomarkers that can accurately predict the responsiveness of colorectal cancer to chemotherapy. This is particularly important given the known toxicity of most chemotherapy. It is known that in approximately 30% of colorectal cancers, the tumor inhibitor of metalloproteinase-3 (*TIMP3*) promoter is hypermethylated, which is predicted to silence the expression of *TIMP3*. *TIMP-3* inhibits a disintegrin and metalloprotease-17 (ADAM-17), a matrix metalloproteinase responsible for the ectodomain shedding of cell surface molecules involved in activation of the epidermal growth factor receptor (EGFR) signaling pathway. ADAM-17 is therefore a key regulator of EGFR ligand activities. It has been previously shown that increased ADAM-17 activity results in increased shedding of amphiregulin, which is an EGFR ligand, and EGFR pathway activation. It has also been shown that high gene expression levels of amphiregulin and epieregulin in primary tumors with wild-type KRAS are more responsive to the EGFR neutralizing monoclonal antibody, cetuximab.

Aim: To determine if aberrant methylation of *TIMP3* silences *TIMP3* expression and results in increased amphiregulin shedding.

Results: In this study, we have shown that in colorectal cancer cell lines, methylation of the *TIMP3* promoter, as assayed by methylation specific PCR, correlates with decreased *TIMP3* expression and increased amphiregulin secretion. Unmethylated *TIMP3* promoter correlates with robust *TIMP3* expression and decreased amphiregulin secretion. If indeed levels of soluble amphiregulin in the condition media are inversely correlated to *TIMP-3* expression, then these data would suggest that the methylation status of *TIMP-3* may predict

responsiveness to EGFR inhibitor therapy through its effects on amphiregulin secretion and will identify a subset of colorectal cancers with EGFR pathway activation due to TIMP-3 inactivation and subsequent increased ADAM-17 activity.

**COMPARATIVE ASSESSMENT OF THE SAFETY AND
EFFECTIVENESS OF RADIOFREQUENCY ABLATION
AMONG ELDERLY MEDICARE BENEFICIARIES
WITH HEPATOCELLULAR CARCINOMA**

Massarweh N, Park J, Yeung R, Flum D

Introduction: Radiofrequency ablation (RFA) use among patients with hepatocellular carcinoma (HCC) has increased dramatically over the last decade. However, assessments of safety and effectiveness outside specialized centers are lacking. A population-based, comparative evaluation of RFA safety and effectiveness was conducted among a national cohort of HCC patients.

Methods: A cohort study of HCC patients (diagnosed 2002-2005) was performed using linked Surveillance, Epidemiology, and End Results—Medicare data. Early (≤ 90 -day) mortality and readmission as well as survival among patients undergoing RFA, resection, or no treatment were compared using multivariate and propensity score adjusted Poisson and Cox regression models.

Results: Of 2,631 patients (mean age 76.1 ± 6.1 years, 65.9% male), 16% received RFA (49.6%) or resection (50.4%). Early mortality (13.6 vs 18.7%, $p=0.16$) and readmission (34.5 vs 32.1%, $p=0.60$) rates as well as the risk of early death (IRR 1.18, 95% CI 0.99-1.40) and readmission (IRR 1.04, 95% CI 0.69-1.57) were not significantly different comparing patients who received RFA and resection. There was no difference in 1-year survival comparing RFA and resection (72.2 vs 79.7%, $p=0.18$), but beyond 3-years there was a survival benefit among patients undergoing resection (39.2 vs 58.0%, $p<0.001$). Patients receiving RFA as a sole therapeutic intervention at one year did not have a lower hazard of death compared to untreated patients (HR 0.88, 95% CI 0.59-1.31).

Conclusions: In the general community, patients treated with RFA do not have a lower risk of early adverse events compared to those treated with resection with no clear long-term mortality benefit as compared to no treatment when RFA is used as a sole intervention. Although RFA has been described as a safe and effective primary treatment for HCC at specialized centers, this experience may not extrapolate to the general community and requires further evaluation.

**THE USE, SAFETY, AND COST OF BARIATRIC SURGERY
BEFORE AND AFTER MEDICARE'S
NATIONAL COVERAGE DECISION**

Kwon S, Flum D, Macleod K, Wang B, Alfonso-Cristancho R,
Garrison L, Sullivan S

Objective: To determine the impact of the Centers for Medicare and Medicaid Services' (CMS) bariatric surgery national coverage decision (NCD) on the use, safety, and cost of care CMS beneficiaries.

Summary Background Data : In February 2006, the CMS issued a NCD restricting reimbursement for bariatric surgery to accredited centers and including coverage for laparoscopic adjustable gastric band (LAGB).

Methods: A pre/post interrupted time-series cohort study using nationwide Medicare data (2004-2008) evaluating rates of bariatric procedures/100,000 enrollees, 90-day mortality, readmission rate and payments.

Results: 47,030 patients underwent procedures at 928 sites pre-NCD and 662 post-NCD. The procedure rate/100,000 patients dropped after the NCD to 17.8 (from 21.9 in 2005) increasing to 23.8 and 29.1 in 2007 and 2008, respectively. Open RYGB (ORYGB) and laparoscopic roux-en-y gastric bypass (LRYGB) were common pre-NCD (56.0% ORYGB, 35.5% LRYGB) changing post-NCD with LAGB inclusion (12.8% ORYGB, 48.7% LRYGB, 36.7% LAGB). 90-day mortality pre-NCD was 1.5% (1.8% ORYGB, 1.1% LRYGB) and post-NCD was 0.7% (1.7% ORYGB, 0.8% LRYGB, 0.3% LAGB), (p -value<0.001). The 90-day rates of readmission decreased post-NCD (19.9% to 15.4%), reoperation (3.2% to 2.1%) and payments (\$24,363 to \$19,746) (p -value for all<0.001). Differences in outcome and cost were largely explained by a shift in procedure type and patient characteristics.

Conclusions: The CMS national coverage decision to restrict coverage of bariatric surgery had an immediate impact on surgical procedure volume and mix, patient outcomes and Medicare payments.

INTRAOPERATIVE CRYOABLATION DURING LVAD IMPLANTATION REDUCES THE INCIDENCE OF POSTOPERATIVE VENTRICULAR TACHYARRHYTHMIA

Mulloy D, Bhamidipati C, LaPar D, Stone M, Kron I, Mahapatra S, Kern J

Background: The number of patients undergoing left ventricular assist device (LVAD) implantation is rising. Ventricular tachyarrhythmia (VT/VF) after LVAD placement is common, especially among patients with preoperative VT/VF. We postulate that intraoperative epi- and endocardial cryoablation in select patients reduces the incidence of postoperative VT/VF. We investigate the feasibility of our novel approach.

Methods: From January 2009 through September 2010, consecutive patients who underwent LVAD implantation were selected (N=50) and stratified by algorithm (Algo: N=23, NoAlgo: N=27). Patients identified by algorithm as high risk underwent open epi- and endocardial cryoablation via LVAD ventriculotomy at the time of implantation (Cryo: N=7). Demographics, risk factors, intraoperative features and outcomes were analyzed. Case-match subgroup analysis (Cryo: N=7, NoCryo: N=7) investigating the feasibility of cryoablation was examined.

Results: The Algo group comprised 46%, while NoAlgo constituted 54% of patients. Mortality remained low (N=1, 2%) among all LVAD recipients. There were no differences in risk factors between groups or subgroups, although preoperative inotropes were less prevalent within the Algo ($P=0.01$) and Cryo ($P=0.09$) groups. Compared to NoAlgo, the Algo group had a lower incidence of complications ($P=0.09$) and decreased postoperative VT/VF requiring catheter-based ablation ($P=0.10$). Case-match analysis revealed significantly decreased postoperative resource utilization and complications in Cryo patients (Table 1). Cryo patients had no recurrent postoperative VT/VF ($P=0.02$).

Conclusions: Postoperative ventricular tachyarrhythmia in patients with known recurrent VT/VF can be minimized with early risk assessment and treatment. Localized epicardial and endocardial cryoablation in select patients offers promising early feasibility when performed during LVAD implantation. Further prospective analysis is required.

*Shown as N (%) or Median [IRQ]; ICU,

CLOSING THE LOOP: FACTORS ASSOCIATED WITH RE-CHARGED AND RE-PRESENTING

LaRiviere C, Huaco J, Sanchez S, Javid P,

Background: Appendicitis remains the most common pediatric surgical emergency. The consequences of missed appendicitis cases which feature perforation are often re-presentation with more severe

Variable*	Cryoablation N=7 (50)	No Cryoablation N=7 (50)	P
Readmit to ICU	-	3 (42.9)	0.05
Re-intubation	-	3 (42.9)	0.05
Total ICU Stay, hours	165.5 [110.0]	441.5 [318.5]	0.01
Length of Stay, days	26.0 [19.0]	57.0 [31.0]	0.03
Any Complication	4 (57.1)	7 (100.0)	0.05
Total # of Complications	0.9 [0.9]	3.0 [2.4]	0.05
Recurrent Postop VT/VF	-	4 (57.1)	0.02

Intensive Care Unit

ASSOCIATED WITH CHILDREN DISCHARGED WITH APPENDICITIS

Avansino J, Goldin A

most common pediatric surgical emergency. are often re-presentation with more severe

Purpose: To identify patient and provider characteristics associated with missed diagnosis of appendicitis identified at a tertiary-care referral center.

Methods: We performed a retrospective case-control study using results from a statewide surgeon-led quality improvement initiative. We identified all cases of appendicitis from 2007 at a single institution coded as having emergency department or pediatrician visit within seven days of operation. Clinical data from all visits was obtained from chart review. Re-presentation was defined as having been evaluated by a healthcare provider and sent home with subsequent presentation for appendectomy within seven days. We used logistic regression to assess odds of re-presentation.

Results: We identified 119 patients (mean age 9.9 years, 62.7% male). Of these, one patient had missing data and was therefore unable to be included in the analysis. Fifteen percent (18) of the patients were sent home after their initial visit (one after surgical evaluation), and then re-presented (55% with perforation, 45% with non-perforated appendicitis). Imaging during the initial visit was associated with decreased odds of incorrect discharge home (OR 0.22; 95% CI 0.07-0.74). These odds were similar even after adjusting for age, gender, payment type, and ethnicity. There were no significant differences between the patients initially sent home and the patients admitted directly when comparing key clinical variables. Neither initial facility type (ED, primary physician's office) nor initial provider type (pediatrician, emergency care physician) were identified to have a higher odds of discharging patients that ultimately re-presented (OR 1.03; 95% CI 0.35-3.02 and OR 0.61; 95% CI 0.22-1.7 respectively).

Conclusion: Feedback to pediatricians and emergency physicians regarding patients referred to tertiary-care centers is critical for improving regional outcomes. We believe that surgeon-led quality-improvement programs coupled with outreach-education (such as effective use of imaging) are essential to identify characteristics of children at greatest risk for misdiagnosis.

PATTERNS OF ANTIBIOTIC USE AND SURGICAL SITE INFECTION IN AUTOLOGOUS BREAST RECONSTRUCTION

Liu D, Dubbins J, Mathes D

Background: Infection rates for surgical treatment of breast cancer are documented at 3-15% higher than average for a clean surgical procedure. Pre and postoperative antibiotics have been found to be useful in lowering infection rates in other surgical groups, yet there is no current consensus on postoperative prophylactic antibiotic use in autologous breast reconstruction.

Methods: A retrospective review of consecutive patients with autologous breast reconstruction between September 2006 and September 2010 was performed. Specific risk factors for autologous reconstruction were reviewed, including American Society of Anesthesiologists class, obesity, smoking, medical comorbidities, irradiation, and chemotherapy history. Data were collected on the type and duration of prophylactic antibiotics. The incidence of surgical site infections (SSI) was measured using Centers for Disease Control and Prevention criteria.

Results: Two hundred and fifty-six patients with 360 breast free flaps who received both preoperative and postoperative prophylactic antibiotics were analyzed. The overall SSI rate was 17.8% (46 of 256 patients). SSI was correlated with increased age, tobacco use, prior radiation, and immediate reconstruction. The duration of postoperative antibiotic use did not differ statistically in those patients who developed SSI (6.4 days versus 7.6 days, $p = 0.24$). Eighty-six (34%) patients received only 24 hours of postoperative antibiotics, while 170 (66%) patients received more than 24 hours of antibiotics for a median duration of 10 days. There was no statistically significant difference in the overall SSI rate in those who received more than 24 hours of antibiotics (14% versus 24%, $p = 0.055$).

Conclusions: There was no reduction in the overall SSI rate among those who received postoperative antibiotic prophylaxis for more than 24 hours. Due to potential adverse events related to prolonged antibiotic use, this practice is not recommended in the autologous breast reconstruction population.

IMMUNOLOGICAL TOLERANCE TO COMPOSITE TISSUE ALLOTRANSPLANTATION IN A DLA-IDENTICAL CANINE MODEL

Chang J, Miwongtum T, Hwang B, Mathes D, Storb R

Introduction: The use of composite tissue allografts (CTA) has become a clinical reality with the successful transplantation of hand and face. However, the survival of these transplants is dependent on chronic immunosuppression. The purpose of this experiment was to induce tolerance utilizing our mixed chimerism protocol in a large animal model for the simultaneous transplantation of hematopoietic stem cells (HSC) and CTA. We also sought to examine the role of the HSC infusion in our mixed chimerism protocol.

Materials and Methods: Four dog transplants were performed across a DLA matched, minor mismatch barrier. All dogs received 200 cGy of radiation on the day of transplant. They then underwent a CTA transplant (Myocutaneous rectus flap) with intraoperative injection of HSC. Two dogs underwent the same protocol without any HSC infusion. All recipients received post-grafting immunosuppression (35 days of Cyclosporine and 28 days of Mycophenolate Mofetil). They were followed for donor cell chimerism in their peripheral blood. The allografts underwent routine biopsies and were followed clinically. All tolerant animals underwent a donor and third-party skin graft. Finally, they were followed for levels of FoxP3, IL-10, and GranzymeB expression in the CD3+ cell populations derived from their peripheral blood, transplanted muscle and transplanted skin.

Results: All four animals receiving CTA and HSC demonstrate long-term tolerance to their CTA (greater than a year). However, only three dogs had detectable donor chimerism. One dog lost its chimerism at 10 weeks post-transplant but remained tolerant to the allograft. The expression of FoxP3 decreased in the peripheral blood but remained stable in the transplanted muscle and skin. The two dogs that underwent the regimen without any HSC rejected their transplants at 40 to 45 days after the cessation of immunosuppression. All tolerant dogs accepted the donor skin graft while rejecting the third-party skin graft.

Conclusion: This study demonstrated simultaneous transplant of HSC and CTA is feasible and leads to tolerance to both the skin and muscle of the transplant. This tolerance induction appears to be dependent on the administration of HSC but not on the long-term engraftment of the HSC and the persistence of donor cell chimerism.