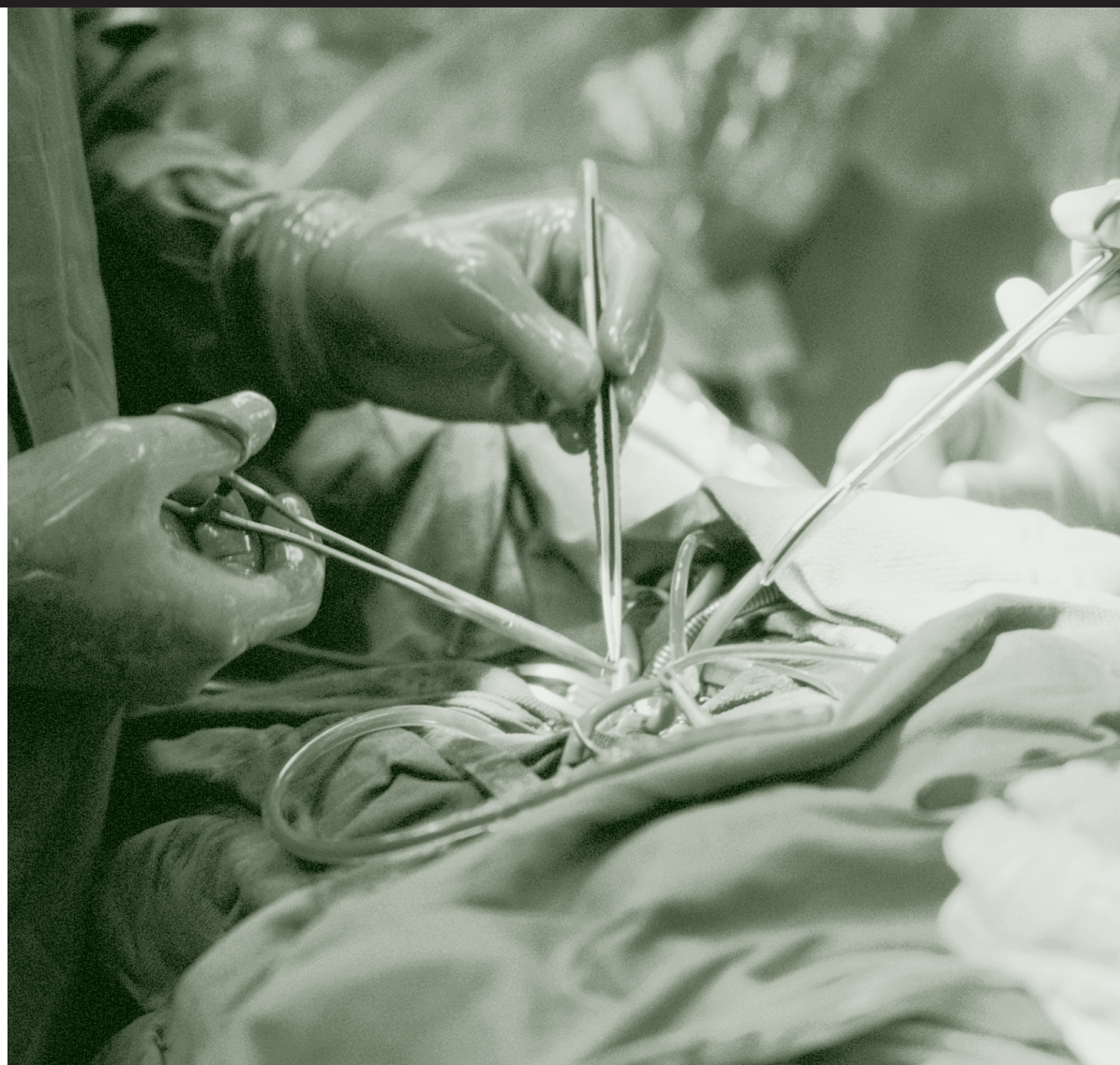
SURGERY RESEARCH

DEPARTMENT OF SURGERY 2009 REPORT



UW Medicine
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Research in the Department of Surgery
University of Washington School of Medicine

2009 REPORT

UNIVERSITY OF WASHINGTON
DEPARTMENT OF SURGERY
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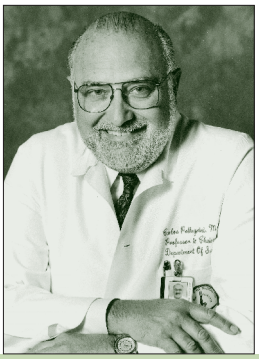

From Isolation to Collaboration: How Surgical Research Is Changing

Surgical researchers used to work in relative isolation from one another as they labored to answer questions in the basic sciences. The past several years, however, have seen major shifts in the type of research performed by surgeons as well as in the structure of the physical surgical laboratories.

First came the recognition that isolation was not conducive to scientific advancement and that efficiency required the creation of larger, multidisciplinary groups to focus on themes. Second, the aim of research began to shift from theory to practice. Translational research — transforming discoveries from the laboratory into clinical applications — became a reality. Third, the idea of improving health in all its dimensions became a focus for many surgeons who started to concentrate on the delivery of surgical care to populations.

More recently, as President Obama awarded $1.1 billion from the American Recovery and Reinvestment Act (ARRA) to the development of Comparative Effectiveness Research, a new avenue was opened that calls for intensification of studies on the relative value of the different preventive, diagnostic and therapeutic modalities on the improvement of health in the population at large. The kind of surgical research performed in our department has evolved, therefore, in its philosophy and in its structure to reflect the changing landscape and resources available.

Furthermore, our ability to protect time for surgeons to devote to their research has been challenged. Indeed, today's hospitalized patient is typically sicker and presents with more severe and multifaceted problems than was the norm 30 years ago. The viability of the hospitals depends more than ever on the ability of surgeons to manage these sicker patients effectively. Technological progress demands constant updates in our training and the surgeon must devote considerable time to practice and study to keep abreast of evolving management options.

Our faculty continue to adapt to these changes and have successfully developed newer platforms of research that reflect the changes outlined above. Unlike yesterday when surgical developments emerged from laboratories where a single investigator delegated responsibilities, today we are moving toward collaborative efforts among academic peers. By embracing these changes in how we conduct research, we allow greater outcomes which will ultimately benefit the patient.

In this book, you will read about some of the exciting ways our department is involved in this developing field. There is no question, however, that although new resources are available, we will continue to rely upon the generosity of concerned donors to help us do what we do best: save and improve lives. If you would like information on how to partner with us, please contact Lynn K. Hogan, Associate Vice President and Chief Advancement Officer of UW Medicine Advancement, at (206) 543-6865 or lhogan@u.washington.edu.

Carlos A. Pellegrini, M.D.
The Henry N. Harkins Professor and Chairman

SELECTED RESEARCH HONORS & AWARDS FROM 2008

SAMAN ARBABI, M.D., ASSOCIATE PROFESSOR, won the *Canizaro Award* for the best paper presented by a new member at the annual meeting of the American Association for the Surgery of Trauma. The title of his paper was "Beta-Blocker Use is Associated with Improved Outcome in Adult Trauma Patients."

The prestigious American Society of Maxillofacial Surgeons Traveling Fellowship was awarded to **CRAIG B. BIRGFELD, M.D.,** ASSISTANT PROFESSOR, to study new treatments in hemifacial microsomia. The fellowship provides funding for further training in craniofacial surgery at participating centers around the world.

The Western Thoracic Surgical Association presented the *Donald B. Doty Educational Award* to **GORDON COHEN, M.D.,** PROFESSOR. This award fosters innovative educational initiatives in cardiothoracic surgery and provides a $10,000 stipend.

LOREN ENGRAV, M.D., PROFESSOR, was awarded a Field Initiated Proposal grant from the National Institute on Disability and Rehabilitation Research/Department of Education to study the efficacy of pressure garment therapy after burns. **DRS. NICOLE GIBRAN, MATTHEW KLEIN & DAVID HEIMBACH** have significant roles executing the grant.

The Department of Defense warded **DAVID FLUM, M.D., M.P.H.,** PROFESSOR, a $1.65 million grant for his work titled "Data Investigation of Bariatric Surgery Outcomes and Economic Savings." The goal of his project is to use collected data and modeling techniques to create a portfolio of research on the economics of obesity and its treatments.

Dr. Flum is also directing a three-year, $1.35 million grant from the Life Sciences Discovery Fund in the Surgical Care and Outcomes Assessment Program/Surgical Outcomes Research Center (SCOAP/SORCE). With this grant, Dr. Flum will expand SCOAP to an additional 30 hospitals across Washington state. By benchmarking member hospitals against each other in the use of various technologies, SCOAP reduces the inappropriate use of health-care technologies to improve their use, effectiveness, and safety.

GREGORY J. JURKOVICH, M.D., PROFESSOR & CHIEF OF HMC TRAUMA SERVICE, has been appointed by the director of the National Center of Injury Prevention and Control, Centers of Disease Control and Prevention (NCIPC-CDC) to a four-year term on the NCIPC Initial Review Group. This 23-member external group provides advice to the CDC on the scientific and technical merits of grants and cooperative agreement applications, similar to the NIH study sections.

MATTHEW B. KLEIN M.D., ASSOCIATE PROFESSOR, received a grant from International Association of Firefighters for his work titled "Long-Term Cost Outcomes of Pediatric Burn Patients."

RONALD V. MAIER, M.D., THE JANE AND DONALD D. TRUNKEY ENDOWED CHAIRT IN TRAUMA SURGERY, was presented with the *2007 Lifetime Achievement Award in Trauma Resuscitation* by the American Heart Association.

Dr. Maier also accepted an invitation to assist the National Institute of General Medical Sciences (NIGMS) develop a five-year strategic plan. The mission of the NIGMS is to support basic research whose results lay the foundation for the diagnosis, treatment, and prevention of disease.

MICHAEL MULLIGAN, M.D., THE UW DISTINGUISHED ENDOWED PROFESSOR IN LUNG TRANSPLANT RESEARCH, was awarded a $1.25 million grant from the National Heart Lung and Blood Institute for his work "Role of TLR-4 In Lung Reperfusion Injury." Lung ischemia reperfusion injury (LIRI) develops in 15-25% of lung transplant recipients and leads to increased recipient mortality. The role of innate immunity in LIRI is not yet known, though bacterial products present in the alveolar space would likely activate it. Donor lungs are frequently colonized or at times mildly infected and whether this should promote or discourage their use for transplantation remains a question. The information garnered from the proposed studies will assist in answering this question.

GRANT O'KEEFE, M.D., M.P.H., ASSOCIATE PROFESSOR, has been named PI on the renewal of the National Institute of General Medical Sciences Post Doctoral training grant in Trauma and Burn Research at Harborview Medical Center. This training program has received funding from the NIH for over three decades.

The American Association for the Surgery of Trauma awarded a $40,000 Research & Education Scholarship to **TAM PHAM, M.D.,** Assistant Professor, for his project titled "Beta Antagonistic Influences on Innate Immune Response in Injured Older Adults."

ANDREW WRIGHT, M.D., ASSISTANT PROFESSOR, received the Association for Surgical Education Research Fellowship for his work titled "Development of a Validated Assessment Tool for Wound Closure." The fellowship is a one-year, home-site fellowship designed to equip investigators with the skills and knowledge needed to plan, implement and report research studies in the field of surgical education.

The Department of Veteran Affairs awarded **PETER WU, M.D.,** ASSISTANT PROFESSOR, a $180,000, three-year Career Development Award to study "Colorectal Cancer Treatment and Cellular Senescence" at the VA Puget Sound. His laboratory will investigate the role of telomerase in regulating therapy-induced senescence and senescence escape in colorectal cancer patients.

BRENDA ZIERLER, PH.D., R.N., R.V.T., ADJUNCT ASSOCIATE PROFESSOR, was awarded a $1.5 million grant from the Department of Health and Human Services for her project titled "Faculty Development: Integrated Technology into Nursing Education & Practice Initiative." The purpose of her study is to create a regional (five-state) multi-institutional nursing collaborative to develop faculty expertise in using simulation, clinical informatics, telehealth and distance learning technologies.

CARDIOTHORACIC SURGERY

GABRIEL S. ALDEA, M.D.

MICHAEL S. MULLIGAN, M.D.

EDWARD D. VERRIER, M.D.

Gabriel S. Aldea, M.D.

· Minimizing Morbidity of Cardiopulmonary Bypass

The William K. Edmark Professor of Cardiovascular Surgery

AWARDS

National Research Service Award in Heart and Vascular Diseases

FUNDING

Alexion Pharmaceuticals, Inc.
Edwards Lifesciences
W.L. Gore and Associates
The Medicines Company
National Institutes of Health

Despite advances in traditional techniques, coronary artery bypass graft cardiac surgery is associated with a mortality rate of 1-4%, as well as a 1-4% incidence of perioperative myocardial infarction (MI) and stroke, or changes in neurological and neuropsychological function. Morbidity is even higher in complex valve and aortic pathologies. Our research efforts are divided into two categories: 1) limit morbidity of CPB, cell salvage and transfusion; and 2) develop alternative therapies for complex aortic and valvular pathologies that do not require CPB.

Clinically, the use of these circuits and techniques reduced the need for homologous transfusion and decreased neutrophil and complement activation, resulting in a reduction in thromboembolic complications, myocardial and pulmonary dysfunction, postoperative morbidity, and cost. The use of heparin-bonded circuits also has resulted in a dramatic decrease in the incidence of perioperative MI to less than 1%, neurological deficits to less than 1%, and pulmonary complications to 1.5%. Compared to previous reports, the incidence of neurological and persistent

We are trying to determine ways to identify individual biological susceptibility prior *to surgery so we can alter surgical technique — either avoid CPB altogether or use a combination of altered equipment, techniques & pharmacological therapy.*

Minimizing CPB morbidity

Much of the morbidity of cardiac surgery is related to manipulation of an atherosclerotic aorta (embolization) and artificial perfusion and to the biological response of the body to artificial perfusion and gas exchange through the non-endothelialized cardiopulmonary bypass (CPB) circuit. These effects may be compounded by the effects of autologous transfusion. Using recent advances in perfusion technology and research in biomaterial sciences we have developed specific surgical techniques that have resulted in the routine application of more biocompatible circuits, such as heparin-bonded cardiopulmonary bypass circuits with alternatives to full anticoagulation protocol. In the laboratory, these techniques have been demonstrated to blunt the inflammatory response to CPB and promote hemostasis.

neuropsychological deficits following CABG was markedly reduced to near baseline.

Figure 1 shows a representative scanning EM at 200-fold magnification of the arterial filter (the last barrier to debris before the blood from the CPB circuit reaches the systemic circulation). This comparison demonstrates dramatic reduction (quantified in 60 patients to be > 80% reduction) in debris and inflammation resulting from the use of biocompatible heparin-bonded circuits with reduced anticoagulation protocol (HBC) compared with conventional non-biocompatible circuits with full anti-coagulation.

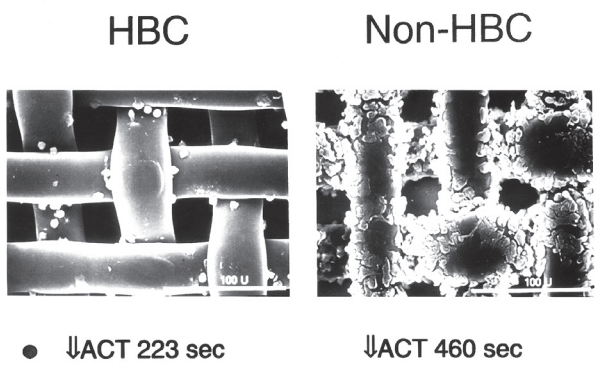

Figure 1: Scanning electron micrographs at 200 fold magnification of arterial filter. Lowest activating times (ACT) in seconds are noted. HBC = heparin-bonded circuits. Non-HBC = control non-heparin-bonded circuits. Heparin bonded circuits (HBC) have been proven to be effective in several research groups, including our own, in preserving platelet function and decreasing inflammation during CPB. However, markers of thrombin generation (PF1.2), inflammation (IL-6, IL-8, elastase, complement), platelet function (ß-thromboglobulin) and neurological injury (neuron specific enolase, S-100b) are all nearly completely blunted when HBC are used and cardiotomy suction is eliminated during CPB. Our results suggest that cardiotomy suction should be eliminated whenever possible. Our results challenge long held precepts that adverse outcomes possibly associated with thrombin generation, inflammation and platelet activation are inevitable whenever CPB is used (Figures 2-4).

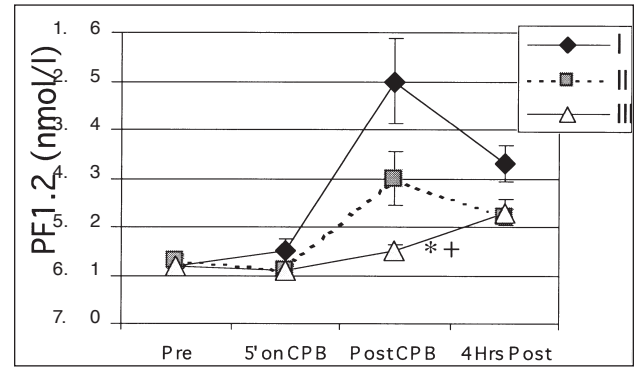

Figure 2: PF1.2 for thrombin generation

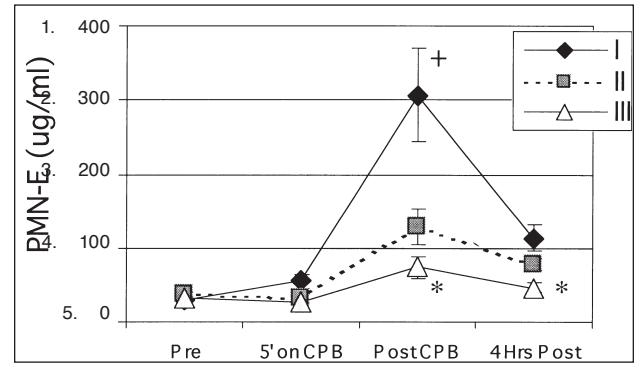

Figure 3: PMN-E for elastase

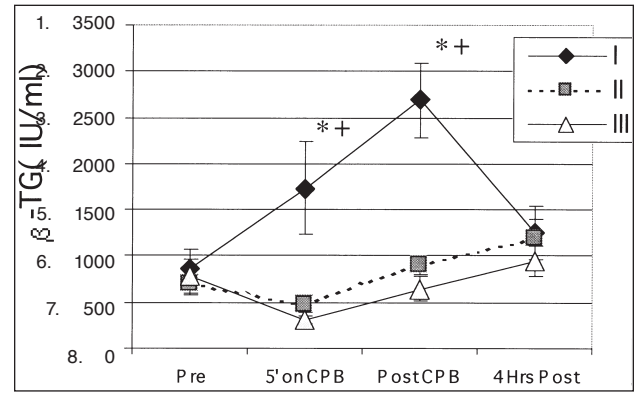

Figure 4: ß-Thromboglobulin for platelet activation

We continue to investigate novel targeted pharmacological interventions as well as further biomaterial modifications of the perfusion surface to further attenuate platelet, neutrophil, and complement activation, and cytokine release.

With the increasing incidence and awareness of HIT(T) we have evaluated alternatives to heparin anticoagulation using the short acting direct thrombin inhibitor Bivalirudin and have demonstrated safety and efficacy. The significance of post CPB HIT antibody conversion on long-term outcomes and the importance of limiting ubiquitous uncontrolled use of UFH is the focus of our future studies.

Our research demonstrates wide differences and individual variability between patients in expressing such responses to CPB, with some patients having a minimal response and others having very accentuated responses to CPB. We are trying to determine ways to identify individual biological susceptibility ***prior*** to surgery so we can alter surgical technique (either avoid CPB altogether or use a combination of altered equipment, techniques and pharmacological therapy); we hope to develop reliable specific biological assays to predict an individual patient's response to artificial perfusion and direct clinical therapy.

We also recognize that both CPB and transfusion may change patients' immunity and immunization and perhaps negatively affect long term outcomes. As part of a three year NIH SCCOR grant and in collaboration with Drs. Nelson and Slichter, we are studying these interactions and the effects of removal of passenger WBC from non-autologous blood on clinical and immunological outcomes of patients undergoing cardiac surgery.

Alternative Therapies (no CPB) for Complex Aortic and Valve Pathologies

In collaboration with Drs. Meissner and Starnes from the Division of Vascular Surgery, we are studying the long-term efficacy of innovative techniques (sole and hybrid) using endovascular stenting to minimize morbidity of complex thoracic aortic pathology (dissections and aneurysms) as part of a multi-center national Gore-TAG trial.

Finally, UWMC has been selected as one of only 10 international sites as part of the international multi-center prospective randomized PARTNERS trial to study the safety and efficacy of percutaneous aortic valve therapy using the Edwards SAPIEN valve. This collaboration between Drs. Larry Dean and Mark Reisman from Cardiology and Drs. Verrier and Aldea from CT surgery will offer therapy for symptomatic aortic stenosis to patients who are not candidates for conventional surgery with careful long term follow-up for this evolving percutaneous (trans-femoral) and trans-apical technology.

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DEPARTMENT CO-INVESTIGATORS

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CO-INVESTIGATORS

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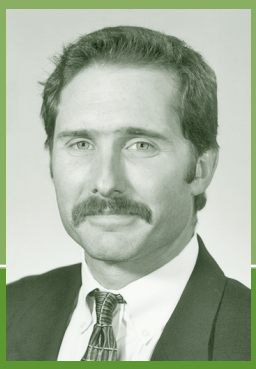
Michael S. Mulligan, M.D.

· Cytokines and Chemokines in Direct Ischemia Reperfusion Injury of Lung and Cardiothoracic Transplant Rejection



The UW Distinguished Endowed Professor in Lung Transplant Research

AWARDS

Caves Award, International Society of Heart and Lung Tranplantation, 2007
Samson Award, Western Thoracic Surgical Association, 2007
Schilling Lecture, University of Washington
Seattle Surgical Society, Best Presentation
Resident Teaching Award 2000

FUNDING

Bayer Corporation
Novartis
PrimeSource Surgical
Thoracic Society Directors Association

Lung transplantation, which was introduced into clinical practice nearly twenty years ago, has become an option for selected patients with end stage lung disease. Refinements in patient selection, perioperative care and immunosuppression have resulted in improved three-year survivals of 70%. Despite these improved outcomes, ischemia-reperfusion, an unavoidable consequence of transplantation, compromises the early and late function of the transplanted lung. Twenty-five percent of transplant recipients experience some degree of reperfusion injury. In addition to acute morbidity, this acute inflammatory injury may compromise the long-term viability of the graft.

Attempts to alleviate immediate reperfusion injury in the grafted lung have focused on improving preservation techniques, minimizing ischemic times and modifying preservation solutions. More recently a number of studies investigated the role of cytokines and inflammatory peptides in the pathophysiology of reperfusion injury. Roles for several cytokines in reperfusion injury in clinical lung transplantation have been postulated for some time and animal studies suggest that these mediators may play a critical role. A number of cytokines have been identified (i.e. TNFα, IL-1β) as important mediators in our animal model of lung reperfusion injury. Inhibition of individual cytokines was found to provide only modest protection from injury however, and has led us to investigate more proximal steps in the proinflammatory signaling cascade initiated by exposure of the lung to oxidative stress.

Reperfusion injury in rat lungs has been shown to be complement-dependent and oxygen radical mediated. It peaks in severity after four hours of reperfusion as assessed by tissue hemorrhage, vascular permeability and accumulation of neutrophils.

A model of hilar isolation for the study of ischemia reperfusion injury of rat lung has been reproducibly established and standardized in our laboratory. A pattern of nuclear factor kappa B (NFkB) and activator protein-1 (AP-1) transactivation has been established and determined to be centrally important to the development of lung injury in our model. We have also found that transcription factor activation is regulated by mitogen-activated protein kinase (MAPK) phosphorylation. MAPK are a group of intracellular signaling proteins activated by mulitiple stimuli, including inflammatory cytokines (TNFα), lipopolysaccharide, radiation, and ischemic injury. They are highly conserved serine/threonine kinases that require dual phosphorylation to become activated. We have characterized the functional significance of two MAPK in ischemia reperfusion injury: the stress-activated protein kinases (SAPK) p38 and c-Jun N-terminal kinase (JNK).

Lung injury as assessed by vascular leakage of ^{125}I labeled BSA has been determined as a measure of injury severity. The permeability index among negative (unmanipulated) controls is consistently 0.09 $\pm$ 0.05. Permeability doubled in animals undergoing only thoracotomy and mechanical ventilation. Ninety minutes of ischemia did not significantly increase mean permeability values; however, four hours of reperfusion resulted in an eight-fold rise in lung permeability to a mean index of 0.75 $\pm$ 0.01 ($p < .001$ compared to controls). In contrast, animals treated with a specific p38 inhibitor experienced a mean 50% reduction in permeability compared to injured controls ($p < .001$) while JNK inhibition reduced lung permeability by 35%. The lungs were also analyzed for myeloperoxidase (MPO) content as a measure of tissue neutrophil accumulation. Increased tissue neutrophil content is detectable after two hours of reperfusion, is significant by three hours and is

marked by four hours. In contrast, lungs from animals treated with p38 and JNK inhibitor demonstrated a 45% and 20% reduction in MPO content respectively compared to four hours reperfused controls. The alveolar macrophage appears to be the key effector cell early in the reaction and we are looking at its response to hypoxia and reoxygenation *in vitro* as well.

In addition, we are currently investigating the role of innate immune receptors in the generation of lung ischemia reperfusion injury. Toll-like receptor 4 (TLR-4), well known to initiate inflammatory signaling cascades in response to lipopolysaccharide, has also been suggested to respond to various other stimuli, including oxidative stress and products from injured and necrotic cells. TLR-4 has also recently been implicated in the modulation of reperfusion injury in other vascular beds. These data suggest that TLR-4 is an excellent candidate for initiating signaling in lung reperfusion injury. Utilizing molecular deletion techniques with short interfering RNA (siRNA) in our animal model, we have found that TLR-4 deletion is profoundly protective from reperfusion injury, reducing vascular permeability and MPO content by over 90% compared with positive controls. Western blotting of whole left lung homogenates detected significant reductions in SAPK phosphorylation with TLR-4 molecular deletion, implicating that SAPK activation in lung ischemia reperfusion injury occurs via a TLR-4 dependent mechanism.

TLR-4 dependent SAPK activation appears to be the key molecular signaling event leading to the generation of lung ischemia reperfusion injury.

In addition to the direct lung ischemia reperfusion projects, we have investigated two *in vivo* models of thoracic transplantation. The first of these models investigates the major impediment to long term survival in lung and heart lung transplantation: chronic rejection, which is histologically defined as obliterative bronchiolitis (OB). OB affects 33–60% of long term lung and heart lung transplant recipients in recent series and more than 60% of patients in prior reports. Clinically, OB is characterized by progressive dyspnea, non-productive cough, reductions in the FEV-1 and mid-expiratory flow volumes. Treatment typically consists of intensification of immunosuppressive therapy or substitution of medications in a standard post-transplant triple medication regimen. Such therapy is at best capable of slowing the rate of progression, but this disease is characteristically progressive and ultimately fatal.

Recent investigations have attempted to define the mediators involved in the development of OB, but these experiments have been limited by the inability to develop a practical and reproducible model. Whole organ transplants are desirable, but such studies are confounded by technical complications, and the costs can be prohibitive. A technically simple model for airway transplantation with histopathologic features of OB has gained acceptance. This technique, originally described in mice and now adapted to rats, produces an experimental OB that is histologically indistinguishable from human OB. We have used this model to investigate the potential role of β-chemokines in the development of experimental OB.

In addition to a variety of other mediators, two of the β-chemokines, MCP-1 and RANTES, were studied for their potential role in the development of obliterative bronchiolitis. Rat tracheas and main stem bronchi were heterotopically transplanted into the subcutaneous tissue of allogeneically mismatched (BN- LEW) or syngeneically matched (LEW-LEW) recipients. Control animals received daily injections of PBS or non- immune rabbit serum; additional animals were treated with polyclonal blocking antibodies against MCP-1 or RANTES. Tissue was explanted at two weeks and examined histologically to quantify change in airway cross sectional diameter and loss of epithelium. Northern and Western blot analysis were performed to measure upregulation of MCP-1 and RANTES mRNA and protein.

Syngeneic control animals demonstrated mild to moderate peri-tracheal inflammation, but near complete preservation of respiratory epithelium and airway cross sectional area. In

contrast, allograft controls demonstrated a dense pan-mural inflammatory response, near complete loss of respiratory epithelium and a 60% reduction in airway cross-sectional area. Animals treated with anti- MCP-1 or anti- RANTES antibodies had more limited histologic changes including only a 12% and 26% reduction in cross-sectional area respectively ($p < .001$). Levels of MCP-1 and RANTES mRNA were also increased in allograft tracheas but not in isografts. These data suggest that MCP-1 and RANTES play important regulatory roles in the development of experimental OB.

A heterotopic rat heart transplant model is also being used to determine the role of CC chemokines in heart allograft function and rejection. This model, which is technically challenging, involves a precise dissection of the donor heart using a 10x operating microscope followed by a hand sewn anastamosis using 8-0 suture. The hearts are explanted at various time points and the laboratory is currently gathering data on the role of chemokine blockade on cytokine expression and abrogation of rejection.

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· Cellular And Molecular Mechanisms of Myocardial Ischemia-Reperfusion Injury

The K. Alvin and Shirley E. Merendino Endowed Professor in Surgery

AWARDS

Thoracic Surgery Residents Association
Socrates Award for Outstanding Surgical Educator

FUNDING

Bayer Corporation
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Cardiovascular disease is the leading cause of death in the United States. Although there are a variety of therapeutic options for patients with cardiac disease, heart surgery is a mainstay of treatment for patients with advanced acquired or complex congenital heart disorders. Despite advances in the techniques of heart surgery, ischemic cardiac injury results in considerable morbidity and mortality. To date, the therapy for acute ischemia of the heart has been largely directed towards re-establishing perfusion of ischemic myocardial, or towards the coagulation system to prevent thrombosis. These therapies have arguably reached an efficacious limit.

Our research focuses on understanding how the myocardium responds to ischemia at the molecular, cellular and physiologic levels. The goal of our research is to translate an understanding of the molecular mechanisms of ischemic cell signaling into applications for clinical practice.

Ischemia-reperfusion Injury: Paradoxically, restoration of blood flow to oxygen-deprived tissue, the mainstay of therapy for ischemia, often causes further myocardial damage (termed "ischemia-reperfusion [I/R] injury"). I/R injury contributes significantly to morbidity and mortality in surgical patients, and is the principal pathogenetic event in stroke, complications of peripheral vascular disease, hemorrhagic shock, and early transplant graft dysfunction. The reperfusion of oxygen-deprived tissue can cause further myocardial injury by inciting a deleterious inflammatory reaction in and around the reperfused tissue. Because restoration of oxygen delivery to ischemic tissue is critical to survival, a substantial amount of research in the last decade has focused on treating or preventing this detrimental consequence of reperfusion. In our laboratory, we examine the molecular mechanisms of regional I/R injury that often complicate cardiothoracic surgical procedures.

Toll-like receptors: Increased expression of Toll-like receptors (TLRs) has been noted in biopsy samples of patients with severe congestive heart failure, suggesting that TLRs may serve a function apart from their classic role in recognizing microbial antigens. TLRs have been identified on cardiac myocytes, but the function of these receptors of innate immunity in the heart is unknown. We believe that TLRs expressed on cardiac myocytes are activated by reperfusion of ischemic myocardium. We postulate that TLR4 activation during ischemia and reperfusion leads to the activation of mitogen-activated protein kinase (MAPK) signaling pathways and specific transcription factors. These DNA-binding proteins can promote the transcription of genes encoding proteins that cause cardiac apoptosis, or that initiate an acute inflammatory process in the myocardium surrounding an infarction.

Research in our laboratory has identified the involvement of innate immunity receptors in the mechanism of ischemic injury. We have examined mice that are genetically engineered to lack Toll-like receptor 4 (TLR4). Compared to wild-type mice, TLR4-null mice develop a significantly smaller infarct after myocardial I/R injury — illustrating that this innate immune signaling pathway plays a role in the pathogenesis of I/R injury.

TLRs can signal through an adaptor protein called MyD88. MyD88-null mice also develop smaller myocardial infarct after I/R injury, indicating that I/R activates a TLR4- and MyD88-dependent signaling event that results in myocardial damage.

In addition, TLR4 is known to signal through MAP kinases. We have pharmacologically inhibited the activity of the MAP kinase p38, resulting in reduced infarct size after ischemia and reperfusion, compared to mice treated

with vehicle alone. Thus, we are able to apply what we are discovering about the basic science of myocardial I/R injury to potential clinical development.

Ischemic preconditioning: Ischemic preconditioning (IPC) of the myocardium is a phenomenon whereby *brief* repetitive periods of transient ischemia and reperfusion substantially protect the heart against subsequent *prolonged* ischemia. Adaptation of the heart to ischemia following IPC is a biphasic phenomenon. There is an early phase of protection that develops within minutes from the initial ischemic insult and lasts 2–3 hours, and a late (or delayed) phase that is acquired 24 hours later and lasts 3–4 days. The enhanced resistance to infarction and myocardial stunning afforded by IPC and the lasting nature of the response has generated considerable interest in this phenomenon as a potential therapeutic adjunct in the treatment of ischemic heart disease in humans.

as well as the translocation of proteins between cellular compartments following cellular stress. Interestingly, HSP60 and HSP70 have been identified as potential ligands for TLR4. In the heart, HSP70 is the primary stress protein responsive to oxidative stress. Increased expression of HSPs in the myocardium increases resistance to ischemia. Our laboratory has shown evidence that IPC is mediated, in part, by the expression of two inducible members of this family, HSP 70.1 and HSP 70.3. Thus, heat shock proteins are potential mediators of the late phase of IPC, and may work through Toll-like receptors.

The balance: Our studies indicate that TLR4 has a detrimental role in prolonged ischemia, but is necessary for the protective effect observed in brief episodes of ischemia. We hypothesize that IPC causes a shift in TLR4-mediated signaling, away from a MyD88-dependant pathway (leading to cellular death), and toward a MyD88-independent

Our research focuses on understanding how the myocardium responds to ischemia and reperfusion at the molecular, cellular and physiologic levels.

The mechanism by which IPC exerts this cardioprotection remains unclear. The classic ligand for TLR4 is LPS (lipopolysaccharide; endotoxin), an integral component of the outer membrane of gram-negative bacteria. Transient activation of TLR4 by LPS in the heart confers functional protection from subsequent I/R injury, indicating that LPS treatment can substitute for ischemia in myocardial preconditioning. We have observed that when TLR4-null mice are treated with ischemic preconditioning, the myocardial infarction size remains large compared to the protection seen in wild-type mice, indicating that TLR4 is necessary for early ischemic preconditioning of the heart. However, MyD88-null mice are responsive to IPC, suggesting that the TLR4 signaling involved in myocardial protections does not require MyD88. Research is ongoing in our laboratory to further elucidate the role of Toll-like receptors in preconditioning.

There is also increasing evidence that endogenous ligands can stimulate TLRs, triggering an immune or inflammatory response. Signals from damaged or stressed cells may initiate an inflammatory response even in the absence of infection. Heat shock proteins (HSPs) are highly conserved molecules that participate in protein folding and assembly,

pathway, leading to the modulation of NFκB activation, ultimately resulting in cellular survival (Figure 1). The regulation of this proposed shift from TLR4-mediated cell death to TLR4-mediated cell survival raises intriguing possibilities for therapeutic intervention, and is an active area of research in our laboratory.

Ischemia reperfusion injury and ischemic preconditioning are critically important in cardiac surgery. Both cyto-destructive (infarction) and cyto-protective (IPC) molecular pathways can be activated following an ischemic event. Our goal is to understand these cellular events so that therapy can be developed to protect against myocardial damage.

Experimental techniques: We utilize cultured cells (cell lines and primary cell isolates) to examine molecular mechanisms that are involved in the response to I/R injury. These studies allow us to examine specific questions about the effects of hypoxia and reoxygenation on molecular pathways in precisely controlled conditions. In addition, cell culture gives us the capability to move DNA sequences into cells in a controlled fashion to deduce cellular mechanisms of activation based on the over-expression of specific proteins. Finally, by employing differential array and DNA microchip technology, we can identify and characterize

novel protein kinases or transcription factors that, in concert with NF-κB, regulate the cellular response to hypoxia and reoxygenation.

We pair these *in vitro* studies with *in vivo* mouse models of myocardial I/R injury and IPC, in which ischemia is induced in mouse hearts by transient occlusion of the left anterior descending coronary artery. Following reperfusion we determine the size of the infarction to quantify the magnitude of cardiac I/R damage. Although these mouse models are technically challenging, they allow for the use of transgenic and gene knockout strains to examine the effects of specific genotypic changes on myocardial I/R injury.

FIGURE 1: TLR4, and possibly other TLRs, are activated by oxidative stress during myocardial I/R injury, either by binding a putative endogenous ligand (eg, HSPs) that circulates in response to myocardial I/R injury or because of physical alterations by oxygen radical species that cause TLR4 activation in the absence of ligand. Receptor dimerization leads to signal transduction via a MyD88 -dependent or –independent pathway, resulting in transcription factor activation (eg, NFκB). NFκB translocates to the nucleus to promote the transcription of genes encoding either cell survival proteins (following IPC) or cell death proteins (following I/R). Thus, ischemia-reperfusion can initiate selective myocardial signaling pathways that result in either myocardial damage or myocardial protection, depending on the nature of the stimulus.

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- Inflammatory Signaling Response to Thermal Injury
- Beta-Blocker Therapy in the Injured Patient

Associate Professor

AWARDS

American Association for the Surgery of Trauma
- **Peter C. Canizaro Award**
- **Junior Faculty Research Award**

Surgical Infection Society Foundation
- **Faculty Research Award**

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National Institutes of Health

Inflammatory Signaling Response to Thermal Injury

Severe thermal insult induces a major disturbance in the homeostatic mechanisms with significant disturbances in hemodynamic, respiratory, and metabolic pathways. Potential post-injury complications include severe sepsis, multisystem organ failure, and death. Since an aberrant systemic inflammatory response appears to be the underlying mechanism for ultimate organ failure, most studies have focused on systemic therapy to control this over-exuberant immune response. However, systemic administration of several anti-inflammatory or immunomodulatory agents, such as platelet activating factor receptor antagonists, anti-TNF antibodies, and IL-1 receptor antagonists, have failed to demonstrate improvement in survival or organ failure. In addition, the systemic administration of immuno-modulators is associated with multiple disadvantages. These agents are not tissue specific and act on multiple organs. In a complex interacting system of cell-specific pathways, systemic inhibition of one pathway may have unpredictable deleterious results.

We therefore propose a new approach which calls for "inflammatory source control." The hypothesis is that burn injury induces dermal inflammation and production of pro-inflammatory mediators, which act as a lasting trigger stimulating the systemic inflammatory response syndrome. Therefore, controlling local inflammatory signaling may attenuate the subsequent complications such as acute lung injury. In this approach, we use topical agents to inhibit post-injury burn wound inflammatory signaling. The agent that we use is a potent inhibitor of p38MAPK, which is a pro-inflammatory signaling pathway that plays a prominent role in the regulation of inflammatory cell responses. The p38MAPK inhibitors are applied to the burn wound using a simple acetone-olive oil vehicle.

Topical p38MAPK inhibition attenuates the burn wound inflammatory response. There is a significantly less pulmonary inflammatory response via reduction of pulmonary neutrophil sequestration, pulmonary cytokine expression, microvascular injury and edema formation. Topical inhibition of p38 MAPK decreased pulmonary collagen deposition and improved pulmonary function with significantly reduced inspiratory and expiratory time. In a burn-pneumonia model, application of p38 MAPK inhibitor to the wound reduced the mortality rate back to sham level (Figure 1). While dermal gene upregulator ATF-2, a downstream p38 MAPK target, was significantly reduced, there was no reduction in pulmonary ATF-2 expression, arguing against significant systemic absorption of the topical inhibitor. These experiments also confirm the strong interaction and dependence on dermal inflammation to drive the systemic inflammatory response.

This novel therapy is practical and fits the current clinical practice of daily application of topical antimicrobial agents to the burn wound.

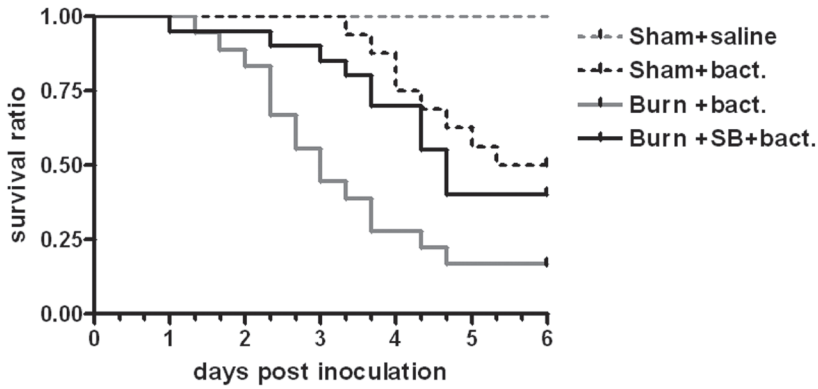

Figure 1: Dermal inflammatory source control improves survival in a burn-pneumonia two hit model.

In summary, topical p38 MAPK inhibition in burn wounds to prevent inflammatory cell activation appears to be an effective strategy to reduce the systemic inflammatory response and end-organ failure. This novel therapy is practical and fits the current clinical practice of daily application of topical antimicrobial agents to the burn wound. Moreover, it is tissue restricted and avoids potential side-effects from systemic administration. I have worked on intracellular inflammatory pathways for the last 10 years, elucidating the mechanism of action of p38MAPK in response to injury. My goal is to continue this investigation and develop an effective practical therapy in severe burns.

Beta-Blocker Therapy in the Injured Patient

Major injury induces a significant sustained release of catecholamines for several days or weeks after trauma. This catecholamine surge is especially increased when there is a significant head injury. The highest concentration of beta-adrenoreceptors is in the cerebral cortex. Activation of these receptors by catecholamines increases cerebral metabolism, glucose and oxygen consumption, which may be beneficial by increasing alertness at times of stress. However, increased cerebral oxygen consumption in the presence of elevated intracranial pressure post-trauma may worsen cerebral ischemia and secondary brain injury (Figure 2). Beta-blockers can break this Trauma-Catecholamine-Head Injury cycle by decreasing the cerebral oxygen requirement, which may attenuate cerebral ischemia and secondary brain injury. Overall, beta-blockers can be beneficial by decreasing hypermetabolism, alleviating cardiac workload and ischemia, and decreasing cerebral oxygen requirement in head injury.

Following our original study in burn patients, we reviewed outcomes for 4,711 trauma patients from 2001 to 2004 and found that 7% received beta-blockers. In the beta-cohort, 45% of patients were on beta-blockers pre-injury. The most common reason to initiate beta-blocker therapy was blood pressure (60%) and heart rate (20%). The overall mortality rate was 5.6%, and head injury was considered to be the major cause of death. After adjusting for age, ISS, blood pressure, GCS, respiratory status, and mechanism of injury, the odds ratio for fatal outcome was 0.3 ($p < 0.001$) for beta-blocker cohort as compared to control. Decreased risk of fatal outcome was more pronounced in patients with a significant head injury. We concluded that beta-blocker therapy is safe and may be beneficial in selected trauma patients with or without head injury. We are planning further studies looking at beta-blocker therapy in trauma patients and their effect on cerebral metabolism.

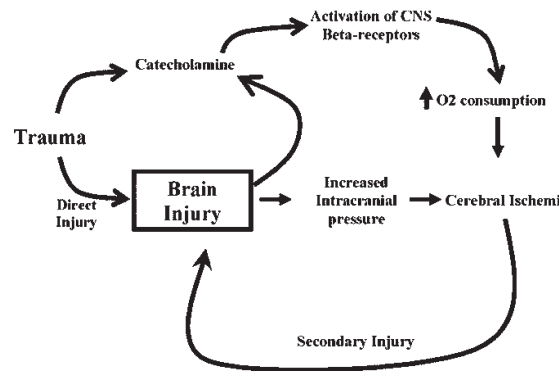

Figure 2: Trauma-Catecholamine-Head Injury Cycle

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Eileen Bulger, M.D.

- Hypertonic Resuscitation for Blunt Trauma
- Prehospital Airway Management & Treatment for Traumatic Brain Injury
- National Variability in Prehospital Care following Injury
- Immunomodulation of the Alveolar Macrophage
- Management of Necrotizing Soft Tissue Infections
- Rib Fracture Management
- Crash Injury Research and Engineering Network (CIREN)

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AWARDS

American Association for the Surgery of Trauma
- Wyeth-Ayerst Research Scholarship Award
- John H. Davis Research Scholarship Award
- Peter C. Canizaro Award

FUNDING

Brain Trauma Foundation
Medic One Foundation
National Institutes of Health
National Highway Transportation and Safety Administration

Based on a strong interest in trauma and critical care, my research has focused on injury prevention, important clinical questions regarding patient management, and elucidating the cellular biology of the systemic inflammatory response. My clinical research has focused on the prehospital care of patients following traumatic injury, including airway management and fluid resuscitation strategies. My laboratory efforts, in collaboration with Dr. Ronald V. Maier & Dr. Joseph Cuschieri, have focused on the immunomodulation of the alveolar macrophage, which plays a key role in the development of the acute respiratory distress syndrome (ARDS). In addition, a collaborative study with Dr. Avery Nathens seeks to explore the predictors of poor outcome following necrotizing soft tissue infection. Additional clinical trials address the pain management options for patients with rib fractures and the development of clinical care guidelines for these patients. To address the injury prevention side of the equation, I have recently become the local PI for the Crash Injury Research and Engineering Network (CIREN), which collects detailed data regarding the biomechanics of injury associated with motor vehicle crashes. These data will allow us to make recommendations regarding automobile design and crash test parameters that will translate into a reduction in occupant injury.

Hypertonic Resuscitation for Blunt Trauma

An evolving body of evidence suggests that resuscitation with hypertonic fluids following injury may improve outcome. The potential benefits of hypertonic resuscitation include more rapid restoration of tissue perfusion, preservation of cerebral perfusion while lowering intracranial pressure for brain-injured patients, and modulation of the inflammatory response at the time of reperfusion, thus lessening the subsequent development of inflammatory organ injury such as ARDS. With the support of the National Heart, Lung, and Blood Institute of the NIH, we have embarked on clinical trials to answer these questions. We recently closed a local trial in which randomized patients received either hypertonic saline/dextran (HSD) or lactated ringers as their first resuscitation fluid, administered by the paramedics at the scene of the injury.

The primary outcome variable was ARDS-free survival within 28 days. Secondary outcomes include mortality, infectious complications, multiple organ dysfunction, and long term neurological function for patients with traumatic brain injury. We have subsequently used the lessons learned from this trial to design a multicenter trial to be conducted by the Resuscitation Outcomes Consortium (ROC). The ROC involves 10 clinical centers in the US and Canada and a data coordinating center based at the University of Washington (PI: Scott Emerson; Co-PIs: Graham Nichol, Eileen Bulger). The Seattle and King County Medic One programs are two of the regional clinical centers (PI: Peter Kudenchuk; Co-PIs: Tom Rea and Eileen Bulger).

The ROC, which is supported by the NIH, Department of Defense and Canadian Institute for Health Research, is charged to conduct prehospital clinical trials of promising therapies for both cardiac arrest and life threatening trauma. The trial of hypertonic resuscitation will enroll nearly 6,000 patients in a three arm trial of HSD, hypertonic saline without dextran and normal saline as the initial resuscitation fluid for a hypovolemic shock cohort and a traumatic brain injury cohort. These trials are designed as definitive Phase III trials to determine the efficacy of this resuscitation strategy. These trials are currently enrolling patients. Investigators from three of the clinical centers including Seattle, San Diego, and Toronto have also submitted an R01 application to conduct detailed studies of the immuno-inflammatory response of patients enrolled in the clinical trial (PI: Bulger).

Prehospital Airway Management & Treatment for Traumatic Brain Injury

Currently supported by two grants from the Medic One Foundation, we have been investigating the airway management strategies employed in Seattle, with a particular focus on the management of patients with anatomy or injuries that make endotracheal intubation particularly challenging. We have reported that with the aid of paralytic agents to facilitate intubation, the Seattle Medic One program has the highest success rate for intubation in the literature at 98.4% and the lowest surgical airway rate at 1.1%. (*J Emerg Med* 2002). We have subsequently established a prospective data collection process to allow us to track the impact of different airway management strategies on patient outcome.

Among injured patients, the group that may benefit the most from early airway control and resuscitation is that of patients with traumatic brain injury (TBI). It has been well established that hypoxia and hypotension contribute to the development of secondary brain injury and worsen outcome following TBI. A single episode of prehospital hypotension has been associated with a two-fold increase in the incidence of adverse outcome (severely disabled, vegetative, or dead) following severe brain injury. With the support of the Brain Trauma Foundation we recently completed a study investigating the relationship between prehospital interventions and outcome following TBI. We identified that patients undergoing prehospital intubation facilitated by neuromuscular blocking agents actually had a better outcome than those intubated without these medications (*J Trauma* 2005).

We next turned our attention to the impact of prehospital ventilation on outcome following TBI. Hyperventilation may lead to cerebral vasoconstriction and thus impair cerebral blood flow. Hypoventilation may lead to cerebral vasodilation and thus raise intracranial pressure. Hyperventilation has been reported to be a common problem following prehospital intubation. We have undertaken a series of studies aimed at defining the optimal ventilation strategy for injured patients. For trauma patients intubated in the prehospital setting, those with an arrival arterial pCO_2 between 30-35mmHg demonstrated improved outcome, which was most marked for those with severe TBI (*J Trauma* 2007). Further studies have examined the impact of correcting patients into a target range in the Emergency Department and our current studies are examining the utility of end tidal CO_2 monitoring for this patient population both in the field and in the ED (*J Trauma* 2008). Taken together, these studies will allow us to design an optimal ventilation strategy for these patients early after injury.

National Variability in Prehospital Care following Injury

In collaboration with Drs. Jerry Jurkovich and Fred Rivara, co-PIs on the National Study of Costs and Outcome for Trauma (NSCOT), we have utilized data collected from 14 geographic regions in the US to assess the variability in prehospital care provided to victims of traumatic injury. We have identified substantial variability in prehospital care among the regions including: prehospital intubation (5-48%), use of neuromuscular blocking agents or sedatives to facilitate intubation (0-100%), surgical airway access (0.1-3.5%), peripheral and central intravenous access (22-95%), and needle thoracentesis (0-5%). Intubation success rates averaged 94% in patients receiving neuromuscular blocking agents vs. 67% for those who did not ($p < 0.001$). This variability persisted even when patients were stratified based on their injury severity and physiology. Understanding this national variability in care and EMS system design is critical to interpreting the various studies in the literature and to designing future multi-center trials.

Immunomodulation of the Alveolar Macrophage

ARDS is a process of acute inflammatory lung injury, which affects a diverse array of surgical and medical patients. The etiology of this process is thought to involve an excessive overexpression of the inflammatory response, leading to the destruction of host tissue. The alveolar macrophage is a key cell in the coordination of this response. Our laboratory has focused on all aspects of this response using endotoxin as a prototypic inflammatory stimulant. In previous studies we have demonstrated that treatment of alveolar macrophages with certain antioxidants, *in vitro*, results in significant inhibition of the macrophage cytokine response. This work was extended to an in vivo model of enteral Vitamin E supplementation in rats with similar results and a recently completed prospective, randomized trial of high dose enteral Vitamin E and C vs. placebo in the surgical ICU.

Recently we have also investigated the use of platelet activating factor acetylhydrolase (PAF AH) *in vitro*. PAF is a pro-inflammatory lipid mediator which has been implicated in several animal models of lung injury. PAF AH is the endogenous enzyme for PAF metabolism. These studies have demonstrated profound inhibition of cytokine production by macrophages treated with PAF AH prior to and following LPS stimulation. With the support of the American Association for the Surgery of Trauma Research Scholarship, we have developed an animal model of ARDS and have begun to test promising modulators of macrophage activation in this model. We

Harborview Medical Center serves as a regional referral center for patients with severe necrotizing soft tissue infection and as a result has seen dramatic increase in the number of these cases over the past several years.

have demonstrated that both PAF-AH and hypertonic saline, when given intravenously, dramatically down-regulate alveolar macrophage activation in response to inflammatory stimuli.

Management of Necrotizing Soft Tissue Infection

Harborview Medical Center serves as a regional referral center for patients with severe necrotizing soft tissue infection and as a result has seen dramatic increase in the number of these cases over the past several years. In an effort to define the morbidity and mortality of this population, we undertook a retrospective review of our experience over a five year period (Anaya et al., *Arch Surg* 2005). In this review we identified clinical predictors of mortality and limb loss based on data available at the time of patient admission. In a subsequent study we incorporated data from patients treated at the University of Texas in Houston and developed a clinical prediction rule which was internally validated. We are also working with the Surgical Infection Society to generate evidence-based guidelines for the management of these patients.

Rib Fracture Management

Rib fractures are a common injury in the blunt trauma population and are often under-appreciated in the setting of multiple injuries. The elderly are particularly susceptible to complications resulting from rib fractures and underlying pulmonary injury. We recently reviewed all patients > age 65 admitted to HMC with rib fractures over the past ten years and compared these to a cohort of younger patients. Of note, there was a nearly linear increase in mortality and complication rates associated with increasing rib fracture number in the elderly group. An elderly patient with only 3-4 rib fractures had a 19% mortality rate and a 31% rate of pneumonia. For an elderly patient with > 6 rib fractures, mortality was 33% with a pneumonia rate of 51%.

The key strategy in the management of these patients involves the ability to obtain adequate pain control to optimize pulmonary status. To determine the best pain management strategy for these patients, we undertook a prospective, randomized trial of thoracic epidural vs. intravenous narcotics. We demonstrated that epidural analgesia decreased the rate of nosocomial pneumonia and shortened the duration of mechanical ventilation (*Ann Surg* 2005). In recognition of the ongoing controversy regarding the indications and contraindications for epidural placement in multiply injured patients, we next conducted a survey of pain service directors at all Level 1 trauma centers in the United States (*Acute Pain* 2008). We plan to use the results of this survey to stimulate the generation of guidelines for the use of thoracic epidural analgesia after injury.

Crash Injury Research and Engineering Network (CIREN)

The Harborview Injury Prevention and Research Center houses one of eight national CIREN centers supported by the National Highway Transportation and Safety Administration. These centers collect detailed injury and crash investigation data following motor vehicle crashes to identify the forces responsible for injury. Some of our current research projects include: examining mechanisms of injury associated with renal injuries, patterns of injury associated with misuse of child restraints, the impact of seat back position on outcome following frontal crashes, the relationship between obesity and lower extremity fractures, the cost of spinal cord injuries associated with rollover collisions, and the development of prehospital triage guidelines.

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Joseph Cuschieri, M.D.

- Toll-Mediated Signaling
- Trauma Induced Mononuclear Cell Reprogramming
- Proposed Mechanism of Lipid Raft Clustering and Reprogramming
- Trauma Induced Phenotypic Alterations
- Class Prediction Based on Cytokine Profiles
- Nosocomial Infections in the ICU

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AWARDS

American College of Surgeons
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Seattle Surgical Society Award
Shock Society Travel Award
Surgical Infection Society Joseph Susan Memorial Award

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Severe injury results in the activation of the innate immune system characterized by the systemic inflammatory response syndrome (SIRS). Although this state may persist, resulting in early development of multiple organ dysfunction syndrome (MODS), the majority of injured patients develop a compensatory response that is characterized by a state of dysregulated immune responsiveness. During this state of dysregulated responsiveness, patients are at increased risk for the development of opportunistic or nosocomial infections. If invasive infection occurs following this state, an exaggerated inflammatory response ensues, leading to the MODS development (Figure 1).

The mechanism responsible for this dysregulated immune activation remains poorly understood. This state has been modeled and characterized by the "two-hit" hypothesis. According to this hypothesis, severe injury results in the reprogramming of innate immune cells so that during subsequent infection an exaggerated host response occurs, resulting in tissue injury. Both the peripheral blood monocyte and tissue-fixed macrophage appear to play critical roles during this state. The primary mechanism in which these cells interact with invading organisms is through the Toll-like receptors (TLRs), a family of pattern recognition proteins. Activation of these receptors by inflammatory factors, such as lipopolysaccharide (LPS), leads to the liberation of various cytokines and chemokines that are in part responsible for eradication of invading organisms. However, when exaggerated, as is the case following severe injury, liberation of the factors leads to subsequent tissue injury and the development of MODS.

The mechanism in which the TLRs are activated and affected by severe injury remains an area of intense investigation. Recently, we have demonstrated that activation of the TLRs, in particular TLR4, requires the formation of a receptor complex with CD14 and other constituents on specialized membrane components termed lipid rafts. In particular, attenuation and augmentation of this receptor complex formation on these membrane platforms results in dysregulated inflammatory mediator liberation. My laboratory efforts, therefore, are to elucidate the cellular mechanisms involved in mononuclear cell reprogramming in patients suffering from MODS and acute respiratory distress syndrome (ARDS) following trauma. If this is accomplished, it would provide the foundation for the development of novel early therapeutic interventions that could be used during the resuscitative period.

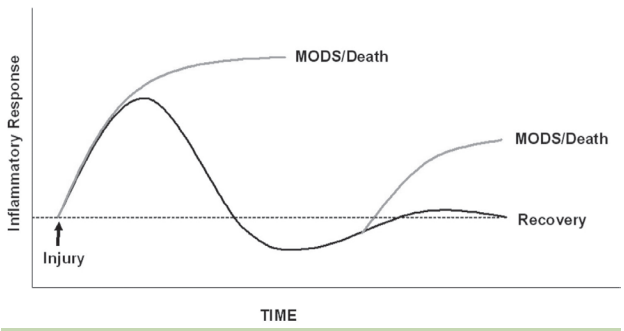

FIGURE 1

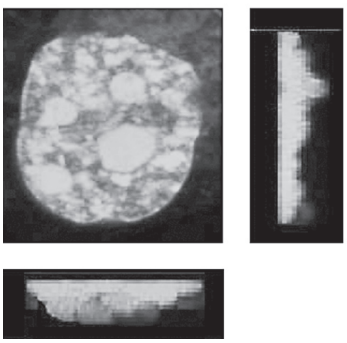

FIGURE 2

Toll-Mediated Signaling

The peripheral blood monocyte and tissue fixed macrophage are activated by pathogen-associated molecular patterns. These are structures that are characteristic of large groups of microorganisms, such as bacterial cell wall components and nucleic acid motifs. Unlike the adaptive immune response, which requires antigen-specific antibodies, innate immune cells are able to respond rapidly to invading organisms without the need for prior exposure.

In mammalian cells, the key component to this response is the family of TLRs. These receptors are responsible for the recognition of the pathogen-associated molecular patterns and lead to the subsequent activation of the monocyte and macrophage. The founding member of the TLR family is the Drosophila protein, Toll, which was initially identified through its ability to control dorsoventral patterning in fruit fly embryos. Recognition of the importance of Toll in the Drosophila innate response prompted exploration for a possible mammalian counterpart.

Currently, a total of 10 human TLRs have been identified that share structural homology and signaling components. All of the described TLRs, except for TLR9, are transmembrane molecules. The extracellular amino termini have variable leucine-rich repeat domains, which are involved in the recognition of pathogen-associate molecular patterns. The intracellular domains contain a conserved Toll/interleukin-1 (IL-1) receptor (TIR) domain. The TIR domain, a defining characteristic of the Toll/IL-1 receptor superfamily, is involved in the association with downstream signaling molecules that mediate the response to TLR stimulation.

Toll-like receptor 4 is part of a complex that recognizes LPS. Lipopolysaccharide is an abundant glycolipid present on the outer membrane of gram-negative bacteria. During Gram-negative infections, the highly conserved lipid A component of LPS activates the immune system, leading to generalized inflammation, manifested clinically as sepsis and septic shock. Lipopolysaccharide released from Gram-negative bacteria is present as an aggregate due to the amphiphilic structure of the molecule. Spontaneous diffusion of LPS monomers from these aggregates to CD14 occurs at a very low rate. However, LPS is transformed into monomers through the action of plasmatic LBP. LBP is a lipid transfer molecule catalyzing movement of phospholipids, in particular LPS monomers from LPS aggregates to CD14. This process results in either cell activation through CD14 or neutralization of LPS. Thus, the rate of either process will determine the response of the host to LPS. Kinetic studies have shown that LPS/LBP complexes bind to CD14 before LPS is transferred to HDL. This suggests that normally LPS first activates immune cells before it is neutralized to prevent overstimulation of the immune system.

Membrane bound CD14 is a 53-kDa glycoprotein present within the plasma membrane via a glycerophosphate inositol (GPI) anchor. CD14 is essential as both a functional receptor and scavenger for LPS. The functional role of CD14 leading to LPS-induced cell activation was initially established using neutralizing antibodies to CD14. Transfection of CD14-negative cells with CD14 greatly enhances sensitivity to LPS. Similarly, mice with a disrupted CD14 gene do not respond to low doses of LPS. Under physiological conditions, LPS-induced cell activation involves the formation of a ternary complex with LBP and CD14 within lipid rafts on the monocytic cell surface leading to cellular activation.

The classical fluid mosaic model proposed by Singer and Nicolson in 1972 has been modified in recent years to accommodate a role for distinct microdomains in the cell membrane, which appear to serve as signaling platforms (Figure 2). The cell membrane is mainly composed of glycerophospholipids, sphingolipids and cholesterol. The headgroups of sphingolipids trigger a lateral association of lipids of this class with one another, which is further enhanced by hydrophobic interactions between the saturated side chains. Cholesterol seems to fill voids between the large glycerosphingolipids, and tightly interacts with sphingolipids, in particular sphingomyelin, by hydrogen bonding. The tight interaction of sphingolipids with one another and with cholesterol results in the segregation of these lipids into discrete membrane structures characterized by a gel-like phase, while glycerophospholipids in the bulk of the cell membrane reside in a more fluid liquid-disordered phase.

These distinct sphingolipid- and cholesterol-enriched membrane microdomains are considered to be floating in an “ocean” of phospholipids, and hence have been termed lipid rafts. In addition to the selective lipid composition, selected proteins are preferentially targeted or constitutively found within the lipid raft. Within mononuclear cells, these modified proteins are composed of saturated acyl-chain proteins, including GPI-anchored proteins, such as CD14, and double acylated proteins. Other receptor proteins, such as the TLRs, are not constitutively found on rafts, but during activation these proteins are recruited into rafts through a mechanism that remains unclear, AZ resulting in the formation of receptor complexes and the presentation of the inciting stimulus.

Severe injury is associated with increased susceptibility to life-threatening infections and sepsis, leading to the development of MODS.

Rafts appear more prominent and more central to the function during activation of the monocyte and macrophage. In resting cells, rafts appear small and unstable, and consensus now suggests that they are smaller than the optical diffraction limit (250 nm). Upon stimulation, the raft-preferring receptors are clustered through a poorly defined mechanism leading to the generation of lipid raft macrodomains, allowing LPS to be briefly released into the lipid bilayer where it finally interacts with the complex of receptors, including TLR4. Due to the abundance of sphingolipids within the raft membrane, it is our hypothesis that sphingomyelinase activation resulting in degradation of lipid raft sphingolipids into the secondary messenger ceramide is the likely candidate involved in lipid raft reorganization within mononuclear cells.

The sphingomyelin pathway is initiated by the rapid hydrolysis of plasma membrane sphingomyelin to the second messenger ceramide via the action of sphingomyelinase. This is believed to result in the reorganization of lipid rafts. Ceramide, which has the unique property of fusing membranes, appears to drive the coalescence of raft microdomains to form large, ceramide-enriched membrane platforms, which exclude cholesterol. Recently, we have been able to demonstrate the formation of these lipid raft ceramide fused macrodomains following LPS stimulation.

The formation of these ceramide-enriched membrane platforms serves to trap and cluster receptor molecules, and potentially exclude other receptor complexes. We have been able to demonstrate that initial binding of LPS to CD14 results in the activation of acid sphingomyelinase resulting in the liberation of ceramide, and the formation of TLR4 raft associated complexes. The mechanism responsible for sphingomyelinase activity, however, remains unresolved but may occur through the activation of phosphatidylcholine (PC)-specific phospholipase C (PC-PLC).

Once this membrane platform is formed, the signaling pathways leading from LPS/CD14 binding to TLR4 complex assembly are not well understood and are important because of the potential for early and selective pharmacological intervention. Although PC-PLC and sphingomyelinase may play a role through the induction of ceramide, the subsequent events leading to TLR4 complex assembly remain for the most part uncertain. Recently, we have been able to shed some light on this mechanism by demonstrating that activation of the PKC isoform, PKC-z, is involved. Although the full effects of PKC-z remain to be elucidated, it appears that the mechanism is ceramide dependent and results in the engagement of integrins and the recruitment of various raft associated proteins.

The high degree of organization observed within lipid raft structures, coupled with their dynamic nature, appears to be important in modulating and integrating signals by providing a signaling microenvironment that is tailored to produce specific biological responses. Changes in protein or lipid composition, size, structure, number, or membrane localization of lipid rafts could potentially affect the functional capabilities of these domains in signaling with important physiological consequences.

Thus, the clustering of lipid rafts and receptor proteins appears to be an efficient means in regulating cell signaling during activation. Additionally, pre-assembly of these factors could be induced following injury and may result in amplification or modulation of signals in a spatially regulated manner. This alteration, induced in part by ceramide content and PKC-z activation, may be involved in not only augmenting signaling but could also negatively regulate signaling by sequestering or excluding signaling components in an inactive state.

Among the proteins that are targeted to form clusters within rafts are those that are anchored in part on the outer leaflet of the membrane and can covalently attach to the GPI-protein, CD14. Examples of such proteins include TLR4, HSP70, HSP90, CXCR4 and CD55. Other proteins that are linked to saturated acyl chains, such as the SRC family of kinases, in particular Lyn, and various integrins, such as Cdc42, CD11b and CD18, are also targeted to rafts and may additionally affect raft morphology and function. Each of these factors plays an important role in external signal recognition and cellular activation. A coordinated pattern occurs, with counter-regulatory components activated to lead to cellular deactivation. The formation of these complexes is induced by factors such as LPS, but the effects of severe injury remain unknown.

Trauma Induced Mononuclear Cell Reprogramming

Severe injury is associated with increased susceptibility to life-threatening infections and sepsis, leading to the development of MODS. Severely injured patients appear to have a dysregulated innate immune response following injury, which appears to be central to the development of these clinical syndromes. The effect of trauma on mononuclear cell phagocytosis, killing of microorganisms, antigen presentation, cytokine production, and induction of cytotoxic effector cells has been characterized. However, the mechanisms responsible remain unknown due to both exaggerated pro- and anti-inflammatory responses. Insight into the mechanisms involved, however, can be determined through *in vitro* modeling of factors induced by severe injury, including PAF, oxidant stress and C5a, and through the induction of tolerance.

Treatment of mononuclear cells with various agents, including PAF, oxidant stress and C5a, results in a heightened responsiveness to subsequently encountered stimuli such as LPS. Critical to this reprogramming is cellular adherence. This is fortunate, since it is difficult to envision an in vivo situation where local tissue injury might occur from stimulation of suspension phase cells.

Common to these various agents is the mobilization of calcium and subsequent activation of CaMK II that we have demonstrated to occur following exposure to each of the reprogramming conditions. Although the cellular source of calcium varies, each factor results in the autophosphorylation and sustained activation of CaMK II. Sustained activation had been previously demonstrated in a number of cell types during sepsis, including cardiac myocytes and smooth muscle cells. Recently, we have demonstrated a similar sustained activation of CaMK II in bronchoalveolar macrophages obtained from injured patients that have gone on to develop ARDS. This is the first example of increased activation of CaMK II following injury, and provides support that cellular alteration of calcium may be an important event in immune cell reprogramming.

In addition to the activation of the regulatory kinase, CaMK II, recent evidence has suggested that sphingomyelinase activation and ceramide production may play additional regulatory roles. In fact, intracellular ceramide levels along with serum TNF-α have been demonstrated to be elevated in patients suffering from severe sepsis. This strong correlation between cell-associated ceramide and serum TNF-α supports the hypothesis that ceramide, along with sphingomyelinase, plays a role in sepsis and subsequent organ dysfunction. Although sphingomyelinase activation and ceramide production may prove to be important following acute injury, this exploration has only just begun.

Desensitization or tolerance is characterized by diminished responsiveness due to repeated stimulation. Lipopolysaccharide has been consistently shown to induce desensitization in mononuclear cells. Cells in the LPS tolerant state respond to a much lesser extent than the initial stimulation resulting in attenuated liberation of chemokines and cytokines. Tolerance has been shown to attenuate several endotoxin mediated components, including IRAK-1, NF-kb and the MAPK. Recently, we have demonstrated that endotoxin tolerance does in fact effect recruitment and formation of the TLR4 complex on lipid rafts. In fact, this attenuation in recruitment of TLR4 and HSP70 during tolerance is reversed by non-specific PKC activation with PMA. This finding is consistent with previous observation that demonstrated reversal of tolerance with PMA administration. Thus, limited recruitment of receptor complexes to the lipid raft receptor platform may underlie the increased risk associated with a subgroup of injured patients at risk for devastating infections.

Putting these data together, we have just begun to demonstrate that cellular reprogramming following trauma is associated with marked alterations in raft protein and lipid composition. These changes in composition place various regulatory proteins in association leading to either enhanced or attenuated activation. Due to these changes, immune cells following injury may predispose these patients to either nosocomial infections or the development of MODS. It is therefore our current goal to evaluate these changes, using various high throughput proteomic and HPLC techniques to categorize them.

Proposed Mechanism of Lipid Raft Clustering and Reprogramming

Based upon our findings, we have developed the following model for lipid raft receptor clustering and severe injury induced reprogramming (Figure 3). Activation is initiated by LPS/LBP binding to CD14 on lipid rafts. This ligand specific binding results in the activation of PC-PLC and the generation of DAG. Liberation of DAG results in the membrane recruitment and activation of sphingomyelinase, leading to lipid raft sphingolipid conversion to ceramide within the lipid raft. Ceramide then results in the clustering of lipid raft proteins through the fusion within lipid rafts leading to increased gel phase fluidity and the activation of various kinases, in particular PKC-z. Activation of PKC-z then potentially leads to the engagement of b2 integrins on lipid rafts, leading to the formation of macrodomains, as well as cytoskeletal changes resulting in lipid raft recruitment of TLR4 components and scaffolding proteins. These cytoskeletal changes are perhaps induced through

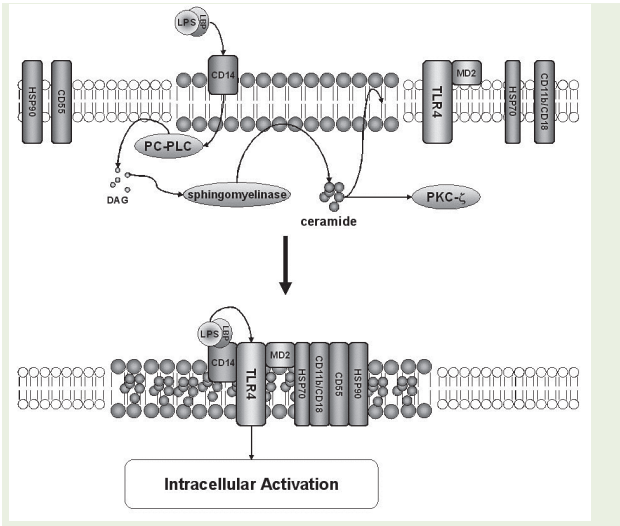

FIGURE 3A

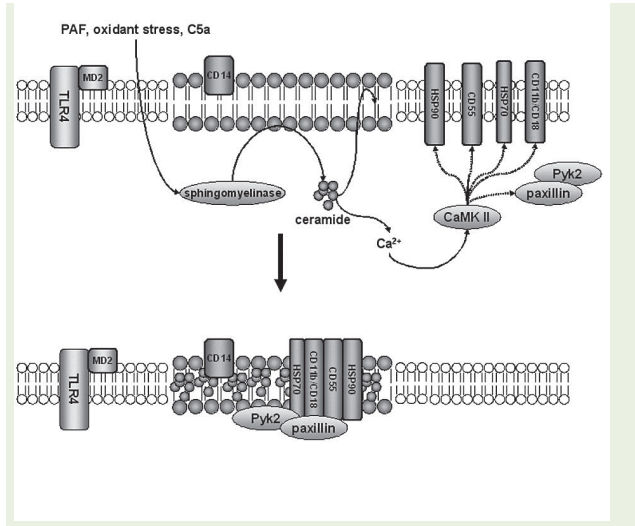

FIGURE 3B

engagement of b2 integrin intracytoplasmic tails of paxillin, Pyk2 and other adapter and scaffolding molecules and kinases. As a result, these adapter proteins are phosphorylated and activated, leading to cytoskeletal reorganization and protein reorganization and recruitment of TLR components (Figure 3A).

Reprogramming following injury is associated with changes in both protein and lipid content within rafts. These changes are due to local generation of ceramide through the activation of sphingomyelinases by reprogramming factors, such as PAF, oxidant stress and C5a. Generation of ceramide leads to calcium mobilization, followed by the sustained activation of CaMK II. Activation of CaMK II, along with lipid raft ceramide fusion, leads to the early mobilization of TLR components, such as HSP70. This clustering and pre-assembly of kinases and scaffolding proteins results in altered signaling induced by subsequent stimuli (Figure 3B).

Trauma Induced Phenotypic Alterations

Peripheral blood CD14 positive monocytes have been recently divided into two subpopulations, namely one with CD16 surface expression but with diminished CD14 expression (CD14+CD16+) and one without any CD16 expression (CD14++CD16-). The population of CD14+CD16+ monocytes normally represents about 10% of monocytes in healthy adults. These CD14+CD16+ cells demonstrate features of differentiated monocytes or tissue macrophages such as increased migration into tissues. They have also been described as "pro-inflammatory" in nature, producing high levels of pro-inflammatory cytokines, increased HLA-DR expression and little to no anti-inflammatory cytokines. Although not previously investigated following severe injury, the percentages and absolute number of CD14+CD16+ monocytes have been shown to be significantly increased in patients with monocytosis associated with cancer, septicemia, acquired immunodeficiency syndrome, and chronic renal failure undergoing dialysis. These findings suggest that CD14+CD16+ cells may play a key regulatory role following severe injury and may therefore be prognostic.

As a result, we have begun to explore changes in the phenotypic makeup of monocytes following injury. We have been able to consistently demonstrate an increase in the number of CD14+CD16+ monocytes. Sustained elevation in the expression of this phenotype following injury is associated with the subsequent development of ARDS and MODS. Although causality has not been examined, these cells do liberate increased levels of pro-inflammatory chemokines and cytokines that may in part be responsible for the development of ARDS and MODS.

The mechanism responsible for the development of this phenotype has, however, remained poorly elucidated. Recently, we have demonstrated that circulating monocytes subjected to reprogramming factors, such as oxidant stress, results in the surface expression of CD16. This increased expression of CD16 appears to be cytoskeletally regulated. Therefore, minimizing changes in cellular architecture following injury by therapeutic interventions, such as hypertonic saline, may become a means leading to improved outcome following injury.

Class Prediction Based on Cytokine Profiles

In addition to the alterations in immune cells following injury, we have recently begun to explore the relative changes in cytokine expression profiles following injury. As a result of our multi-center collaboration with the Host Response to

Injury and Inflammation consortium, we have examined the early and sustained changes in cytokine expressions following severe injury. To date, we have demonstrated that early elevation in IL-6 to 350 pg/ml within the first 24 hours is predictive of the development of MODS. Although mortality was not predicted by this cytokine profile, patients with elevation in IL-6 were demonstrated to have prolonged ventilator requirements, ICU LOS, hospital LOS, and risk for infection (Table 1).

Similar effects appear to occur with other mediators in a time dependent fashion. These alterations following initial injury may serve to be predictive of poor outcome, and potentially more importantly serve to distinguish future therapies based on innate immunity. Specific therapies targeted at different immune responses would lead to directed individual therapy, rather than non-specific disease based therapy.

	Group 1 (IL-6<350pg/ml) N=47	Group 2 (IL-6>350pg/ml) N=32	p value
IL-6 (pg/ml)	160.3 ± 13.45	1450 ± 390.7	0.0028
Age (yr)	34.64 ± 1.58	32.00 ± 1.62	ns
Gender (% Male)	20(42.5)	25 (78.1)	0.0014
Initial Base Deficit	-8.43 ± 0.529	-8.59 ± 0.67	ns
ER Lowest SBP	87.89 ± 3.33	86.06 ± 4.15	ns
APACHE II score	25.13 ± 0.96	29.75 ± 1.11	0.0026
ISS	28.49 ± 1.90	33.69 ± 2.32	ns
RBCs first 24hrs	1987 ± 181.8	4124 ± 605.4	0.0018
ICU LOS	8.25 ± 0.88	16.28 ± 2.11	0.0011
ICU VENT days	5.72 ± 0.83	13.41 ± 1.86	0.0005
Hospital LOS	16.79 ± 1.99	28.66 ± 4.06	0.0120
Mortality	2 (4.2)	2 (6.2)	ns

TABLE 1

Nosocomial Infections in the ICU

The overall effects of this dysregulated immunity following injury clearly predispose patients to increased risk for the development of nosocomial infections and eventual organ dysfunction. Ventilator associated pneumonia remains the most common infection in the critical injured patient. Recently we have been able to demonstrate that these infections which occur at a rate of 15-20 infections/1000 ventilator days are associated with severe chest injury and the patient nutritional status. Although the severity of chest injury can not be changed post-injury, the nutritional status of the patient can be optimized to diminish this risk. Recently, we have investigated the effect of immediate enteral nutrition on a severely injured cohort of patients with trophic feeds initiated within 36 hours of injury. In this cohort of patients, immediate enteral nutrition was associated with a diminished risk of ventilator associated pneumonia, nearly reducing the risk in half.

Although the risk of infection remains high in patients with severe injury, infections by multi-resistant organisms remains an even higher concern. Infections due to MRSA or acintobacter are common and associated with very poor outcomes compared to other infectious organisms. Thus, an attempt to minimize this risk is essential. Recently, we have begun to use 2% chlorhexadine washes in the ICU. This strategy has led to a significant reduction in the colonization of patients with both MRSA and acintobacter. Additionally, initiation of this daily wash has been associated with a reduction in nosocomial infections caused by these organisms.

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Nicole Gibran, M.D.

- Burn Wound Repair
- Cytokine Response to Thermal Injury
- Neuroinflammatory Response to Wound Repair
- Response to Burn Injury: Role of Melanocortin 1 Receptor in Wound Healing

FUNDING

National Institutes of Health

- **National Institute of Diabetes, Digestive and Kidney Diseases**
- **National Institute of General Medicine Sciences**

WA State Association of Fire Fighters Burn Foundation

Wound repair constitutes an essential component of every surgical subspecialty. The health care system spends millions of dollars annually to apply the latest "goo *du jour*" onto wounds. But in spite of all we know about response to injury, we still do not offer good solutions to patients with chronic non-healing wounds or with hypertrophic scars and keloids. Our collective efforts have been focused on understanding the response to cutaneous injury for wounds with either insufficient or exuberant responses.

Burn Wound Repair

With increased patient survival following burn injuries, rehabilitation and problems associated with scarring such as hypertrophy and itching become important. Since early civilization, we have been adapting topical treatments for wounds. While the growth factors that we apply to wounds today are more sophisticated than the honey, wine, oil or resins that were used in ancient medical practices, we still do not know what the growth factors do or when they should be applied.

Valuable studies over the past 30 years have augmented our understanding of the progression of repair from an acute injury through coagulation, inflammation, blood vessel formation, fibrogenesis and epithelialization, and finally to remodeling. Nevertheless, we still do not fully understand normal wound repair and thus how to therapeutically modulate repair in compromised wounds.

We designed our basic science efforts to define cellular and extracellular inflammatory processes in normal burns. Our aim has been to better understand what deviations result in non-healing wounds or in abnormal scars in order to know when to perturb the healing process with a repair accelerant. We have studied the temporal and spatial localization of dermal inflammatory cells, basic fibroblast growth factor, macrophage chemoattractant protein-1, and collagenase during repair. Collectively, our data support the theory that the skin itself is a component of the immune system and that non-inflammatory cells may contribute to the initiation and maintenance of the inflammation at the wound site. Furthermore, these studies have accented the notion that inflammatory mediators at the wound site are present at specific phases in the repair process, and that interventions with exogenous mediators must be timely.

Inflammatory Responses to Thermal Injury

With introduction of early excision and grafting and improved critical care, mortality following burn injury has dramatically decreased over the past 30 years; future improvements in survival will require innovative pharmaceutical and wound coverage interventions. For the past five years we have collaborated in a multicenter, multispecialty effort to understand host responses to injury. After five years and a renewal in 2006, we are positioned to correlate clinical data with corresponding genomic and proteomic analyses from patients with severe burn injuries. Ability to predict patients who are likely to develop multi-organ failure or die after a severe injury is the first step in understanding potential targets for therapeutic intervention.

Neuroinflammatory Responses to Wound Repair

Our lab has been dedicated to defining neuroinflammatory responses to wound repair. The sensory nerves in skin regulate pain transmission, but also a local inflammatory response within the wound bed. We have identified normal temporal and spatial distribution of pain fibers in human burn wounds.

Our aim has been to better understand what deviations result in non-healing wounds or in abnormal scars in order to know when to perturb the healing process with a repair accelerant.

We have demonstrated that patients with sensory deficits due to both spinal cord injury and diabetes mellitus have a dramatic reduction in cutaneous sensory nerves, especially in the wound beds. We have also recently determined that activity levels of neutral endopeptidase, a membrane bound enzyme that degrades substance P, is elevated in the wounds and skin of patients and mice with diabetes. Therefore, it was not a surprise to us that exogenous substance P shortens time to healing in a model of delayed wound repair in diabetic mice. We have also observed increased levels of the enzyme neutral endopeptidase in skin and wounds from diabetic mice. We have shown that increased glucose and fatty acids increases neutral endopeptidase levels in cultured endothelial cells. Most interestingly, this increase can be inhibited with antioxidant treatment.

Following injury, sensory nerves are absent within the injury site. With time there appears to be a transient abnormal increase in neuroinflammatory mediator within the wound that eventually approaches normal. These findings are important because itching, which is mediated by neuropeptides, is a major complaint of patients with thermal injuries. Hypertrophic scars have elevated levels of substance P and decreased neutral endopeptidase activity compared to uninjured skin and normal scars.

Our lab is focused on determining endothelial cell derived signals that govern nerve cell differentiation. Sensory nerve-derived neuropeptides stimulate endothelial cells following injury to round up, proliferate and synthesize adhesion molecules and cytokines. These studies are currently focused on intracellular signaling pathways that mediate substance P-mediated changes to the endothelial cell. Activated endothelial cells stimulate reinnervation of the injury site. We have defined this process to be a neuro-endothelial axis and believe that it may contribute to the pathophysiology of hypertrophic scar formation. Our latest effort has been to determine the mechanism by which substance P upregulates an inflammatory response. We have evidence that change in substance P-induced cell shape with the accompanying reorganization of the cytoskeleton may be an intermediary step. Most recently we have focused on the role of nitric oxide synthase as means of mediating substance P activity. In wound repair it appears that some cellular responses to substance P involve nitric oxide but others are independent of the reactive oxidative species.

Response to Burn Injury: Role of Melanocortin 1 Receptor in Wound Healing

A new direction of our laboratory is to demonstrate a role for the melanocortin 1 receptor in hypertrophic scar formation using both genetic and cell biological approaches. The infrastructure for this project involves creating a unique repository of DNA samples from affected individuals linked to a database of detailed phenotypic (i.e., clinical) information from the same individuals. The resulting DNA bank of will also be available for future genomic study. We will evaluate the DNA for polymorphisms in the MC1R gene and correlate that with tendency for scarring. As a mechanistic corollary we will also use RNAi technology to test our hypothesis at the cellular level by investigating the role of the melanocortin 1 receptor in fibroblast responses implicated in hypertrophic scar formation.

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When the response to injury is normal, wounds heal without complication. However, disruption of normal responses to injury can result in either excessive scarring or chronic wounds. In order to develop therapeutic approaches to improve adult wound healing it is necessary to understand the role of cell signaling pathways in the coordination of inflammation, angiogenesis, re-epithelialization and tissue remodeling. In our effort to understand the molecular mechanism of wound repair, our laboratory is focused on determining the role of bone marrow-derived cells in wound healing and on elucidating the function of both Wnt signaling and age-associated cell signaling during normal adult wound healing. Understanding normal wound healing will help us better understand and treat aberrant healing processes.

Origin of Cells in a Healed Wound: Bone Marrow

Normal wound repair has been thought to involve the proliferation and migration of local terminally differentiated cell types into the wound from the adjacent uninjured tissue. However, recent evidence suggests that cutaneous repair also involves recruitment of non-resident, undifferentiated cells from distant sources, such as the bone marrow. Populations of progenitor cells have been identified as valuable sources of uncommitted cells that are capable of reconstituting multiple cell types in various tissues, including skin. This population of cells may play a critical role in the induction of tissue regeneration at sites of injury. The ability to manipulate these cells may provide a previously unrecognized means of therapeutic intervention in patients with non-healing wounds.

The most studied progenitor cell type is the hematopoietic stem cell (HSC) from the bone marrow. By creating chimeric mice that express green fluorescent protein (GFP) only in their bone marrow cells, we have found that HSCs migrate to sites of dermal injury, differentiate into several cell phenotypes, and incorporate into the cutaneous wound for the long term. The majority of these bone marrow-derived cells resemble undifferentiated dermal fibroblasts with occasional dendritic type cells and endothelial cells (Figure 1). These findings suggest that bone marrow derived cells in the wound not only participate in the inflammatory response, but are also an important source of cells for reconstituting the dermis. We are currently investigating this unique role of bone marrow-derived cells in wound repair by determining how these cells interact with the epidermal keratinocytes and dermal fibroblasts in the skin. We have evidence that bone marrow-derived cells induce dermal fibroblasts to migrate in response to injury.

Wnt Genes in Adult Wound Repair

Wnt genes encode cysteine-rich glycoproteins, which are secreted to activate signaling pathways that regulate cell proliferation, migration and differentiation during embryonic development and in the adult organism. During embryogenesis, Wnt signaling regulates both gastrulation and patterning of the body axes. In the adult organism, Wnt signaling regulates the maintenance of adult stem cell populations in the intestine. In addition, perturbation of Wnt signaling has been linked to a number of human diseases including cancer, which suggests this pathway has a role in maintaining the function of adult organs and tissues.

By 2030, one in five people in the United States will be over the age of 65 and as a consequence elderly adults will represent the majority of patients suffering acute injury and chronic wounds.

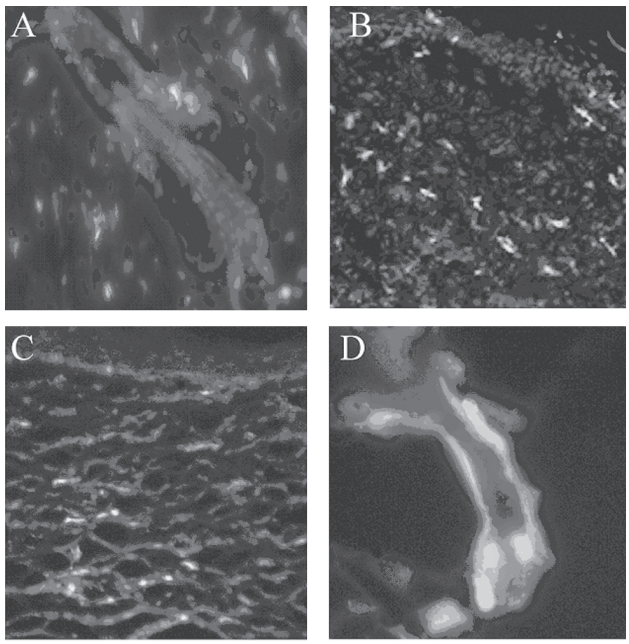

Figure 1 A: Normal skin showing numerous green cells of bone marrow origin in normal mouse skin.

Figure 1 B: At 30 days after wounding, healing skin shows a large number of dermal green cells, which (C) persist at 90 days after the wound has healed (D). Note the numerous green cells that line a blood vessel in a healed wound, demonstrating the potential for bone marrow cells to form microvessels (brighter signal seen in black & white).

Little is known about the role of Wnt signaling in cutaneous wound repair. However, it is clear that the Wnt signaling pathway is important in hair follicle morphogenesis and pigment formation in normal uninjured skin. Work in our laboratory is focused on the temporal and spatial regulation of endogenous Wnt genes during cutaneous wound repair. We have determined that the expression of nine Wnt genes is increased in response to cutaneous injury. We are also investigating the role of Wnt-4 signaling in the regulation of terminal differentiation of epidermal keratinocytes.

The Aged Response to Injury

By 2030, one in five people in the United States will be over the age of 65; as a consequence, elderly adults will represent the majority of patients suffering acute injury and chronic wounds. This is a significant medical challenge because the aged response to injury is impaired, with increased inflammation and delayed wound closure. Aged trauma and burn patients require longer hospital stays and have higher mortality compared to younger patients. In order to develop novel therapeutic approaches for the aged trauma and burn patient, it is necessary to understand how age alters the molecular responses to injury. We are investigating how cell signaling pathways that regulate aging also impact inflammation, re-epithelialization and tissue remodeling during wound repair.

A major theory of aging is that it results from a lifelong accumulation of molecular and cellular damage resulting from inadequate stress responses and repair mechanisms. Our overall hypothesis is that genes known to delay aging by activation of stress responses are required in the response to injury, and age-related changes in these genes results in impaired wound healing. The FoxO transcription factors are good candidate genes for investigating this hypothesis, as these proteins promote longevity by up-regulation of genes important in the stress response. We are currently testing our hypothesis that activation of FoxO transcription factors is required for normal cutaneous repair and that age-associated changes in FoxO activity lead to altered response to injury with age.

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- Washington State Trauma Registry and Central Region CQI
- Post-Traumatic Stress Disorder in Trauma Patients
- Field Triage Document and Implementation Toolkit

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FUNDING

Centers for Disease Control and Prevention
- **National Center for Injury Prevention and Control**

National Highway Traffic Safety Administration
National Institute of Mental Health

National Study on Costs and Effectiveness of Trauma Care

The University of Washington and Johns Hopkins University have been collaborating on the largest extramural grant ever awarded by the National Center for Injury Prevention and Control of the Centers for Disease Control and Prevention (CDC) for the study of injury. This project, titled "The National Study on Costs and Effectiveness of Trauma Center Care," has as its principle investigator at Johns Hopkins University Dr. Ellen MacKenzie, Professor of Health Policy, Senior Associate Dean for Academic Affairs in the School of Public Health, and Director of the Johns Hopkins Center for Injury Research. The Principle Investigators at the University of Washington are Dr. Gregory J. Jurkovich, Professor of Surgery, Chief of Trauma at Harborview, and Director of the Acute Care Section of the Harborview Injury Prevention and Research Center (HIPRC) and Dr. Fred Rivara, George Atkins Professor of Pediatrics and past Director of Harborview Injury Prevention and Research Center.

The purpose of this $4.8 million, direct-cost grant is to examine variations in trauma care, and outcomes from trauma care, in designated trauma centers compared to non-trauma centers across the United States. Specific outcomes to be addressed include mortality, morbidity, functional outcome, and quality of life status. Estimates of costs associated with care will also be conducted at Level I Trauma Centers, Level II Trauma Centers, and non-trauma centers.

The specific aims of this research project are to:

- Examine variation in trauma care between trauma centers and non-trauma centers;
- Examine the relationship between treatment received and mortality, complications, & functional outcome;
- Estimate the costs of care at trauma centers vs. non-trauma centers; and
- Describe the relationship between cost and effectiveness of care.

The study has carefully selected 14 regions of the country and 80 hospitals from which we recruited patients. These locations were selected based on data from the Area Resource File, the American Hospital Association, and trauma center designation databases. These hospitals were selected to represent a wide range of volumes and hospital characteristics in these 14 regions. We identified lead physicians for the study at each of these hospitals and collected comprehensive data from each institution on available resources for the care of trauma patients. In addition to IRB approval by Johns Hopkins and the University of Washington, we sought and obtained IRB approval (and annual renewals) from each of these 80 hospitals. From this initial total we ended with 18 Level 1 trauma centers and 51 non-trauma center hospitals in 12 states.

We hired skilled nurses to serve as regional coordinators in each of these 14 regions and undertook rigorous training of them in patient identification procedures and chart abstraction to guarantee high quality data collection. We collected ongoing data on all hospital discharges for trauma in each of the study hospitals for 15 months, and developed new software to identify eligible patients on the basis of

injury severity, age and body region injured. We developed a sophisticated sampling algorithm and instituted this to identify 8,000 trauma patients for the study.

We contracted with Westat, one of the leading survey research firms in the world, to conduct follow-up phone interviews at 3 and 12 months after injury. We spent a great deal of time developing, piloting and revising measures to determine functional outcomes at these follow-up times. We culled the literature, consulted our National Advisory Committee, consulted experts and developers of measures to come up with the most comprehensive, sensitive group of indicators of functional outcome. We have completed all three-month and 12-month patient interviews, for an 80% follow-up rate.

We developed software for chart abstraction, trained our regional coordinators in it, and have abstracted about 2,000 fields of chart data. We have obtained charts from transferring hospitals as well as charts on re-hospitalizations.

To determine costs of care, we have obtained hospital bills on each of the study patients and abstracted them using the UB-92 standard format. To supplement the CDC funds for this project, we wrote a grant and were funded by the National Institute on Aging to obtain Medicare data on the study patients aged 65 and older. We obtained data from MarketScan to determine national data on professional fee costs for trauma.

The products from this study will be remarkable. Just a few of them are:

- Determination of for which types of patients and kinds of injuries trauma center care has better outcomes than care in non-trauma centers.
- The most complete data available on the cost of trauma, payor mix and how these vary by type of hospital.
- Relationship between cost of trauma care and outcome.
- We will be able to recommend the best measures to be used for examining functional outcome of trauma.
- Determination of the types of hospital resources which make the most significant impact on outcome from trauma.
- Determination of the types of pre-hospital resources which make the most significant impact on outcome from trauma.
- Relationship between volume of trauma care and outcome for a wide variety of injury problems.
- Determination of how transfer status affects outcome.
- Understanding of how trauma systems interact with trauma center status of hospitals to influence outcomes.

Data collection for this study is complete, and includes 1,104 patients who died in hospital and 4,087 patients who were discharged alive. Our first major publication is in the *New England Journal of Medicine*, focusing on the mortality advantage seen in trauma centers compared to non-trauma centers. We used propensity-score weighting to adjust for observable differences between patients treated at trauma centers and those treated at hospitals without a trauma center. We have demonstrated a 20% reduction in in-patient deaths at trauma centers vs. non-trauma centers (7.6% vs. 9.5%) and a 25% one-year death rate reduction (10.4% vs. 13.8%). The life-saving beneficial effects of trauma center care is most evident in the younger (age < 55), more severely injured patients (AIS 4-5), with a relative risk of death within 30 days of injury between 0.67 and 0.78 (CI <1.0). Vexing questions remain on why this dramatic beneficial effect is not seen in the elderly, and will be the focus of further studies. Ongoing evaluation of this data set has determined that the best functional outcomes for lower extremity fractures are also obtained in Level I trauma centers. Disappointingly, we have been unable to show any variation in the functional outcome following head injury based on type of acute care provided. Cost effectiveness evaluation is ongoing.

Washington State Trauma Registry and Central Region CQI

Washington State now has a trauma system that has been in place for over eight years. Previous studies (See Nathens et al.) have suggested that it takes about this length of time for a trauma system to mature, and to show benefits in life-saving effects of trauma center care. Central Region (conforming geographically to King County) is one of eight designated trauma and emergency medical regions in the state, and has been collecting trauma registry data for the past eight years.

The Central Region Quality Assurance Committee oversees the collection and analysis of these data, in an effort to analyze and improve trauma care and outcomes in the Central Region. This committee, along with personnel from the Harborview Injury Prevention Center and the State Department of EMS and Trauma Care, is analyzing the data in an effort to address a variety of trauma system issues which remain largely unanswered in today's trauma systems. These include such questions as, "How long is too long in the pre-hospital phase of care?"; "How many patients and of what severity are essential to maintain skills and good outcome?"; and "When should you bypass the closest lowest level trauma center for the highest level trauma center?"

Studies conducted at Harborview have also demonstrated that injured adolescents represent a high-risk pediatric population, with almost 40% reporting no source of primary care, 30% showing signs of PTSD, 11% with high depression symptom levels, and 17% with problem alcohol use.

Ongoing or recently completed data analysis includes the outcomes of elderly patients with hip fractures in Central Region trauma and non-trauma hospitals, the distribution of the most severely injured patients (ISS > 15) within the regional trauma centers, Airlift Northwest landing zone delays by site location, the outcome on non-operated splenic injuries, and an assessment of preventable mortality in the region. A comparison of Central Region trauma patient outcomes to a national reference, the Major Trauma Outcome Study, reveals a significantly lower mortality for both adult blunt and penetrating trauma patients treated in the Central Region compared to this national norm.

Post-Traumatic Stress Disorder in Trauma Patients

A valued addition to the Department of Psychiatry at Harborview Medical Center is Dr. Doug Zatzick. He has a special interest in post traumatic stress disorder (PTSD) in trauma patients, and is responsible for initiating cooperative studies between surgery, pediatrics, and psychiatry on the assessment and treatment of PTSD in trauma patients. PTSD occurs in 20-40% of patients over the course of the year after physical injury. Youth admitted to the hospital for physical injury are at increased risk for recurrent traumatic life events; identifiable risk factors appear to be assault injury and history of injury prior to inpatient admission. Further, in a study comparing PTSD at Harborview and UC Sacramento, 58% of 269 randomly selected injury survivors who were screened for PTSD, depressive, and peritraumatic dissociative symptoms demonstrated high levels of immediate posttraumatic distress and/or alcohol abuse/dependence. Regression analyses identified greater prior trauma, non-white ethnicity, and site as significant independent predictors of high levels of posttraumatic distress. Early mental health screening and intervention procedures that target both PTSD and alcohol use should be developed for acute care settings.

Studies conducted at Harborview have also demonstrated that injured adolescents represent a high-risk pediatric population, with almost 40% reporting no source of primary care, 30% showing signs of PTSD, 11% with high depression symptom levels, and 17% with problem alcohol use. The burden of these largely unrecognized and untreated medical psychiatric issues is likely to include significant recidivism.

A growing body of clinical trials research suggests that PTSD may be efficaciously treated with psychotherapeutic and psychopharmacological interventions. Also, there is now evidence that pediatricians can successfully detect and intervene with youth and their families who are suffering from psychosocial disturbances. An additional aim of the investigation is to elucidate the clinical, family and community infrastructures available to support the implementation of psychosocial interventions for injured youth with PTSD. The overarching goal of the proposed investigation is to provide preliminary data that will inform the development of a larger scale R01-funded randomized intervention trial targeting PTSD and posttraumatic functional impairment among injured adolescents.

In a remarkable blend of basic molecular science and clinical care, some researchers are beginning to investigate the gene expression signatures on peripheral blood cells (monocytes), and preliminary work suggests that such genetic expression is distinct and recognizable and predictive of those who go on to develop PTSD and those who do not. (Shefi et al., *Molecular Psychiatry* 2005).

Triage of Trauma Patients from the Field

The CDC and National Highway Traffic Safety Administration (NHSTA) have asked me to chair a diverse working group of individuals representing health care providers from emergency medicine, prehospital care, surgical care, and bioengineering to revise, disseminate, and implement national guidelines for the triage of injured patients from the field. This working group has revised the "Field Triage Document" as published by the American College of Surgeons Committee on Trauma Resource Document of Care of the Injured Patient. The next step is wide dissemination of these guidelines, and the development of a "tool kit" for implementing these guidelines in trauma system design across the country.

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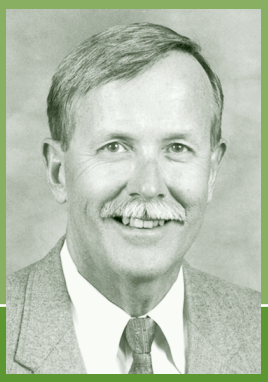
Ronald V. Maier, M.D.

- Harborview Injury Prevention and Research Center
- Clinical Trials in the Surgical Intensive Care Unit
- Modulation of the Excessive Inflammatory Response to Biomaterials
- Modulation of the Trauma-Related Macrophage Inflammatory Response to Prevent ARDS, MOFS and Death
- Genomic Controlled Phenotypic Response to Severe Injury



The Jane and Donald D. Trunkey Endowed Chair in Trauma Surgery

AWARDS

American Trauma Society
- William S. Stone Award

National Institutes of Health
- NIGMS Institutional NRSA

Shock Society
- Scientific Achievement Award

FUNDING

Centers for Disease Control
- National Center for Injury Prevention and Control

Engineering Research Center (ERC)

National Institutes of Health
- Institutional National Research Service Award
- National Heart, Lung and Blood Institute
- National Institute of General Medical Sciences

National Science Foundation

Trauma remains a major cause of death and morbidity in America. It is the number one cause of mortality among 1–45-year-olds and is the overall number one cause of loss of productive years of life in America. Death due to injury occurs in three peaks: 1) at the scene; 2) during the acute resuscitation phase; and 3) late, after one to two weeks of ICU support, secondary to multiple organ failure and sepsis. My research focuses on each of these phases. Prevention provides the best means to minimize deaths at the scene. Trauma system developments and improvements in acute care, including early resuscitation, will reduce early deaths and minimize subsequent morbidity. Finally, elucidation of the genomic and molecular responses to severe injury will identify treatment modalities to prevent the autodestructive inflammatory response causing organ dysfunction and death following trauma.

Harborview Injury Prevention and Research Center

I am Senior Advisor of the Harborview Injury Prevention and Research Center (HIPRC). HIPRC is linked closely with the Northwest Regional Trauma Center at Harborview Medical Center. The goal of HIPRC is to diminish the impact of trauma on people's lives and to draw on the effectiveness of the Northwest Regional Trauma Center's injury prevention and trauma treatment programs. Established at HMC in 1985, HIPRC is a component of the University of Washington and the Schools of Medicine and Public Health.

Current projects include identifying the risk factors for injury while developing new techniques for the application of epidemiology in the field of trauma research. Further goals are to develop and utilize systematic, high-quality data systems to document the types, causes, treatment and consequences of injuries in a wide variety of settings. A particular focus is on assessment of outcomes and the impact of trauma system development. In addition, development and assessment of new, more effective means to resuscitate and treat injured patients along the entire spectrum of care from prehospital to rehabilitation is ongoing. Following are examples of current investigations.

EVALUATION OF THE EFFECT OF STATE FIREARM LEGISLATION ON FIREARM MORTALITY

Firearm-related mortality continues to comprise approximately twenty percent of all injury-related deaths in this country, despite the implementation of "preventive" legislation regulating handguns. Numerous handgun laws have been enacted, and the ultimate effect of such legislation on firearm violence is questionable and highly debated. We have investigated whether a "shall issue" law permitting unrestricted carrying of concealed handguns, a minimum age of twenty-one for private purchase, a minimum age of twenty-one for possession, a mandatory registration law, restricting purchases to "one gun a month," or a ban on "junk guns" would reduce firearm-related mortality.

We have reviewed vital statistics for the entire United States from 1979-1998, looking at total and firearm, homicide and suicide death rates. "Shall issue" and mandatory registration laws were associated with a respective 17% and 21% increase in homicide rates. Mandatory registration and a ban on "junk guns" reduced firearm suicide rates. Individual gun legislation varies in regard to the effect on firearm mortality. Permitting unrestricted carrying of concealed weapons through "shall issue" laws increases firearm and total homicide rates. Implementing laws restricting the purchase or possession of handguns by persons younger than twenty-one years of age reduces firearm homicide and firearm suicide rates in youths.

RELATIONSHIP BETWEEN TRAUMA CENTER VOLUME AND OUTCOME

The premise underlying regionalization of trauma care is that optimal outcomes can be achieved at greatest efficiency if care is restricted to relatively few dedicated trauma centers. Implicit in this premise is that higher patient volumes will lead to greater experience and this experience translates into better outcomes. This relationship appears to hold for other areas of surgical care involving complex procedures but, in contrast, there is no such relationship when less complex procedures are evaluated. Previous studies evaluating the relationship between institutional volume and outcomes in trauma patients are difficult to interpret because of multiple logistical issues.

Two distinct cohorts of trauma patients are being evaluated, including penetrating abdominal injury and multisystem blunt trauma with a minimum head injury and lower extremity long bone fracture, treated at 31 academic Level I or Level II trauma centers across the United States, participating in the University Health System Consortium. Results indicate a strong association exists between trauma center volume and outcome, with significant improvements in mortality and length of stay, but only when the volume exceeds at least 600 cases per year, and these benefits were only evident in patients at the highest risk for adverse outcomes and not in the vast majority of lesser-injured patients.

Clinical Trials in the Surgical Intensive Care Unit

We are performing multiple ongoing trials based on the pathophysiologic response of the severely injured patient, many in conjunction with the Division of Pulmonary and Critical Care in the Department of Medicine. In particular, clinical studies and associated basic investigations are focused on acute respiratory distress syndrome (ARDS), which affects critically ill and injured patients.

ARDS is largely responsible for the prolonged intensive care unit and hospital stay, and contributes significantly to mortality in these patients. Management is primarily supportive while the underlying disease process stabilizes and resolves. Attempts to reduce the consequences of ARDS have focused upon 1) pharmacologic manipulation of the inflammatory response, and 2) modifying positive pressure ventilation techniques to reduce the potential iatrogenic ventilator-associated lung injury. Examples of current studies are:

LOW TIDAL VOLUME VENTILATION IN ARDS

The mortality rate from acute lung injury and ARDS is approximately 40-50%. Traditional approaches to mechanical ventilation use tidal volumes of 10-15 ml/kg of body weight. These volumes are much larger than those in normal subjects at rest, but are frequently necessary to achieve normal values for partial pressure of arterial carbon dioxide and pH. Since atelectasis and edema reduce aerated lung volumes, inspiratory airway pressures are often excessively high to achieve these parameters, suggesting the presence of excessive distension, or "stretch," of the remaining aerated lung.

Thus, this traditional approach to mechanical ventilation exacerbates or perpetuates lung injury and, in contrast, the use of lower tidal volumes during ventilation reduces or prevents this deleterious process. Previous uncontrolled studies suggest that lower tidal volumes may improve survival. However, this approach may necessitate acceptance of significant acidosis and decreased arterial oxygenation, or increased levels of positive end-expiratory pressure (PEEP). A clinical trial in conjunction with the ARDS Network tested whether lower tidal volumes during mechanical ventilation in patients with acute lung injury improved ARDS severity and/or survival. The trial has been stopped after enrollment of 861 patients because mortality was lower in the group treated with lower tidal volumes. Mean tidal volumes were 6 cc/kg vs. 12 cc/kg, with a subsequent reduction of mean plateau pressures to 25 cm compared to 34 cm of water. Thus, in patients with acute lung injury and ARDS, mechanical ventilation with a lower tidal volume and, subsequently, a lower mean plateau pressure results in decreased mortality.

Modulation of the Inflammatory Response

The potentially auto-destructive excessive immuno-inflammatory response is thought to contribute to the initiation and progression of ARDS and to ultimately affect patient outcome. Work at Harborview Medical Center (HMC) has shown a high incidence of Vitamin C and potential Vitamin E deficiency in trauma patients admitted to the HMC intensive care unit. A study of patient admissions to HMC found that 64% of patients had plasma Vitamin C levels below the reference range and 23% of patients had plasma Vitamin C levels less than 0.20mg/dL, indicating Vitamin C deficiency as defined by the World Health Organization. Reports from other institutions document a low plasma Vitamin C concentration in 28-83% of select hospitalized patient populations and 12-21% in a random sample of all new hospital admissions.

Our HMC study demonstrated that supplementing 3 grams/day of Vitamin C and 3000 IU/day of Vitamin E in patients with initially low levels resulted in plasma levels within the normal reference range within seven days. Patients not receiving supplements remained in the low or below the reference range. The significance of Vitamin C deficiency in these patients is illustrated by a study of 78 patients with 105 fractures of the mandible treated at HMC: those patients who had fracture complications (infection, malunion) had significantly lower serum Vitamin C concentration than those with good fracture outcomes. In addition, patients with ARDS have been shown to have high levels of oxidants and suppressed levels of antioxidants, such as Vitamin C and Vitamin E, in bronchoalveolar lavage (BAL) specimens.

We hypothesize that routine supplementation of Vitamin C and E will protect against oxidant-induced injury in severely injured and stressed patients, and avoid the diffuse insult predisposing to ARDS and other organ dysfunction, and also secondary nosocomial infections such as ventilator-associated pneumonia and wound infections. In a prospective observational study, all trauma admissions to the HMC surgical ICU had three grams of Vitamin C or 3,000 IU of Vitamin E, divided over three doses per day, started at the time of admission. Otherwise, care was standard and the populations were followed to determine the incidence of ARDS, duration in the ICU, mortality and infectious complications. In addition, we studied BAL samples for evidence of oxidant injury and cytokine production.

The results show that the treatment with anti-oxidant supplementation on admission to the surgical ICU produced a 50% reduction in evidence of oxidant injury in the BAL solution, along with a 50% reduction in the production of inflammatory mediators, while having no detrimental effect on the production of antibacterial mediators of the immune system. Concomitant with this decrease in intrapulmonary inflammatory response, there was a decrease by 50% in the incidence of ARDS and a significant decrease in length of stay and ventilator days in these critically ill patients. Concomitant with this decrease in development of ARDS and inflammation was a 50% reduction in mortality in the treated population.

Modulation of the Excessive Inflammatory Response to Biomaterials

The production and release of potent inflammatory mediators by tissue-fixed macrophages coordinate and orchestrate a series of biologic events that lead to either normal wound healing or abnormal chronic granulation and typical "foreign body" reaction. The goal of the experiments performed in conjunction with the University of Washington Engineered Biomaterials (UWEB) program funded by the NSF is to define the cell signaling processes that control the pro-inflammatory phenotype of the macrophage in response to various biomaterials and cause the subsequent chronic inflammatory response that leads to non-healing and extrusion of biomaterials.

Preliminary experiments demonstrated that adherence by the macrophage to various surfaces primes the macrophage for activation. Subsequent steps in the inflammatory response lead to multi-nucleated giant cell formation and subsequent capsule formation, secretion of extracellular matrix, vascular budding, and fibroblast proliferation with thick collagen deposition. Prevention of the pro-inflammatory phenotype may well equate with prevention of foreign body reaction. In current studies, we are investigating coating of biomaterials with various molecules. These include osteopontin and various anti-inflammatory agents, such as anti-oxidants, including Vitamin E and components of the extracellular matrix, such as hyaluronic acid derivatives, to test the subsequent response of adherent macrophages to inflammatory stimuli, such as endotoxin.

In addition, we are studying materials of various selected pore sizes to minimize cell spreading and to test spatial structural impact on macrophage response to inflammatory stimuli. End-product analysis of inflammatory mediators, such as TNF, procoagulant activity and IL-8, along with the normally produced anti-inflammatory mediators, IL-10 and PGE2, are monitored. These mediators exist in a delicate balance and time sequence to produce normal, as opposed to abnormal, wound healing and chronic inflammation.

The ultimate goal is to modulate the surface characteristics of biomaterials so that they may be adapted as "compatible" and elicit a normal host response and normal wound healing with incorporation of the biomaterial — "true healing."

Modulation of the Trauma-Related Macrophage Inflammatory Response to Prevent ARDS, MOFS and Death

The last major area of investigation is based on the aberrant host immuno-inflammatory response to trauma and sepsis. This auto-destructive response is thought to be responsible for the induction and persistence of the "malignant systemic inflammatory response" underlying ARDS and multiple organ failure syndrome (MOFS). ARDS and MOFS are the major determinants of late death following trauma.

The primary etiology of ARDS and MOFS leading to late mortality following trauma is the clinical "sepsis syndrome," or systemic inflammatory response syndrome (SIRS). This diffuse inflammatory response causes disseminated tissue injury and subsequent organ dysfunction. The long-lived, highly diverse tissue-fixed macrophage is a crucial central coordinator of both the normal and the aberrant host immuno-inflammatory response. The macrophage is both primed and activated by a multitude of stimuli during the inflammatory response.

Until now, therapeutic approaches have focused on control or inhibition of single components of the overall inflammatory response. However, since the inflammatory response is replete with redundancy and feedback amplification mechanisms, it is appealing to take a broader approach to control the inflammatory response and subsequent injury to multiple diffuse organ beds. To achieve this goal in these basic laboratory investigations, we are focusing on the cellular and molecular mechanisms involved in macrophage signaling and activation by inflammatory stimuli and the subsequent production of multiple inflammatory cytokines.

The goal is to develop therapeutic interventions based on controlling these intracellular transduction pathways and to modulate the over-aggressive macrophage response and the subsequent auto-destructive immuno-inflammatory response. Currently, we are studying the manipulation of cellular signal transduction mechanisms that control inflammatory mediator genes by altering the intracellular levels and release of calcium, the regulation of levels of cyclic AMP and the delineation of regulatory protein kinase signal transduction pathways, particularly the MAP kinase family, including ERK1/2, JNK and ρ38. In addition, we are investigating signaling processes activated through formation of focal adhesion complexes induced by adherence of the monocyte/macrophage as critical to the host inflammatory cell response.

We hypothesize that routine supplementation of Vitamin C and E will protect against oxidant–induced injury in severely injured and stressed patients and avoid the diffuse insult predisposing to ARDS and other organ dysfunction.

A major focus is on the ability of anti-oxidants, such as vitamin E, or cytoskeletal spatial disruption with agents, such as cytochalasin D, to modify the cellular response to inflammatory stimuli. Recent investigations have also demonstrated that hypertonic preconditioning similarly disrupts the signaling pathways in the macrophage. Hypertonic saline has been shown to produce an adequate resuscitation for the severely injured while limiting the excessive inflammatory response. Recent investigations have confirmed that hypertonic saline led to a reduction in ERK1/2 phosphorylation with no effect on ρ38. This was correlated with an inhibition of stress fiber formation in the macrophages and appears to link the necessity for cytoskeletal polymerization for optimal MAP kinase signal transduction and inflammatory mediator production. Thus, hypertonic saline early in the response of the host to reperfusion injury could lead to a reduction in subsequent organ injury and failure. Elucidation and control of these macrophage cellular mechanisms will permit development of future safe therapies to prevent ARDS, MOFS and death in the critically ill surgical patient.

Genomic Controlled Phenotypic Response to Severe Injury

To better understand the pathophysiologic phenotype in the severely injured patient, a collaborative study has been developed, funded by the NIH-NIGMS for a "Glue Grant," a consortium and large-scale project grant. The intent is to study the entire human genomic response across time to the severe stress of injury, resuscitation and subsequent nosocomial infections. To enable this, the technological developments necessary for reproducible, high quality isolation of RNA and analysis via microarray chips have been developed through this consortium. The analysis of gene expression data in clinical medicine has been plagued by a lack of critical evaluation of accepted methodologies for the collection, processing, and labeling of RNA.

Whole blood obtained from healthy subjects was either untreated of stimulated ex vivo with SEB. Blood samples were also collected from trauma patients, but were not stimulated ex vivo. Total RNA was isolated from the whole blood with the PAXgene proprietary blood collection system or from isolated leukocytes. Biotin cRNA was hybridized to Affymatrix GeneChips. Correlation coefficients for gene expression measurements and replicates from healthy subjects using both techniques are excellent. Unsupervised analyses, including hierarchal cluster analysis, however, revealed that the RNA isolation method resulted in greater differences in gene expression than stimulation with SEB or among different trauma patients. The intraclass correlation as a measure of signal-to-noise ratio of the difference between SEB-stimulated and unstimulated blood from healthy subjects was significantly higher in the leukocyte-derived samples than in whole blood. Thus, the isolation of RNA from whole blood using the buffycoat is critical to the validity of the microarray analyses. For ongoing studies, the buffycoat and subpopulations are being employed to analyze the serial sequence of genomic responses in the severely injured to identify patterns predictive of trajectory and subsequent outcome.

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- Pathophysiology of Post-Injury Infection and Organ Failure
- Nutritional Support in the Intensive Care Unit

Professor

FUNDING
National Institute of General Medical Sciences

Severe traumatic injury results in biochemical and physiological changes that often lead to the development of nosocomial infection (pneumonia, wound infections, etc.) and remote organ (lung, kidney, liver) failure. Excluding those patients who succumb to their injuries and die in the immediate (≤ 1 hour) or early (≤ 24 hours) post-injury period, infection and organ failure (MODS; multiple organ dysfunction syndrome) are leading causes of death. Furthermore, infection and organ failure contribute to prolonged and resource intensive hospital stays. However, if these complications are not lethal, they do not appear to result in major long-term disabilities.

There have been considerable recent advances in characterizing the human genome, and we have now progressed from the notion of knowing the structure (the initial goal of the Human Genome Project was to sequence the entire human genome) to now understanding the function of our genetic material. Second, failure to consider individual variability, in the form of gene polymorphisms, may have reduced our ability to detect beneficial effects of novel therapies.

We are interested in both of these related phenomena and our research program aims to characterize genetic influences on the risk of and outcome from injury-related nosocomial infection and organ failure and to better characterize the nature of the inflammatory response to tissue injury. Our research program is directed at understanding the genetic basis for human variation in inflammatory responses and how these differences influence the clinical course of sepsis. We are also focusing on pathways that have traditionally been not considered "inflammation-related," but appear to have important influences on how the inflammatory and innate immune responses are regulated in humans.

TLR4 genetic variation influences the severity of post-traumatic sepsis

Genetic variation in the innate immune response likely contributes to the marked variation seen in the risk of and outcome from infectious diseases, including sepsis. Epidemiologic studies have demonstrated a strong familial association with death from infectious disease in general and, more specifically, an association between a familial "anti-inflammatory" response and death from meningococcal sepsis. The role of specific genetic differences in conferring risk is less certain, with many examples of discordant observations regarding numerous genetic variants. Examples of conflicting observations have primarily concerned single nucleotide polymorphisms (SNPs) in genes involved in the innate immune response, such as tumor necrosis factor–alpha (TNFα), lipopolysaccharide binding protein (LBP) and CD14. LPS is a major component of the outer wall of gram-negative bacteria, serving as the key ligand for immune cell recognition and activation in response to infection. Innate immune cells, such as macrophages and monocytes, recognize endotoxin by a specific receptor complex, which contains CD14, LPS binding protein (LBP), and Toll-like receptor 4 (TLR4). Recognition by this receptor complex leads to the activation of specific mitogen-activated kinases (MAPK), including p38, and the synthesis and release of pro-inflammatory cytokines, including TNFα, interleukin-1β (IL-1β), and IL-6. TLR4 is central to LPS signaling. Its role is highlighted by animal and *in vitro* studies that have identified mutations in the TLR4 gene associated with hypo-responsiveness to LPS and hyper-susceptibility to infection by gram-negative bacteria.

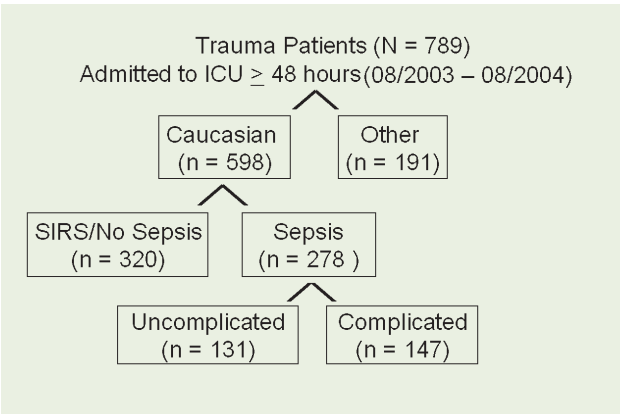

Figure 1: Trauma Cohort 5-08

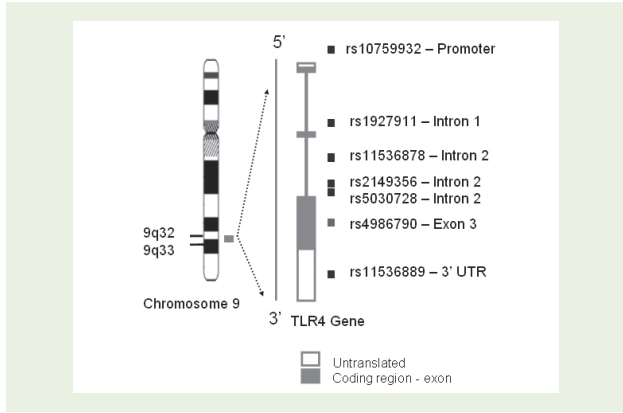

Figure 2: TLR4 Gene SNPs

To determine the relationship between TLR4 genetic variation and sepsis, 598 Caucasian trauma patients were admitted to the intensive care unit for ≥ 48 hours. Sepsis developed in 278 patients and complicated sepsis developed in 147 (Figure 1). Patients with complicated sepsis had a higher mortality and longer ICU length of stay compared to patients with SIRS or uncomplicated sepsis alone. Respiratory failure (ARDS or ALI) was the most common organ dysfunction, and occurred among almost all patients (132, 90%) with complicated sepsis. After adjusting for age, ISS, and clinical risk factors for complicated sepsis (sex, initial base deficit, blood transfusions, and severe thoracic trauma), carriage of the TLR4 896 G-allele was associated with a decreased risk of complicated sepsis (OR 0.29, 95% CI 0.12-0.69, p = 0.005). This observation is similar to our previous finding in a smaller, independent cohort of trauma patients, but does conflict with other published reports.

We sought to further characterize how variation in the TLR4 gene might influence the risk of complicated sepsis. In order to do so, DNA samples were genotyped for six "haplotype tagging" SNPs (htSNPs) (Figure 2). These additional markers of TLR4 variation were evaluated in the context of the previously described risk factors by including them in the multivariate logistic regression analysis. Including these htSNPs markedly changed the results of our initial analysis and demonstrated that the risk of complicated sepsis associated with TLR4 genetic variation was associated with two htSNPs rather than the +896 variant. Additional examination of the TLR4 haplotypes demonstrated that the +896 SNP, exists in association with (is linked to) the haplotype tagged by htSNP4. In this large cohort of severely injured trauma patients, the decreased risk for complicated sepsis seems more strongly associated with carriage of htSNP4 than carrier status of the specific TLR4 G896A variant. Our observations indicate that TLR4 variation is indeed associated with sepsis severity after traumatic injury. We are looking for additional, potentially causative variation which may be responsible for differences in inflammatory signaling and clinical outcomes.

Additional studies of MAPK phosphatase (MKP-1/DUSP1) as a potential mediator of epinephrine induced immune suppression

Cyclic AMP (cAMP) is a prototypic intracellular second messenger with many effects. It is a common pathway for a number of extracellular signaling molecules that signal through G-protein coupled receptors. Epinephrine is one such signaling molecule that increases levels of cAMP via the G-protein-coupled β-adrenergic receptor. Sympathetic activation with local and systemic release of adrenergic mediators such as epinephrine is an important component of the immediate stress response that leads to increased intracellular cAMP in those cells and tissues expressing the β-adrenergic receptor. Data indicate that stimulation of β2 adrenergic receptors (β2AR) increases intracellular cAMP and decreases production of proinflammatory cytokines, such as TNF-α, while increasing production of others, such as the anti-inflammatory cytokine IL-10. These changes in the balance of inflammatory responses may have important implications for an individual's ability to respond to

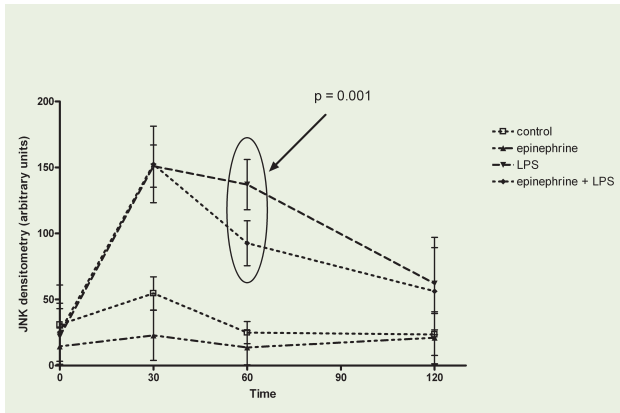

Figure 3: JNK Time Course

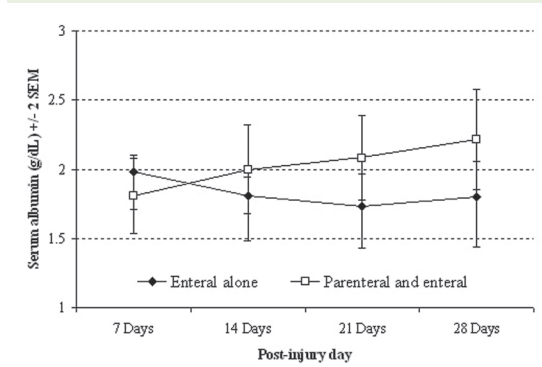

Figure 4: Albumin TPN

infection during times of stress, such as acute traumatic injury. The intracellular mechanisms leading to these effects, particularly to the suppression of TNF-α release, are unclear.

Using Affymetrix GeneChips, and applying Ingenuity Pathway Analysis, we have identified a potential role for the MAP kinase phosphatase MKP-1 (also known as DUSP1). We have conducted additional experiments in which we have examined the activation and subsequent deactivation of MAP kinase proteins in innate immune cells. In our initial experiments, we observed that MAPK activation was not influenced by β-adrenergic inhibition. However, based upon the results of gene expression analysis, which suggested that MKP-1 may be important, we performed additional experiments looking at both phosphorylation and subsequent dephosphorylation of p38 and JNK. Both of these are intracellular signaling molecules important to inflammatory activity. The results of our experiments are shown in Figure 3 and demonstrate that, while JNK phosphorylation/activation is not influenced by β2AR stimulation, JNK dephosphorylation occurs earlier and to a greater degree (see the 60 minute time point) in the presence of epinephrine stimulation. We conclude that adrenergic activation influences inflammation through MAPK dephosphorylation, reducing TNF-α gene transcription and translation.

Critically ill patients are often treated with adrenergic and anti-adrenergic agents that likely influence inflammatory cell activation and possibly also clinical outcomes. Furthermore, it is possible that manipulation of inflammatory signaling by epinephrine (by blocking or enhancing) can be exploited to restore homeostasis in critically ill patients and minimize complications such as septic shock and remote organ failure. It is possible that the phosphatase pathway represents an important regulator of innate immunity that is related to epinephrine and cyclic AMP.

Nutritional support in the intensive care unit

This arm of our research program is focused on an important clinical aspect of managing critically ill patients: the role of artificial nutritional support. Severely injured patients have marked metabolic derangements, generally characterized by increased substrate utilization and protein catabolism. Bench and clinical research has provided ample evidence supporting the notion that specialized nutritional support is beneficial and improves important clinical outcomes in the critically ill. However, marked differences exist in the application of the evidence to the care of these patients. The results of a recent survey of nutritional support practices in intensive care units (ICU) indicate that few patients receive more than 50% of estimated caloric or protein requirements in the initial 5 days in the ICU and that intake is quite variable from patient to patient. Our focus here is on the use of parenteral nutritional (PN) support. Although well-studied in patients undergoing elective surgical procedures, there is little evidence supporting the use of PN in severely injured patients. In this setting, most published studies comparing parenteral and enteral routes have concluded that enteral nutrition (EN) leads to better outcomes than PN. However, the relative benefits and potential harm of PN in the context of partial EN are not known. It is assumed that any detrimental effects

The objective of our research program is to develop new knowledge in the areas of the biology of innate immunity and inflammation in the context of severe traumatic injury and infection. We also aim to learn how we can better apply existing therapies to critically ill patients.

of starvation might be overcome by parenteral support in patients unable to tolerate full enteral support. This assumption is the basis for recommendations by several organizations that include the administration of PN when adequate enteral nutrition cannot be reliably achieved within 7 days of injury in critically ill patients. It is unknown whether earlier institution of PN would be beneficial particularly in circumstances where enteral support may be partially successful but usual caloric targets are not achieved. There are conflicting recommendations regarding the use of supplemental PN when nutritional support targets are not reached. Some suggest that underfeeding is associated with adverse outcomes whereas others note that there is no advantage conferred by supplemental PN and recommend that PN not be used in combination with EN. We analyzed a large multi-institutional database and asked whether initiating PN in the initial 7 days post-injury beneficially influences outcomes.

In the initial analysis of 567 severely injured patients, those who received early PN (95/567, 17%) had more severe injuries, had a greater severity of shock and received more red blood cell transfusions in the first 12 hours than patients who did not receive early PN. Abdominal injuries were more severe (higher abdominal AIS score, a greater percentage with hollow viscus injuries and treatment with laparotomy and/or an open abdomen) in those receiving early PN. Patients received PN for a median of 4 days (25th – 75th percentile = 2 – 9 days). A substantial number (63/95) of patients receiving early PN received little or no enteral support during the first post-injury week. Of the entire 567 patients, those receiving early PN were more likely to develop a nosocomial infection than those who did not receive early PN (relative risk (RR) = 2.1; 95% CI = 1.6 – 2.6, $p < 0.001$).

We then limited our analysis to a total of 249 (44% of the original 567 patients) who were "enteral tolerant" and received at least 1000 kcal/day on at least 1 day during the first week. Of these, 217 (87%) received only EN during the initial week and 32 (13%) received additional PN. The median day of PN initiation was 3 days post-injury (IQR: 2 – 5 days). The risk of nosocomial infection was higher in the group receiving early EN + PN (RR = 1.6, 95% CI = 1.2 – 2.1, $p = 0.005$). After adjustment, combined EN + PN remained associated with an increased risk for nosocomial infection when compared to patients receiving EN alone. Considering the association between EN + PN use and specific infections, the largest increase was observed in the risk of blood stream infections (RR = 2.8, 95% CI = 1.5 – 5.3, $p = 0.002$). Enteral tolerant patients who received additional PN had an approximate 2-fold increased risk of death before (RR = 2.3; 95% CI = 1.0-5.2, $p = 0.06$) and after adjustment (aOR = 2.7; 95% CI= 0.8-8.8, $p = 0.10$). Other adverse outcomes were more common in patients receiving combined nutritional support.

There was no evidence that patients receiving EN + PN had higher blood glucose concentrations nor were they more likely to be "hyperglycemic" when using different serum glucose concentrations to define hyperglycemia (110 g/dL or 130 g/dL, for example). The maximum serum glucose concentration measured during the first week was similar in patients who did and did not receive early PN (165 ± 28 g/dL versus 164 ± 34 g/dL, p-value = 0.5). There was no difference in the maximum serum albumin between groups during the first week, with serum albumin in both groups generally below normal (2.0 versus 1.8 g/dL, $p = 0.11$). However, and of considerable interest, the maximal albumin did indeed eventually reach higher levels in patients receiving combined support as shown in Figure 4. Although

these data do suggest a beneficial biochemical effect of PN that is manifest by serum albumin concentrations, this was not accompanied by an improved clinical outcome.

Our data suggest that parenteral nutritional support, when commenced during the first post-injury week along with a moderate amount of enteral calories, does not provide measurable clinical benefit and is associated with an increase in the risk of nosocomial, particularly blood stream, infections.

Summary

Our research program aims to understand the genetics and biology of critical illness, particularly in severely injured patients. We hope to be able to use our knowledge of host genetic influences on infection risk and outcomes, to enable us to focus and test better treatments. We are also interested in understanding how our available treatments (such as parenteral nutritional support) are beneficial, and in some circumstances, harmful.

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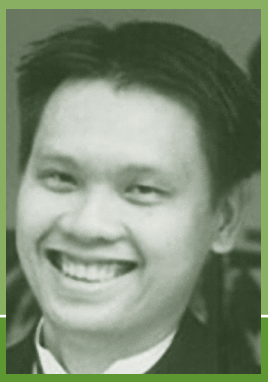
- Older adults' responses to trauma and burn injury
- Infections in surgical patients



Assistant Professor

AWARDS

American Burn Association

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John A. Hartford Foundation Centers for Excellence in Geriatric Medicine

- AAST Foundation Research Scholarship

With the aging of the US population, traumatic injury in older adults is expected to reach epidemic levels and has already created a major impact on trauma systems. At our Level I Trauma and Regional Burn Center, falls surpassed motor vehicle crashes as the leading cause of trauma deaths for the first time in 2006. The mean age in this group of patients was 69 years old. Approximately 10% of burn patients admitted to our institution are over 60 years of age, but they account for 45% of hospital deaths. Appropriate treatment strategies are therefore critical in order to improve outcomes for injured older adults.

Older adults' responses to traumatic injury

We seek to better understand why outcomes in older injured adults remain far inferior to those of younger patients. Although trauma centers deliver superior care for the injured, a recent national study found that patients aged ≥ 55 did not similarly benefit. Thus, better stratifications of outcomes and improved understanding of aged responses to injury are necessary to develop effective treatment strategies. Our recently completed analysis of the National Burn Repository (NBR) demonstrated age-dependent differences in resource utilization and mortality risk within the older burn population. Our ongoing projects using the NBR focus on practice variations in the care of older patients, and the impact of complications and comorbidities on their post-injury outcomes.

Our translational program aims to delineate unique aspects of the response to injury in older adults. Cohort studies have indicated an association between beta-adrenergic blockade and decreased mortality after traumatic brain injury. In burn patients, beta-antagonist treatment markedly reduces post-burn hypermetabolism. We propose that beta-adrenergic blockade may influence innate immune responses, and confer protective effects in older patients. We are evaluating the impact of aging and beta adrenergic antagonist exposure on monocyte activation, a key initiating event in innate immunity. We are conducting a prospective observational study of trauma patients (younger adults and older subjects) admitted to our Harborview Medical Center (HMC). In this study, peripheral blood monocytes (PBMC) from enrolled subjects will be isolated and activation assays will be performed with and without exposure to the non-selective beta antagonist propranolol. Results of this project will be used to further explore aging and beta-antagonist effects on downstream PBMC gene expression.

Older adults admitted for burn and trauma injuries present an opportunity for the screening of chronic conditions that could affect their future health. Osteopenia and osteoporosis constitute a so-called "silent disease" since their consequences do not manifest until very late in the disease process. Hip and vertebral fractures associated with osteoporosis are a significant morbidity in older adults and often will severely limit the return to pre-injury function. The prevalence of chronic bone loss in the injured older patient population has not been well-described to date. We postulate that older adults who suffer traumatic injury represent a particularly high-risk group since they may be medically underserved, and may be more apt to risk-taking behaviors. The purpose of our pilot project is to obtain an estimate of the prevalence of this disease in older injured adults admitted to HMC using DEXA screening and

Our recently completed analysis of the National Burn Repository demonstrated age-dependent differences in resource utilization and mortality risk within the older burn population.

correlative laboratory tests. These tests will be repeated at a 2 year follow-up interval, in order to provide an estimate of the impact of traumatic injury and its treatment. Data gained will form the basis of an intervention proposal.

Infections in surgical patients

Ventilator-associated pneumonia (VAP) represents the most common nosocomial infection in mechanically ventilated patients in the Surgical Intensive Care Unit (SICU), with reported prevalence rates four times higher than in medical ICUs. Hence, the accurate diagnosis and treatment of suspected VAP constitute high priorities in surgical critical care. Many tools and guidelines developed for the general ICU population remain to be studied and validated in the injured patient population. In our first study, we documented that the Clinical Pulmonary Infection Score (CPIS) did not increase the clinician's ability to predict VAP in burn patients, a finding similar to previous findings in trauma patients. Therefore, a quantitative strategy (i.e. bronchoscopy) to diagnose VAP in burn patients is preferred. In our second project, we have evaluated the practice of selective empiric antibiotic treatment while awaiting definitive bronchoscopy results in critically ill trauma patients with suspected VAP. Our results indicated that this selective strategy is not associated with adverse outcomes and constitutes good antibiotic stewardship. Our current project attempts to define treatment failure in Methicillin-Resistant Staphylococcus Aureus (MRSA) pneumonias in critically ill patients.

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PEDIATRIC SURGERY

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· Outcomes Research in the Pediatric Patient

Assistant Professor

FUNDING

CHRMC Academic Enrichment Fund Award
CHRMC Clinical Outcomes Steering Committee Award

Clinical outcomes research in pediatric surgery is a relatively uncharted field. As a subspecialty, we treat a huge variety of diseases rarely. Our research focus at Children's Hospital has been to develop a model for approaching a surgical diagnosis, questioning the assumptions upon which our current practice decisions are based, and identifying appropriate historical and appropriate new processes and outcomes in order to improve the care that is delivered to our patients.

We have begun with the model of Gastroesophageal Reflux Disease in infants and children. Currently, this is one of the most common diagnoses in pediatrics, resulting in a significant over-prescription of anti-acid medications among children. Many children, however, fail medical management of their GERD and are ultimately referred to surgeons for anti-reflux procedures (ARPs). While the diagnostic algorithm to identify and treat this disease in adults is standardized, the pathway in children is variable and controversial. This variability stems largely from the multitude of etiologies and symptoms, the fact that most of the patients are not able to communicate their symptoms directly, the lack of evidence that specific symptoms are truly attributable to GERD, and the variability with which these aspects of the disease are reported in the literature.

Our task in addressing this problem therefore has several layers. First, we must provide evidence that the historical model of diagnosis and treatment does not provide the best outcomes. Several recent publications and pending publications have demonstrated this to be the case. For example, a population-based study that we performed suggests that though most children that undergo ARPs are hospitalized at lower rates after the operation with reflux-related diagnoses, children that have their surgery at age four or greater, and children that are developmentally delayed, may in fact be hospitalized at rates that are higher afterwards. This starkly contrasts previously held beliefs that developmentally delayed children are the population that would benefit most from this operation.

Second, we must identify which symptoms are reliably attributable to GERD in infants and children, and subsequently we must learn how to reliably confirm the diagnosis. Symptoms that are associated with GERD in children can be broken down into several categories: mild esophageal symptoms, severe esophageal symptoms, and extra-esophageal symptoms. Unfortunately, many of these "symptoms" are observed behaviors, and not necessarily directly attributable to GERD. For example, regurgitation is a normal physiologic event in infants. The natural history of this is progression over the first year of life such that up to 70% of children will "spit-up" at least twice each day. This prevalence should diminish to about 7% by 12 months of age. The transition from normal regurgitation to the diagnosis of GERD occurs when a child develops symptoms such as pain, weight loss and failure to thrive, and feeding aversion. Many studies, however, report greater than 90% resolution of symptoms among children who have ARPs, yet when evaluated post-operatively by objective measures of GERD such as pH studies, up to 30% continue to have "silent" reflux. In fact, the use of objective studies to confirm the diagnosis of GERD is extremely variable. A recent publication evaluating over 7,000 ARPs from 7 major hospitals confirmed that only 68% of patients have an upper GI study, and only 54% of patients undergo pH testing prior to surgery. Many of these operations, we can conclude, are therefore performed based on symptoms rather than objective measures of the presence

Gastroesophageal Reflux Disease in infants and children is one of the most common diagnoses in pediatrics, resulting in a significant over-prescription of anti-acid medications among children.

of this disease, and given that approximately 60% of these operations are occurring in children under one year of age, it is likely that it is not the patient who is reporting these symptoms directly. This confirms the need to identify appropriate measures of outcomes.

Third, we must identify the best measures of the success of our medical and/or surgical interventions. Another study that is currently pending publication demonstrates that the rate of ARPs is incredibly variable across free-standing pediatric hospitals nationwide. Compared to other common operations such as appendectomy, pyloromyotomy, and gastrostomy tube placement, the rate of ARPs over the past six years has increased eleven-fold over these other common procedures in some hospitals, and decreased over forty-fold in others. This suggests that the indications for this operation have grown increasingly controversial over time as pediatric surgeons have started to evaluate more objective measures of outcomes. At present, there are no population and disease-specific measures of quality of life. A major part of our research effort has been to develop such a tool for not only evaluating the quality of life of children with GERD, but, as importantly, measuring the impact of this disease on the patient and his/her family and caregivers. In the course of our study, we identified that GERD impacts not only a child's health, but also the ability of the family to enjoy dinner together, to vacation, and to perform daily tasks. Many families of children with severe GERD find that they cannot go out into public without eliciting significant social scrutiny and significant planning ahead with extra clothing and special formulas, and have therefore learned many coping mechanisms that we have identified. Once we have validated this tool, we will use this tool to measure the impact that medical and surgical intervention have on these important consequences of this disease. Additionally, we are studying the relationship between specific symptoms and this quality of life tool as well as the various objective measures of GERD that are available to us such as UGI studies, pH tests, manometry, nuclear medicine studies, and esophagoscopy.

This effort is being addressed by a multidisciplinary team including pediatric surgery, pediatric gastroenterology, and pediatric pulmonary medicine, and several other disciplines. We have created an algorithm for the diagnosis of children with GERD, and we are prospectively following patients over time in order to identify the natural history of this disease in children, the impact of specific underlying medical conditions on this natural history, the association of each of the objective measures available to us and our QOL tool with specific symptoms, and tracking the impact of medical and surgical interventions.

In addition to the research described above, we are also evaluating several other surgical conditions that affect infants and children. We are looking at the variety of treatment options for pediatric empyema, and creating an algorithm for its diagnosis and treatment. We are looking into infants with gastroschisis in order to identify co-morbidities and treatment options that can improve outcomes such as time on TPN, time to full enteral nutrition, time in the hospital, and factors associated with re-hospitalizations, development of short bowel syndrome and need for transplantation. We are also studying infants with biliary atresia in order to identify factors that are associated with success of the Kasai Portoenterostomy, and factors leading to failure and ultimately liver transplantation.

Appendicitis is one of the most common surgical emergencies in children. Historically, this has been treated with aminoglycoside-based triple antibiotic therapy. A recent study identified that single-agent therapy is equivalent in terms of length of stay, and the occurrence of post-operative complications and re-admissions. Additionally, we are studying the efficacy of ultrasound imaging (US) in the diagnosis of appendicitis, and establishing new sonographic criteria for diagnosing this disease.

Several other projects are also in progress with other disciplines at Children's Hospital and Regional Medical Center. Along with members of the Department of Anesthesia, we have initiated a randomized trial of regional anesthesia versus conventional practice for post-operative pain-control in children undergoing umbilical herniorrhaphy. In addition to the above project on appendicitis, we are also working with our colleagues in radiology to establish new criteria for the sonographic diagnosis of hypertrophic pyloric stenosis.

Pediatric surgery remains an open field in terms of outcomes research that is at once exciting and daunting. We face particular challenges in that many of the outcomes that are easily quantifiable in adults are one step further removed in that we often cannot communicate directly with our patients. Additionally, many of the conditions that we treat are so rare, that true outcomes will not be validated unless we can organize multi-institutional studies. Though this type of collaboration may be challenging given the strong history and variability within this field, our department remains energetic about initiating this type of collaboration, and participating in studies initiated elsewhere.

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Kenneth W. Gow, M.D.

· Pediatric Surgical Oncology

Associate Professor

Childhood cancer is one of the leading causes of mortality in the pediatric population. Over the last several decades, however, survival rates have been steadily improving, with surgeons playing an important role in this upward trend. Specifically, surgeons insert central venous lines, biopsy for diagnosis, resect tumors, and follow-up to assess for complication or recurrence. We are striving to study each of these areas of contribution.

Central Venous Catheters

Central venous catheters (CVC) are one of the most common procedures performed in children. For children with cancer, these catheters are key to effective administration of chemotherapy. These catheters were developed at the Fred Hutchinson Cancer Research Center in Seattle [1, 2]. We are currently reviewing important steps in the insertion and the management of CVCs. With improvement in the process of insertion, we have succeeded in creating an enhanced system of delivery. We are also planning on standardizing other aspects of catheter insertion and management to try and reduce remaining rates of thrombosis and infections.

Biopsy

In terms of obtaining tissue for diagnosis, surgeons have two goals. First, surgeons need to be able obtain sufficient tissue. Second, they must do so via the least invasive means. Some situations require additional, specialized tools for securing the proper tissue. We have initiated a sentinel lymph node biopsy program that focuses on guided lymph node biopsy for patients with skin neoplasms and sarcomas, thereby establishing optimal staging for these patients [3]. Further, we have also been using ultrasound guidance to allow for better identification of some lesions, which previously have been difficult to isolate. One condition that might particularly benefit from ultrasound guidance is deep-seated lung lesions, where we have pioneered the application of minimally invasive thoracoscopic ultrasound (MITUS) [4]. This is just one of the many ways that we have utilized minimally invasive surgical techniques to improve biopsies, and ultimately outcomes, in children with cancer [5, 6]. Our center has been prominent in advocating the role of MIS for biopsy of masses in children as well as resection [7].

One condition that might particularly benefit from ultrasound guidance is deep-seated lung lesions, where we have pioneered the application of minimally invasive thoracoscopic ultrasound.

Resection

As discussed above, the role of MIS in resection of childhood solid tumors is a new area that we are helping to advance [7], specifically as applied to thoracic and abdominal sites. Excellent results have been obtained, and we continue to push the envelope in this area. Also, because some tumors have indistinct margins, we are also studying new tools and techniques that may further help pinpoint the tumor and allow for complete resection. Such cutting-edge tools include a hand-held PET probe and beta probes that optimize real-time intra-operative localizations. Other investigational areas include the use of "tumor paint," an injectable agent being pioneered by investigators at the Seattle Cancer Care Alliance (SCCA) [8].

Support

While surgeons play an important role in obtaining tissue for diagnostic and therapeutic purposes, we also serve as a valuable resource for supportive care during therapy. Support includes evaluation of patients having complications during chemotherapy, radiotherapy, or hematopoietic stem cell transplantation (HSCT). We have recently reviewed the Extracorporeal Life Support Registry to review the use of ECLS for children with neoplasms [9] or HSCT.

Follow-up

While we have made remarkable progress in the treatment and management of children with cancer, there has been a rise in long-term survivors. This has led to a relatively newer area of study, in which patients are tracked for long-term functional outcomes following therapy, as well as for the development of secondary malignancies.

Children's Oncology Group

While we study our local results, we actively participate in the Children's Oncology Group (COG), which is the national organization that develops the protocols for childhood cancer. Children's Hospital and Regional Medical Center is well represented throughout all of the major study groups in COG, which gives us the footing to review previous databases and to raise questions for future study.

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Robert S. Sawin, M.D.

· Neuroblastoma in the Pediatric Patient

The Herbert E. Coe Endowed Chair in Pediatric Surgery

AWARDS

Robert E. Condon Surgical Resident Competition
Wisconsin Surgical Society

FUNDING

U.S. Army, Madigan Army Medical Center
• Dept. of Clinical Investigation

Neuroblastoma is the most common solid malignancy affecting children. Despite treatments involving aggressive regimens of chemotherapy, and even bone marrow transplantation, the mortality for neuroblastoma remains 40 to 50%. The biology of an individual neuroblastoma tumor varies, with advanced stage tumors manifesting very different molecular and genetic features from those with early stage disease.

Perhaps the most intriguing feature of neuroblastoma is the well-documented spontaneous maturation of highly malignant tumors to a more differentiated benign variant, called ganglioneuroma. An understanding of this maturation process, including the molecular signals that trigger that change, might engender therapeutic methods that harness that maturation process.

Our laboratory effort has focused on a particular peptide growth factor, gastrin releasing peptide (GRP), which is expressed in both adult and pediatric tumors that are derived from neural crest cells. Our work has shown that GRP and its receptor, GRP-R, are both expressed in abundance by neuroblastoma cells in culture and by tumor cells removed from children. Our cell culture studies have also shown that inhibitors of GRP retard neuroblastoma growth.

We are presently working collaboratively with the Clinical Research Institute at Madigan Army Medical Center to define the quantitative differences of GRP and GRP-R expression in neuroblastoma as compared to ganglioneuroma. Our hypothesis is that these differences account for the virulence of the behavior of a given tumor. If verified, this observation would suggest that GRP antagonists might be useful clinically to stimulate maturation of neuroblastoma cells.

Our cell culture studies have also shown that inhibitors of GRP retard neuroblastoma growth.

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John Waldhausen, M.D.

· Surgical Treatment Review Improves Children's Healing Process

Professor

Pediatric surgery is in general a very clinically oriented field, though there is an increased emphasis on research in our division. At CHRMC most of our research activity has been oriented toward what we do in the operating room and on the hospital ward; however, new faculty have background in and are focusing on tissue engineering and outcomes research. It is important to examine the way we practice surgery and by either randomized prospective trial or by retrospective review determine how we can make changes that will benefit our patients. These studies may involve a wide spectrum of both congenital defects and problems encountered in the older child.

The treatment of Hirschsprung's disease, for example, as well as that of other congenital anomalies, has experienced a trend towards one stage surgical repair in the neonate rather than traditional delayed or multiple stage repairs. One of our recent submissions for publication detailed the technique and reported the results of our use of the transanal Swenson performed in the first several days of life. This technique, in which the Swenson is performed through the anus, thus avoiding a large abdominal dissection, had not previously been described. There are several advantages of the one stage repair. Colostomy is avoided, along with its potential complications, which in the infant may approach a rate of 20%. The length of hospital stay is decreased and hospitalization for colostomy closure is avoided entirely. In theory, long term function may be improved by earlier development of neural connections controlling anal sphincter function.

Minimally invasive surgery (MIS) is becoming an increasingly important technique in the treatment of pediatric surgical disease. MIS has often been advocated in both adult and pediatric patients based on its appeal to the patient or consumer rather than by any rigorous trial. In one attempt to correct this problem, several years ago an attempt was made at a national level with NIH funding to examine the efficacy of MIS in the pediatric oncology patient. The questions asked dealt with safety and accuracy in obtaining tissue for histologic diagnosis. Though this study never came to fruition at a national collaborative level, we examined our own results at CHRMC to determine whether both laparoscopy and thoracoscopy were useful, accurate ways to obtain tissue. We examined patient outcome and treatment of disease based on decisions made from tissues obtained by MIS techniques. MIS was found to be an excellent, accurate method with no adverse or inappropriate clinical decisions made based on the tissues obtained.

Many MIS procedures take special skills and advanced training in order to become proficient. Often these techniques are espoused to the surgical community with little regard as to what experience is needed to be able to reasonably perform the operation. Few MIS procedures in children are encountered as often as some of those in adults, so that the ability for any one pediatric surgeon to become very experienced may be limited. Some of our studies helped to establish a learning curve with laparoscopic splenectomy and pyloromyotomy so that other surgeons learning how to do the operation might know what to expect in the early stages of learning the procedure. We have also recently examined outcomes and results of both open and laparoscopic pyloromyotomy in order to determine the efficacy of the laparoscopic approach. Future projects will involve outcomes research in pediatric gastroesophageal reflux and Nissen fundoplication.

Few minimally invasive surgery procedures in children are encountered as often as some of those in adults, so that the ability for any one pediatric surgeon to become very experienced may be limited.

Other studies have answered simple questions about everyday clinical situations such as whether a period of water seal is needed to safely remove chest tubes in children. We have evaluated our use of ERCP in children when symptoms or studies suggested common duct gallstones and tried to discern useful protocols or pathways to help determine when ERCP should be performed preoperatively rather than after cholecystectomy and intraoperative cholangiogram. Our goal was to avoid unnecessary ERCP and the general anesthetic needed to perform it in children.

Ongoing collaborative efforts with colleagues in other divisions such as orthopedics have enabled us to expand the use of minimally invasive surgery for conditions such as pediatric scoliosis by doing thoracoscopic exposures as well as thoracoscopic anterior fusion and instrumentation. A joint effort with orthopedics and pulmonary medicine has allowed us to be part of a national collaborative study on the use of the expandable titanium rib, used to treat children suffering from thoracic insufficiency syndrome. Prior to the development of this device no good method existed for the treatment of this condition. It is hoped that the use of the expandable rib will allow us over time to expand the thorax of children with Jeune's syndrome or thoracic insufficiency from other congenital problems such as scoliosis, fused ribs or congenital diaphragmatic hernia. Children's was an FDA study center for the evaluation of this device and we are taking lead roles in determining the efficacy of this treatment.

Each of us in pediatric surgery does a high volume of clinical work and it is important to step back on occasion to examine how well one is doing and to question whether something could be done better. This has been our primary focus and the underlying intent of these and many other projects conducted in our division.

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PLASTIC SURGERY

RICHARD A. HOPPER, M.D., M.S.

MATTHEW B. KLEIN, M.D.

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Associate Professor

- Cleft Lip and Palate
- Syndromic Severe Midface Hypoplasia
- Craniosynostosis

FUNDING
CHRMC Craniofacial Endowment Fund
KLS Martin Ltd.

Craniofacial surgery is a relatively new subspecialty of Plastic Surgery, being officially initiated at the 4th Congress of the International Confederation for Plastic and Reconstructive Surgery in Rome in 1967. Since then it has become an active field of clinical and basic science research with the goal of improving the treatment of a broad spectrum of reconstructive procedures of the cranium and face. Our research is focused on the treatment of three specific birth defects affecting children: cleft lip and palate, syndromic midface hypoplasia, and craniosynostosis.

Sequential radiographic imaging is being used to learn how the facial bones adjust, remodel, and grow after they have been advanced such a large distance.

Clinical Research

CLEFT LIP AND PALATE

Children born with a cleft lip and/or palate require intensive multi-disciplinary care from the day they are diagnosed to the time they stop growing. In the first year of life the children undergo two fundamental operations; repair of their cleft lip and nose deformity at age three months, and repair of their palate at age one year. The goal of research in this field is to optimize these two operations so that the need for multiple secondary surgeries during early childhood and adolescence is minimized.

One way to optimize surgeries is to ensure that the infants are well nourished in preparation for the stress and post-operative healing of the procedure. From clinical experience we have identified a sub-group of infants with cleft lip and palate who do not gain weight and grow appropriately, despite standard of care feeding and nutritional intervention. If these infants can be identified before they demonstrate failure to grow, their diets could be tailored to prepare them for surgery.

We have initiated a study to measure the metabolic rates of infants with cleft lip and/or cleft palate using indirect calorimetry, and to compare these with clinical measurements such as weight gain, growth, and diagnosis. The study is taking place at the Craniofacial Center at Children's Hospital and Regional Medical Center. It enrolls 30 children a year in the study and follows them during the first year of their life, before and after each of their surgeries. The goal of the study is to create new guidelines for the nutritional care of infants with cleft lip and palate based on their individual needs.

SYNDROMIC SEVERE MIDFACE HYPOPLASIA

Children born with syndromes such as those described by Apert, Crouzon, and Pfeiffer can have such poor growth of their upper facial skeleton, or midface, that it compromises the closure of their eyelids and therefore the protection of their vision, the airway of their nose and therefore their ability to sleep, and the relationship between their upper and lower jaws and their ability to chew. The recognized surgical treatment of these children is to separate the upper facial

skeleton from the rest of the skull, known as a LeFort III osteotomy, then to move the upper face forward and secure it in place with bone graft harvested from the child's ribs.

The limitations of this traditional Lefort III advancement are that some of the child's ribs need to be removed and, because of the tightness of the skin and muscle overlying the upper facial skeleton, the face can usually only be moved forward around one centimeter. Repeat Lefort III operations or inadequate advancements were therefore not uncommon in children with severe midface hypoplasia, or restricted growth.

Over the past ten years, a technique known as distraction osteogenesis has been used to treat severe midface hypoplasia (Figure 1). This involves performing a Lefort III osteotomy, but instead of advancement and bone grafting, the incisions are closed and a skull-based distraction device is attached to the upper facial bones with wires. Over the next two to three weeks, the midface is slowly moved forward at a rate of 1 mm a day. This slow advancement allows the skin and muscle to adjust, such that advancements of up to three centimeters are possible. Once the advancement is complete, the device remains in place for two months while the fibrous tissue that has formed in the bone gap turns into solid bone. Bone grafts are therefore not needed.

Since midface distraction osteogenesis is a relatively new technique in evolution, we are actively researching ways to improve the process at the Craniofacial Center of Children's Hospital. A prospective Institutional Research Board (IRB)-approved study is underway to examine the psychosocial impact of the three month-long procedures on the patients and their families, and to suggest interventions to minimize the stress. Pre- and post-operative extensive sleep studies are being performed on all the children undergoing the procedure to examine the effect on quality of sleep. Sequential radiographic imaging is being used to learn how the facial bones adjust, remodel, and grow after they have been advanced such a large distance. Timing of how long it takes the new bone to form behind the advanced facial bones is also being studied to determine the optimum time to remove the distraction device.

Basic Science Research

CRANIOSYNOSTOSIS

Craniosynostosis is early fusion of one or more of the growth sutures of an infant's skull, resulting in a progressive deformity of the child's skull shape. In some cases craniosynostosis can also result in deviation of the position of the eyes and face, or can restrict the expansion of the brain as it grows. The majority of affected infants have isolated craniosynostosis with no family history of the birth defect and no other medical problems. Unfortunately, the current treatment of craniosynostosis is to subject these otherwise healthy infants to a joint neurosurgery and craniofacial plastic surgery operation with the need for blood transfusions and the risks of severe morbidity, or in rare cases, mortality. The ideal treatment of isolated craniosynostosis would be to prevent the suture fusion from occurring by blocking the responsible abnormal molecular pathway.

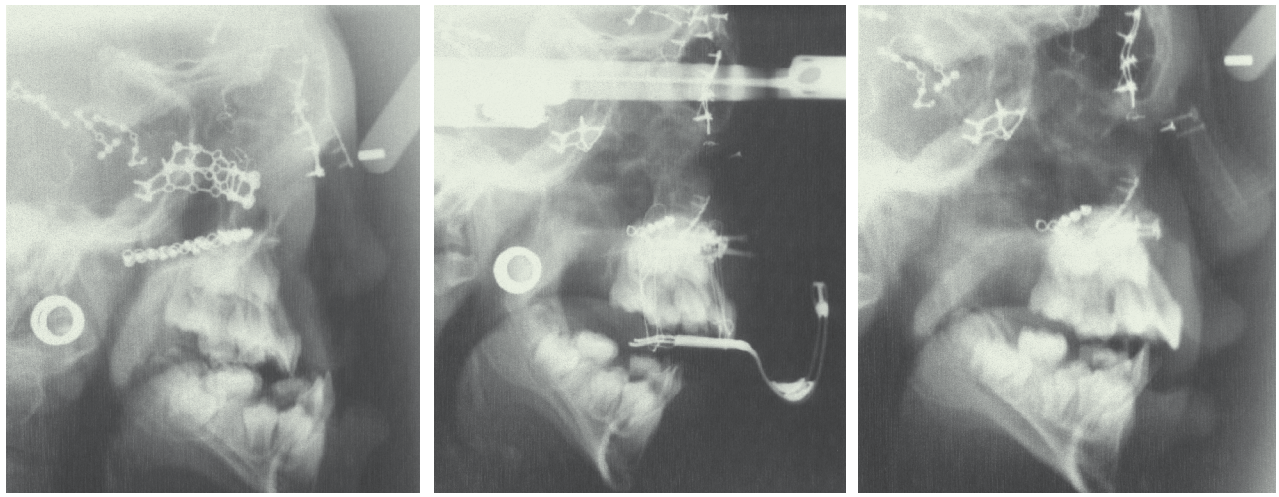

Figure 1: Lateral Cephalograms of a child undergoing midface distraction osteogenesis with an external device. (Left) Before the operation, the child is having problems sleeping due to constriction of her nasopharynx, problems with dry eyes due to lack of cheek protection, and a problem chewing due to her upper jaw being well behind her lower jaw. (Middle) The facial bones have been separated from the skull and the external distraction device has slowly advanced them over a period of two weeks. This process is not painful, but involves frequent follow-up visits and parent support. (Right) After removal of the device, the advanced bone has healed in a favorable position, with a small overcorrection to allow for future mandible growth.

There is a reliable sex ratio to the presentation of isolated craniosynostosis that has not been explained. Early closure of the sagittal or metopic sutures, both midline sutures, occurs predominantly in males. In contrast, coronal suture fusion is more common in females. Our theory is that there is a subgroup of individuals with craniosynostosis whose bone cells, or osteoblasts, are more susceptible to the *in utero* effects of sex hormones. Both testosterone and estrogen are present in the uterine environment, and from research on osteoporosis in the elderly, both are known to increase osteoblast differentiation into mineralized bone.

With IRB approval, we have been collecting bone samples from children undergoing craniofacial surgery for craniosynostosis and creating osteoblast cultures from them. Now that we have established primary cell lines representing different types of craniosynostosis and different sexes, we are examining the effect of different concentrations of sex hormones on osteoblast growth, differentiation and selective gene upregulation. Our goal is to identify patients whose osteoblasts have an increased susceptibility to the effects of sex hormones and to determine the molecular reason for this susceptibility.

Osteoblasts cultured from fused sutures grow faster than osteoblasts cultured from open, or patent, sutures. The prevailing theory is that osteoblasts around fused sutures are abnormal, however our alternate theory is that there are cells within normal sutures that serve to inhibit the growth of surrounding osteoblasts to prevent premature suture closure. In craniosynostosis, this normal inhibitory mechanism is lost, and fusion occurs. To test this theory, we have cultured sub-populations of cells grown from fused and open sutures in the same individual. We are examining differences in gene expression among these sub-populations and how one population can affect the growth of the other.

Osteoblasts do not exist in isolation in the skull. Bone healing involves a complex coordination between osteoblasts and adjacent blood vessel, or endothelial, cells. A collaborative project with Dr. Geoff Gurtner at New York University Medical Center examined the interaction of rat cranial osteoblasts with endothelial cells in the presence of pulsed electromagnetic fields (PEMF).

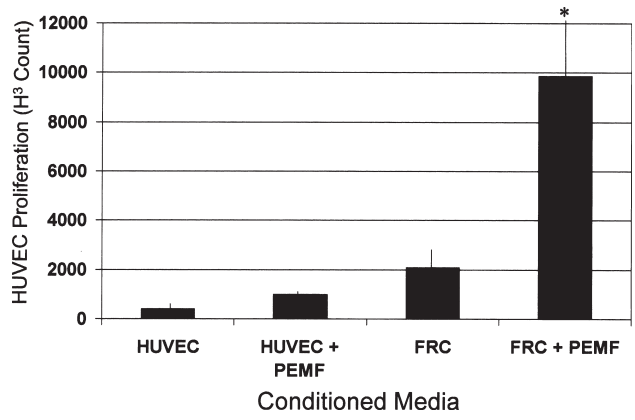

Figure 2: The effect of media from osteoblasts and endothelial cells stimulated with a pulsed electromagnetic field (PEMF) on endothelial cell proliferation. When human endothelial cells (HUVEC) were grown in media from fetal rat osteoblasts stimulated with PEMF (FRC+PEMF), their rate of proliferation increased significantly (* $p < 0.05$) compared to media from unstimulated osteoblasts (FRC) and stimulated or unstimulated HUVEC.

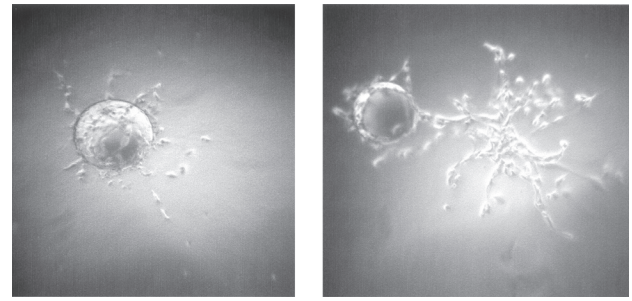

Figure 3: The effect of pulsed electromagnetic fields (PEMF) on endothelial cell tubulization. Endothelial cells attached to microspheres formed significantly more tubules when exposed to PEMF (left) than when not exposed the PEMF (right).

We found that when the osteoblasts were stimulated with PEMF, they secreted a protein that increased the growth rate of endothelial cells almost fivefold (Figure 2).

This dramatic increase in blood vessel growth does not appear to be due to the well known vascular endothelial growth factor (VEGF), therefore the next phase of the project is to identify the protein responsible. PEMF was also shown to increase directly the formation of early blood vessels, or tubules, by the endothelial cells (Figure 3). These two observations help us to understand better the beneficial effects of PEMF on bone healing, and may eventually lead to ways to create the same effect without the use of cumbersome electromagnetic devices.

As an exciting extension of our continuing work on craniosynostosis osteoblasts and osteoblast-endothelial cell interactions, we are collaborating with Professor Patrick Stayton of Bioengineering to use the technique of micropatterning to examine and manipulate cell-cell interactions in a controlled fashion.

OTHER CO-INVESTIGATORS

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Matthew B. Klein, M.D.

- Organization and Delivery of Burn Care
- Psychosocial and Functional Outcomes of Older Adults Following Burn Injury
- The Impact of Resuscitation Fluid Volume Received on Outcome
- Development of Validated Patient Reported Outcome Measurement Tools for Burn Survivors

FUNDING

National Institutes of Health
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Washington State Council of Firefighters Burn Foundation

The David and Nancy Auth – Washington Research Foundation Endowed Chair for Restorative Burn Surgery

Advances in critical care and surgical management have significantly improved survival following burn injury. Today, survival following extensive burn injury has become the rule rather than the exception. Accordingly, the emphasis of burn care and research has shifted towards optimizing the outcome of burn survivors. Within the overall theme of burn injury outcomes, my research has evolved into four domains: organization and delivery of burn care, psychosocial and functional outcomes of older adults following burn injury, the impact of fluid resuscitation volumes on outcome, and the development of validated instruments for assessment of burn outcomes.

Organization and Delivery of Burn Care

Burn care is a resource-intensive endeavor requiring specialized equipment and personnel. While the optimal national structure for delivery of burn care has long been debated, the need for organized systems and quality measures of burn care has received increased attention in light of recent concerns for mass casualty disaster planning. Our research in this domain has focused on the concept of regionalization of burn care; that is, a system in which a single center provides care over a defined geographic area as exists currently in the Pacific Northwest. The initial study performed on this topic was published in the international journal *Burns* in December 2006. This was a retrospective cohort study comparing the outcomes of patients transferred to our regional burn center with those of patients admitted to the burn center directly from the field. While there have been numerous previous studies demonstrating worse outcomes for trauma patients transferred to trauma centers from preliminary care facilities, there were previously no studies examining the outcome of transferred burn patients. In this study, we found that there was no difference in outcome between patients transferred to our burn center from a preliminary care facility and patients admitted directly from the field—a critical requisite for the delivery of burn care over a large geographic area in which patients will often receive initial care at a hospital without a burn center.

The next project in this domain examined the complications that occurred during the long-distance transport of a cohort of patients admitted to the UW Burn Center from 2000-2003, and the manuscript was published in the *Journal of Burn Care and Research* in 2007. This study demonstrated that patients can be transported safely and efficiently over long distances to a regional burn center. This finding has important implications for the organization of burn care nationally, given the decreasing number of American burn centers and the decreasing number of burn surgeons. In addition, these findings also have important implications for national disaster planning that must rely on safe and efficient triage and transport of burn injured patients in a mass casualty event.

Ongoing studies in this domain include an analysis of the geographic distribution of burn centers relative to population density and an analysis of population access to burn centers by ground and air transport utilizing two different geography databases. In addition, studies comparing the outcomes of patients treated at verified burn centers (verified by the American College of Surgeons/American Burn Association Verification Committee) with those treated at non-verified burn centers are underway, utilizing data from the national Healthcare Utilization Program National Inpatient database. Similar studies have been performed for non-burn trauma patients but have not been done for burn patients.

These findings have important implications for national disaster planning that must rely on safe and efficient triage and transport of burn injured patients in a mass casualty event.

Psychosocial and Functional Outcomes of Older Adults Following Burn Injury

Older adults are at increased risk for burn injury for a number of reasons and are at increased risk for adverse outcomes. The majority of the literature on elderly burn patients has focused merely on factors that influence survival, with less attention on the psychosocial and functional outcomes of those patients that survive their injuries. We have recently completed a study using data from the National Institute on Disability and Rehabilitation Research multicenter database examining the long-term functional outcome, health-related quality of life and psychological distress in a cohort of burn patients age 55 and older. A second project examining the impact of extent of burn injury and pre-injury comorbidities on morbidity and outcome utilizing data from the UW Burn Registry is currently underway. The findings from these two studies will be used to design an interventional strategy aimed at improving the outcome of older adults following burn injury. In addition, we are examining national trends in older adult burn injury and outcome utilizing the American Burn Association National Burn Repository.

The Impact of Resuscitation Fluid Volume Received on Outcome

Fluid resuscitation is a critical component of the acute care of a burn patient. There has been a recent trend towards larger volumes of fluid being administered following burn injury that has been purported to increase injury complications. In 2004 we published two studies examining the trend over the past 30 years towards larger volumes of fluid administered to burn patients: " Is Supra-Baxter Resuscitation in Burn Patients a New Phenomenon? " and "'Opioid Creep' Is Real and May Be the Cause of 'Fluid Creep.'" The first paper reported that the volume of fluids administered to a cohort of patients in 2000 was significantly higher than that administered to an age and injury-matched cohort from 1977. In the subsequent paper we examined the potential role of increased opioid administration over the same time period.

In 2006 we reported on the complication of orbital compartment syndrome in patients who received large volumes of fluid resuscitation in a manuscript published in the *Journal of Trauma*. This clinical study demonstrates the association between large fluid volumes received and the possible development of orbital compartment syndrome in a group of severely burned patients. Detection and treatment of orbital compartment syndrome can be critical to the prevention of ocular complications including decreased vision.

To better examine the factors influencing the need for large volumes of fluid resuscitation and to verify the long hypothesized association between increased fluids received and risk for adverse outcome, we analyzed the data collected as part of the multicenter NIH-funded "Inflammation and Host Response to Injury" project. The results of this study are reported in a manuscript that was published in *Annals of Surgery* in 2007. This is the first manuscript in the burn literature utilizing prospectively collected multicenter data to demonstrate an association between large volumes of fluid received and increased risk of adverse outcomes including mortality.

Ongoing studies in this research domain are focused on development of better statistical models that can predict adverse outcome based on fluids received, and plans are underway to try to develop an interventional study that will utilize alternatives to narcotics in the early post-injury period which may reduce fluid volume requirements.

Development of Validated Patient Reported Outcome Measurement Tools for Burn Survivors

Effective assessment of the impact of burn injury on psychosocial and functional outcomes and development of effective intervention and rehabilitation strategies are contingent on the availability of reliable burn-specific outcome measurement tools. Traditionally, burn research studies have relied on functional and psychosocial assessment surveys, which have been developed and validated using non-burn survivor populations. The validity of these tools for burn survivors has not been assessed.

As the first step in this project, we are performing a systematic review of the literature to determine which patent reported outcome (PRO) instruments have been used for burn outcome studies and which of these have been previously validated. We will then develop a concept bank of issues that are critical to burn survivors' functional, psychosocial and community integration aptitude. This concept bank will be developed from focus groups with burn survivors themselves as well as from focus groups with burn providers. It will also be used in the content validity assessment of currently available PROs and as the first step in the development of a PRO validated for burn survivors.

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DEPARTMENT CO-INVESTIGATORS

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· Immunologic Tolerance and Long-Term Survival without Chronic Immunosuppression

Assistant Professor

Worldwide there are currently 28 patients and 38 hand allografts that have been performed, with survival times from two to nine years. More recently, two separate French groups and a Chinese group have performed partial face transplants. All of these transplants are dependent on chronic immunosuppresion, with their attendant side-effects including malignancy and opportunistic infection. In addition, all of these transplants have demonstrated episodes of rejection of the skin that required treatment with steroids, monocolonal antibodies or an increase in the overall level of immunosuppression.

Our long-term goal is to devise practical methods for inducing immunologic tolerance that would allow for the long-term survival of these complex tissue organs without the need for chronic immunosuppression. This would significantly improve the risk-benefit ratio and allow for the more widespread use of CTA in the reconstruction of lost limbs and severe facial deformities. One method of inducing tolerance to an allograft is to establish a state of mixed chimerism. In a mixed chimera, the donor's immune system has become tolerant not only of the alloantigens expressed by host tissues, hematopoietic and immune cells. In turn, the recipient's cells are tolerant of the alloantigens on the surface of the donor's tissue and hematopoietic cells.

I. Establishment of stable immunologic chimerism in a canine model

We have established a stable dog leukocyte antigen (DLA)-identical marrow grafts. Two major advances achieved by this approach are:

1. Total destruction of the recipient's immune system is not necessary, as is practiced in conventional bone marrow transplantation. Instead, lower dose non-myleoablative conditioning is achieved with 2 Gy total body irradiation (TBI) before and a short course of immunosuppression with mycophenolate mofetil (MMF) and cyclosporine (CSP) after transplantation.
2. MMF and CSP are synergistic and capable of controlling residual host-vs.-graft (HVG) reactions and ameliorating graft-vs.-host disease (GVHD). This has allowed allogeneic hematopoietic cell transplantation (HCT) without severe organ toxicities and myeloablation characteristic of traditional high-dose conditioning regimens. The approach has been translated successfully into the clinic to treat patients with malignant and nonmalignant blood disorders.

Our long-term goal is to devise practical methods for inducing immunologic tolerance that would allow for the long-term survival of these complex tissue organs without the need for chronic immunosuppression.

II. Induction of immune tolerance to renal allografts using mixed chimerism

Using this preclinical canine model, with mixed chimerism, renal allografts were transplanted accompanied by bilateral native nephrectomies. With five-year follow up, we found normal renal function in all recipients and no histological evidence of acute or chronic rejection. This tolerance of the transplanted kidney did not extend universally to donor skin grafts. Two of four animals rejected their donor skin grafts in a delayed fashion. Yet, the loss of the donor skin graft did not affect the tolerant state of the kidney. This phenomenon has been noted before in tolerance derived from the engraftment of donor marrow. This has been labeled "split tolerance" where the animal is tolerant to a renal allograft but not a skin graft.

III. Successful Induction of Donor-Specific Tolerance to a Composite Tissue Allograft

We have recently extended this work, showing that this donor-specific tolerance can be induced in our composite tissue allograft (CTA) model. We developed a canine model for CTA with a myocutaneous rectus allograft. The myocutaneous rectus allograft is based on the deep inferior epigastric artery and vein. This vessel also gives off a superficial artery that directly supplies the skin. This allows for the allograft to be transferred as either a muscle allograft, skin allograft or a composite allograft of both muscle and skin. The allograft is harvested from the donor animal and transplanted into a subcutaneous pocket in the recipient. The artery is anastomosed to the femoral artery and the vein anastomosed to the femoral vein.

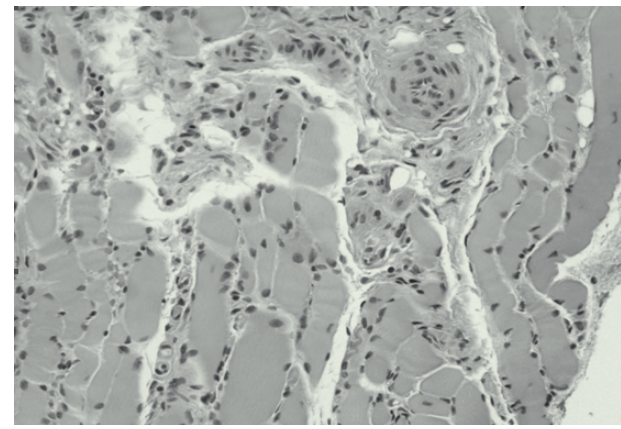


FIGURE 1: G244 Muscle bx Pod 41 days

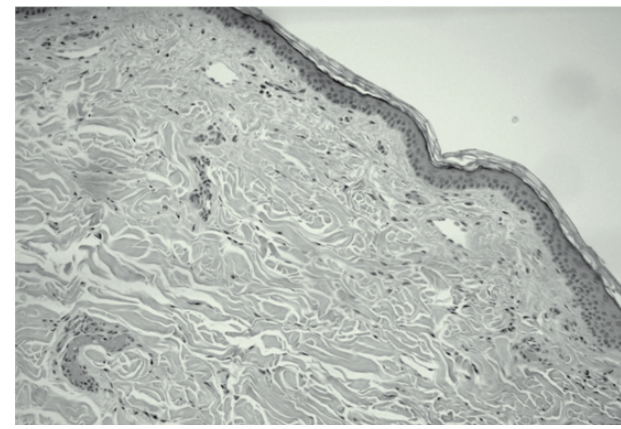


FIGURE 2: G767 Skin bx Pod 56 days

Mixed chimerism was achieved via a non-myleoablative conditioning with 2 Gy total body irradiation (TBI) and bone marrow transplant. After the injection of the bone marrow a short course of immunosuppression with mycophenolate mofetil (MMF) and cyclosporine (CSP) is given. The animals are then followed for the presence of donor cells by VNT-PCR. After stable mixed chimerism was established for greater than three months post-transplant the animals were ready for composite tissue transplantation.

The transplants were performed after establishment of stable mixed chimerism. The donor allografts were transplanted without any further use of immunosuppression. Currently, three animals transplanted with a complex myocutaneous tissue allograft have accepted their transplant for 60, 100 and 117 days respectively. The allograft were followed both clinically for evidence of rejection and through protocol biopsies. Thus far, the allografts appear normal with excellent hair growth. In addition, the biopsies have not demonstrated any evidence of rejecton. In contrast, a control animal was transplanted without any immunosuppression and the transplanted tissue was rejected in 15 days. This animal was not chimeric and the allograft became erythematous and swollen around day 11. The biopsies taken from the allograft have demonstrated signs of rejection. All animals are mixed chimeras. G767 is post-operative day 117 and has 49% donor granulocytes and 52% donor mononuclear cells. G244 is post-operative day 100 and has 98% donor granulocytes and 98% donor mononuclear cells. G500 is post-operative day 60 and has 41% donor granulocytes and 77% donor mononuclear cells. Thus, far we have not noted any evidence of skin rejection as was noted in the kidney allograft model. Histologic analysis has demonstrated minor but stable perivascular infiltrate but no signs of acute rejection (See Figure).

We have also begun to examine the allografts for the expression of Foxp3 in both the peripheral blood and in the allograft. Thus far, we have followed the peripheral blood of one of the dogs from the time of transplant and noted a drop in Foxp3 expression in the peripheral blood with a rise in expression of Foxp3 in the transplanted muscle.

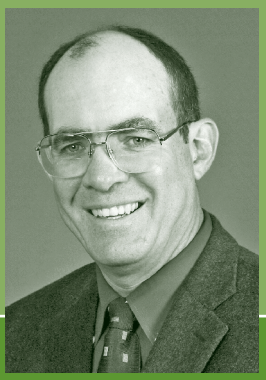
TRANSPLANT SURGERY

JAMES D. PERKINS, M.D.

JORGE D. REYES. M.D.

James D. Perkins, M.D.

· Clinical Outcomes Research in Transplantation



Professor

Clinical outcomes research examines specific illnesses and therapies and evaluates whether current practices are truly effective. Questions are asked about such factors as medication dosages, operative techniques, information management, and infection control. Based on the results of these studies, protocols may be adjusted to give improved results. Improved results in the field of transplantation might take the form of higher patient survival rates, lower rejection rates concomitant with low infection rates, more effective use of immuosuppressive therapy, or shorter hospital stay.

Below are some of the clinical outcomes research projects we are currently pursuing in the Division of Transplantation.

- ***Quality Control Studies in Liver Transplantation*** (James Perkins, M.D.; Jorge Reyes, M.D.)
- ***Liver Transplantation at Extremes of Body Mass Index*** (Andre Dick, M.D.; James Perkins, M.D.)
- ***An Automated Information System to Reduce Immunosuppressive Toxicity*** (Esther Park; James Perkins, M.D.)
- ***Factors Predict Prevention of Hepatocellular Carcinoma Recurrence After Liver Transplantation*** (James Perkins, M.D.; Jorge Reyes, M.D.; Oliver Lao, M.D.; Jennifer Weissman)
- ***Pediatric Liver Re-Transplantation*** (Oliver Lao, M.D.; Jorge Reyes, M.D.; Patrick Healey, M.D.; James Perkins, M.D.)
- ***Clostridium Difficile Infection in Solid Organ Transplant Patients*** (Steven Pergam, M.D.; Ajit Limaye, M.D.; James Perkins, M.D.; Connie Davis, M.D.)
- ***Surgeon Fatigue in Transplantation*** (Jeffrey Halldorson, M.D.; James Perkins, M.D.)

Research Opportunities and Resources

The field of transplantation is rich in possibilities for both basic science and clinical research. We are most fortunate in the Division of Transplantation at the University of Washington to have not only interesting questions to pursue, but also the resources available and an environment conductive to investigation. Our statistical expertise, together with our custom-designed clinical transplantation database, allows us to perform multiple clinical outcomes research projects per year as ideas are developed. Our transplant fellows are afforded an excellent opportunity to learn research methods and receive guidance and encouragement through faculty mentorship. Our most important resources are our people – our gifted faculty and fellows who ask important questions and persevere until they find answers. We look forward to the answers our research will bring in order to improve the lives of patients who can benefit from transplantation.

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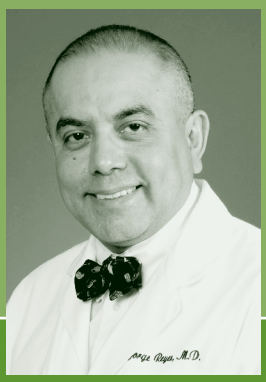
Jorge D. Reyes, M.D.

- Mechanism of organ transplant tolerance and rejection: The role of regulatory T cells (Treg), dendritic cells (DC), and costimulatory molecules on tolerance induction
- Assessing the safety of immunosuppression withdrawal in liver transplant recipients



Professor and Chief
Transplant Surgery

New immunosuppressive drugs improve the short-term survival of organ transplant recipients. However, long-term survival remains comparatively poor. This is likely due to the fact that immunosuppressive strategies are not tolerogenic. Transplant tolerance is likely to arise not from improved immunosuppressive regimens, but from improved understanding of the normal mechanisms that generate and maintain self-tolerance, and the ability to manipulate these mechanisms for the prevention and treatment of transplant rejection.

The overall goal of our research is two fold:

1) **Basic science investigations:** To define mechanisms of peripheral tolerance induction in order to develop new strategies to guide clinical therapy in transplant recipients. I am currently focusing on studying the cellular and molecular basis of immune mechanisms of organ transplant tolerance and rejection using our unique mouse orthotopic liver transplant (OLTx), heterotopic heart transplant (HTx), skin transplant (STx), or islet transplant (ITx) models. Our research uses the characteristics of TCR transgenic or gene knockout mice and costimulatory molecule blocking reagents to define and characterize the dominant factors involved in organ transplant tolerance induction. These factors include T cell subsets (including T regulatory cells [Treg]), the signals or pathways between antigen presenting cells (APC) (such as dendritic cells [DC]) and alloreactive T cells, both locally (in grafts) and systemically (in the spleen and lymph nodes), and the cytokines which modulate T cell activations and differentiations.

The goals of our research are:

- to further ascertain the mechanisms of organ transplant tolerance and rejection;
- to examine the ability of liver APCs to induce Treg and liver transplant tolerance, *in vivo* and *in vitro*, and to study the cytokines and costimulatory molecules that modulate this activity;
- to assess and maximize the therapeutic potential of DC and Treg in promoting tolerance induction in organ transplantation.

Mechanisms of murine spontaneous liver transplant tolerance and the role of regulatory T cells

It has been previously demonstrated that murine liver grafts are accepted spontaneously across all MHC barriers and induce donor-specific tolerance without immunosuppressive therapy (hepatic tolerance). The tolerance induced by a liver allograft can further induce the tolerance of subsequent organs such as a heart or kidney from the same donor origin. The tolerance is transferable to the naïve syngeneic mice by spleen or liver graft infiltrating cells obtained from long-term liver allograft recipients. Despite *in vivo* hyporesponsiveness to the liver allografts and to subsequent grafts from the same donor, *in vitro* mixed lymphocyte response (MLR) and cytotoxic lympholysis (CTL) assays showed unimpaired antidonor reactivity (split tolerance).

By contrast, livers from donors treated with Flt3 ligand (FL), which dramatically increases hepatic functional mature DC, are rejected acutely. This switch from tolerance to rejection is associated with marked reduction in apoptotic activity of graft infiltrating T cells, enhancement in costimulation between donor APCs, major DC and recipient T cells, and increased production of IL-12, IFN-γ,

We believe that these studies will provide better understanding of the mechanism of transplant tolerance and rejection, and facilitate novel therapeutic strategies to combat organ rejection and even autoimmune disorders such as diabetes.

and IL-10. The mechanism of liver tolerance continues to be extensively investigated and is considered by many to be due to the tolerogenicity induced by liver DC. Apoptosis of mature T cells in the liver, but with persistence of their precursors in the periphery, was suggested to be the explanation for split tolerance.

However, apoptosis alone cannot explain liver-induced tolerance to subsequent other organ grafts from the same donor strain. The liver tolerance seems to be an active process and one which is mediated by regulatory T cells. We hypothesize that inducing activated T cell apoptosis and Treg production are both critical to liver tolerance. Liver immature DC may be a key factor to induce Treg cell production and mediate activated T cell apoptosis. Co-stimulation between donor DC and recipient T-cells contribute to the T cell immune deviation, alloreactive T cell apoptosis, and function of regulatory T cells. To test our hypothesis, we treated liver donors or recipients with depleting anti-CD25 mAb. For the first time, we confirmed that depletion of recipient, but not donor, CD4+CD25+ regulatory T cells prevented spontaneous liver transplant tolerance. It was associated with enhanced anti-donor immune responses (MLR, CTL, NK activities, and Th1 cytokines IL-2 & IFN-γ production) and decreased alloreactive T cells, particularly in CD8 T cell apoptosis.

This suggests that recipient $CD4^+CD25^+$ regulatory T cells play a very important role in spontaneous liver transplant tolerance induction, and this Treg may mainly affect the indirect pathway of antigen recognition. Further studies on other potential mechanisms of $CD4^+CD25^+$ Treg on liver tolerance induction are undertaken in our laboratory.

The role of costimulatory molecules on tolerance induction

T cell activation requires two distinct signals: Signal 1 is antigen specific, mediated via the T cell receptors, and delivered in the context of donor MHC class II; Signal 2, the costimulatory signal, is not antigen specific. Costimulatory molecules, in particular the B7/CD28 super family, have recently been extensively studied. A number of new members have been discovered and characterized, including B7/ CD28, B7/ CTLA4, CD40/CD40L, and most recently PD-L/ PD-1, B7H / ICOS, OX40L /OX40, 4-1BBL/4-1BB, CD30L/CD30, and Tim3L /Tim3. It has already been known that B7/CTLA4, PD-L /PD-1, and Tim3L /Tim3 interactions provide a negative signal to the T cell, inhibit T cell activation and IL-2 production, and induce tolerance. On the other hand, B7/CD28, B7H/ICOS, CD40L/ CD40, 4-1BBL/4-1BB, and OX40L/OX40 interactions provide a positive signal to the T cells, promote T cell proliferation and IL-2 production, and induce immunity. Each of these costimulatory pathways may function independently or cooperatively with each other.

To examine the mechanistic relationships among these signals and precisely assess which signal is critical for transplant tolerance induction and rejection, our approach was a comprehensive investigation of their molecular constituents and functions on the alloimmune response. Using a model of orthotopic liver transplantation and heterotopic heart transplantation in mice with a costimulatory pathway deficiency, we analyzed the expression profiles of those genes and the outcome of the allografts. These studies on the role of these new accessory molecules and their effect on tolerance induction, activated T cell apoptosis, and possible promotion of Treg may provide crucial implications for designing a target for a trial of DC, antibody, or gene based therapy in patients receiving organ transplants.

We have recently tested costimulation blockade on liver DC and T cell interaction by using CTLA4 Ig and anti-CTLA4 mAb. The results showed that blocking both B7-CD28/ B7-CTLA4 signals using CTLA4 Ig promoted liver allograft survival from FL pretreated donors. It was associated with increased alloreactive T cell apoptosis in the liver graft and recipient spleen, and increased IL-10, decreased IFN-γ levels in the recipient serum. In contrast, blocking CTLA4 signal using anti-CTLA4 mAb, which was defined as a negative signal to the T cells, broke the liver spontaneous tolerance and induced liver allograft acute rejection. This was associated with decreased alloreactive T cell apoptosis in the liver grafts and recipient spleens, and increased IL-2, IFN-γ, decreased IL-4 production, and decreased the $CD4^+CD25^+$ regulatory T cells in the recipient spleens.

The role of dendritic cells (DC) in organ transplantation

DC, professional antigen presenting cells of the immune system, have been considered as having the potential to either stimulate or inhibit immune responses. Exploiting the immune-regulatory and tolerogenic capacities of DC holds great promise for the treatment of cancer, autoimmune disease, and prevention of transplant rejection. We have reported that liver immature DC play a critical role in the liver transplant spontaneous tolerance. We also reported that the immunoregulatory cytokine IL-10 induces Treg both *in vivo* and *in vitro* and promotes heart allograft survival in mice.

A recent report revealed that DC is capable of inducing $CD4^+CD25^+$ Treg which express CTLA4 and produce immunosuppressive cytokines IL-10 and TGFβ, down-regulating alloimmune responses. Costimulation between donor DC and recipient T-cells may not only contribute to T cell immune deviation and alloreactive T cell apoptosis, but also may lead to production of regulatory T cells. Thus, treating the allograft recipient with immature donor DC in the presence of IL-10 or TGFβ may drive regulatory T cell generation *in vivo* and promote organ transplant tolerance. We will challenge DC-treated recipients with allogeneic heart transplants or islet transplants (in NOD mice or STZ treated diabetes mice) to assess the therapeutic potential of DC-induced alloantigen specific tolerance.

We believe that these studies will provide better understanding of the mechanism of transplant tolerance and rejection, and facilitate novel therapeutic strategies to combat organ rejection and even autoimmune disorders such as diabetes.

2) Translational studies: Liver transplantation has progressed significantly since the first successful clinical trials performed in 1963, and has depended on improvements in organ preservation, immunosuppressive drug therapy, and improvements in surgical and peri-operative care. Survival has improved dramatically with the introduction of newer immunosuppressive drugs; however, though rejection of the transplanted organ is no longer a significant threat, the potential for infection and/or toxicity from drug therapy remains so given the lifelong need for treatment. Therefore, the ultimate goal is the acceptance of the transplanted organ without the need for lifelong drug therapy, a state called "tolerance."

In order to prevent organ rejection, patients receiving liver transplants currently require life-long treatment with immune system-suppressing medications to prevent the rejection of the transplanted liver. However, these medications can cause long-term side effects, such as infection, kidney problems, diabetes, and cancer. In patients infected with hepatitis C virus (HCV), these medications may increase the risk of HCV infection in the transplanted liver. The purpose of this study is to determine whether a slow withdrawal of immune system-suppressing medications is safe. The study also looks at whether slow withdrawal will help reduce the long-term side effects of immune system-suppressing medications and decrease the chance for HCV infection of the new liver in transplant patients with HCV. During and after the withdrawal phase, participants are closely monitored for liver allograft function, signs of rejection, levels of HCV in the blood and liver, and for the response of the immune system to the withdrawal of immunosuppression.

This is a Phase II NIH sponsored trial and may give us insights into the mechanisms for the achievement of tolerance, as well as when to withdraw immunosuppression.

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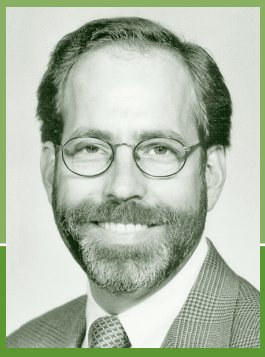
· The Breast Health Global Initiative (BHGI)



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Purpose and Specific Aims

The Breast Health Global Initiative (BHGI) is an ongoing public-private global health alliance devoted to medically underserved women, co-sponsored and co-led by the Fred Hutchinson Cancer Research Center and Susan G. Komen for the Cure. Benjamin O. Anderson, M.D., BHGI Chair and Director, founded BHGI in 2002. Dr. Anderson is Director of the Breast Health Clinic, Breast Care and Cancer Research Program and Professor of Surgery at the University of Washington and the Seattle Cancer Care Alliance. He is a Full Member in Epidemiology in the Division of Public Health Sciences at the Fred Hutchinson Cancer Research Center.

Mission: The *Breast Health Global Initiative (BHGI)* strives to develop, implement and study evidence-based, economically feasible, and culturally appropriate guidelines for international breast health and cancer control for low and middle income countries to improve breast health outcomes.

Through a series of invitation-only global summit consensus conferences (2002, 2005, 2007), BHGI has published consensus-based guidelines addressing **Health Care Disparities** (June 2003), **Resource Stratification** (Jan 2006) and **Guideline Implementation**. The completed BHGI resource-sensitive guidelines define comprehensive evidence-based pathways for coordinated step-by-step quality improvement in breast cancer early detection, diagnosis, and treatment.

Now in the phase of **guideline implementation**, BHGI is developing three critical and interrelated areas:

- Dissemination and Implementation (D&I) Research
- Education and Training
- Technology Application and Development

This implementation will take place through the development of **BHGI Learning Laboratories** in low and middle income countries (LMCs) in which information transfer can take place in an environment of collaborative learning, study and analysis.

Objectives

The goal of the BHGI is to improve breast cancer outcome among women from underserved communities through the successful dissemination and implementation of BHGI guidelines. The principle target is LMCs, although the same principles apply to underserved populations in high-income countries.

Background

Among women, breast cancer is the most common cause of cancer-related death worldwide, with case fatality rates highest in LMCs. Globally, breast cancer is the most common cancer among women, comprising 23% of all female cancers that are newly diagnosed in more than 1.1 million women each year. Over 411,000 deaths each year result from breast cancer annually, accounting for over 1.6% of female deaths from all causes. Projecting to 2010, the annual global burden of new breast cancer cases will be 1.5 million and an ever-increasing majority will be from

The foundation of the plan will be the creation of international learning laboratories, creating parallel laboratories in different parts of the world to develop and test modules that will form the basis for program expansion within low- and middle-income countries and regions.

LMCs. Approximately 4.4 million women diagnosed with breast cancer in the last five year are currently alive, making breast cancer the single most prevalent cancer in the world. Despite the common misconception that breast cancer is predominantly a problem of wealthy countries, the majority of breast cancer deaths in fact occur each year in developing rather than developed countries.

Low resource countries have generally not identified cancer as a priority health care issue because infectious disease is the predominant public health threat in such settings. Nonetheless, resources are inevitably spent on cancer treatment when patients seek medical care for what is typically advanced-stage disease. Cancer becomes an increasing problem in such countries as the control of communicable diseases improves and life expectancy increases. However, obstacles to improving cancer care arise from multiple sources, including deficits in public knowledge and awareness, social and cultural barriers, challenges in organizing health care, and insufficient resources.

Guideline development. Evidence-based guidelines outlining optimal approaches to breast cancer detection, diagnosis, and treatment have been well-developed and disseminated in several high resource countries. These guidelines define optimal practice, and therefore have limited utility in LMCs. Optimal practice guidelines may be inappropriate to apply in LMCs for numerous reasons, including inadequate personal resources, limited health care infrastructure, lack of pharmaceuticals and cultural barriers. Hence, there is a need to develop clinical practice guidelines oriented towards LMCs, specifically considering and adapting to existing health care resources.

Co-sponsored by the Fred Hutchinson Cancer Research Center and Susan G. Komen for the Cure, the Breast Health Global Initiative (BHGI) strives to develop evidence-based, economically feasible, and culturally appropriate guidelines that can be used in nations with limited health care resources to improve breast cancer outcomes. The BHGI held three Global Summits to address *health care disparities* (Seattle 2002), *evidence-based resource allocation* (Bethesda 2005) and *guideline implementation* (Budapest 2007) as related to breast cancer in LMCs. Modeled after the approach of the National Comprehensive Cancer Network (NCCN), BHGI developed and applied an evidence-based consensus panel process now formally endorsed by the Institute of Medicine to create resource-sensitive guidelines for breast cancer early detection, diagnosis, treatment, and health care systems, as related to breast health care in LMCs. The BHGI guidelines are intended to assist ministers of health, policymakers, administrators, and institutions in prioritizing resource allocation as breast cancer treatment programs are implemented and developed in their resource-constrained countries.

The Global summit series: 2002, 2005, 2007

A series of invitation-only BHGI global summit consensus conferences (2002, 2005, 2007) were the foundational basis of development and publication of the BHGI consensus-based guidelines addressing **Health Care Disparities** (June 2003), **Resource Stratification** (Jan 2006) and **Guideline Implementation**.

GLOBAL SUMMIT 2002 (SEATTLE): HEALTH CARE DISPARITIES

("International Breast Health Care: Guidelines for Countries with Limited Healthcare Resources," *Breast Journal*, May/June 2003: Vol. 9, Suppl. 2)

CONSENSUS STATEMENTS:

Early Detection Panel; Diagnosis Panel; Treatment Panel.

The first biennial Global Summit was held in Seattle, October 2002, to establish the first breast health Guidelines to address how care may best be provided in countries where significant gaps in health care resources exist. Guidelines development followed consensus panel analysis of evidence-based breast care modeling. 38 breast cancer experts representing 17 countries and nine world regions on three panels created Guidelines for breast cancer in countries with limited health care resources based upon definitions created by the World Health Organization (WHO) for

national cancer programs. The breast health care Guidelines were published as a supplement publication in *The Breast Journal* in 2003 and have been made freely available in an unrestricted fashion on the Internet for world-wide access (http://www.fhcrc.org/science/phs/bhgi/).

GLOBAL SUMMIT 2005 (BETHESDA):

RESOURCE STRATIFICATION

("Guidelines for International Breast Health and Cancer Control," Breast J 2006:12, Suppl 1:S117-120)

CONSENSUS STATEMENTS:

Early Detection and Access to Care Panel; Diagnosis and Pathology Panel; Treatment and Resource Allocation Panel, Health Care Systems and Public Policy Panel, and individual articles relevant to breast health care and cancer control in LMCs.

The U.S Office of International Affairs, NCI was the official host organization of the 2005 global summit. 67 participants from 33 developed and developing countries participated in the meeting to update and expand on the BHGI guidelines published in 2003. The 2005 BHGI panels outlined a stepwise, systematic approach to health care improvement in the areas of early detection and access to care, diagnosis and pathology, treatment and resource allocation, and health care systems and public policy, as they relate to breast health care in limited-resource settings. A tiered system of resource allotment was defined using four levels—basic, limited, enhanced, and maximal—based on the contribution of each resource toward improving clinical outcomes.

- **Basic level** — Core resources or fundamental services necessary for any breast health care system to function.
- **Limited level** — Second-tier resources or services that produce major improvements in outcome such as survival.
- **Enhanced level** — Third-tier resources or services that are optional but important, because they increase the number and quality of therapeutic options and patient choice.
- **Maximal level** — Highest-level resources or services used in some high resource countries that have lower priority on the basis of extreme cost and/or impracticality.

During this analysis, a number of key points were identified and/or demonstrated:

- Early breast cancer detection improves outcome in a cost effective fashion assuming treatment is available;
- The effectiveness of early detection programs require public education to foster active patient participation in diagnosis and treatment;
- Clinical breast examination combined with diagnostic breast imaging (breast ultrasound with or without diagnostic mammography) can facilitate cost-effective tissue sampling techniques for cytological or histological diagnosis;
- Breast conserving therapy with partial mastectomy and radiation requires more health care resources and infrastructure than mastectomy, but can be provided in a thoughtfully designed limited resource setting;
- The availability and administration of systemic therapy are critical to improving breast cancer survival;
- Estrogen receptor testing allows patient selection for hormonal treatments (tamoxifen, oophorectomy) which is both better for patient care and allows proper distribution of services;
- Chemotherapy, which requires some allocation of resources and infrastructure, is needed to treat node-positive, locally advanced breast cancers, which represent the most common clinical presentation of disease in low-resource countries;
- When chemotherapy is unavailable, patients presenting with locally advanced, hormone receptor negative cancers can only receive palliative therapy.

The Guidelines Tables that delineate cancer detection, diagnosis and treatment resources and services within an organized stratification schema are published in a January/February 2006 supplement to the *Breast Journal* and are available online (http://www.fhcrc.org/science/phs/bhgi/). These tools can be used to communicate programmatic needs to hospital administrations, government officials and/or health care ministries. It is the thesis of the BHGI that these works create a framework for change, by defining practical pathways through which breast cancer care can be improved in an incremental and cost-effective fashion.

GLOBAL SUMMIT 2007 (BUDAPEST):

GUIDELINE IMPLEMENTATION

"Guidelines for International Breast Health and Cancer Control—Implementation."

CONSENSUS STATEMENTS:

Early Detection Panel; Diagnosis Panel; Treatment Panel, Health Care Systems Pane l, focus group articles and individual articles relevant to breast health implementation in LMCs.

The American Society of Clinical Oncology (ASCO) was the official host organization of the meeting, held October 1-4, 2007 in Budapest, Hungary. The 2007 summit format fundamentally adhered to the 2005 Summit structure. 100 experts in medicine, science, advocacy, policy, public health,

health economics, sociology, and health communications from 40 countries came together in Budapest to address implementation issues of "best practices with limited resources." Preceding the Global Summit and in association with the Breast Health Global Initiative, Susan G. Komen for the Cure hosted and held a Global Breast Cancer Advocates Summit, *Ignite the Promise*, September 29-30, 2007 in Budapest (www.komen.org).

The 2007 BHGI summit focus shifted, from development and expansion of the 2006 *Guidelines for International Breast Health and Cancer Control*, to address effective implementation and integration of breast health care interventions described in the Guidelines. BHGI Global Summit panels addressed early detection, diagnosis, treatment, and health care systems.

Based upon reallocation of existing resources and incorporation of a breast health care program with existing programs to potentially improve outcomes in a cost-sensitive manner and infrastructure, the 2007 Global Summit will improve breast health services and ultimately breast cancer survival for women in geographic areas where resources are limited.

The aim of the health systems panel discussion was to identify effective, efficient and feasible approaches to cancer care delivery that promote high quality care in a climate of resource limitations, using breast cancer as a model disease that needs to be addressed in limited resource countries. Specifically, the panel:

- Outlined breast health interventions for early detection, diagnosis and treatment proven successful in the setting of limited resources;
- Defined programmatic approaches that support key breast health interventions that can be replicated in communities where resources are limited ;
- Provided guidance on how to overcome obstacles to implementation of breast health interventions when resources are limited;
- Fostered collaboration, coordination and integration of breast health services among clinical communities, public health researchers and advocacy and non-governmental organizations by defining optimal interventions that will effect real benefit;
- Supported the testing, implementation and development of new international and national pilot research, demonstration and technology development projects for breast health care in areas with limited resources;

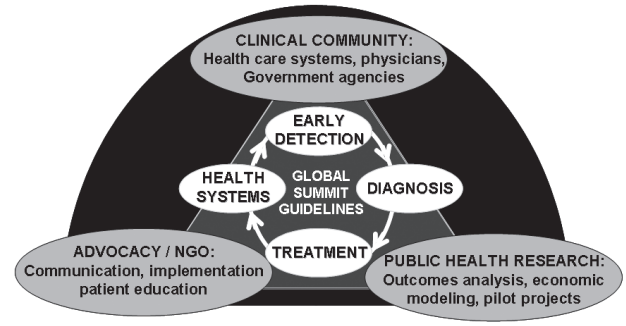

FIGURE 1: BHGI functions as a structure for linkage among stakeholder groups to implement change.

- Created the next iteration of the clinical guidelines (*Guidelines for International Breast Health and Cancer Control – Implementation*), the result of the global summit conference, which will be disseminated to educate policy makers, providers and patients about how breast cancer can be effectively treated in areas where resources are constrained; and
- Promoted the further development and use of economic modeling tools that will benefit countries and individual communities in determining cost-effective strategies to improve breast health outcome.

BHGI Organizational Linkages

The BHGI is a structure for linkages through interdisciplinary communication, cooperation and alliance-building via the Global Summits, ongoing communications, the Web site, and pilot research and demonstration projects between the clinical community, advocacy groups and NGOs, and public health sciences (Figure 1):

Guideline Dissemination: Publication and Distribution

The BHGI Guidelines have been disseminated worldwide and made freely available for downloading via the Internet from the Fred Hutchinson Cancer Research Center (FHCRC) and Komen for the Cure Web sites, as well as from BHGI international network organizations and other world health and medical organizations, governmental agencies, non-governmental organizations (NGOs), health ministries, patient advocacy groups, health related organizations, foundations, societies and associations.

The Guidelines are identified on PubMed and other medical literature search engines. The 2005 Guidelines published in 2006 are available through the **Agency for Healthcare Research and Quality-AHRQ National Guideline Clearinghouse** (**AHRQ-NGC**). The 2008 Guidelines will similarly be made freely available, and submitted to the AHRQ-NGC.

In their 2007 report, *Cancer Control Opportunities in Low- and Middle-Income Countries*, the Institute of Medicine (IOM) provides a detailed summary of BHGI methodology, which the editors identify as a model approach for developing resource-sensitive guidelines that could be applied to other cancers or chronic diseases for which effective treatments are available. A systematic evaluation of these outcomes awaits realization of implementation of the guidelines in a range of LMCs.

Guidelines do not in and of themselves improve outcomes for women. Implementation is the critical step by which the value of the guidelines may be measured. The next step for BHGI is to implement the evidence based Guidelines in LMCs to improve breast cancer outcome and to provide a framework for improvement in cancer outcome for all treatable cancers. Thus, the completion of guidelines development now leads into the next phase of program development: Guidelines implementation.

BHGI Five-Year Implementation Plan

THREE KEY FOCUS AREAS

1. DISSEMINATION & IMPLEMENTATION (D&I) RESEARCH
2. EDUCATION AND TRAINING PROGRAMS
3. TECHNOLOGY ASSESSMENT, APPLICATION AND DEVELOPMENT

To achieve Guidelines implementation, BHGI has outlined a five-year plan with three key focus areas: dissemination & implementation (D&I) research, education and training programs, and technology assessment, application and development. The foundation of the plan will be the creation of international **learning laboratories**, creating parallel laboratories in different parts of the world to develop and test modules that will form the basis for program expansion within low- and middle-income countries and regions.

1. DISSEMINATION & IMPLEMENTATION RESEARCH

Dissemination & Implementation (D&I) is a new, growing area of research to study how knowledge can be transferred to successfully achieve improved health outcomes, such as reduced morbidity from breast cancer. The 2007 BHGI Summit included discussions on knowledge transfer that will be continued over the next five years. Through the D&I program component, BHGI will be able to accelerate the successful adaptation and implementation of the Breast Health Global Initiative Guidelines for breast cancer care in low- and middle-income countries by identifying the attributes most relevant to organizational and behavioral change in health systems.

To do this the first necessary component was Guideline Development, which is now complete. The next component, **Implementation-Readiness Assessment**, is in process. Readiness assessment will build on the success of BHGI Guideline resource stratification and continue to use evidence-based research and expert consensus opinion. The BHGI will use evidence-based research and expert consensus to identify qualitative and quantitative tools that can be used to determine the readiness of a health care system to implement BHGI guidelines at the next level of care.

The 5-year program will develop and pilot-test implementation-readiness assessment approaches to create a readiness assessment package for free distribution globally. No such comprehensive package is available at this time; the few individual tools that are available address only parts of the process and have not been tested or adapted for LMCs. Assessment packages can be used to inform the design of optimal national guideline implementation strategies. These implementation-readiness assessment packages will include evaluation components for utility, validity, and feasibility.

Combining implementation-readiness assessment with the BHGI Guidelines and quality indicators will facilitate the implementation of breast cancer care guidelines and identify successful and sustainable programs to improve the care of breast cancer patients.

2. EDUCATION AND TRAINING

The second component of the BHGI five-year plan is Education and Training. Public education is mandatory to improve breast health outcome in LMCs. Regardless of resource availability, breast health outcomes cannot improve unless women understand the benefits of early detection and are willing to undergo timely diagnosis and treatment. BHGI will collaborate with Komen for the Cure and other organizations involved in public education and advocacy to support outreach efforts.

The BHGI professional education and training program will be developed with partnering organizations and key stakeholders throughout the world to create professional education and training curricula to ensure successful, coordinated and sustainable programs.

EDUCATION AND TRAINING PROJECTS

Development of Training Curricula for LMCs: Professional education and training programs for breast health care exist in many international settings. However, these efforts primarily target the education of health care providers in high-income countries about novel (and usually expensive) drugs and technologies used in the delivery of cutting edge care. These professional educational efforts typically do not address the specific needs of health care providers in LMCs where infrastructure is lacking or dysfunctional. BHGI will collaborate with organizations and agencies working on improving breast health care to develop curricula that is appropriately selected for target LMCs.

BHGI Learning Laboratories established in collaboration with sponsoring institutions in LMCs will become a venue for education and training. BHGI Learning Laboratories will create unique environments for information transfer, collaborative learning, study and analysis. A key principle in success of these learning centers is the recognition that experts coming from high-income, middle-income and low-income countries all have information, experience and skills to share. While experts from high-income countries may have expertise in the application of cutting-edge diagnostic tools or therapies, experts from LMCs have expertise in the reality of health care delivery in limited resource settings. Real world problem solving will require a collaborative approach using mutual knowledge transfer from all participants. Currently in process of development is the establishment of the first Learning Laboratory in Kumasi, Ghana.

3. TECHNOLOGY ASSESSMENT, APPLICATION & DEVELOPMENT

BHGI technology efforts will be focused in two areas: application of currently available technology and development of novel technology.

APPLICATION OF CURRENTLY AVAILABLE TECHNOLOGY

Even in low-income settings, some basic level technology is necessary to provide cancer care. Existing technology in imaging (ultrasound, mammography, x-ray) tissue sampling (minimally invasive needle biopsy) and pathology (histopathology and/or cytology, immunohistochemistry) are necessary resources for effective cancer care. BHGI guidelines will be used in LMC settings to identify needed standard technology infrastructure.

Sharepoint 2007 Portal for Breast Health Communication

International partnership addressing health issues in LMCs requires the development and application of low-cost communication tools to facilitate information transfer between partner organizations and also to make key information generally available to the public. In 2005, BHGI developed a Web site on the Fred Hutchinson Cancer Research server (www.fhcrc.org/science/phs/bhgi/) to facilitate outside communication regarding BHGI activities. The BHGI Web site serves as an information portal, providing access for downloading BHGI publications and materials. In 2007, BHGI developed a customized Internet portal to facilitate dialogue, information exchange and manuscript preparation for the 2007 Global Summit participants. This BHGI portal was written using the Microsoft program **Sharepoint 2003** and has become a hub of much of the international work of the Global Summit, facilitating organization of the Summit and the ensuing international collaborative writing of the *Guidelines for International Breast Health - Implementation*.

The BHGI Internet Sharepoint communication portal will be further developed and expanded, updating the BHGI portal to **Sharepoint 2007**, the newest iteration of this program. The BHGI Sharepoint 2007 portal will provide **open access** to **outside users** for dissemination of all BHGI health information. The **password protected work areas** will be improved in Sharepoint 2007 to facilitate **project management**, internal **data collection** and organization, and **networking** among international partners. In addition, the 2007 portal will provide the opportunity to create **eLearning** projects based on the results of the D&I research projects discussed above.

DEVELOPMENT OF NOVEL TECHNOLOGY

While some tools commonly used in high-income countries are unaffordable in LMCs, other simpler tools are available and can be applied. Special collaborations with technology companies can be formed for the development of modular diagnostic clinics that integrate clinical evaluation, basic imaging, tissue sampling and histopathological assessment to make accurate cancer diagnoses and prepare for treatment. Projects are currently in planning stages through BHGI collaborating organizations.

Pilot programs will be considered to test technology applications in imaging and pathology, and to develop measures of effectiveness. BHGI past research and discussions have laid the groundwork for these important next steps in technology assessment, application and development in LMC and regions.

During the five-year plan, BHGI will use science and expert consensus and develop multiple partnerships to help define the necessary tools, processes and timeframes for development. In the process, BHGI will define what a successful, sustainable learning laboratory looks like, identify laboratory locations and partners in diverse regions of the world, develop curricula, and pilot test methodologies to create sustainable learning modules.

In conjunction with the BHGI alliance and key stakeholders, a global meeting will be held in the next 18-24 months (date to be determined) to present the progress of the BHGI Implementation Plan.

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· Surgical Care and Outcomes Assessment Program

Professor

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The Surgical Care and Outcomes Assessment Program (SCOAP) is a prospective, surveillance system model in which Washington State's surgical community can work towards a common goal of improving patient care and health outcomes. SCOAP delivers timely data on appropriate use, effectiveness and safety of common surgical procedures to clinicians and hospitals and facilitates quality improvement initiatives to incorporate these data to reduce inappropriate care, readmissions, and re-interventions. The primary aims of the Life Sciences Discovery Fund SCOAP project are to increase the SCOAP network by 30 additional Washington State hospitals; promote public reporting of SCOAP safety, use and outcomes data; and assess the health economic impact of variability in surgical healthcare processes and outcomes, as well as demonstrate the positive health economic impact of appropriate healthcare utilization.

Background and Significance of Outcome Variability

Across hospitals in our state there is significant variation in the outcomes of nearly every surgical procedure. For example, administrative data reveal a five-fold difference in rates of death within 30 days of surgery for the treatment of obesity and similar variability in operative complications after colon resection and other major surgical interventions. There is also substantial variation in the use of evidence-based processes of care aimed at preventing errors and assuring quality. Across statewide hospitals, one in five patients who should receive antibiotics within an hour of an operation fail to receive them, one in three patients undergoing major surgery fail to receive appropriate agents to prevent blood clots, and one in five patients who are on heart protective medication fail to have that medication restarted after the stress of surgery. These processes of perioperative care are essential to the safe delivery of emerging interventions and healthcare technology.

As one of the largest purchasers of healthcare, the State of Washington is impacted significantly by the cost of this variability. Adverse outcomes such as re-interventions and reoperations increase healthcare costs and keep workers out of the workplace. Our group worked with the Washington State Health Care Authority (HCA) to calculate the potential financial benefits of a reduction in variation of adverse outcomes across the state. $300 million/year is spent on inpatient general surgery inclusive of 73,000 patient days. Costs savings of over $30 million/year — comprising 1,200 complications/year and 7,000–8,000 hospital days/year — could be avoided if one standard deviation of inter-hospital variation was reduced.

The Surgical Clinical Outcomes Assessment Program (SCOAP)

Investigators at the University of Washington and Foundation for Healthcare Quality have been working in partnership with the healthcare stakeholders from across the State to develop such a surveillance system for in-hospital procedures. The Surgical Care and Outcomes Assessment Program (SCOAP) focuses on diagnostic and interventional surgical technology associated with commonly performed and/or rapidly emerging surgical procedures. SCOAP tracks and feeds back data to hospitals and clinicians within a collaborative environment of clinicians, quality improvement personnel, administrators and other stakeholders from across the state.

We predict a reduction in statewide healthcare expenditures associated with more appropriate use of new technology and a reduction in readmissions/reinterventions.

SCOAP encourages the appropriate and effective use of surgical care and then tracks individual hospital level outcomes to direct QI towards evidence-based standards of care. SCOAP is a voluntary activity driving best practices in both small and large hospitals across the state. In its pilot years, SCOAP has been funded in part by the statewide chapter of the American College of Surgeons, early-adopting hospitals, and a stakeholder group including the Health Care Authority, DSHS and insurers. In its first two operational years, SCOAP created a surveillance system at 19 hospitals. In January 2008, the University of Washington received a $1.3 million dollar grant from Washington State's Life Sciences Discovery Fund to expand SCOAP into 30 additional hospitals across the state, promote public transparency in healthcare safety and efficacy, and to demonstrate the cost-effectiveness of appropriate surgical healthcare.

Hospital Participation

By the end of 2007 there were 19 SCOAP hospitals in Washington and Oregon (Legacy Hospital System, Portland). With the support of the Life Sciences Discovery Fund grant, enrollment in SCOAP has increased to 30 hospitals by June 2008. These hospitals are in both rural and urban environments across the state, include large and small hospitals/catchment areas, and represent approximately 70% of general surgical care in the state. The number of hospitals involved in SCOAP increases each quarter through active outreach by University of Washington and Foundation for Healthcare Quality investigators and the SCOAP management committee.

Publicly Reporting Healthcare Use, Safety and Efficacy

With support from the Life Sciences Discovery Fund, University of Washington and Foundation for Healthcare Quality investigators are developing a public access website within the SCOAP website (www.surgicalcoap.org) that will provide links to a subset of the aggregate SCOAP data for hospitals with explanatory lay-language hyperlinks and action items such as "What can you do about this information?" Within time, the website will change from aggregate, anonymous data listing to hospital identified data for public transparency about Washington State hospital safety performance. SCOAP process of care metrics will be informative to the public and provide accountability to patients.

The Health and Economic Impacts of Inappropriate and Appropriate Surgical Care

In a pilot study of SCOAP, Department of Social and Health Services of Washington State (DSHS) evaluated administrative claims data for SCOAP procedures for 90 statewide hospitals over a three year period. The median payment per procedure, length of stay and average outlier and readmission rates were calculated for each procedure across all hospitals in the state (2003-2006). Differences between individual hospitals' performance on these DSHS metrics and the median of all hospitals for that procedure were calculated. If the percent difference was a negative number, then for that parameter the hospital would be considered a "DSHS cost saver." When this linkage of SCOAP and DSHS data was completed (September 2006), there were 10 hospitals with at least one full quarter of SCOAP data. The report concluded that DSHS paid over 39 million dollars in recent years for care related to these index procedures at hospitals in the state. Among all statewide hospitals performing these procedures, there was

a high rate of cost outliers and it appeared that much of this cost and outlier status would be reduced by minimizing preventable adverse events such as reoperative surgery for operative complications, postoperative myocardial infarction because of beta-blocker withdrawal and pulmonary embolism because of failure to give appropriate prophylaxis. 87% of SCOAP hospitals were DSHS "cost savers" for at least one of the parameters of at least one of the operations they performed. SCOAP hospitals represented centers that were "better performers" from a healthcare utilization perspective than non-SCOAP hospitals. This DSHS report concluded that SCOAP promotes better health care utilization and saves healthcare dollars.

With the support of the Life Sciences Discovery Fund, we plan to conduct further health economic evaluations of SCOAP data collected over several quarters and at several hospitals. We predict that SCOAP participation and increased utilization of SCOAP process of care measures for new/emerging healthcare technology will be associated with a lower rate of in-hospital and post-discharge adverse outcome. To investigate this hypothesis we will make comparisons of SCOAP vs. non-SCOAP hospital procedures using a relational database including all Washington State hospital procedures using the Comprehensive Health Abstract Reporting System (CHARS). CHARS was established in the State of Washington in 1984 and is administered by the Department of Health. CHARS is one of the few hospital discharge datasets nationwide that is constructed to allow for longitudinal evaluations of outcome based on unique patient identifiers. We also predict that hospitalizations at SCOAP hospitals and "higher use" SCOAP hospitals (for process of care metrics) are associated with lower healthcare costs per operation than those at non-SCOAP hospitals or "lower use" SCOAP hospitals, respectively. Working with leading health economists at the University of Washington, a deterministic, payer-oriented model will be used to determine the cost saving associated with SCOAP participation and high-levels of SCOAP performance. The model is based on reduction in adverse safety outcomes (readmissions, re-interventions) and uses SCOAP performance data based on SCOAP participation for at least one year.

Future Directions

Expanding SCOAP through the LSDF will increase participation to 48 hospitals and emphasize its focus on innovative/emerging procedures and technologies. This will create an "aviation-like" surveillance system around new and emerging technology and will improve quality of care, promote the safe use of new technology and improve cost-effectiveness of our system. We predict a reduction in statewide healthcare expenditures associated with more appropriate use of new technology and a reduction in readmissions/reinterventions. Through public access to SCOAP reports we anticipate increased healthcare consumer confidence and greater satisfaction with Washington State healthcare legislation and legislative activity. Building on these successes, SCOAP aims to include all statewide hospitals, additional emerging healthcare technologies and procedures, and medical treatments beyond the in-hospital arena (e.g. cancer care, chronic care, and outpatient services).

SCOAP's future will be in continually redirecting its focus on safety, effectiveness, appropriateness and cost-effectiveness to emerging and high impact targets (i.e. new and emerging technology) and initiating quality improvement activities at every healthcare setting in the state. One of the responses to the SCOAP initiative has been to produce a safety and quality checklist for the operating room. The checklist is used at the start of surgery as part of an extended "time out" and after surgery as part of a debriefing. The goal is to have the checklist deployed in all Washington State hospitals by the end of 2009, and SCOAP will begin to facilitate this and other like quality improvement outreach projects into the Washington State community at large.

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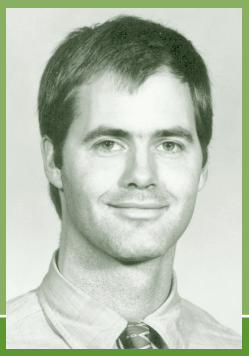

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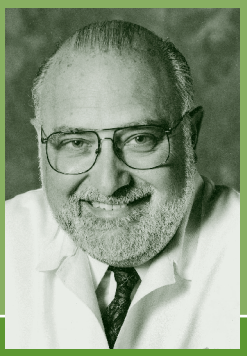

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Multichannel Intraluminal Esophageal Impedance

Multichannel Intraluminal Impedance (MII) is a new technology available for the detection of bolus presence within the esophageal lumen. This has potential applications for measuring esophageal motility (bolus moving from mouth to stomach) and reflux (bolus moving from the stomach retrograde up the esophagus). Based on ionic flow current, it has the capability of detecting the bolus presence characteristics (liquid, gas or mixed) as well. The catheter has multiple pairs of sensors distributed along the esophagus (figure 1); with continuous monitoring, the direction of propagation (oral or aboral) is determined.

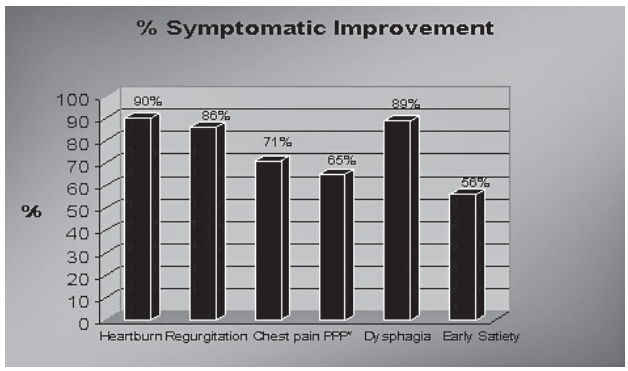
Esophageal Motility

Traditional measurement of esophageal motility consisted of manometry only, which measured the contraction of the esophageal muscle while swallowing. The addition of impedance gives an objective measurement of whether the swallowed material (usually water) moves completely through the esophagus. With this test we also have the patient swallow a viscous material that theoretically "tests" the motility of the esophagus more than water. When we investigated patients with GERD before antireflux surgery, we found that in 278 water swallows, 5% had normal esophageal motility and incomplete bolus clearance, as well as 9% with abnormal manometry and complete bolus clearance from the esophagus. When challenging esophageal motility with viscous material, our results showed that in 252 swallows, 6% had normal manometry but incomplete bolus clearance and 5% had abnormal manometry and complete bolus clearance. These results coincide with those obtained by other investigators, specifically those patients with incomplete bolus clearance and "normal" manometry tracings and vice versa. This phenomenon was unrecognized before this new technology.

Combined Multichannel Intraluminal Esophageal Impedance and Manometry does not Predict Post-operative Dysphagia after Laparoscopic Nissen Fundoplication

Laparoscopic Nissen fundoplication (LNF) is an effective treatment for GERD, although side effects such as dysphagia may occur. Manometry is used to evaluate peristaltic disorders, but alone is not effective in determining which patients may be at risk for postoperative dysphagia. MII is a relatively new technology that allows us to evaluate the transit and clearance of swallowed air, liquid and viscous material from and within the esophageal lumen. Combined MII and manometry is considered an advanced tool for esophageal function testing since it provides simultaneous evaluation of esophageal contraction (manometry) and bolus transit (MII); thus revealing the functional aspects of esophageal motility. Because of the relationship between dysphagia and esophageal clearance, we hypothesized that the addition of MII to manometry may detect those patients most at risk of developing postoperative dysphagia.

We prospectively evaluated 69 patients undergoing LNF. All patients completed a pre-operative symptom questionnaire, MII/manometry, and 24-hour pH monitoring. We defined post-operative dysphagia as occurring more than once a month with a severity ≥ 4 (0-10 scale).



* *PPP: postprandial pain*

FIGURE 1: **Impedance and manometry tracing showing complete bolus transit (MII) with a normal manometric tracing.**

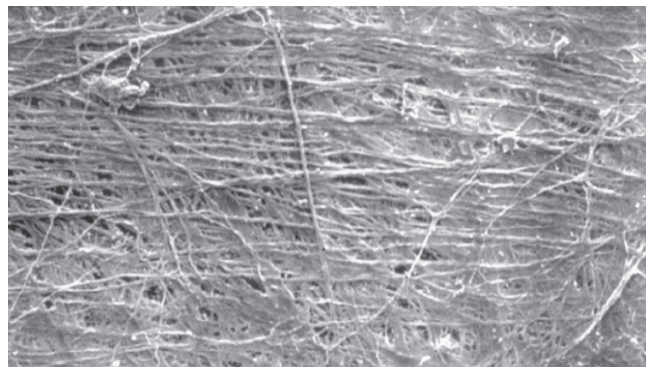


FIGURE 2: **Small intestinal submucosa (SIS).**

Thirty patients (43%) reported pre-operative dysphagia, but there was no significant difference in manometric (LES pressure and relaxation, peristalsis) and MII findings (esophageal transit and clearance) between patients with pre-op dysphagia (n = 30, 43%) and those without (n = 39, 57%). Among patients with pre-operative dysphagia only nine (30%) had persistent post-operative dysphagia. After LNF, only three (8%) out of 39 patients without preoperative dysphagia developed new dysphagia. Patients with post-op dysphagia had similar manometric and MII findings to those who did not develop dysphagia. (Table 1)

We concluded that neither manometry nor MII appear to be effective in predicting dysphagia before and after Nissen fundoplication.

The Role of Non-Acid Reflux in Respiratory Disease

Gastroesophageal reflux disease (GERD) is frequently implicated as a contributing factor to respiratory diseases, particularly asthma. Currently, 24-hr pH-monitoring is the gold standard for evaluation of reflux, although as typically performed, it is limited to the measure of acid reflux events reaching up to 15 cm above the lower esophageal sphincter. Multichannel Intraluminal Impedance monitoring (MII) is a relatively new technique which can be used in conjunction with traditional pH-monitoring to study both acid and non-acid reflux events. In impedance-pH monitoring, reflux is measured throughout the esophageal body up to the level of the pharynx. Using this technique, pharyngeal reflux episodes can be readily demonstrated even in healthy subjects without GERD. The purpose of our recent retrospective review of impedance-pH studies performed at UWMC was to investigate the relationship between gastroesophageal reflux, both acid and nonacid, and respiratory disease, as well as to test the hypothesis that patients with underlying respiratory disease, such as asthma, have more reflux episodes that reach the level of the pharynx compared to other patients referred for workup of GERD.

All impedance-pH studies performed at the University of Washington between 2003 and March 2007 were retrospectively reviewed together with manometric and outpatient medical records. Of these, 99 were studied off of acid suppression therapy and had no history of antireflux surgery, meeting inclusion criteria. Sixty-four patients had typical GERD symptoms and no dominant airway symptoms, while 35 were diagnosed with respiratory disease (asthma = 26, COPD = 3, IPF = 6). Manometric parameters, total reflux episodes (acid and non-acid), and pharyngeal reflux episodes were compared between patients with respiratory disease and those without respiratory disease using Student's T-test, with $p < 0.05$ considered significant.

No differences between patients with and without respiratory disease were observed in manometric parameters, including LES resting pressures (mean ± SD, 13.1 ± 1.8 mmHg *vs.* 16.5 ± 1.5) or effective peristalsis (82 ± 4% *vs.* 84 ± 4%). Distal esophageal acid exposure was also similar between the two groups, as were total numbers of acid and non-acid reflux episodes. In contrast, the total number of reflux episodes (and acid episodes) reaching the level of the pharynx was significantly greater in the respiratory disease group (Table 2).

We concluded that the addition of impedance to pharyngeal pH monitoring differentiates patients with respiratory disease from more typical GERD patients. Since the non-acid pharyngeal reflux episodes demonstrated by this technique are potential aspiration events, this both improves our understanding of the factors which may contribute to respiratory disease and may be important in directing appropriate therapy in these patients.

TABLE 1: Manometric and impedance findings between patients with and without post-operative dysphagia

	Post-operative dysphagia (n=12)	No post-operative dysphagia (n=57)	*p*-value
LESP (mmHg)*	20.4 ±12.4	13.7 ±10.7	.354
% Peristalsis	80 ±32	88 ±18	.685
% LBT**	78 ±30	79 ±24	1
Liquid BTT† (secs)	6.4 ±1.4	7.0 ±1.4	.838
%VBT††	66 ±33	64 ±29	.526
Viscous BTT (secs)	7.4 ±2	7.8 ±2	.561

* *Lower esophageal sphincter pressure*
** *Liquid bolus transit*
† *Bolus transit time*
†† *Viscous bolus transit*

TABLE 2

	% pH < 4	Total reflux episodes	Acid episodes	Non-acid episodes	Pharyngeal reflux episodes	Pharyngeal acid episodes	Pharyngeal non-acid episodes
No Resp. Dis.	4.8 ± 0.9	34 ± 3	17 ± 2	16 ± 2	4 ± 0.7	1 ± 0.3	3 ± 0.5
Resp. Dis.	4.3 ± 0.8	38 ± 4	21 ± 4	16 ± 2	7 ± 2.0	2 ± 0.9	5 ± 1.5
p-value	0.773	0.470	0.342	0.998	**0.042**	**0.041**	0.144

The Role of Pepsin the Pathogenesis of GERD-Related Laryngitis

It is estimated that 3-10% of the US population has significant symptoms of laryngopharyngeal reflux (LPR) for which they seek treatment, costing over $1 billion per year. Most laryngeal diseases associated with LPR are thought to develop following direct contact of the laryngeal epithelium with gastric refluxate, containing acid, pepsin and bile acids. To date, diagnosis and treatment has focused on the acid component of the refluxate. This is because the traditional focus of GERD was on acid, and also because it was thought that pepsin and bile acids would not cause injury at higher pH. Thus, patients with LPR are prescribed proton pump inhibitors (PPI's) to increase the pH of the refluxate.

This focus, however, appears misguided for several reasons. One, PPI therapy appears to have limited ability to protect these patients from LPR-induced damage. Two, more sophisticated testing methods such as multi-channel intraluminal impedance-pH monitoring (MII) have demonstrated a strong association of non-acidic reflux with laryngeal symptoms and injury. Three, our team of investigators have data which supports a role for pepsin in reflux-attributed laryngeal injury and disease, independent of the pH of the refluxate. Of most significance, we have recently demonstrated that pepsin is taken up by human laryngeal epithelial cells by receptor-mediated endocytosis in patients with a clinical diagnosis of LPR.

We believe that further investigation of the relationship between laryngeal uptake of pepsin and LPR may yield substantial insight into the pathogenesis of this disease and perhaps a better diagnostic test for LPR (which is lacking). Laparoscopic Nissen fundoplication (LNF) is effective in halting all components of gastroesophageal reflux by creating a mechanical barrier. In theory, this should be the most effective therapy currently available to stop pepsin from reaching the larynx.

Therefore, we propose a study of patients with clinical LPR who would have laryngeal pepsin checked before and after LNF, along with physiologic reflux studies (pH-MII) and standardized symptom outcomes. Our hypothesis is that LNF will result in the elimination for pepsin from the laryngeal epithelium. If true, this would further validate the role of GERD, and more specifically pepsin, in the pathogenesis of LPR. Furthermore, if the presence and elimination of pepsin correlates with a clinical response to LNF, there may be a role for pepsin screening as a diagnostic test and predictor of therapeutic response.

Surgical Treatment of Achalasia

DOR VS. TOUPET FUNDOPLICATION: MULTI-CENTER RANDOMIZED TRIAL

The development of gastroesophageal reflux is essentially guaranteed after a well done Heller myotomy. Our experience has shown that there is no way to maximally relieve the dysphagia of achalasia and at the same time prevent GERD. For this reason, most surgeons add a partial fundoplication to this procedure. The most common fundoplications are a Dor (anterior) and Toupet (posterior) fundoplication. The theoretical advantage of the Toupet is that it holds the edges of the myotomy open (possibly better relief of dysphagia) and is considered a better antireflux procedure, while the Dor fundoplication is placed over the exposed mucosa of the esophagus, thus buttressing a microperforation, should it occur.

A group of four major esophageal surgical centers have organized a multi-center randomized trial to answer whether one of these fundoplications is superior to the other in this situation. They are performed fairly equally around the world at this time and we hope to definitively determine whether there is a difference.

Epiphrenic Diverticulum

MINIMALLY INVASIVE TREATMENT OF EPIPHRENIC DIVERTICULUM.

Epiphrenic diverticula are those that occur in the distal esophagus. They represent herniation of the superficial layers of the esophageal wall through the muscular layer as a result of increased intraluminal pressure. This is a very rare entity for which the cause is not well known, although in the majority of the cases an underlying neuromuscular disorder is present, causing an increased intraluminal pressure.

There are controversies regarding the ideal surgical treatment and approach of epiphrenic diverticula. Historically, the standard operation for the treatment of epiphrenic diverticula has been a thoracotomy (big incision in the chest), resection of the diverticula and myotomy (cutting the distal sphincter of the esophagus to decrease the intraluminal pressure). Since the introduction of minimally invasive surgery for the treatment of gastro-esophageal reflux disease in 1991, a variety of esophageal diseases have been approached using this technique. Although epiphrenic diverticula is rarely seen in most clinical practices, these patients are now being referred and repaired with increased frequency in those centers performing minimally invasive esophageal surgery. Several authors have reported in the literature their modest experience in the treatment of epiphrenic diverticula using a minimally invasive approach. We began treating epiphrenic diverticula using a minimally invasive approach 11 years ago. Based on our vast experience using this technique and high volume of patients, we are reviewing our treatment outcomes with minimally invasive surgery for epiphrenic diverticula.

We are going to undertake a retrospective assessment of all consecutive patients who underwent minimally invasive treatment of epiphrenic diverticula at the University of Washington from 1997-2007.

We are conducting this study to determine the short-term (operative time, blood loss, rate of conversion to conventional surgery, complications, length of stay, hospital mortality) and long-term (symptoms improvement, need of reoperation and endoscopic dilation, overall satisfaction with the operation) outcomes with this approach.

Paraesophageal Hernia

REPAIR OF PARAESOPHAGEAL HERNIAS WITH SMALL INTESTINAL SUBMUCOSA (SIS)

Laparoscopic paraesophageal hernia repair (LPEHR) is associated with a high recurrence rate. Repair with synthetic mesh lowers recurrence, but can cause dysphagia and visceral erosions. This trial was designed to study the value of a biologic prosthesis, small intestinal submucosa (SIS) (Figure 3) in LPEHR.

Patients undergoing LPEHR (n = 108) at four institutions were randomized to primary repair (1º) (n = 57) or primary repair buttressed with SIS (n = 51) using a standardized technique. The primary outcome measure was evidence of recurrent hernia (≥ 2cm) on an upper gastrointestinal series (UGI), read by a study radiologist blinded to the randomization status, six months after operation. At six months, 100 (94%) completed clinical symptomatic follow-up and 94 (90%) had a UGI.

Peri-operative Outcomes: The SIS group had larger anterior-posterior hernia diameter, but similar esophageal length. Operative times (SIS 202 min vs. 1º 183 min, p=0.15) and peri-operative complications did not differ. There were no operations for recurrent hernia nor mesh-related complications.

TABLE 3

	Heartburn Pre-op	Heartburn Post-op	PP pain pre-op	PP pain post-op	Dysphagia Post-op	A-P Hernia Diameter	Recurrent Hernia (≥ 2cm)
1º	5.1	0.4*	4.4	1.1*	0.7	5.8 cm	25%
SIS	5.2	1.0*	4.9	1.5*	1.5	6.4 cm†	7%†

** Pre-op vs. Post-op, p<0.01*
† 1º Repair vs. SIS p<0.05
Symptoms score: visual analog scale (0-10)

Primary Outcome: At six months, three patients (7%) developed a recurrent hernia > 2 cm in the SIS and 12 patients (25%) in the 1º group.

Secondary Outcomes: Both groups experienced a significant reduction in all measured symptoms (heartburn, dysphagia, regurgitation, chest and post-prandial (PP) pain) after operation. There was no difference between groups in either pre or post-operative symptom severity.

This trial has demonstrated that adding a biologic prosthesis during LPEHR reduces the likelihood of recurrence, without mesh related complications or side effects.

This year we are doing a follow-up of this study to assess if the benefit of SIS is durable. We are assessing all patients in the trial with the same questionnaire and UGI (now an average of four years after their operation). This second stage of the trial will not only test the durability of biologic mesh for PEH but the durability of biologic mesh in general. This is a big question, now with the explosion of biologic mesh materials on the market now, with little data concerning their long-term effectiveness.

Esophageal Cancer

OUTCOMES OF LAPAROSCOPIC ASSISTED ESOPHAGECTOMY FOR ADENOCARCINOMA OF THE ESOPHAGUS

The incidence of esophageal adenocarcinoma has increased more rapidly than any other gastrointestinal malignancy in the last decade. The prognosis of patients with esophageal cancer remains poor. Only 56% of the patients who present with esophageal cancer have resectable disease, with an overall five-year survival rate of 10%. Esophageal resection remains the gold standard, not only in providing the optimal chance for cure, but also the best palliation for dysphagia. However, the conventional open operations are quite invasive, with a morbidity of 50% and a mortality of 5-10% in high-volume centers.

Laparoscopic procedures offer an advantageous alternative to conventional open operations, such as less operative trauma than experienced with thoracotomy or manual blind and blunt transhiatal esophagectomy; less perioperative blood loss; shorter ICU stay. Furthermore, a minimally invasive procedure does appear to offer the potential for a more radical mediastinal resection, under direct vision, when compared with transhiatal esophagectomy. However, controversy still exists about what is the best approach to and extent of the dissection. At the University of Washington, we started performing laparoscopic-assisted esophagectomy in 1995 for tumors of the distal esophagus and gastroesophageal junction. We conducted this study to determine the short-term (complications, length of stay, pathologic staging, lymph node harvest, blood loss, etc.) and long-term (cancer free survival and overall survival) outcomes with this approach.

Since 1995, 72 patients with esophageal adenocarcinoma underwent laparoscopic-assisted transhiatal esophagectomy at the University of Washington using the aforementioned technique. The mean operative time was 321 + 73 minutes and the blood loss 318 + 239 ml. The median ICU stay was one day (1-35), whereas the hospital stay was nine days (7-58). One patient (1.4%) died within 30 days postoperatively.

The most common complications were: anastomotic leak in 14 patients (all but one were managed non-operatively), pneumothorax in 18 patients (only six patients requiring drainage), pleural effusion in nine patients, atrial fibrillation in eight, wound infection in seven (all managed in the outpatient setting), transient recurrent nerve paralysis in six, deep vein thrombosis in four and pulmonary embolism in three patients. In the long-term follow-up 13 patients reported anastomotic stricture requiring dilation. The overall long-term survival was 85% at one year, 68% at three years and 63% at five years.

Our results support that the laparoscopic-assisted transhiatal esophagectomy is a safe and feasible procedure with decreased morbidity and mortality and with good survival rate. Therefore, this approach should be included in the armamentarium for the treatment of esophageal adenocarcinoma.

Natural Orifice Transluminal Endoscopic Surgery (NOTES) represents a new paradigm shift, which may significantly change the management of gastrointestinal and intraabdominal diseases. It is an exciting time, and not since the wave of laparoscopic surgery 20 years ago has there been such a radical change in the way we think about surgery.

High-Resolution Esophageal Manometry (HRM)

HRM is a recently developed tool in the evaluation of esophageal motility which utilizes many closely spaced pressure-recording sites along a manometry catheter in order to display a relatively continuous profile of esophageal motor activity from the upper esophageal sphincter, along the length of the esophageal body, and across the lower esophageal sphincter. A recording device produces color-contour plot, with time on x-axis, esophageal length on y-axis, and pressure represented by a color scale. Data between recording sites is interpolated to demonstrate pattern and pressure gradients. The result is a more complete and detailed picture of esophageal motility, with potentially better and more accurate characterization of esophageal function than standard manometry.

The Swallowing Center of the University of Washington has contracted to purchase a cutting-edge system that combines HRM with MII, which became available in the spring of 2008. Preliminary proposals for research investigation using this new equipment include:

EVALUATION OF DYSPHAGIA USING HRM-IMPEDANCE

Because HRM illustrates a continuous motility pattern along the entire esophagus and provides a detailed display of pressure across the LES and proximal stomach, it may reveal abnormalities at specific loci of bolus retention when combined with MII, and may be able to elucidate previously uncharacterized causes of dysphagia. All patients presenting with dysphagia as the primary complaint would be eligible. After completion of a detailed dysphagia questionnaire, combined HRM/MII would be performed and compared to results from control groups of patients with heartburn and healthy volunteers. We would then identify and localize any areas of bolus retention on MII and correlate with peristaltic activity in both dysphagia subjects and control groups.

FACTORS RESPONSIBLE FOR FUNDOPLICATION FAILURE

Because HRM is able to illustrate separation of high-pressure zones (HPZ) much more clearly than standard manometry, it is potentially a highly useful tool in determining the etiology of recurrent reflux after fundoplication. All post-fundoplication patients would be evaluated using HRM + MII, pH monitoring, EGD, and UGI, and would complete a detailed symptom questionnaire. Subsets of patients having recurrent reflux symptoms, dysphagia, and gas bloat would be defined. HRM/MII results between asymptomatic patients and patients with recurrent symptoms would be compared, examining the length of the HPZ, the HPZ "pressure profile," and the presence of a single vs. a dual HPZ, possibly indicating recurrence of a hiatus hernia, or a "slipped" fundoplication.

Combined detailed motility and bolus clearance patterns in patients complaining of dysphagia would be defined, attempting to determine where in the esophagus clearance fails, and what the motility characteristics are in that segment. Furthermore, HRM findings would be compared to information obtained from EGD, pH monitoring, and UGI, to correlate HRM data with these more traditional investigations in patients with recurrent reflux after fundoplication. Finally, we hope to determine specific factors associated with a "successful" fundoplication, such as a certain minimum length of HPZ, postoperative motility patterns, or postoperative bolus clearance patterns.

In addition to these planned investigations, collaboration with other centers that will begin using this new technology at the same time both in the U.S. and internationally has already been initiated. This will increase the potential to perform additional studies and permit more rapid subject accrual.

Natural Orifice Translumenal Endoscopic Surgery

Natural Orifice Transluminal Endoscopic Surgery (NOTES) represents a new paradigm shift, which may significantly change the management of gastrointestinal and intraabdominal diseases. The idea, as the name implies, is to access and perform procedures in the abdominal cavity via a natural orifice (e.g. mouth or anus) using an endoscope. The theoretical advantages of NOTES include reducing operative pain and morbidity, as well as avoiding wound infections, hernias, and adhesions. Furthermore, NOTES might offer advantages for patients in whom conventional transabdominal or laparoscopic procedures are unattractive, e.g., morbidly obese patients and patients with extensive scars, burns, or infections in the abdominal wall.

The first animal experience with NOTES was published in 2004 by Kalloo et al., who demonstrated the feasibility and safety of a per-oral transgastric endoscopic approach to the peritoneal cavity with long-term survival in a porcine model.

Our main goal for NOTES research at the UW is to assess the feasibility and safety of new devices and tools as well as different surgical procedures, most of which are being done via traditional surgical methods but instead using a translumenal approach. We have developed relationships with gastroenterology and bioengineering colleagues to form a NOTES research group. This group's goal is to develop and test the next generation of instruments that will make more advanced flexible endoscopic and NOTES procedures not only possible, but safe and effective. It is an exciting time, and not since the wave of laparoscopic surgery 20 years ago has there been such a radical change in the way we think about surgery.

EVALUATION OF THE GASTRIC MOTILITY AFTER NOTES

There are potential disadvantages with NOTES, which were issued by the Natural Orifice Surgery Consortium for Assessment and Research (NOSCAR). Some are technical, like limitations in instrumentation to operate via current flexible endoscopes. There is also the potential for gastric leak and/or intraabdominal contamination and infection. We and some other centers around the world are actively involved in trying to work out these problems. Few, however, are involved in the study of long-term effects of NOTES, such as the investigation of gastric motility and function after NOTES.

The primary aim of this study is to test the hypothesis that transgastric endoscopic surgery does not affect gastric motility. Using EGG and radiography with swallow of barium-impregnated rings, we want to evaluate the effects of transgastric endoscopic surgery on the gastric motility of dogs from electrophysiological and mechanical points of view. We are also actively involved in the development of instrumentation and equipment to make this feasible. While we currently do not know what role NOTES will have within the field of surgery, it clearly has the potential to revolutionize it similar to how laparoscopy did 20 years ago.

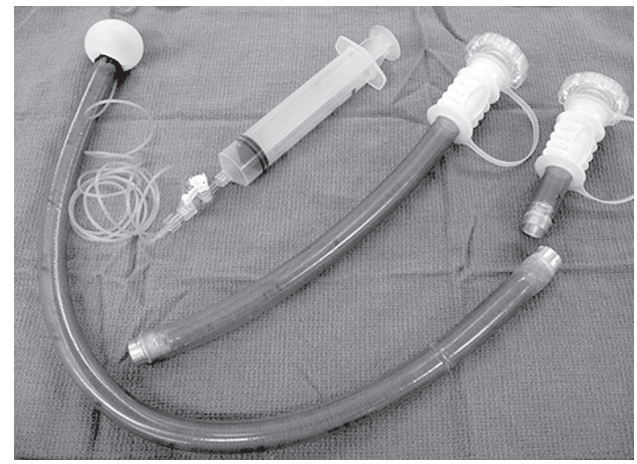


FIGURE 3

A SEGMENTED BALLOON-TIP OVERTUBE FOR PERITONEAL ACCESS IN NOTES

NOTES applications often require repeated insertion and withdrawal of endoscopes and accessories into the peritoneal cavity after achieving initial access to the peritoneum. To achieve this, an extended overtube can be advanced across the luminal wall at the time of initial peritoneal access. However, the length of overtube required for this restricts the working length of the endoscope (length of endoscope minus the length of the overtube). We have developed a segmented overtube system that allows for maximal working length once peritoneal access has been achieved.

The overtube (see Fig 3) consists of the following components modified from the Guardus® overtube (US Endoscopy): 1. A single distal overtube section (60 cm) with an inflatable balloon on the distal end used to secure the tip of the overtube after peritoneal access. A threaded connector is attached to the proximal end; 2. Two proximal sections of two lengths (30 cm and 5 cm) which are interchangeable via a threaded connector; 3. A single inner tube (90 cm) used for peritoneal access only. The 30 cm proximal section is attached to the distal section (total length of 90 cm) and the inner tube is inserted along with the endoscope. The endoscope is then advanced along with the tip of the overtube into the peritoneum using the standard needle knife/balloon dilation technique. The overtube balloon is then inflated securing the tip within the peritoneum. The

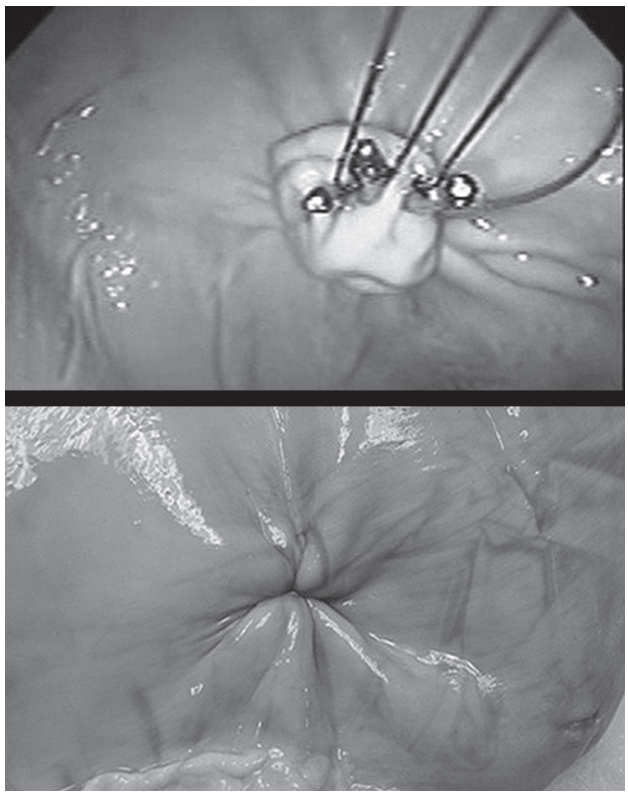


FIGURE 4: Image of Closure. Endoscopic view on top; full thickness visual below.

entire overtube can then be reduced. The endoscope and inner tube are withdrawn and the longer proximal section of the overtube is detached and replaced with the shorter proximal segment (5 cm). The operator now has approximately 40 cm of working length with the endoscope and has direct access into the peritoneum via the overtube.

Results: Preliminary *in vivo* studies using this overtube system have been performed resulting in stable access to the peritoneal cavity for NOTES procedures. The overtube system allows for rapid insertion and withdrawal of the endoscope and the ability to deliver accessories and materials into the peritoneum without sacrificing the working length of the endoscope.

This overtube system is relatively simple to build using existing commercially available components and can potentially be modified for human anatomy to maintain a sterile conduit into the peritoneum.

RETRACTED CLIP-ASSISTED LOOP CLOSURE FOR GASTROTOMY IN NOTES

A reliable method for gastrotomy closure will be essential for NOTES to become viable clinically. Several methods have been reported; however, simple methods using existing endoscopic accessories have been ineffective. Specialized devices are in development but are not widely available. We have developed a novel, simple method for gastric closure that uses existing endoscopic accessories with very minor modifications. We report preliminary data on a new method of gastrotomy closure using modified clips and endoloops.

A standard NOTES gastrotomy with needle-knife incision followed by balloon-dilation with a 20 mm diameter balloon was performed in 12 ex vivo pig stomachs. Gastrotomies were closed using the conventional hand-sewn technique in 6 specimens and using the new retracted clip-assisted loop closure technique in 6 specimens. The retracted clip-assisted loop closure technique involves deploying 3-4 Resolution® clips (modified by attaching a 90 cm length of suture string to the end of each clip) along the margin of the gastrotomy with one jaw on the serosal surface and the other jaw on the mucosal surface. The attached strings are exteriorized through an overtube. With the endoscope external to the subject, an endoloop is then passed through the endoscope channel, opened, and the strings are threaded through the open loop and advanced into the stomach. Retraction is then applied to the strings, causing the gastric wall to tent. The endoloop is then secured below the tips of the clips, completing a full thickness gastrotomy closure. An air leak test was performed via insufflation with the endoscope. Fluid leak pressure was then measured for each specimen.

The retracted clip-assisted method achieved an air-tight seal in 100% of the specimens. Endoscopic image of the appearance of the closure is provided (upper image). On visual inspection 2/6 appeared to achieved a full thickness closure (lower image). The leak pressure ranged from 16-88 mmHg (mean 37 mmHg). Results improved as the investigators gained experience with the technique. In comparison, the leak pressure for the hand-sewn technique ranged from 67-103 mmHg (mean 81 mmHg).

The retracted clip-assisted gastrotomy closure technique is a promising new technique for NOTES gastrotomy closure that uses existing endoscopic accessories with minor modifications and warrants further investigation.

TRANSGASTRIC VENTRAL WALL MESH FIXATION IN A PORCINE MODEL

After Kalloo et al. published the first animal experience with NOTES, other transgastric peritoneal procedures were performed in the porcine model, such as tubal ligation, cholecystectomy, gastrojejunostomy, splenectomy and oophorectomy with tubectomy.

We assume that NOTES might represent a feasible and potentially less invasive approach to ventral wall hernia repair. Fong et al. have described in an abstract the placement of a synthetic mesh into the peritoneal cavity in a porcine model using a transcolonic approach and transcutaneous magnetic handling. We consider that the transgastric approach would be more adequate because of the sterile nature of the stomach.

Ten pigs will be used for this study. An endoscopic needle knife is used to create a 20 mm gastric incision. Upon entering the peritoneal cavity, a 5 cm x 5 cm synthetic mesh (Parietex mesh, Covidien) is introduced through the scope into the abdomen and fixed to the anterior abdominal wall using previously placed sutures that are retrieved percutaneously with an Endoclose device (Covidien). After finishing with the fixation of the mesh, the scope is withdrawn into the stomach and the gastrotomy is closed with 2-4 sutures as necessary.

Ten days later, the animals are euthanized and necropsy is done in all the pigs. The abdominal cavity is examined, looking for adhesions or evidence of intra-abdominal infection. The abdominal wall into which the mesh was placed and the stomach are explanted. The attached mesh is assessed, regarding the tissue in growth into the mesh by routine histopathologic exam. The transfacial suture placement is also going to be assessed by gross inspection. A piece of the mesh is removed and sent to microbiological studies to assess quantitatively and qualitatively the growth of bacterial colonies using direct exam with Gram stain and culture in aerobic and anaerobic mediums. The main goals of this study are to assess the: feasibility of placement of a synthetic mesh into the abdominal cavity using a transgastric approach in a porcine model; tissue ingrowth into the mesh (peritonealization); and mesh contamination, if any.

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- Objective Assessment of Surgical Skills
- Operating Room of the Future

FUNDING

Defense Advanced Research Projects Agency (DARPA)
U.S. Army Medical Research & Material Command
- **Telemedicine & Advanced Technology Research Center**

Objective Assessment of Surgical Skills

There is a totally new paradigm in surgical education and training based upon surgical simulation. A national consortium of surgical training centers will define new metrics and outcome performance measures, establish criterion levels of performance, validate efficacy of simulators as educational tools and then train residents to criterion and evaluate the performance in the operating room.

The conceptual change is to train residents (in the future) not for a given time, but rather to a given criterion level, a level which reduces errors to the absolute minimum and provides maximum quality, especially for patient safety. The above will be implemented by using the Minimally Invasive Surgery Trainer – Virtual Reality (MIST-VR) and the Xitact Laparoscopic Cholecystectomy simulator, in addition to other systems such as the "Blue Dragon" that are described elsewhere.

This new educational system will initially be implemented and validated at UWMC, then expanded to the WWAM I region, and finally to a national level.

Operating Room of the Future

Recent introduction of robotic systems into clinical surgery indicates a fundamental new direction for surgeons. Research will be conducted to integrate robotics into an entirely new concept for the operating room – one which decreases the number of personnel required, increases efficiency and quality control, and which incorporates the robotic system into the hospital information system. In addition, the robotic systems will be used to train, objectively assess and certify competence of surgeons.

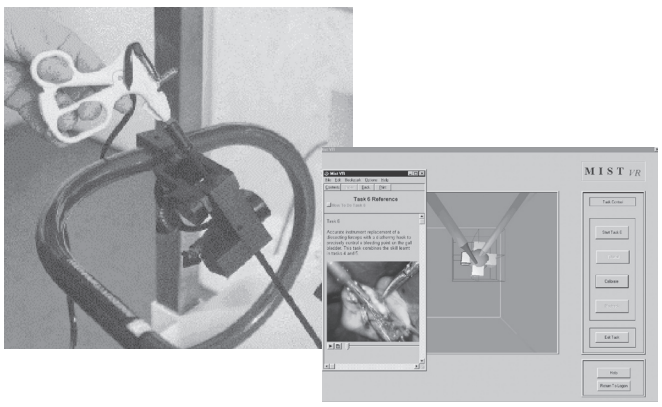

FIGURE 1: MIST-VR basic surgical skills simulator illustrating the image on the simulator screen, and the input handles for tracking motion

FIGURE 2: Xitact Laparoscopic Cholecystectomy simulator illustrating the portable system and video image.

Recent introduction of robotic systems into clinical surgery indicates a fundamental new direction for surgeons.

FIGURE 3: Zeus surgical robotic system

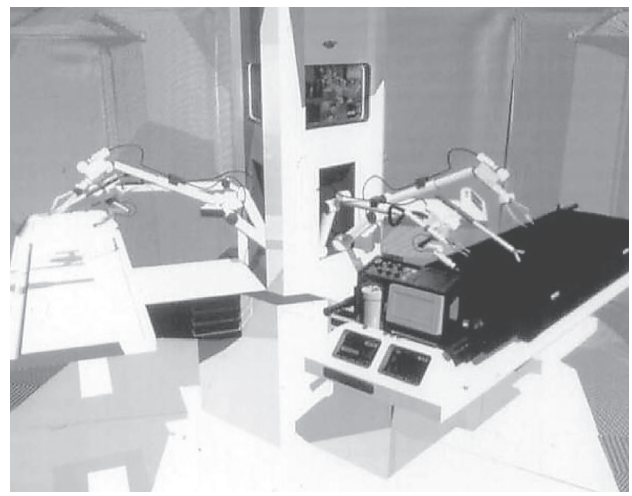

FIGURE 4: OR of the future – concept drawing from Integrated Medical Systems

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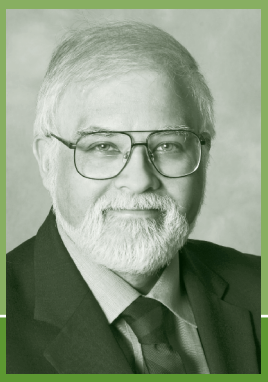

Professor

- ISIS Sponsored Procedure-specific Research Projects:
  - Simpraxis Laparoscopic Cholecystectomy Trainer
  - Central Venous Catheterization (CVC) Trainer

Under the sponsorship of the Institute for Surgical & Interventional Simulation (ISIS), we are moving forward this year with two research studies that evaluate the benefit of simulation-based training on performance.

The first study, the Simpraxis Laparoscopic Cholecystectomy Trainer, is a computer-based, cognitive trainer that introduces laparoscopic cholecystectomy in depth to a novice resident. We are targeting residents starting their second year. In working through the trainer, the pathophysiological and anatomic basis for the procedure is reviewed as are the indications for surgery. The surgeon must then set up the patient and positioning, chose instruments, and carry out the procedure in a step by step process that is of sufficient granularity that the student's in-depth knowledge of the procedure is directly challenged. Decisions that must be made on a minute by minute basis, anatomy that must be recognized and registered accurately, and instrument changes for specific steps

Cognitive Procedural Map

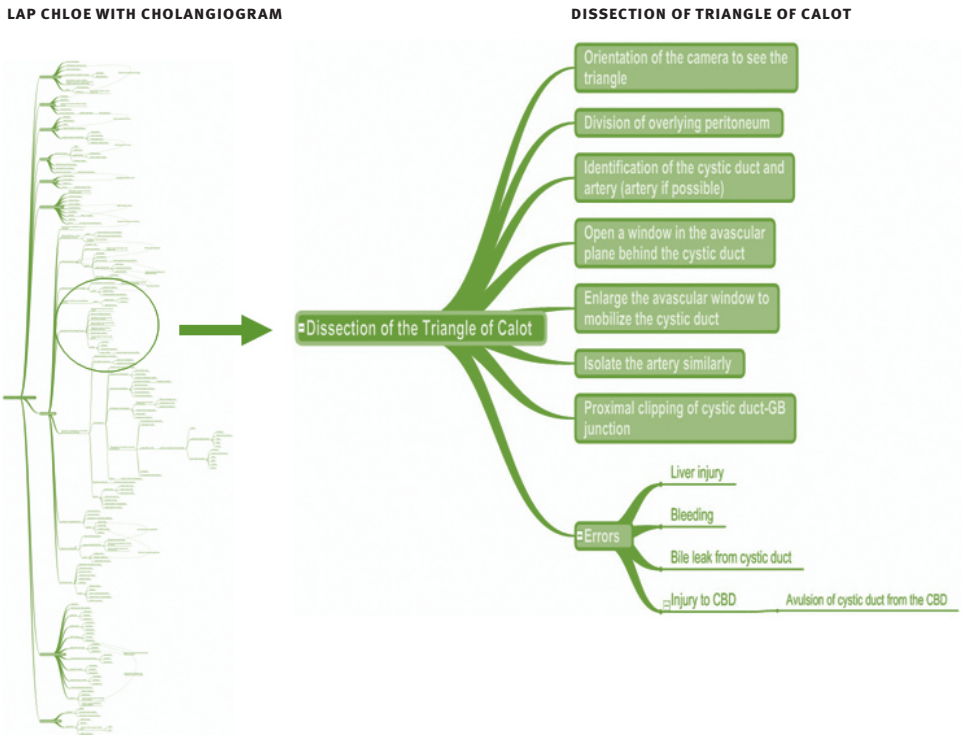

Figure 1: Graphic depiction of the cognitive map used to develop the Laparoscopic Cholecystectomy trainer.

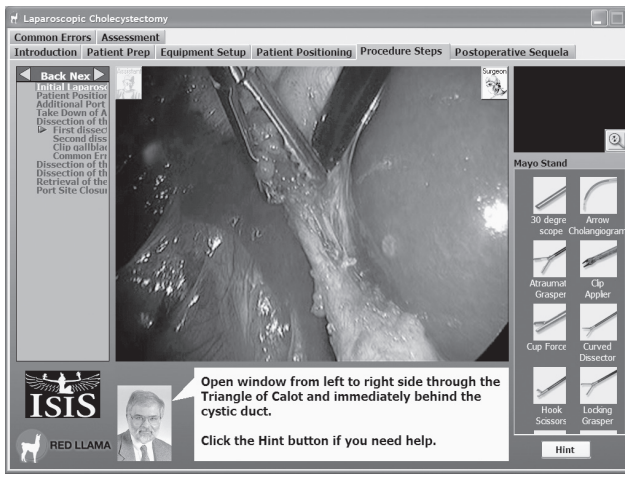

Figure 2: **A screen-shot of the Simpraxis Laparoscopic Cholecystectomy trainer.**

in the procedure provide the trainee with an experience that closely mimics the clinical decision making process of a procedure. Since laparoscopic cholecystectomy is one of the most common general surgical laparoscopic procedures carried out, this process of detailed decision making for specific parts of the procedure serves as a basis for training in laparoscopic surgery that has broad application to other laparoscopic procedures in general surgery. A map of the procedure, a so-called cognitive map serves to organize the information presented through the trainer (Figure 1).

In the research study designed to provide validation of this trainer, we have developed a crossover training design that randomly designates junior residents interested in participating to one of two groups, a study group and a control group. Both groups receive a standard orientation to laparoscopic cholecystectomy as provided to all residents currently in our EVATS training program. This includes an orientation to scoring in the trainer and scoring during subsequent surgery. Each resident will be provided with a unique ID labeled on a USB thumb drive. This will be used to access the trainer and capture data from training sessions. The control group receives no further training outside the operating room. The study group receives access to the Simpraxis trainer (Figure 2) and is expected to complete the entire trainer, a process that takes about two hours. We expect that the trainer will be used to completion at least once by the study group but many will chose to spend more time working through the different modules of the trainer to master it and improve their scores for timing, errors, and the anatomy and procedural quizzes imbedded in the trainer.

In the OR, attending surgeons will be blinded to the status of the residents other than knowing that the resident is participating in the study. Scoring for the procedure will be carried out by the attending at the end of the procedure using a modified GOALS methodology (Global Operative Assessment of Laparoscopic Skills - Figure 3), a validated instrument for scoring of laparoscopic surgical performance.

Table 1. Global Operative Assessment of Laparoscopic Skills

Domains	Anchor descriptors				
	1	2	3	4	5
Depth perception	Constantly overshooting target, hits backstop, wide swings, slow to correct		Some overshooting or missing plane but corrects quickly		Accurately directs instruments in correct plane to target
Bimanual dexterity	Use of one hand, ignoring nondominant hand, poor coordination between hands		Use of both hands but does not optimize interactions between hands to facilitate conduct of operation		Expertly uses both hands in a complementary manner to provide optimal working exposure
Efficiency	Uncertain, much wasted effort, many tentative motions, constantly changing focus of operation, or persisting at a task without progress		Slow, but planned and reasonably organized		Confident, efficient and safe conduct of operation, maintaining focus on component of procedure until better done by another approach
Tissue handling	Rough, tears tissue by excessive traction, injures adjacent structures, poor control of coagulation device (recoil), grasper frequently slips off		Handles tissues reasonably well, with some minor trauma to adjacent tissues, eg, coagulation of liver, causes unnecessary liver bleeding, occasional slipping of grasper		Handles tissues very well with appropriate traction on tissues and negligible injury of adjacent structures. Uses energy sources appropriately but not excessively
Autonomy	Unable to complete entire procedure, even in a straightforward case and with extensive verbal guidance		Able to complete operation safely with moderate prompting		Able to complete operation independently without prompting
Level of difficulty	Easy exploration and dissection	Intermediate between 1 and 3	Moderate difficulty (eg, mild inflammation, scarring, adhesions, obesity, or severity of disease)	Intermediate between 3 and 5	Extremely difficult (eg, severe inflammation, scarring, adhesions, obesity, or severity of disease)

Adapted from Vassiliou et al, American Journal of Surgery, [2] with permission.

Figure 3: **This scoring sheet represents the GOALS(see text) portion of the clinical scoring for performance,a validated system for measuring laparoscopic surgical capability.**

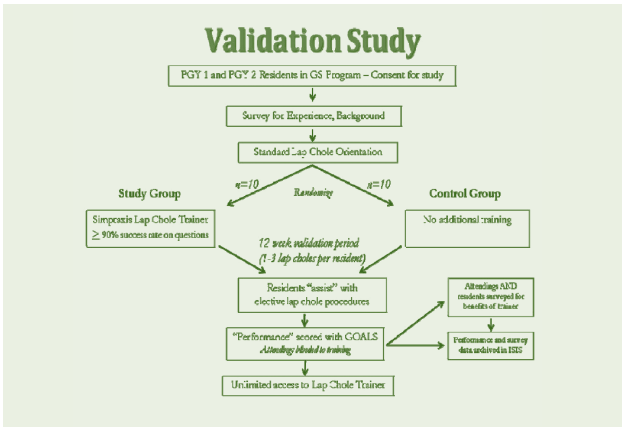

Figure 4: **Outline of research protocol for evaluation of the Laparoscopic Cholecystectomy trainer.**

Skills in identifying anatomy, tracking the steps of the procedure, and accurately setting up each stage of the procedure with appropriate positioning and instruments will be compared between residents who have completed the training and those going through out standard type of training. At the conclusion of the experimental period, the control group will have access to the training program in an unlimited fashion. A flow chart of the protocol is included (Figure 4).

With this study, we hope to demonstrate the value of this cognitive trainer – so called because the purpose of the trainer is to inform thought processes around the procedure – in improving safety and efficiency of laparoscopic cholecystectomy. By correlating the best performance of subjects on the trainer to their surgical scores (in a blinded fashion), we intend to also show that greater facility in the trainer due to greater practice time and achieved scores translates to improved performance in the OR, at least early in the learning curve.

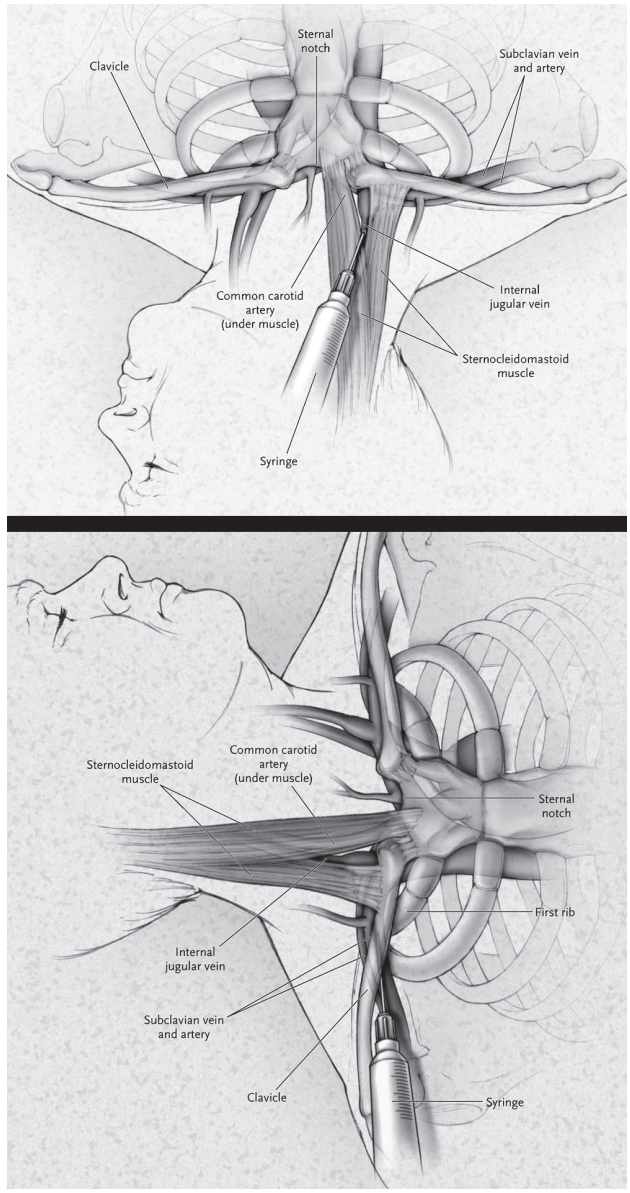

Figure 5a and b: **Anatomy of the internal jugular (a) and subclavian veins (b) in the neck and upper torso. The CVC trainer includes these images and the capability to start at the skin and strip off superficial layers to expose the vascular anatomy.**

Skills in identifying anatomy, tracking the steps of the procedure, and accurately setting up each stage of the procedure with appropriate positioning and instruments will be compared between residents who have completed the training and those going through our standard type of training.

In the second study, training for the procedure of central venous catheterization (CVC)has been developed as a combined cognitive and physical simulator. The key steps of the training are:

1. Orient all practitioners placing CVC to the indications and risks of the procedure
2. Orient to the anatomy of the central venous system (subclavian and internal jugular veins)(Figure 5a and b)
3. Instruct on the use of ultrasound and ultrasound anatomy (Figure 6)
4. Establish a standard procedure for patient identification, preparation, equipment setup, sterile technique with full barrier precautions, ultrasound-guided access to the venous system, catheter placement, catheter security, and dressing.
5. Define optimal management for a comprehensive set of complications or problems with catheter placement.
6. Using the Simulab CentralLineMan™ simulator, to practice and demonstrate the entire procedure from sterile technique to ultrasound guided needle, guidewire, and catheter placement (Figure 7).

Part of this training will be carried out using a cognitive simulator available on the web in a Adobe Flash™-based training program with video, audio, photographs, and extensive diagrams that guide the novice and experienced practitioner through the entire procedure (Figure 8). The physical simulator, CentralLineMan, will be located in ISIS and other locations.

The purpose of this trainer is to standardize training in CVC across disciplines and specialties and to address significant quality issues that have been identified nationally and at our medical centers around infection risk, risks of pneumothorax, and the risks of arterial cannulation. To measure the effects of training on safe use of CVCs, we have put into place as part of this training effort a quality tracking system for all CVCs. We have established a template in the electronic medical record, ORCA, that will standardize information captured during catheter placement, tracking, and removal. Information captured in this format will be searchable so that experience with CVC placement can be tracked over time. Nursing staff will be available to assist in the placement of CVCs at UWMC and HMC, providing a standard level of support in preparing patients and equipment for CVC placement, monitoring patients during CVC placement, and then tracking catheters until removal. To facilitate this effort, each

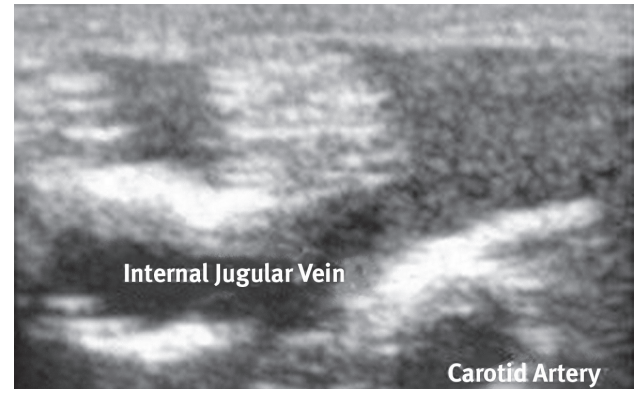

Figure 6: Ultrasound appearance of the internal jugular vein and carotid artery side by side, as visualized during needle advancement into the vein.

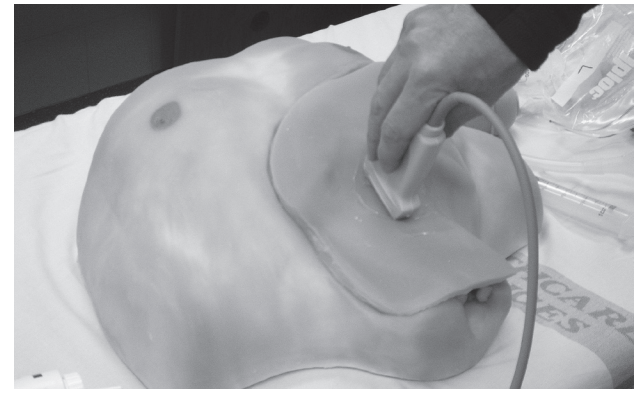

Figure 7: CentralLineMan™ is a physical simulator for CVC placement into the internal jugular and subclavian veins that is ultrasound capable, permitting guided placement of catheters using visible anatomic and ultrasound anatomic guidelines.

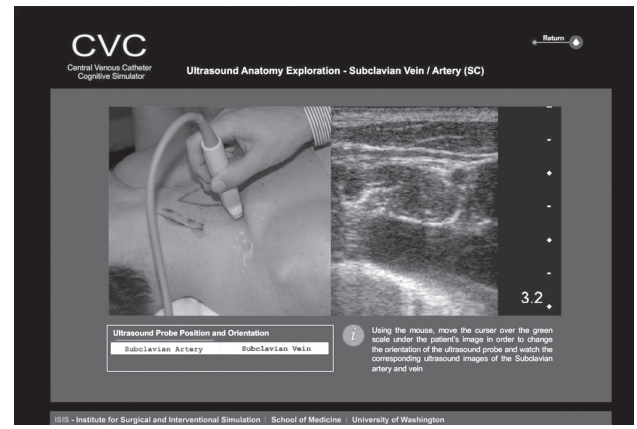

Figure 8: ISIS CVC Trainer. This is an interactive, web-based portal for cognitive training in CVC placement that has been developed through ISIS. This image shows the ultrasound probe placement and image in an interactive format.

CVC will have a unique tracking number (UTN) in the CVC kit. This number will be used to uniquely identify each introducer sheath and/or triple lumen catheter being inserted in the ICU, radiology suite, OR, and on the ward. The UTN will become part of the medical record and be used as an index term in the structured documentation.

The research aspects of this training system derive from the deployment of an enterprise-wide standardized protocol for CVC placement. Tracking and standardized documentation will occur before all training is complete. By July of 2009, all attending physicians and residents placing CVCs will have been offered an opportunity to complete the training. Those needing this technical skill as part of their professional portfolio of skills will be asked to complete an entire procedure of CVC placement in simulation without error, in order to be certified for CVC placement. After May of 2009, all CVCs being placed at UWMC or HMC will be performed by residents and attending physicians who have been credentialed through this process. Tracking of the number, location, indications, circumstances, duration, and complications of CVC placement (including wastage of catheters by failed attempts) over time will provide important validation for enterprise-wide, standardization of simulation-based training for key procedures.

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· The role of the TSC1/2 complex in tumor development

Professor

AWARDS

LAM Foundation Senior Investigator Award

FUNDING

National Institutes of Health
LAM Foundation
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Sackler Foundation

Over the last several decades, the study of hereditary tumor syndromes has laid a solid foundation for the genetic basis of cancer. While the number of patients suffering from these syndromes is small, the identification and elucidation of the underlying genetic pathways have shown to be of broad relevance to many forms of sporadic human cancers.

Investigations have found that the majority of hereditary tumors involve mutations of certain tumor suppressor genes. This latter class of genes has diverse functions including cell cycle regulation, DNA repair, apoptosis, protein degradation, cell-cell interaction, and signal transduction. However, a common feature of these genes is the "two-hit" genetic mechanism to inactivate their function during tumorigenesis. In the case of hereditary cancers, the first hit is inherited as a germline mutation of one of the alleles of the tumor suppressor gene, and the second hit is an acquired somatic mutation of the remaining allele of the same gene. This results in the loss of function of the tumor suppressor, thus creating a setting to promote tumor development.

One of the latest examples comes from the study of the tuberous sclerosis complex (TSC), an autosomal dominant disorder affecting more than 50,000 Americans. As a member of the phakomatoses, TSC is characterized by the appearance of benign tumors involving many organ systems, most notably the central nervous system, kidney, heart, lung, and skin. While classically described as "hamartomas," the pathology of the lesions is diverse, with features of abnormal cellular proliferation, growth (size), differentiation and migration.

Occasionally, TSC tumors progress to become malignant lesions (i.e., renal cell carcinoma). The genetic basis of this disease has been attributed to mutations in one of two unlinked genes, *TSC1* and *TSC2*. The protein products of these genes are found to negatively regulate the mTOR pathway, which controls protein synthesis, among other functions. Many human cancers have been found to exhibit abnormal activation of the PI3K/Akt/mTOR pathway, and recent clinical studies showed a therapeutic advantage in patients treated with an mTOR inhibitor. The key areas of current investigation focus on the elucidation of the molecular mechanisms of mTOR-related tumorigenesis, and the involvement of this pathway in liver cancer.

Growth Factor and Energy Metabolism in TSC Tumors

Studies in *Drosophila* have revealed a novel role of hamartin and tuberin in the PI3K/mTOR signaling pathway that is pivotal to the cellular response to growth factors (e.g., insulin) and nutrients. Genetic screens in mosaic flies for cell size control identified loss-of-function mutants of the *Drosophila* homologs of TSC1 and TSC2 that exhibit increased cell size in a cell-autonomous fashion. Conversely, over-expression of dTSC1 and dTSC2, but neither alone, effectively rescued this phenotype (i.e., reduced cell size). Genetic epistatic experiments in flies showed that the effects of dTSC1 and dTSC2 were dominant over dInR and dAkt but not dTor and dS6K. Biochemical studies confirmed a negative regulatory role of the hamartin-tuberin complex in mTOR-dependent protein synthesis.

The current model suggests that tuberin inhibits mTOR activity by serving as a GTPase activating protein for Rheb, a Ras-related protein, and consequently reduces p70S6K and 4E-BP1-dependent protein translation (Figure 1). Upon growth factor stimulation of PI3K, downstream activation of Akt results in phosphorylation of tuberin and releases its inhibition on mTOR. In TSC tumors, cells have lost TSC1 or TSC2 activity, thus resulting in uninhibited cell growth associated with elevated levels of mTOR and p70S6K activities. Indeed, pharmacologic blockade of mTOR with

If indeed hamartin and tuberin act on distinct molecular targets in various pathways, how may their function be regulated?

rapamycin, an immunosuppressant drug, causes profound anti-tumor response *in vivo*. However, it is not currently known how up-regulation of mTOR results in tumor formation, nor do we understand the mechanisms of tumor response to rapamycin.

Other unanswered questions include the physiologic role of TSC1/TSC2 in cellular metabolism, the function of PI3K/mTOR pathway in tumor initiation, and the long-term efficacy of rapamycin in TSC pathology. These issues are being addressed using various cellular and *in vivo* models of TSC.

The β–catenin pathway and the TSC genes

At present, not all of the TSC phenotype can be explained by one pathway. Our lab has explored the role of the TSC genes in the Wnt/β–catenin pathway. The latter has been implicated in the regulation of cell proliferation, differentiation, and migration. The Wnt family of secreted growth factors acts on multiple signaling cascades among which the β–catenin canonical pathway is best understood for its role in various human cancers (e.g., colon, skin, liver). β-catenin is a highly conserved 95-kD protein involved in cell-cell adhesion and intracellular signaling. In its latter role, β-catenin shuttles from the cytosol to the nucleus upon Wnt stimulation, where it binds the LEF/Tcf family of transcription factors to activate downstream target genes such as cyclin D1 (Figure 1).

Our observations showed that renal tumors derived from our TSC animal model expressed high levels of β–catenin and cyclin D1. In 293T renal epithelial cells, expression of TSC1 and TSC2 reduced β–catenin levels by promoting its degradation. Correspondingly, TSC1/TSC2 inhibited β–catenin dependent activity of the LEF/Tcf transcription factors. Evidence suggested that TSC1 and TSC2 act at the level of the β–catenin degradation complex by associating with its components (i.e., GSK3, Axin) in a Wnt-dependent manner. Collectively, the TSC proteins likely function in multiple pathways giving rise to the diverse manifestations of the pathology resulting from their inactivation (Figure 1). Efforts to demonstrate *in vivo* participation of these pathways and their relative contribution to the disease phenotype are currently our focus of investigation.

The Role of TSC1/2 in Microtubule Organization and Function

If indeed hamartin and tuberin act on distinct molecular targets in various pathways, how may their function be regulated? One possible mechanism for separating multiple activities within the cell could be on the basis of unique subcellular localization of the proteins. Since signaling complexes function as modules, the context in which they interact with other proteins depend on their localization. For example, insulin stimulation of PI3K leads to localized increased concentration of PIP3 at the plasma membrane. This, in turn, recruits Akt from the cytosol to the membrane where it becomes activated.

In studying the subcellular localization of hamartin and tuberin, we found that they indeed reside in multiple compartments (i.e., cytosol, microsome, cytoskeleton). Of particular interest is the vesicular component in which tuberin was previously shown to interact with rabaptin-5 to modulate endocytosis. Biochemical analyses showed that the microsomal fraction of TSC2 belongs to the lipid raft domains and interacts with caveolin-1, a cholesterol-binding, structural protein of caveolae. Cells devoid of tuberin have mis-localized caveolin-1 and reduced formation of caveolae at the plasma membrane.

Recent studies point to a role of tuberin in regulating the transport of proteins such as caveolin-1 from the Golgi apparatus to the membrane. The molecular mechanism mediating this function of tuberin and the consequence of faulty protein trafficking in tumorigenesis remain to be elucidated.

Genetic Modifiers and Phenotypic Heterogeneity

One of the unexplained observations of the TSC syndrome is the variability in disease severity. This so called phenotypic heterogeneity can be seen in related individuals carrying the same genetic mutations, thus implicating the presence of other modifying factors.

Using animal models of TSC, we studied the influence of genetic background on tumor size and found that a specific TSC2 mutation, when placed into two unrelated strains of rats, produced vastly different disease burden. By means of quantitative trait analysis, a genetic modifier was identified and mapped to rat chromosome 3.

It appears that this locus affects tumor size without significant influence on tumor multiplicity, suggesting a role in tumor progression rather than initiation. The identity of this gene and its function are currently being sought.

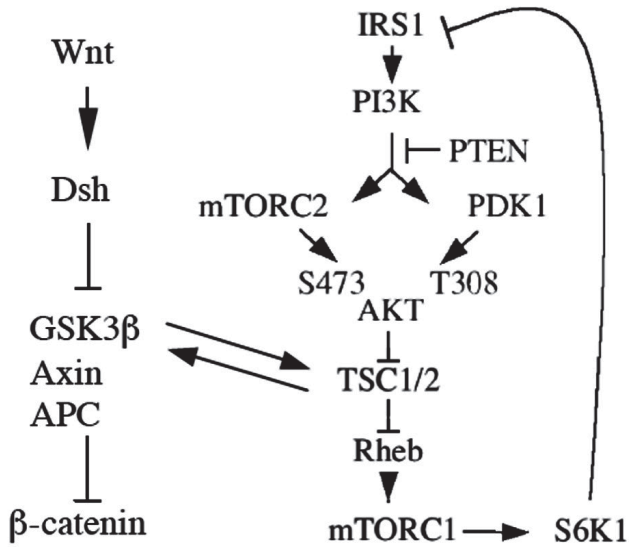

Figure 1: Model of TSC1/TSC2 pathway

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VAPSHCS / GENERAL SURGERY

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· Mechanisms of Injury Control after Hepatic Ischemia-Reperfusion

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FUNDING
VA Merit Review Grant

Liver failure is often preceded by a period of inadequate tissue and cellular perfusion (ischemia). Hypoxia/re-oxia initiates early activation of Kupffer cells, producing a wave of reactive oxygen species (ROS) and proinflammatory cytokines, in particular TNFα, IL-1β, and IL-6, as well as chemokines. These cytokines, together with reactive oxygen species, act in a paracrine manner on adjacent hepatocytes and endothelial cells, resulting in direct cytotoxic effects. Hepatocytes (HC), in turn, amplify the Kupffer cell (KC) response by expressing and releasing mediators such as IL-6 to further activate neighboring cells. Cytokines released into the circulation may also initiate secondary organ injury, setting the stage for multiple organ failure. Activated neutrophils are recruited by chemokines to the sites of primary and secondary injury. Working in concert with activated compliment, these mediators exacerbate the initial injury through microcirculatory vasoconstriction and release of additional reactive oxygen products. This late phase may continue to progress, culminating in liver necrosis with attendant organ failure, or resolve with resumption of normal liver function and a return to homeostasis.

While the progression of injury following liver ischemia-reperfusion has been well described, the mechanisms of regulation contributing to injury control and ultimate resolution are less well understood. Therapeutic strategies to improve outcomes have been aimed at blocking individual components of this widely redundant inflammatory cascade prior to the onset of IR. To date, however, laboratory successes have not translated to clinically relevant therapies. Further, given that many patients present for treatment after the pro-inflammatory phase of injury is well underway, a more realistic approach would focus on understanding the mechanisms of inflammation regulation and control.

Understanding the mechanisms of cellular signaling that precede, trigger and control the inflammatory response to an injury could be key to effective clinical modulation of ischemia-reperfusion injury and its complications.

Suppressor of Cytokine Signaling Control of Inflammation

A regulated response to injury requires both active inflammation, with the expression of pro-inflammatory cytokine and chemokine mediators and neutrophil activation and trafficking, and active inflammation control. The spectrum of pro- and anti-inflammatory cytokines that contribute to this dynamic process utilize common cell signaling pathways to mediate their effects. One key pathway involves the Janus family of tyrosine kinases (JAK-Tyk) and the signal transducers and activators of transcription proteins (STATs). The JAK-STAT pathway requires cytokines to form a ligand-receptor complex that phosphorylates the cytoplasmic portion of the cytokine receptor. This receptor-associated Janus kinase (JAK) then forms a docking site for signal transducer and activator of transcription (STAT) and the resulting complex allows tyrosine phosphorylation of the STAT with formation of an activated dimer or tetramer. The STAT dimer/tetramer translocates to the nucleus and binds with a specific DNA sequence and/or other transcription factors to effect targeted gene transcription.

In addition to sustaining cytokine signaling, STAT-mediated cell signaling also induces the expression of Suppressors of Cytokine Signaling (SOCS) proteins that serve as classic negative feedback mechanisms for cytokine expression. Numerous cytokines important to acute inflammation activate cells through JAK-STAT, including TNFα, IFNγ, IL-1, IL-6, IL-10 and erythropoietin. These mediators are, in turn, controlled, at least in part, by SOCS proteins.

Understanding the cellular signaling mechanisms that initiate the hepatic inflammatory response and trigger its regulation is central to the development of effective strategies for injury control after ischemia–reperfusion.

Our current work focuses on determining the role of JAK/STAT signaling and SOCS-mediated negative regulation on the evolution of liver injury and resolution. We hypothesize that SOCS1 and SOCS3 are essential to the evolution and ultimate resolution of liver IR, cooperatively delimiting cytokine/chemokine-mediated primary and secondary injuries through negative regulatory cross-talk between cells as well as distinct intracellular signaling pathways. Using a murine model of hepatic IR, we are exploring the protective effects of SOCS-induction with erythropoietin as well as the injurious effects of SOCS1 or SOCS3 conditional deletion from hepatocytes on liver IR severity. We have shown that while SOCS3 expression is consistent across a broad range of IR injury from mild to severe, SOCS1 expression directly parallels the severity of ischemic injury. These data suggest that SOCS1 provides a second tier of cytokine regulation when SOCS3 alone is insufficient. Deletion of either protein from hepatocytes appears to be tolerated when injury is moderate but loss of SOCS1 dramatically compounds injury severity when ischemia is prolonged. These data support the concept that while SOCS1 and SOCS3 share some functionality, they do not appear to be interchangeable.

Erythropoietin – A Potential Injury Protection Strategy

Like many of the pro-and anti-inflammatory mediators important to hepatic IR, erythropoietin (rhEPO) also signals through JAK-STAT. Erythropoietin is a glycoprotein hormone vital to the differentiation of committed erythroid progenitor cells. Over the last decade a variety of non-hematopoietic effects have been attributed to exogenous rhEPO, in particular protection after ischemia in a variety of tissues, including brain, heart, kidney and, most recently, liver. This protection has been observed with pre- and post-injury treatment and has generally been ascribed to induction of anti-apoptotic genes. A less well explored function of rhEPO is its capacity to induce several suppressors of cytokine signaling (SOCS1, SOCS3 and Cis), primarily through STAT5 and STAT3.

We have shown that rhEPO primarily induces Cis-mediated regulation in erythroid progenitor cell lines, Socs3 appears to be dominant rhEPO-induced SOCS regulatory mechanism in normal liver. rhEPO induces both Socs1 and Socs3 early in reperfusion after severe IR. Like many of the cytokines important to IR, rhEPO is known to signal

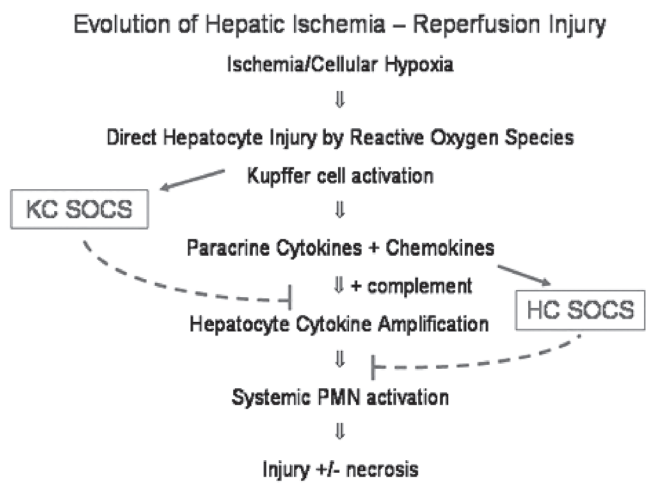

Figure 1: Evolution of Liver Injury and Resolution

through STAT3 as well as STAT5. STAT5 is significantly blunted by rhEPO while STAT3 signaling is sustained. This occurs in the absence of major pro-inflammatory mediators important to IR that signal predominantly through STAT3. Socs3 is known to selectively regulate IL-6 associated STAT3 signaling but not that utilized by IL-10. Active negative regulation of pro-inflammatory mediators coupled with sustained anti-inflammatory cytokine signaling mechanisms, would alter the balance of the response to severe IR and inhibits injury progression.

Our next phase of study will focus on the role of SOCS-mediated cytokine regulation in non-parenchymal liver cells, in particular Kupffer cells, utilizing mice with inducible deletion of SOCS1, SOCS3, or both regulatory genes in all liver cells. We hypothesize that rhEPO's direct affect is primarily on Kupffer cells, setting the stage for prompt regulation of the initial cytokine burst, without which the amplification of IR injury through targeted pro- and anti-inflammatory secondary gene responses in neighboring hepatocytes cannot proceed.

SUMMARY OF SIGNIFICANCE: Furthering our understanding of the cell signaling events that define and control the acute inflammatory responses to primary and secondary injury will foster the development of treatment strategies important to promoting injury progression, resolution and healing. Our long-term goal is to identify and potentially exploit the natural inflammatory control mechanisms as a novel avenue for clinical management of ischemia-reperfusion injuries.

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· Rural Surgery

· General Surgery Manpower and Demographics

FUNDING

CHRMC Academic Enrichment Fund Award
CHRMC Clinical Outcomes Steering Committee Award

Rural Surgery

General surgeons are a crucial component of the medical workforce in rural areas of the United States. Any decline in their numbers could have profound effects on access to adequate health care in such areas. A study group from the University of Washington involving the Department of Surgery, the Department of Family Medicine and the Washington, Wyoming, Alaska, Montana, Idaho (WWAMI) Rural Health Research Center funded by a grant from the Federal Office of Rural Health Policy set out to characterize the general surgery workforce in rural America. We hypothesized that the rural areas of the United States are relatively undersupplied with general surgeons.

Our method was to cross-reference the general surgeons listed in the 2001 American Medical Association Physician master-file with the ZIP code version of the Rural-Urban Commuting Area (RUCA) classification system. General surgeons were classified as self designated practitioners of general surgery/abdominal surgery/critical care surgery/trauma surgery, clinically active and 62 y/o or younger. These are the same criteria used by Jonasson et al. in their landmark surgical manpower papers from the 1990s.

RUCAs use census tracts to define rurality, based on community populations and work commuting patterns. We grouped the RUCA classifications into the following categories. An "urban" area is defined as a metropolitan core with a population of greater than 50,000. A "large rural" area has a town core with a population of 10,000–50,000. A "small/isolated rural" area has a town core with population of 2,500–10,000 or areas without an urban core of 2,500. The characteristics of the practitioners in these areas that we examined were: surgeon to population ratios, age, gender, US/Canadian medical school versus International Medical Graduate, and board certification.

We found that in 2001 there were 17,243 clinically active, non-federal general surgeons in the United States. The overall general surgeon to population ratio was 6.4 general surgeons per 100,000 population. Females constituted 10.6% of the general surgeons. 80.1% were graduates of US or Canadian medical schools. General surgeons in urban areas were more likely to be female (11.7%) than those in large rural (6.1%) or small/isolated rural areas (7.3%). General surgeons 50 years and older were significantly more likely to be located in small/isolated rural areas than in urban areas (51.6% versus 42.1%, $p < 0.001$). International medical graduates were more likely to be located in small rural areas than urban areas (25.2% versus 20.1%, $p < 0.001$). Board certification was not reported for 4.7%. 79.1% of surgeons were located in urban areas, 11.3% in large rural areas and 9.5% in small/isolated rural areas. The number of general surgeons per 100,000 was 6.4 nationally, 6.53 in urban areas, 7.71 in large rural areas and 4.67 in small/isolated rural areas.

Overall, the 22.4% of the American population living in the nation's rural areas are served by 20.8% of the nation's general surgeons. At first glance it would seem that rural America is adequately supplied with general surgeons. However, when you compare the large rural areas with 7.71 surgeons per 100,000 with small/isolated rural with 4.67 surgeons per 100,000 it becomes apparent that not all rural areas have equal access to surgeons. How much this will affect a given area depends upon its proportion of residents living in small/isolated rural areas. Within our WWAMI

General surgeons 50 years and older were significantly more likely to be located in small/isolated rural areas than in urban areas. International medical graduates were more likely to be located in small rural areas than urban areas.

district the percentage of the population living in small/isolated rural areas area as follows: Washington 7.8%, Wyoming 41.3%, Alaska 27.3%, Montana 38.2% and Idaho 26.8%.

The question remains whether there is a shortage of rural general surgeons at present. Several definitions of "adequate" supply have been proposed over the years, including 7.1 general surgeons per 100,000 proposed by Jonasson and Kwakwa in 1996 and an HMO benchmark of 5–6 per 100,00 proposed by Goodman et al. Neither of these takes into account actual adequacy of provision of surgical care. However, the number of general surgeons per 100,000 population is, on average, markedly lower in small/isolated rural areas than in large rural and urban America. In some areas the number is as low as 2 or 3 surgeons per 100,000. When you compound that with obstacles of distance from medical centers, geography and weather – especially in the American West – a shortage of rural general surgeons is probably present in many small/isolated areas of this country. The fact that our data show that these surgeons are more likely to be male and elderly than their urban counterparts suggests that this problem will only worsen.

General Surgical Manpower

The second phase of our study examined the demographic trends of general surgeons over the past 25 years in order to ascertain whether the apparent gap between urban and rural general surgical manpower is static or worsening and to determine what factors contribute to it. What we found surprised us. The overall supply of general surgeons per 100,000 population has declined by 25% over the past 25 years. The overall rural ratio dropped by 21%, but the urban ratio dropped by an even greater 27%. Factors contributing to this decline include: the fixed number of graduating general surgery chief residents per annum since 1997 despite an increase in population of 1% per annum during this period; increasing numbers of graduating general surgery residents (as much as 70–80% in some studies) choosing to sub-specialize rather than go directly into the practice of general surgery; and, in urban areas, increased penetration of health maintenance organizations (HMOs), which tend to employ fewer surgeons per 100,000 patients than are found in areas without HMOs. Whether this decline in the supply of general surgeons relative to the population signifies a "shortage" of surgeons and a concomitant decrease in access to surgical care is difficult to measure. However, it raises a legitimate concern of whether there will be an adequate number of general surgeons to care for an increasingly elderly population, with its attendant increase in demand for surgical care.

Conclusions

The relative undersupply of general surgeons will have different effects on the healthcare system depending on location. In many urban areas, recent publications by the Institute of Medicine and the American College of Surgeons cite problems with staffing and availability of general surgeons for emergency services. In remote and isolated rural areas, recent research reveals serious problems with recruiting partners for and replacement of rural general surgeons – who are already, on average, closer to retirement age than their urban counterparts.

Solutions to the problem in urban areas include having surgical hospitalists cover emergencies and consults in shifts (already done in the academic setting at UCSF and in private settings in Spokane). Larger urban centers may come to employ a member of the evolving specialty of acute care surgeons as promulgated by Gregory Jurkovich of Harborview. For rural areas, measures will include better preparation for and recruitment for rural practice. This would be fostered by more rural rotations for general surgery residents.

Such rotations exist at OHSU and Cooperstown, and we will be developing a similar program here at the UW under the leadership of Drs. Carlos Pellegrini and Karen Horvath. Rural hospitals will also have to consider offering significant financial bonuses and /or debt relief, as well as guaranteed locums coverage, if they hope to lure graduating general surgeons to more remote rural areas.

Other issues in rural surgery that we plan to examine are: training and education, volumes/outcomes, quality of surgical care and recruitment/retention. It is our hope that this research will serve to inform the decision making processes of the government agencies, private bodies and educational institutions whose policies and plans will determine how surgical care is provided to rural residents in the future.

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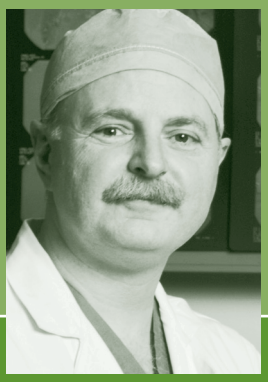
Michael Sobel, M.D.

· Heparin, Platelets, and Vascular Cells



Professor and Chief
Veterans Affairs PSHCS

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Located at the Veterans Administration Puget Sound Health Care System, the Vascular Research Laboratories are led by Michael Sobel, M.D., Errol Wijelath, Ph.D., and supported by other Ph.D.s and postdoctoral trainees. The principal focus of Dr. Sobel's research group is understanding the structure-function relations of heparin's interactions with vascular proteins and cells. Heparins are a family of structurally heterogeneous sulfated polysaccharides. Heparin is best known for its anticoagulant properties, which are exerted by heparin binding to the plasma protein antithrombin-III. But beyond their conventional anticoagulant actions, heparins have a wide range of other biological effects, antiproliferative and anti-inflammatory as well as stimulatory actions on some vascular cells. And while the interaction between heparin and antithrombin-III is known to depend on a well defined structural domain — the heparin pentasaccharide — heparin interactions with other proteins and cells have not been as well characterized. In part, the structural complexity of carbohydrates and heparin in particular has hindered efforts to better understand its structure-function relations. Also, the biological effects of heparins have often been contradictory or confusing, due to the complexity of the biological models used.

The interactions between platelets and heparin have been especially confusing. The autoimmune-mediated phenomenon of heparin-induced thrombocytopenia is one aspect of heparin-platelet interactions. But apart from this unusual immune reaction, Dr. Sobel's laboratories have found that heparin directly influences platelet function by at least two separate mechanisms.

Heparin Interactions with von Willebrand Factor

Using biophysical methods, binding assays, and molecular modeling, we demonstrated that heparin binds to a specific domain of von Willebrand factor (vWf) [1, 2]. This plasma protein is essential for normal platelet hemostatic function, and mediates the adhesion of platelets at sites of vascular injury (especially under high shear, arterial conditions). When heparin binds vWf it interferes with the platelet hemostatic properties of the protein. Specific sub-species of heparin were purified that bound vWf with especially high affinity. Through scientific collaborations with Dr. Yasuo Suda, a carbohydrate polymer chemist in Japan, a structurally defined disaccharide motif was identified that was responsible for heparin's binding to vWf. A refined heparin with high affinity for vWf (and low affinity for antithrombin-III) was effective at preventing arterial occlusion in an animal model of platelet-vWf-dependent arterial thrombosis [3, 4]. This work holds future promise for developing novel antithrombotic heparins that interfere with vWf-mediated platelet adhesion, rather than retarding plasma coagulation.

Heparin Binds Directly to the Platelet Integrin

Heparin also has a contradictory, direct stimulatory effect on platelet function. In related work, it was shown that heparin binds directly to the platelet surface, and that one of the important binding sites may be the platelet fibrinogen receptor, GpIIbIIIa (integrin αIIbβ3). Unlike vWf, which mediates platelet adhesion at high shear rates, the fibrinogen receptor is responsible for platelet aggregation and clumping at lower shear rates. Through physiological studies of platelet aggregation, photoaffinity cross-linking, and cell-signaling work, heparin was found to bind to this platelet integrin, and enhance its binding of fibrinogen [5].

Heparin Modulates β3 Integrins

How does heparin activate or enhance integrin function in the platelet? To see whether these effects were unique to the platelet integrin (αIIbβ3), the K562 cell line was transfected with different integrins, and the effects of heparin on integrin-mediated cell adhesion were studied. Surprisingly, the effect of heparin on integrin function depended on the integrin subunit. A stimulatory effect was observed in all β3 containing integrins (αIIbβ3, αV β3) but the type of α subunit did not seem to be as important. The effect of heparin was structure-specific, as other glycosaminoglycans and low molecular weight heparins showed no enhancement of adhesion (6). Because integrins are such ubiquitous receptors in vascular cells, a detailed understanding of precisely how heparin modulates these receptors may lead to novel drugs to modulate thrombosis and vascular healing.

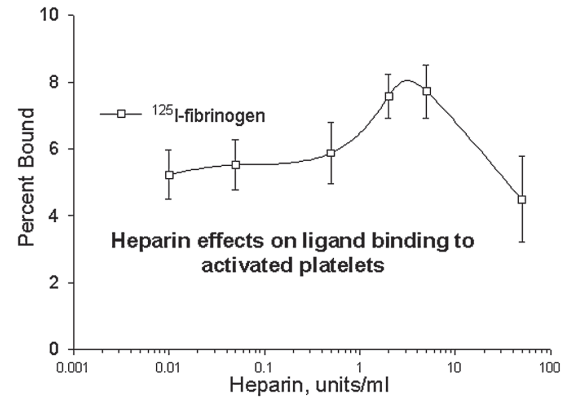

FIGURE 1: ^{125} **I-Fibrinogen binding to thrombin-activated platelets was measured over a range of heparin concentrations. At concentrations of 2 and 5 units/ml heparin, fibrinogen binding was significantly increased.**

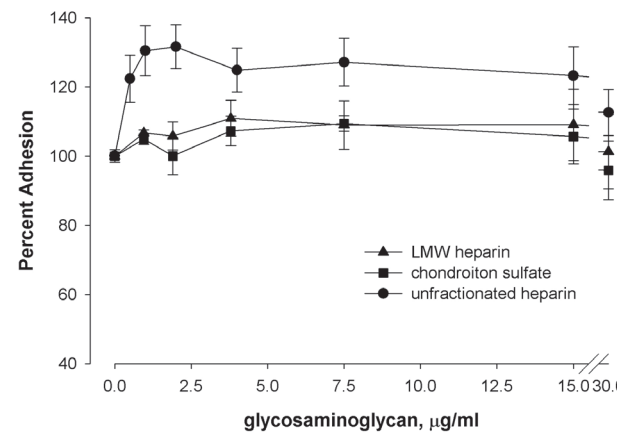

FIGURE 2: **Thrombin activated platelets.**

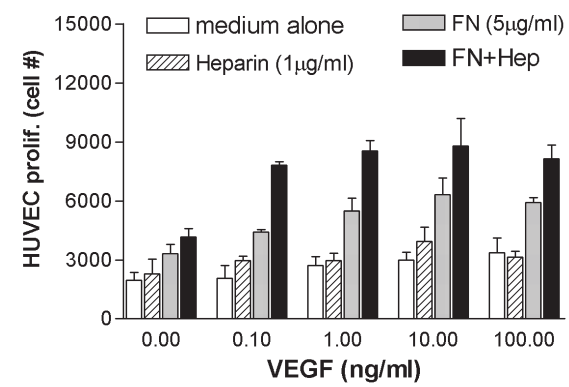

FIGURE 3: **Adhesion of K562 avb3 cells to vitronectin. Unfractionated heparin enhances integrin-mediated adhesion, but other glycosaminoglycans do not.**

Because integrins are such ubiquitous receptors in vascular cells, a detailed understanding of precisely how heparin modulates these receptors may lead to novel drugs to modulate thrombosis and vascular healing.

Heparin Modulation of Endothelial Cell Migration and Proliferation

Matrix proteins and growth factors (and their respective cellular receptors — integrins and receptor tyrosine kinases) are key actors in angiogenesis and vascular healing. Integrins and growth factor receptors work together to enhance the extracellular signals from each pathway, leading to increased endothelial cell proliferation and migration. Vascular Endothelial Growth Factor (VEGF) and fibronectin appear to have a unique complementary relationship. In a recent publication, VEGF was shown to preferentially bind to fibronectin over other matrix proteins [7]. Platelets actually release pre-formed VEGF/fibronectin complexes, and these complexes have significantly more potent mitogenic effects than VEGF or fibronectin alone on endothelial cells. Heparin further supports the synergistic biological effects of VEGF/fibronectin. Once again, heparin (and cell-surface heparan sulfate proteoglycans) may be playing a key role in modulating the extracellular assembly of specific ligands on their cellular receptors.

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Peter C. Wu, M.D.

· Gastrointestinal Cancer Treatment and Cellular Senescence

Assistant Professor

AWARDS

Society of Surgical Oncology
• James Ewing Research Award

FUNDING

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VA Career Development Award

Advanced gastrointestinal (GI) cancers treated with chemotherapy and radiation exhibit disappointingly low 5–30% complete response rates. The majority of tumors are limited to only partial responses and surgery continues to be the mainstay treatment for most GI cancers despite poor overall survival rates. For example, chemoradiotherapy for advanced rectal cancer often results in detectable tumor volume reduction following early treatment, but is often succeeded by tumor progression despite additional therapy.

Cellular senescence has long been described for primary tissues grown under culture conditions. This "aging" associated physiological arrest has been shown to limit the replicative lifespan of cells in response to gradual erosion of the telomere. Replicative cellular senescence can also result from oncogenic signals. For example, *ras*-induced senescence has been increasingly recognized as a tumor suppression mechanism in carcinogenesis and accounts for the proliferative arrest observed in many benign tumors. Malignant tumors are characterized by their ability to bypass replicative senescence, but can be induced into a state of cell cycle arrest following multimodal therapy, termed **therapy-induced senescence.**

Mounting evidence now suggests that therapy-induced senescence is a prominent solid tumor response to therapy and it most reasonably accounts for early provisional treatment responses by prolonged cell cycle arrest. However, certain senescent cancer cells are capable of escaping senescence and resuming cell division, leading to eventual tumor progression. Therapy-induced senescence is predicted to be a telomere-independent process since telomere erosion is not expected to occur given the lack of cell doubling.

Surprisingly, we discovered that massive telomere loss does indeed occur in senescent cancer cells following chemotherapy. Furthermore, we have also found that senescent cells that escape replicative arrest are able to partially recover their telomere loss. Based upon these observations, we propose the hypothesis that **modulation of telomerase activity regulates escape from therapy-induced senescence in colorectal cancer**. Therapy-induced cellular senescence is a novel paradigm of cancer therapy response that has recently been validated *in vivo* through work done by our laboratories and others. We aim to define the role of telomerase in regulating therapy-induced senescence and senescence escape in colorectal cancer.

This project is a key component of an ongoing effort to elucidate molecular mechanisms of therapy-induced senescence and identify markers that can reliably predict treatment response and reveal key checkpoints that could be targeted to block senescence escape and enhance clinical treatment responses in patients diagnosed with locally advanced or metastatic GI cancers.

Current Understanding of Cellular Senescence and Cancer

A requirement for the malignant transformation of tumor cells capable of infinite cell division is bypass of the physiological program of cellular senescence that limits the replicative lifespan of normal cells. In the lifespan of somatic cells, progressive loss of telomere length occurs with each successive cell division, reaching a critical shortening which has been shown to trigger a p53-mediated replicative arrest signal. Human diploid fibroblasts enter a state of replicative arrest after 60–80 population doublings, which has been termed *Hayflick's limit* or mortality stage 1 (M1).

It has been well established that the replicative lifespan of these cells can be extended beyond this limit through inactivation of the p53 and other pathways. Immortalized cancer cells appear to bypass the M1 checkpoint through mutational inactivation or oncogenic viral targeting of these pathways. After bypassing M1 restriction, telomeres progressively shorten with each successive cell division until a critical second restriction point is encountered, termed M2.

How, then, do tumor cells keep dividing? Encountering this barrier provokes either of two mechanisms to avoid reaching a critical threshold of telomere loss that will result in cell death. One mechanism tumor cells utilize to preserve telomere length is over-expression of telomerase which catalyzes telomere repair, and the other involves activation of an alternative telomere-lengthening mechanism. Both of these mechanisms enable tumor cells to bypass the M2 restriction and thereby regain capacity for unlimited cell division and immortalization.

Despite having bypassed both M1 and M2 stages, cancer cells can still undergo terminal growth arrest in response to anti-cancer drugs or ionizing radiation. This telomere-independent response, termed therapy-induced senescence, is believed to overlap with the physiologic cellular senescence program. Senescent cells in replicative arrest are characterized by morphologic alterations, including enlarged and flattened cell shape with increased cytoplasmic granularity, nuclear polyploidy, and characteristic expression of the senescence marker, b-galactosidase (SA-b-gal, see Fig. 1). We have shown that therapy-induced senescence can be reliably induced in various tumor cell lines following exposure to a variety of chemotherapeutic agents, which suggests that therapy-induced senescence represents a primordial cellular stress response of epithelial cancer cells to anti-cancer drugs.

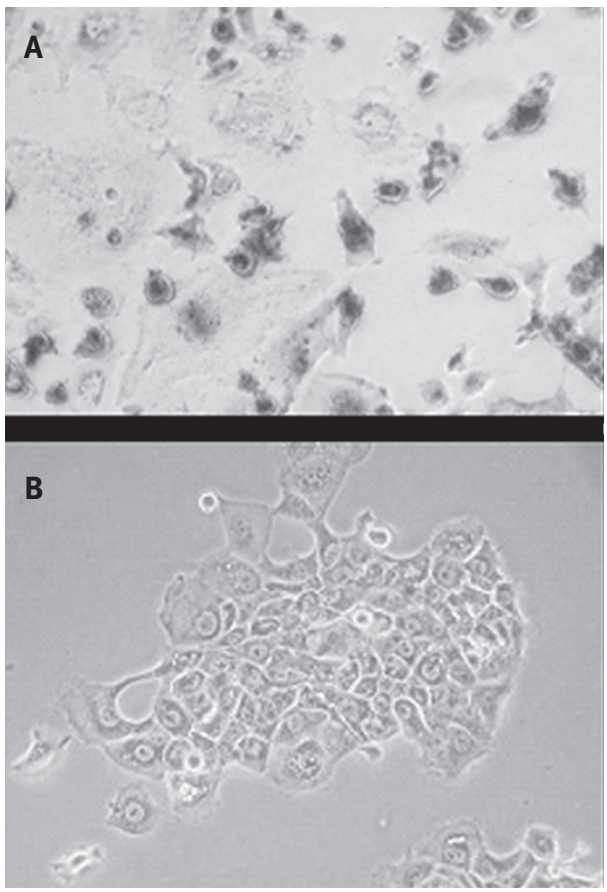

Figure 1: Light microscopy of blue-appearing tumor cells in therapy-induced senescence stained with X-gal. (A) H1299 lung carcinoma cells. (B) Bx-PC3 pancreatic carcinoma cells

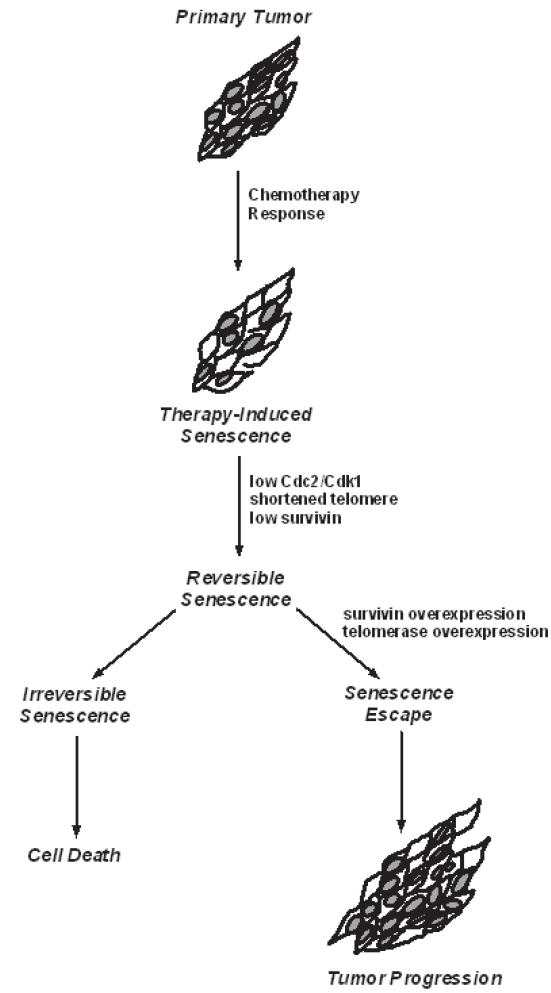

Figure 2: Hypothesis – modulation of telomerase activity regulates escape from therapy-induced senescence in colorectal cancer.

Therapy-Induced Senescence Occurs *In Vivo* and Represents Tumor Response to Treatment

The demonstration of therapy-induced senescence *in vivo* has been reliably shown in xenograft and transgenic murine models. Evidence of senescence has also been shown in a reported outside study of post-treatment tumor samples obtained from breast cancer patients treated with a neoadjuvant multi-agent chemotherapy. SA-b-gal expression, a reliable marker for senescence, was found in 41% of tumors resected from patients treated with chemotherapy, but in only 10% of untreated patients. Similar findings have been published in our laboratories showing evidence of chemotherapy-induced senescence in human lung cancer patients treated at the VA. Furthermore, this study also showed that tumor senescence markers correlated with clinical tumor response. Lung cancer patients in whom tumors were identified in a state of therapy-induced senescence showed minimal tumor response to treatment and evidence of tumor progression. By comparison, patients without evidence of tumor senescence showed improved responses to therapy.

Clinical Response of Solid Tumors to Multimodality Therapy is Best Described by Therapy-Induced Senescence

The anti-tumor effects of chemotherapy have been commonly attributed to two forms of programmed cell death, apoptosis and autophagy. For most solid gastrointestinal cancers, however, these mechanisms cannot account for the modest (20–40%) disease response to chemotherapy observed weeks to months after treatment. Even in patients demonstrating near-complete responses to chemotherapy and/or radiotherapy, any remaining residual viable tumor cells will regain proliferative capacity resulting in cancer recurrence. For example, chemoradiotherapy used to treat locally advanced rectal cancer patients frequently produces detectable tumor volume reduction that is later overcome by tumor progression despite ongoing therapy.

The relatively slow onset of solid tumor responses to chemotherapy and the lack of a consistent correlation with apoptosis in numerous studies suggest that other pathways regulating cell death may predominate. Moreover, similarities in observed response rates regardless of the particular chemotherapeutic agent applied to specific cancers suggest that chemotherapy drugs may mediate their effect through non-specific drug/target mechanisms. The therapy-induced senescence model closely parallels the clinical observations of gastrointestinal malignancies treated with chemotherapy. Given that most solid tumors recur following therapy, it follows that some cancer cells undergo cell cycle arrest as a result of senescence *in vivo* and retain the ability to escape senescence in order to reproliferate, resulting in cancer progression.

Cdc2/Cdk1 Regulates Therapy-Induced Senescence and Escape from Senescence

The cyclin-dependent kinase, Cdc2/Cdk1 is a key control point that determines senescence phenotype. In order to examine key aspects of therapy-induced senescence and senescence escape, we have used the p53-null, p16-deficient NCI-H1299 carcinoma line as a model. H1299 cells exposed to moderate doses of camptothecin were shown to arrest in G2/M and enter a senescent state. Allowing recovery time, occasional senescent cells (frequency of 1:100,000 cells) were able to "escape" cell cycle arrest and form proliferating or "escape" colonies.

Analysis of these escape colonies showed that Cdc2/Cdk1 was aberrantly over-expressed compared to low-level expression observed in senescent cells. Furthermore, these cells were found to be dependent upon Cdc2/Cdk1 kinase activity to sustain viability, such that blocking Cdc2/Cdk1 via a selective inhibitor or competitive siRNA translated into rapid cell death. Specific inhibition of Cdc2/Cdk1 was also found to effectively abrogate escape from therapy-induced senescence. These findings suggest that down-regulation of Cdc2/Cdk1 mediates induction of senescence and its aberrant over-expression is essential for escape from senescence.

The Cdc2/Cdk1 Effector Protein, Survivin, Inhibits Apoptosis and is an Important Determinant of Clinical Outcome

Survivin, a 16.5 kDal nuclear protein, is the smallest member of the human inhibitor of apoptosis protein (IAP) family. Survivin is expressed in a cell cycle-dependent manner and levels are markedly increased during mitosis. Survivin protein is stabilized by undergoing phosphorylation mediated by Cdc2/Cdk1 kinase and appears to play a crucial role in mitotic spindle association and inhibition of caspase-9-mediated apoptotic activity. Administration of the chemotherapy drug Taxol, a microtubule inhibitor agent, in HeLa cells activates a survival checkpoint by up-regulation of Cdc2/Cdk1 resulting in activation and accumulation of survivin.

The relatively slow onset of solid tumor responses to chemotherapy and the lack of a consistent correlation with apoptosis in numerous studies suggest that other pathways regulating cell death may predominate.

Conversely, suppression of survivin activation with the Cdc2/Cdk1 kinase-inhibitor flavopiridol enhances adriamycin-induced apoptotic cell death. Survivin knockout has been shown to be embryonic lethal and fibroblasts derived from these animals exhibit catastrophic defects in microtubules, centrosomes, spindle poles, and in mitotic spindle microtubule formation. These results collectively suggest a critical role for survivin in cellular mitosis. Survivin has been found to be over-expressed in many types of human cancers; has been associated with unfavorable clinical prognosis in cancers of the breast, esophagus, stomach, pancreas, and colon; and has been shown to correlate with therapy resistance in a variety of clinical settings.

Survivin Enhances Telomerase Activity

In normal human cells, telomeres or nucleoprotein complexes located at the chromosome ends progressively shorten by 50–200 bp with each successive cell division through the loss of terminal DNA sequences. Telomeres are maintained by telomerase, a ribonucleoprotein polymerase that contains hTERT, a catalytic subunit providing reverse transcriptase activity. hTERT expression mainly determines telomerase activity and is expressed at high levels in embryonic stem cells and germ cells which decreases during differentiation and disappears in fully differentiated somatic cells.

However, through unknown mechanisms, hTERT is reactivated in 80–95% of cancer cells. When telomere shortening reaches a critical threshold, cells are either prompted to enter into protective cell cycle arrest (i.e. senescence) or undergo apoptotic cell death. In contrast, cancer cells possess the ability to maintain and preserve telomere length and undergo sustained proliferation. Survivin has been shown to upregulate telomerase activity by augmenting the expression of human telomerase reverse transcriptase (hTERT) by phosphorylation of Sp1 and c-myc proteins that enhance binding to hTERT promoter. These findings support the concept that survivin enables cancer cells to escape senescence by promoting telomerase activity.

Telomeres and Cancer Senescence

Telomeres stabilize chromosomes and may act as a "mitotic clock" that determines the maximum replicative capacity of somatic cells. In humans, the telomere terminus is composed of 4–15 kbp of the hexanucleotide repeat TTAGGG, followed by a single-strand nucleotide overhang that loops back upon itself forming a "t-loop," which is associated with telomere DNA-binding factors that function to preserve telomere integrity. Additionally, complex nucleoprotein structures also serve to protect the telomere ends. Loss of the "t-loop" and terminal nucleoprotein complex, termed "uncapping," exposes the telomere to degradative shortening.

Our finding that HCT116 senescent colorectal cancer cells suffer massive telomere loss suggests that telomere integrity is rapidly compromised following camptothecin exposure by a mechanism that is unrelated to replication-dependent telomere attrition. The mechanism of such massive telomere shortening has not been thoroughly examined. Since telomere dysfunction in these situations is likely related to disruption of end structures *in vivo*, we propose that telomere uncapping leads to exonucleolytic degradation of telomere DNA in therapy-induced senescence.

Senescent escape cells that are able to recover their telomere length may regain replicative capacity. Survivin has been shown to enhance telomerase activity via up-regulation of Sp1 and c-Myc mediated telomerase gene transcription in colon carcinoma cells and telomerase up-regulation has been observed in senescence-resistant breast cancer cells treated with adriamycin. It follows that survivin has a protective function in senescent escape cells by inhibiting apoptosis and promoting escape by up-regulating hTERT and promoting telomere lengthening.

Current Laboratory Objectives

We have shown *in vitro* that cancer cells exposed to chemotherapy can enter a state of reversible replicative arrest (i.e. therapy-induced senescence) characterized by shortened telomeres and low levels of survivin protein. While the majority of cancer cells will transition to irreversible senescence, small numbers of senescent cancer cells can escape cell cycle arrest. Cancer cells that escape senescence and reenter the cell cycle are presumably a major contributor to cancer progression. Though independent observations have been made regarding the protective effect of senescence and telomerase on cancer cell survival and negative impact on clinical prognosis, the relationship between the two has yet to be established. Our preliminary studies suggest that telomerase expression modulates escape from senescence. The purpose of our current work (Fig. 2) is to ask: "Does telomerase regulate senescence status during colorectal cancer treatment?"

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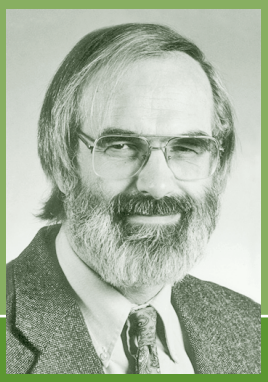

Research Professor Emeritus

- Tissue Pulsatility Imaging of Brain
- Atherosclerotic Plaque Neovascularization
- Ultrasound Reading Center for Carotid Stenosis

FUNDING

National Institute of Biomedical Imaging and Bioengineering

The purpose of this work is to use noninvasive low cost methods to characterize the vascular perfusion of tissues for diagnosis. Since the wide clinical use of arterial and venous plethysmography to diagnose arterial and venous obstructive diseases, there has been controversy about whether the primary volume changes in tissue were due to changes in the volume of the major arteries and veins or to changes in the volume of the microcirculation.

Tissue Pulsatility Imaging of Brain

Traditional methods of plethysmography measured volume changes in an entire body part, including both major vessels and micro-vessels. The introduction of photo-plethysmography, showing both pulsatile changes in skin reflectivity with the cardiac cycle and phasic changes in reflectivity with the respiratory cycle, supported the concept that the volume changes were, at least in part, volume changes in the arterioles and venules. We have extended the study of the volume changes in arterials and venules to deeper tissues using ultrasonic strain measurement methods.

Tissue can be considered a composite structure of cells, interstitium, matrix and microvessels. We consider the cells and matrix to be of constant volume over periods of a minute. The interstitium is an extravascular, extra-cellular space containing saline and is a space which may expand with edema over periods longer than a minute. The intravascular volume has three regions, venular at low pressure, capillary and arteriolar at high pressure. Images of tissue are comprised of "voxels," small volumes resolved by the method.

In ultrasound imaging, voxels are nearly cubes, 1 mm on each dimension. Microvessels (arterioles, capillaries and venules) are too small to resolve in 1 mm voxels. In each voxel the spacing of microvessels is about 0.1 mm, so there are about 1000 capillaries in each voxel. About 1% of tissue volume is capillaries, 1% is arterioles and small arteries, and 3% is venules and small veins. About 5% of each imaging sample volume contains blood in the microcirculation. Normally the tissue pressure is below the venous pressure, leaving the veins fully inflated. However, in regions at an elevation above the heart (above the right atrium) the veins and venules are collapsed. Whenever the local tissue pressure is below the local venous pressure, the venules are inflated; when the tissue pressure exceeds the venous pressure, the venules collapse.

We are developing ultrasonic and optical methods to measure percent volume changes in tissue. These methods can be used to determine both whether the microvascular volume in tissue is normal, elevated (from tumor angiogenesis), or reduced (from ischemia). They can also be used to detect elevated interstitial tissue pressures by determining the local venous pressure at which the local tissue volume changes from deflated to inflated. Of particular interest are tissues at high pressures, such as arterial walls and atherosclerotic plaques on those walls.

Transmural pressure is the difference between intravascular pressure and the surrounding tissue pressure. A model made of balloons representing the microcirculation and columns of fluid representing the venous pressure, arterial pressure and interstitial pressure demonstrates the principle that the tissue composite does not have a linear volume change as the tissue pressure rises, but there are sudden changes. As the interstitial pressure exceeds the venous pressure, the veins and venules collapse, reducing the tissue volume by 3% suddenly. Then, the volume changes little with increases in pressure until the arterial pressure is reached. At those pressures, the volume of the tissue pulsates, as arterial pressure rises above and drops below the interstitial

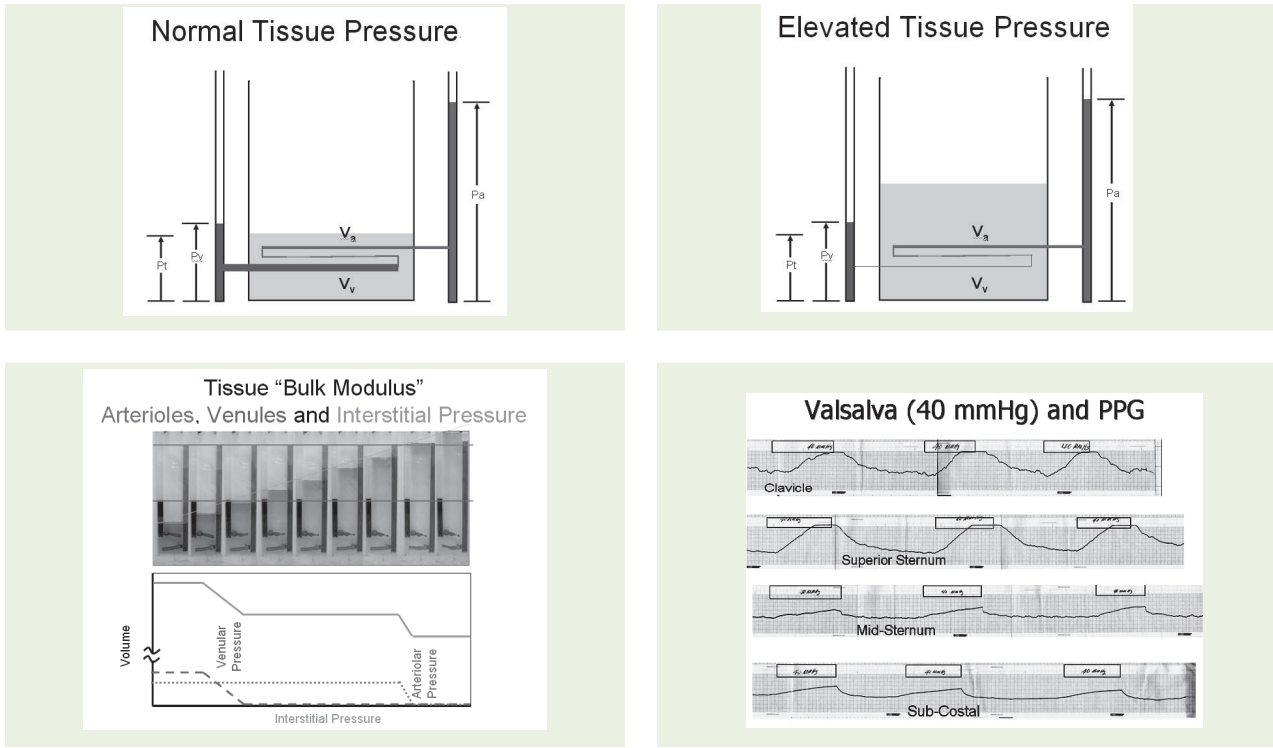

FIGURE 1: Tissue Pressure and Volume
UPPER LEFT When tissue pressure is normal, venules are inflated. **LOWER LEFT** As interstitial pressure increases above arterial pressure, the arteries and arterioles also collapse. **UPPER RIGHT** When tissue pressure is elevated, venules are collapsed. **LOWER RIGHT** Upper 2 tracings show the inflation of skin venules above the right atrium when a Valsalva maneuver raises central venous pressure; lower 2 tracings show the compliance of inflated of skin venules below the right atrium when a Valsalva maneuver raises central venous pressure.

pressure. This latter effect is the cause of large pulsations under a blood pressure cuff, because the blood pressure cuff, of course, controls the interstitial pressure.

Atherosclerotic Plaque Neovascularization

Applying these principles to the microcirculation providing nourishment to the cells within an atherosclerotic plaque, we are conducting a study of the effect of Bernoulli pressure depression due to high intra-stenotic velocities on carotid plaque pulsatile strain.

The vasa vasorum are a network of tiny arteries and veins on the outer wall of major blood vessels which penetrate into the wall to supply oxygen and nutrition to the vascular wall and remove metabolic waste products. In addition, these vessels penetrate through the arterial wall and into neovessels in the atherosclerotic plaques to provide nutrients and oxygen to the abnormal cells forming the plaque. The blood in the intraplaque neovessels is squeezed out in systole and inflates the neovessels in diastole.

We are developing an ultrasound examination method that can be performed through the skin of the neck to measure the strain (deformation) of these plaques due to the filling and emptying of the neovessels as the arterial pressure rises and falls with the cardiac cycle. By determining the arterial pressure when the neovessels inflate, the pressure in the vasa vasorum can be determined; the inflation volume is equal to the neovascular volume. Atherosclerotic carotid artery plaques which have a large neovascular volume are vulnerable to rupture, causing a stroke. The ultrasonic measurement developed in this project will differentiate plaques vulnerable to rupture from those that are stable. In this study we will measure the plaque strain in patients to provide a distribution of normal and abnormal strain values in the people who have stenotic atherosclerotic carotid artery plaques.

We expect the plaque strain waveform to deviate from the artery diametric waveform if the stenosis causes an arterial pressure drop; the waveform strain will mimic the pressure drop waveform and will be expansion in the proximal region of the plaque and contraction in the distal region of

Brain imaging studies show that people have many infarcts in other regions of the brain such as those associated with decision making, language, perception and memory which might not cause recognizable symptoms and might be missed on conventional neurological examination.

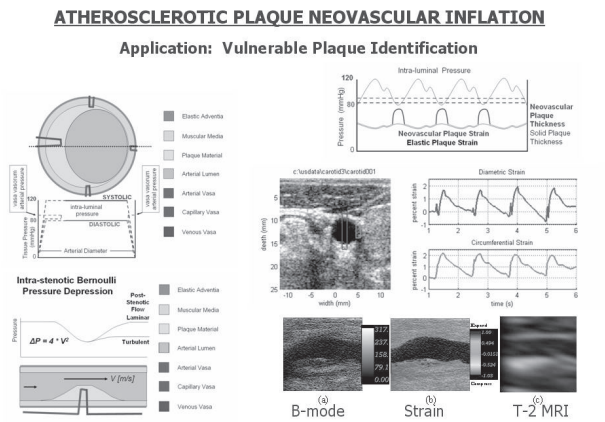

FIGURE 2: Carotid Plaque Vascularity Theory—A vulnerable atherosclerotic plaque is expected have a pulsatile strain waveform with a sudden inflations component in the upper right.

MIDDLE RIGHT WAVEFORMS: UPPER: measured arterial diameter pulsatile waveform, **LOWER:** measured tangential wall strain waveform.

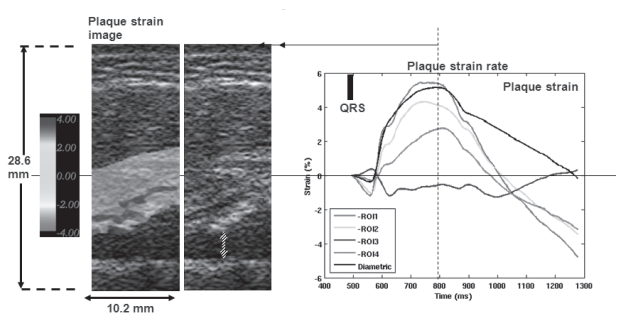

Image Computing Sciences Laboratory, 2007, Vijay Shamdasani

FIGURE 3: Inverse Plaque Strain Waveform at different locations in the plaque compared to the diametric waveform (black). If vascularization is absent and shear strain is absent, then the inverse radial plaque strain waveform will match the diametric strain waveform according to theory. Deviations indicate either neovascularization or shear strain.

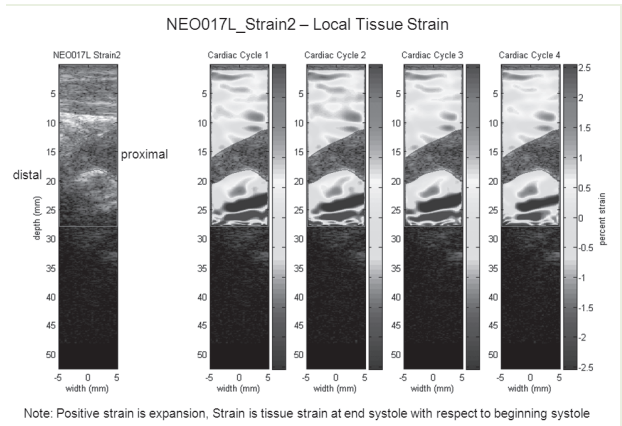

FIGURE 4: Reproducibility of Plaque Strain between cardiac cycles.

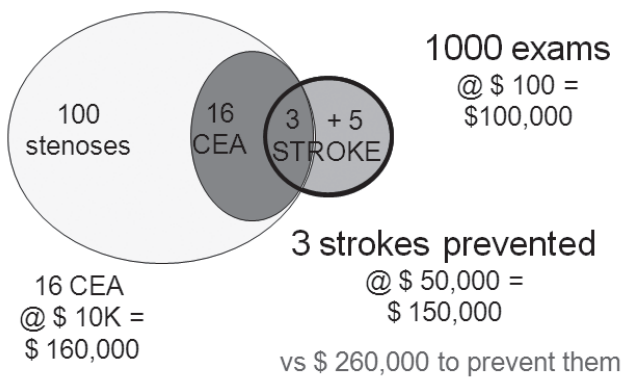

FIGURE 5: 1000 examinations for carotid stenosis yield 16 cases with severe carotid stenosis; 3 of those will have carotid embolic stroke in 2 years if untreated. This project is designed to differentiate those who will have embolic stroke from those who will not. Nearly half of strokes come from carotid artery plaques (3 in this example). Other causes of stroke (5 in this example) include hemorrhage into the brain, emboli from the heart and paradoxical emboli from the venous system through a Patent Foramen Ovale (PFO).

the plaque. We expect the central plaque to exhibit an additional expansion during very high systolic velocities.

The presence of excessive shear strain indicates a mechanically weak plaque subject to shear stress. A peak systolic expansion strain indicates excessive neovascularization **plus** Bernoulli pressure depression tending to rupture the plaque.

Ultrasound Reading Center for Carotid Stenosis

Currently, all patients with high blood velocity in a carotid stenosis are recommended for treatment with endarterectomy or stent. If left untreated only 20% of those cases will have stroke.

Among the factors that increase the risk of stroke are symptoms of Transient Ischemic Attack (TIA), also called "mini stroke." These are symptoms of sudden loss of sensation or motor control on one side of the body. From brain imaging studies, infarcts in the motor or sensory cortices in the brain can be associated with those symptoms. However, brain imaging studies show that people have many infarcts in other regions of the brain—such as those associated with decision making, language, perception and memory–which might not cause recognizable symptoms and might be missed on conventional neurological examination. Such infarcts, if detected, might help to improve identification of people with embolizing carotid plaques or other sources of emboli. We are attempting to conceive of low cost methods of screening to identify these "silent infarcts" without brain imaging.

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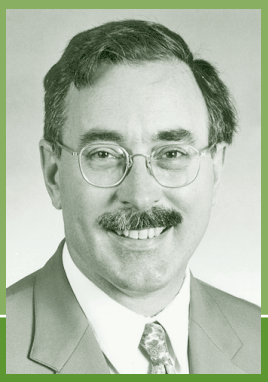
· Regulation of Vascular Smooth Muscle Cell Growth



The V. Paul Gavora – Helen S. and John A. Schilling Endowed Chair in Vascular Surgery

AWARDS

National Heart, Blood and Lung Institute MERIT Award

National Institutes of Health

- **Vascular Surgery/Cardiology Training Grant**

FUNDING

National Institutes of Health

Vascular surgical procedures are designed to rebuild diseased blood vessels and improve blood flow. While these procedures restore the circulation, they also cause injury. This injury induces a wound healing response that in some instances is associated with accumulation of scar tissue (intimal hyperplasia) and significant luminal narrowing (e.g. 20-40% of coronary arteries treated by angioplasty). Smooth muscle cells living in the arterial wall proliferate in response to injury and are largely responsible for the intimal hyperplasia (Figure 1). The primary objective of our laboratory is to understand the factors that stimulate and inhibit the growth of smooth muscle cells, and to develop new strategies for the pharmacological control of intimal hyperplasia.

REGULATION OF INTIMAL HYPERPLASIA IN DAMAGED ARTERIES: We use the rat carotid artery stripped of its endothelium by the passage of a balloon embolectomy catheter as a simplified model of vascular repair after endarterectomy or angioplasty. As in human arteries, the response to injury in rat carotid arteries involves a series of events leading to intimal hyperplasia. Medial smooth muscle cells start proliferating at 24-48 hours. They begin to migrate into the intima at four days, and they continue to proliferate and to synthesize matrix for several weeks before resuming the resting state. The net result is a substantial increase in wall mass.

The critical issue is to define the factors that start and stop this process. We have been studying heparin as a paradigm for drugs that inhibit smooth muscle cell proliferation and migration. Since heparin-like heparan sulfates secreted by endothelial cells and resting smooth muscle cells can inhibit growth, they may play a role in maintaining the growth-arrested state in normal arteries. The current experiments are designed to test the hypothesis that heparin inhibits smooth muscle cell growth by interfering with the activation of the EGF and FGF receptors.

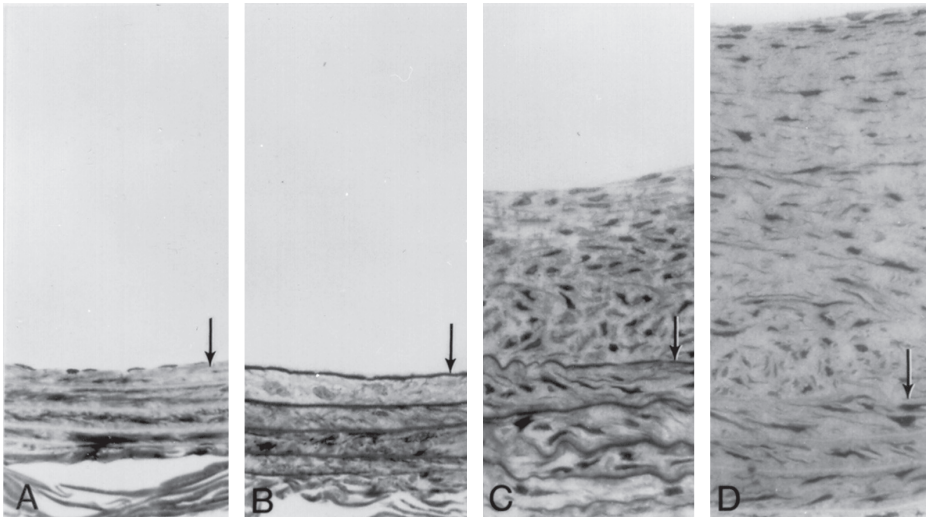


Figure 1: This series of photographs shows how a normal rat carotid artery (panel A-histologic cross-section) responds to injury. Angioplasty of the artery removes the surface endothelium (panel B). By two weeks (panel C), smooth muscle cells have migrated from the media into the intima (region above the elastic layer marked by the arrow) and have begun to proliferate (intimal hyperplasia). The thickening of the wall reaches a maximum by three months (panel D).

Since heparin-like heparan sulfates secreted by endothelial cells and resting smooth muscle cells can inhibit growth, they may play a role in maintaining the growth-arrested state in normal arteries.

Recent studies in the laboratory have defined a novel pathway of smooth muscle cell activation which depends on these receptors. Thrombin can induce cell growth by interacting with its G-protein coupled receptor. In rat smooth muscle cells, the activated thrombin receptor in turn causes the release of heparin-binding EGF-like protein (HB-EGF) from the cell membrane, and the released HB-EGF then binds to the EGF receptor to induce a cell response. Blockade of the EGF receptor with specific antibodies inhibits cell growth and suppresses intimal hyperplasia in balloon-injured rat carotid arteries. In human smooth muscle cells, thrombin treatment induces the release of endogenous FGF and activation of the FGF receptor, instead of the EGF receptor. FGF mediates the cellular stimulus induced by not only thrombin but also PDGF and Factor Xa. We are currently pursuing experiments designed to understand "crosstalk" between growth factor and cytokine pathways. In particular, we are investigating the function of two membrane-associated heparan sulfate proteoglycans, syndecan-1 and syndecan-2, which may be involved in FGF signaling and smooth muscle growth control.

NITRIC OXIDE AND SMOOTH MUSCLE PROLIFERATION: Nitric oxide (NO) is the principal arterial vasorelaxant. It is also an inhibitor of smooth muscle cell growth and injury-induced intimal hyperplasia. The mechanism of action has not been delineated although, in part, it depends on intracellular cyclic GMP and the activation of a cGMP-dependent protein kinase (PKG). We are currently studying a downstream target of NO and PKG, vasodilator stimulated phosphoprotein (VASP). Overexpression of VASP mutated to prevent phosphorylation by PKG makes cells unresponsive to NO, while overexpression of VASP mutated to prevent phosphorylation by PKC makes the cells sensitive to NO but unresponsive to serum. Thus, VASP may prove to be pivotal in the response of smooth muscle cells to growth stimulants and inhibitors, and pharmacological manipulation of this pathway might be a fruitful approach to controlling the arterial response to injury.

REGULATION OF SMOOTH MUSCLE GROWTH IN GRAFTS BY BLOOD FLOW AND PDGF: We have found that smooth muscle cell proliferation and neointimal hyperplasia in primate PTFE grafts are exquisitely regulated by changes in blood flow. Normal blood flow promotes neointimal hyperplasia, while high blood flow suppresses it or induces it to shrink (atrophy). In the grafts, smooth muscle cells proliferate where endothelial cells are present, whereas in injured arteries they proliferate only where the endothelium is missing. Thus, depending on the physiological state, endothelial cells can have a positive or a negative effect on smooth muscle cell growth. Using molecular arrays, we are attempting to define the molecules altered by changes in blood flow that might regulate smooth muscle cell proliferation. We have recently identified bone morphogenetic protein-4 (BMP-4), a member of the TGF-β family, by array analysis. BMP-4 is expressed by endothelium, is upregulated by increased shear stress, and inhibits growth and at times kills smooth muscle cells. Other genes identified in this array experiment include tissue-type plasminogen activator, ADAMTS-4, and hyaluronidase-2; these genes may play a significant role in matrix degradation during cell death and tissue atrophy.

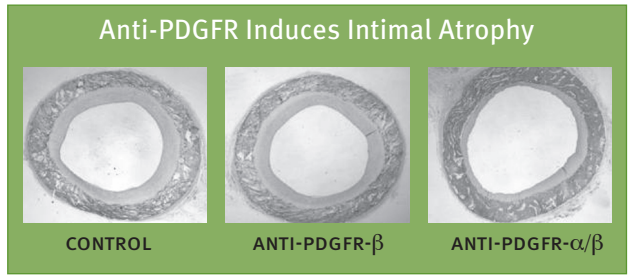


FIGURE 2: Histological cross-sections of normal flow PTFE grafts at 2 weeks following initiation of treatment with vehicle control, blocking antibodies to PDGFR-β, or blocking antibodies to both PDGFR-α and PDGFR-β. (H&E staining, 16X).

Recent experiments using a mouse monoclonal antibody that recognizes and blocks the beta form of the PDGF receptor (PDGFR-β) have demonstrated conclusively that intimal hyperplasia in grafts as well as in injured arteries depends on PDGF. In collaboration with Celltech Ltd. and ZymoGenetics, Inc., this antibody has been genetically engineered to resemble a human immunoglobin; this "humanized" antibody has been tested in a human trial for the prevention of restenosis after coronary stent angioplasty and *failed*. We are astonished by this result and, in consequence, have gone back to the laboratory to investigate it further. Blockade of both PDGF receptors may be necessary. When we block both PDGFR-β and PDGFR-α, we not only suppress intimal thickening but we induce ca. 50% intimal atrophy (Figure 2) by two weeks. This novel finding indicates to us that restenosis might be a pharmacologically reversible process.

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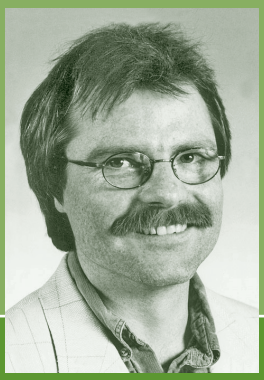
Günter Daum, Ph.D.

· A role for sphingosine-1-phosphate receptor-2 in smooth muscle cell differentiation and restenosis



Research Associate Professor

FUNDING
National Institutes of Health

Restenosis is the cause for the unacceptably high failure rate (20-30%) of surgical interventions, such as vein grafts, stents, and angioplasty, to restore blood flow in occluded vessels. Restenosis is characterized by loss of luminal area due to negative remodeling (decreased vessel cross-sectional area) and intimal hyperplasia (accumulation of intimal smooth muscle cells (SMCs) and extracellular matrix). The introduction of stents prevents negative remodeling but not intimal hyperplasia. Stents allow local delivery of growth inhibitory drugs, and the use of rapamycin (sirolimus) is the most promising approach to date to inhibit stent restenosis. However, not all vascular occlusions are suitable for stenting. In addition, a systemic approach to prevent restenosis is still desirable since such treatment would be less invasive and possibly less expensive.

Despite tremendous research efforts, it is still unclear which factors drive the formation of restenotic lesions in humans. Since intimal cells exhibit a smooth muscle phenotype, i.e. they express SMC-restricted genes, the current paradigm is that medial SMCs become activated and migrate towards the lumen, where they proliferate and produce matrix. To what extent adventitial cells or blood borne stem cells contribute to intimal formation is unclear. We also do not know whether a developing intima after vein grafting or arterial stenting is regulated by the same factors. Common to all growing intimae is that cells proliferate; the critical question is why? One should not forget that excessive intimal growth after arterial injury does NOT occur in 70% of patients. Thus, one may ask the question, what "goes right" in these patients? SMCs in normal arteries are extremely quiescent. For these cells to be able to proliferate, they must de-differentiate. Logically, after the healing process, SMCs must re-differentiate to become quiescent again. It is our key hypothesis that the window of de-differentiation determines whether intimal lesions develop or not.

A role for sphingosine-1-phosphate receptor-2 in SMC differentiation and restenosis: Serum-response-factor (SRF) plays a key role in SMC differentiation. SRF is a transcription factor that in concert with SMC-specific co-factors of the myocardin-like family of proteins regulates the expression of SMC-specific genes. Potent activators of SRF are bioactive lipids, such as lysophosphatidic acid, sphingosylphosphorylcholine and sphingosine-1-phosphate (S1P). Our work focuses on S1P, which is recognized by SMCs through three receptors, S1P1, S1P2, and S1P3. All S1P receptors are G protein-coupled receptors that are linked to different G alpha subunits and thus, activate different signal transduction pathways.

Genetic ablation of S1P receptors in mice revealed that S1P1 is required for arterial development, whereas mice without S1P2 or S1P3 develop normally. S1P2 was initially of interest to us since it is the only S1P receptor that activates the small GTPase Rho, which is required for SRF-dependent expression of SMC differentiation genes. To investigate whether S1P2 expression affects the response to arterial injury, we compared lesion formation after ligation of the left common carotid in wild-type and S1P2 knock-out mice. The difference between the two mice was dramatic. Wild-type mice did not form significant lesions, whereas S1P2-deficient arteries developed large lesions between 2 and 4 weeks after injury (Fig. 1). In both arteries, injury induced proliferation of medial cells. This event was transient in the wild-type artery, whereas it was continuous in the S1P2-deficient artery (Fig. 2-3).

This observation suggests that the onset of SMC activation is similar in both arteries, and that S1P2 in the wild-type vessel is responsible for the transient nature of SMC activation. Consistent with our hypothesis, we found that S1P induces SMC differentiation genes in wild-type but not in S1P2-deficient SMCs. We are currently investigating the molecular mechanisms of this process and our goal is to define a role for S1P2-induced stimulation of SRF in our mouse injury model.

Conclusion: Our work suggests that S1P2 regulates an SRF-dependent differentiation program in SMCs that terminates SMC proliferation and migration after injury, and thus prevents intimal growth. We consider stimulation of SMC differentiation an intriguing possibility to limit intimal formation because such approach should have few side effects as it lacks general cytotoxicity.

Common to all growing intimae is that cells proliferate; the critical question is why?

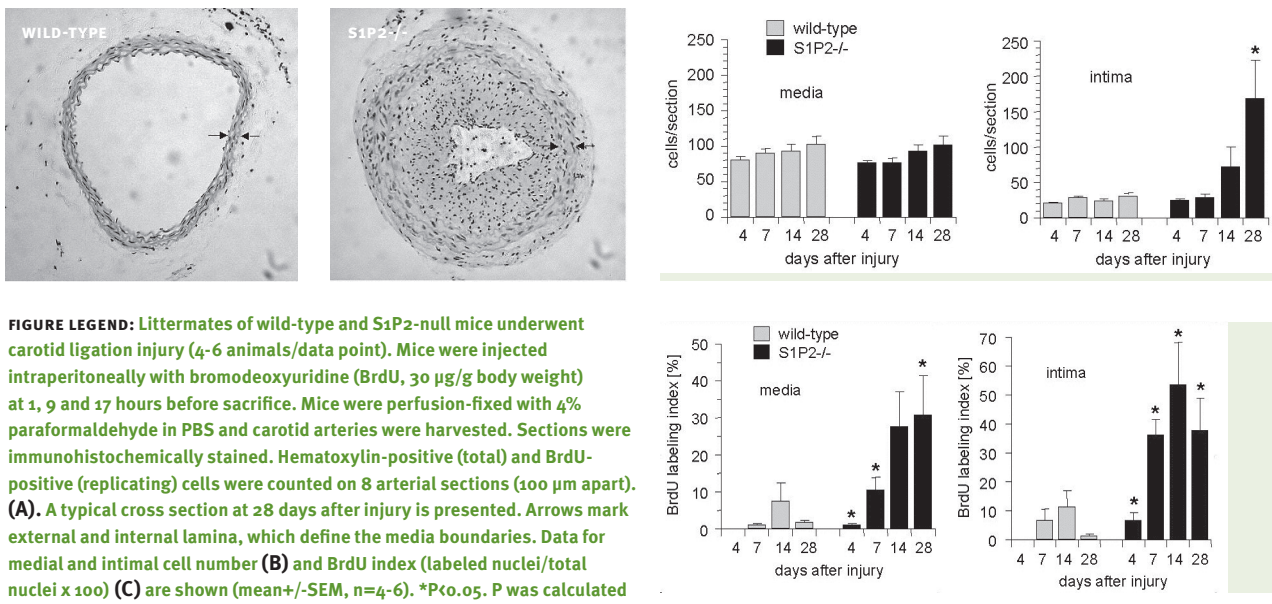


FIGURE LEGEND: Littermates of wild-type and S1P2-null mice underwent carotid ligation injury (4-6 animals/data point). Mice were injected intraperitoneally with bromodeoxyuridine (BrdU, 30 µg/g body weight) at 1, 9 and 17 hours before sacrifice. Mice were perfusion-fixed with 4% paraformaldehyde in PBS and carotid arteries were harvested. Sections were immunohistochemically stained. Hematoxylin-positive (total) and BrdU-positive (replicating) cells were counted on 8 arterial sections (100 µm apart). **(A).** A typical cross section at 28 days after injury is presented. Arrows mark external and internal lamina, which define the media boundaries. Data for medial and intimal cell number **(B)** and BrdU index (labeled nuclei/total nuclei x 100) **(C)** are shown (mean+/-SEM, n=4-6). *P<0.05. P was calculated by unpaired t-test and indicates significance of difference between wild-type and S1P2 null mice at a given time point after injury.

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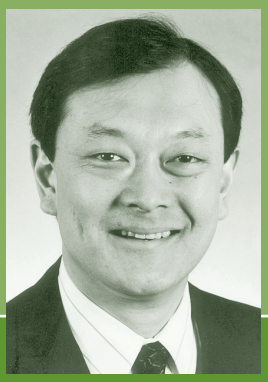
Thomas Hatsukami, M.D.

· Magnetic Resonance Imaging of the High-Risk Atherosclerotic Plaque



Professor

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Introduction

Cardiovascular disease (CVD) is the number one cause of death worldwide and is a leading cause of long-term disability. It is estimated that the annual cost for the care of victims of CVD is over $390 billion per year in the U.S. alone. Most CVD events, such as heart attack and stroke, are atherosclerosis-related. Traditionally, the degree of vessel lumen narrowing has been used to identify the high-risk atherosclerotic plaque. However, there is increasing evidence that the structure, composition, and inflammatory activity of the atherosclerotic lesion are more important markers of the vulnerable plaque. Progress in understanding how vulnerable plaques develop has been hindered by our inability to serially examine these critical characteristics of the diseased vessel wall in a non-invasive fashion.

The mission of our research group is to advance high-resolution magnetic resonance imaging (MRI) technology for accurate, non-invasive examination of atherosclerosis. Our laboratory is organized along five core functions: 1) Imaging Physics: develop novel image acquisition techniques; 2) Histology: provide the histological gold-standard for validation of MRI findings; 3) Imaging Software: build custom-designed tools that permit more efficient, reproducible, quantitative image analysis; 4) Clinical Studies: apply MR imaging techniques to understand mechanisms leading to development of the vulnerable plaque; and 5) Reading Center: provide training, quality control, and image analysis for multi-center clinical trials using MRI.

Validation

Significant improvements in MR image quality have been made possible by a combination of hardware development and novel image acquisition sequences (Figure 1). The accuracy of this high-resolution MRI technique has been extensively validated by comparing pre-operative carotid MRI findings to matched histological sections of the excised plaque. We have shown that MRI can categorize carotid plaque types according to established American Heart Association histological classification criteria (Table I), with a weighted Kappa of 0.79, indicating very good agreement between MRI and histology (*Circulation* 2002, 106:1368).

Furthermore, we have shown that MRI can accurately identify the presence and precisely quantify the size of critical features of the vulnerable plaque, as defined by an expert panel (*Circulation* 2003, 108:1664). These features include the degree lumen narrowing and overall plaque burden (*Circulation* 1998, 98:2666; *Magnetic Resonance in Medicine* 2000, 44:968), fibrous cap thinning and rupture (Figure 2: *Circulation* 2000, 102:959), the lipid-rich necrotic core and intraplaque hemorrhage (Figure 3: *Arteriosclerosis, Thrombosis and Vascular Biology* 2005, 25:234), and the degree of neovasculature and inflammatory cellular infiltration of the plaque (Figure 4: *Circulation* 2003, 107:851; *Radiology* 2006, 241:459) .

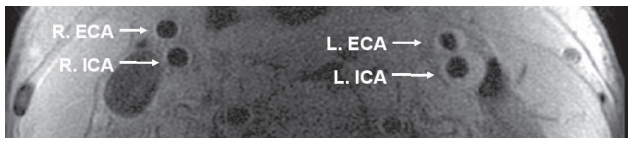

Figure 1: MRI of right and left internal and external carotid arteries demonstrating good suppression of flow artifact and clear delineation of the lumen and outer boundary of normal (right) and diseased (left) carotid arteries. Note evidence of compensatory (expansive) enlargement on the left side. The cross-sectional area of the lumen is similar on both sides, yet there is significantly greater plaque burden on the left. ICA = internal carotid artery; ECA = external carotid artery.

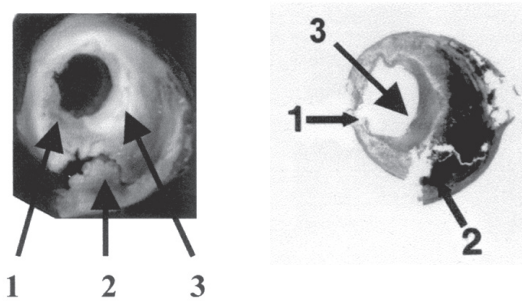

Figure 2: Example of a common carotid plaque with fibrous cap rupture and intraplaque hemorrhage. Photo of gross section of common carotid artery (left panel), trichrome stained histological section (middle panel), and corresponding TOF MR image (right panel). Arrow 1 indicates an area of cap rupture, arrow 2 = intraplaque hemorrhage, and arrow 3 = area of thick, collagen-rich fibrous cap. The thick cap appears as a dark band adjacent to the lumen on MRI. The dark band is absent, and there is adjacent hyperintense signal in the region of cap rupture.

Lesion Type	Definition
I–II	Isolated foam cells or small foam cell layers
III	Pre-atheroma: small extracellular lipid pools
IV–V	Atheroma/Fibroatheroma: confluent lipid core with surrounding fibrous tissue
VI	Complicated lesion: surface defect, hemorrhage or thrombus
VII (Vb)	Predominantly calcified plaque
VIII (Vc)	Predominantly fibrotic plaque

Table I: Modified American Heart Association (AHA) classification scheme for describing atherosclerosis lesion types.

Automated Quantitative Image Analysis

Analysis of the MR images is a time-consuming process, with approximately 70 high-resolution images generated for each artery. In order to perform large-scale clinical studies, automated, quantitative image analysis tools are needed, which would improve reproducibility and efficiency. Our lab has developed a probability-based segmentation method that utilizes morphological information, such as local wall thickness, coupled with active contours to limit the impact from noise and artifacts associated with *in vivo* imaging (Figure 5).

In experiments involving 142 sets of multi-contrast images from 26 subjects undergoing carotid endarterectomy, segmented areas of the lipid-rich necrotic core, calcification, loose matrix and fibrous tissue on MRI agreed with areas on the corresponding histological section with correlations (R^2) of 0.78, 0.83, 0.41 and 0.82, respectively. In comparison, areas outlined by expert MRI readers blinded to histology yielded correlations of 0.71, 0.76, 0.33 and 0.78, respectively (*Magnetic Resonance in Medicine* 2006, 55:659).

Clinical Studies

With funding from the National Institutes of Health, we have enrolled over 300 individuals over the past seven years in a prospective study, where participants undergo high-resolution MRI examination of their carotid arteries every 18 months. This study has demonstrated that arteries with intraplaque hemorrhage are associated with more rapid progression in overall plaque and lipid-rich necrotic core size (Figure 6: *Circulation* 2005, 111:2768). The percent change in wall volume over 18 months was 6.8% amongst those with intraplaque hemorrhage, compared to –0.15% for those without hemorrhage (p= 0.009). The lipid-rich necrotic core increased by 28.4% in plaques with hemorrhage, compared to –5.2% in those without hemorrhage (p= 0.001). Furthermore, those with intraplaque at baseline were much more likely to develop *new* plaque hemorrhages during follow-up, compared to controls (43% versus 0%, P=0.006).

In a prospective MRI study to test the hypothesis that specific carotid plaque features are associated with a higher risk of subsequent ipsilateral TIA or stroke, 154 participants underwent a baseline carotid MRI examination, and were called every 3 months to identify symptoms of new-onset transient ischemic attack (TIA) or stroke. Twelve cerebrovascular events that were judged to be carotid-related occurred during a mean follow-up period of 38.2 months. Cox regression

Over 16.7 million people die of cardiovascular disease each year — one person every two seconds. Our primary goal is to develop and validate high-resolution imaging methods that will improve our ability to identify individuals at highest risk.

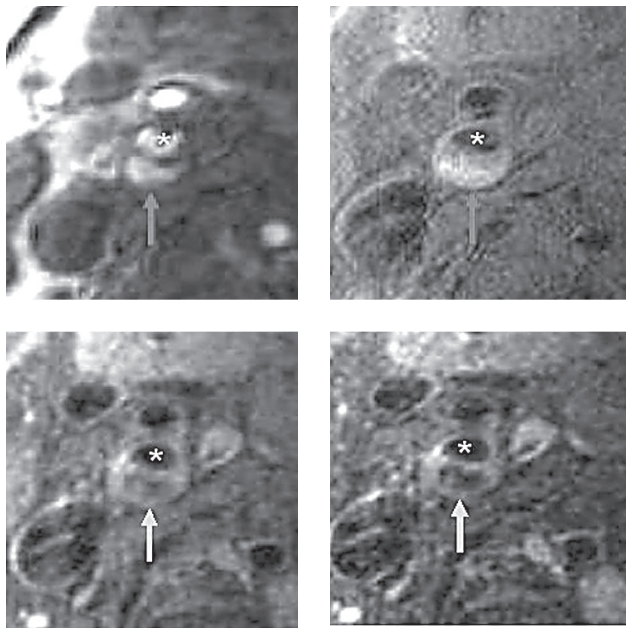

Figure 3: Example of an AHA Type VI (complicated) lesion with acute hemorrhage into the lipid-rich necrotic core. The asterisks indicate the lumen of the internal carotid artery. Early intraplaque hemorrhage, seen on the corresponding histological cross-section on the right, is identified by a hyperintense (bright) signal on time-of-flight (TOF) and T1-weighted (T1W) MR images, and relatively hypointense (dark) on the proton density- (PDW) and T2-weighted (T2W) images.

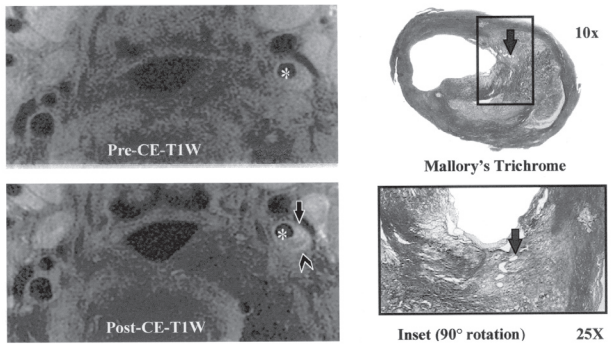

Figure 4: Pre-gadolinium contrast enhanced T1-weighted image of common carotid artery in left upper panel, post-contrast enhanced T1W image in left lower panel, and corresponding 10X and 25X trichrome stained histological sections. Note the enhancement seen in the shoulder region (arrow) in the post-contrast enhanced image. This enhancing region demonstrates abundant development of neovasculature and inflammatory cell infiltration on the corresponding histological section.

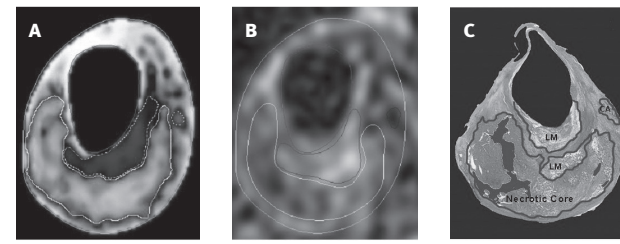

Figure 5: Segmentation results showing (a) automated quantitative image analysis tool; (b) manual outline by expert reviewer; and (c) corresponding histology section demonstrating a large necrotic core, loose matrix (LM) and a small area of calcification (CA). The dark regions within the necrotic core on the histology specimen are artifacts due to sectioning.

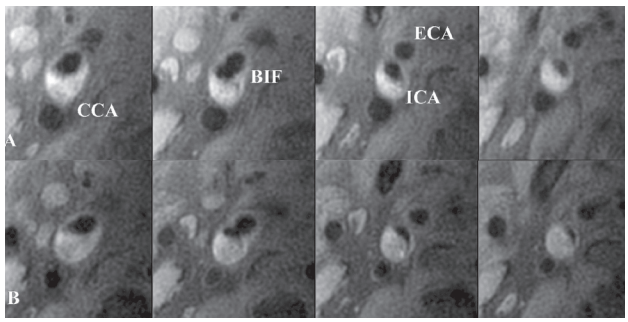

Figure 6: T1-weighted images of progression of atherosclerosis associated with intraplaque hemorrhage in the right carotid artery. Each column presents matched cross-sectional locations in carotid artery from baseline MRI (A) and MRI obtained 18 months later (B). Lumen area was decreased, and wall area was increased in each location on the second examination. CCA=common carotid artery; BIF= common carotid bifurcation; ICA=internal carotid artery; and ECA=external carotid artery.

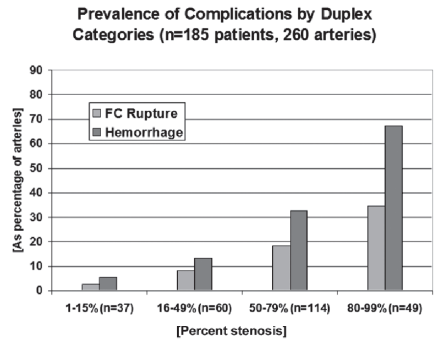

Figure 9: Prevalence of MRI-identified fibrous cap (FC) rupture and intraplaque hemorrhage by degree of stenosis in carotid plaques (n=260) of asymptomatic volunteers. The degree of stenosis was determined by duplex ultrasound.

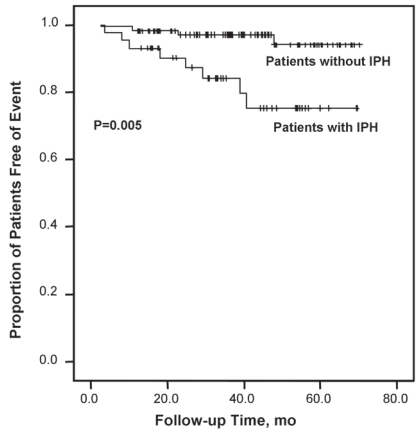

Figure 7: Kaplan-Meier survival estimates of the proportion of subjects remaining free of ipsilateral TIA or stroke with (lower curve) and without (upper curve) IPH. IPH=intraplaque hemorrhage.

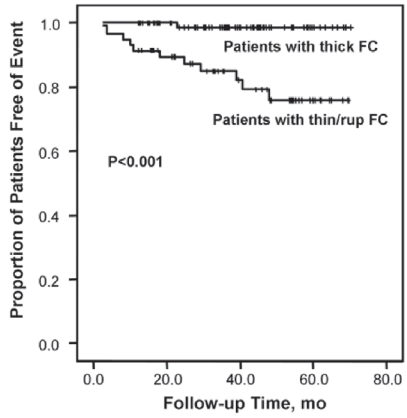

Figure 8: Kaplan-Meier survival estimates of the proportion of subjects remaining free of ipsilateral TIA or stroke with (lower curve) and without (upper curve) thin/ruptured fibrous cap. FC=fibrous cap.

analysis demonstrated significant associations between ischemic events and presence of a thin or ruptured fibrous cap (hazard ratio, 17.0; $P<0.001$), intraplaque hemorrhage (hazard ratio, 5.2; $P=0.005$), and larger mean necrotic core area (hazard ratio for 10 mm^2 increase, 1.6; $P=0.01$) in the carotid plaque. Figures 7 and 8 demonstrate Kaplan-Meier survival estimates for ipsilateral event-free survival among patients with and without intraplaque hemorrhage and thin/ruptured fibrous cap, respectively (*Stroke* 2006, 37:818).

In a recent review of 260 carotid MRI examinations performed in asymptomatic subjects, the prevalence of arteries with intraplaque hemorrhage or fibrous cap rupture was assessed across a range of luminal stenoses. The findings shown in figure 9 indicate that up to a third of subjects with asymptomatic 50-79% stenosis have evidence of plaque disruption or intraplaque hemorrhage. Surprisingly, disruption or hemorrhage was noted in approximately 10% of asymptomatic individuals with only 16-49% carotid stenosis.

Conclusions

Magnetic resonance imaging is a promising tool for studying the pathophysiology of human atherosclerosis progression and regression in vivo. In addition to precisely assessing plaque burden, MRI is capable of accurately classifying disease according to established AHA criteria, and identifying critical plaque features such as the fibrous cap and neovasculature. A better understanding of disease mechanisms and factors leading to more rapid progression will permit identification of high-risk individuals for more aggressive treatment, and potentially lead to the development of novel methods for therapeutic intervention.

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- Healing of Prosthetic Vascular Grafts
- Preventing Dialysis Access Failure
- Factors Involved in Restenosis
- Endovascular Repair of Abdominal Aortic Aneurysms

Professor
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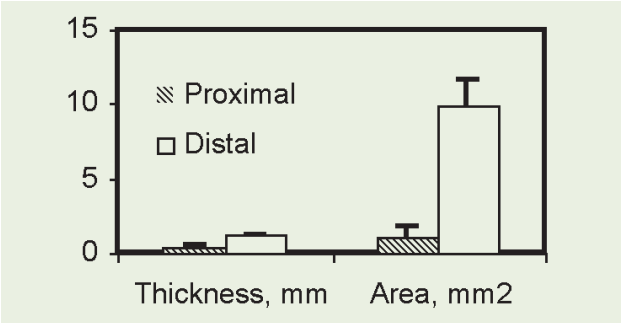
FUNDING
National Institutes of Health

Healing of Prosthetic Vascular Grafts

Vascular surgery has made tremendous advances in the last few decades. Bypass grafts, angioplasty, stents, and stent grafts are now standard treatment for arterial insufficiency and aneurysm disease in peripheral arteries. However, long-term success of these procedures, particularly small diameter prosthetic devices, is limited due to thrombosis and restenosis. Part of the problem is the lack of a natural, non-thrombogenic, biologic lining. Prior work in the Clowes lab has shown that increased porosity of standard polytetrafluoroethylene (PTFE) bypass grafts when placed in non-human primates can allow complete endothelialization of the graft surface by capillary ingrowth along the length of the graft. We tested these grafts in humans, placing composite bypasses made of standard and high-porosity PTFE in the femoropopliteal position (Figure 1). Unfortunately, there was no evidence of capillary ingrowth as measured by the surrogate marker labeled platelet activity. Subsequent studies have shown that capillary ingrowth may be dependent on species, age, and the nature of the surrounding tissues. In collaboration with Dr. Sobel, we are studying ways to enhance graft healing by addition of natural matrix components and growth factors (such as vascular endothelial growth factor) using a canine model of PTFE grafts placed in the carotid position. These grafts behave like those in our clinical trial; they fail to endothelialize. It is hoped that pretreatment will provide a more robust response and endothelialization.



GRAPH 1: Sheep Eight Week Data

Preventing Dialysis Access Failure

Effective renal dialysis requires several hundred milliliters per minute of blood flow. To accomplish this, a fistula is created between an artery and vein, typically in the arm (Figure 2). This provides a high-flow conduit just under the skin surface where it can be accessed by needle puncture. Unfortunately, these fistulae have a high failure rate, even higher than other vascular grafts. Re-operation for failed access is a major cause of morbidity, prolonged hospital stay, and increased cost in the treatment of renal failure. Most access failures are caused by intimal hyperplasia at the venous end of the graft. This is very surprising since in animal models we have found that increased blood flow reduces wall thickening after placement of prosthetic arterial grafts.

We have studied this problem in an animal model. PTFE grafts like those used in humans are placed in the necks of sheep, and measurements are made of the narrowing at the junction of the graft and native vessels. We have found that standard grafts fail within two to three months due to narrowing, which is much more pronounced at the venous end (Graph 1). Active thrombosis along the graft surface, particularly at the venous end, appears to be a major contributing factor. Thickening is greatly reduced if the grafts are sewn into an artery instead of a vein, even if blood flow is increased by creation of an artery-to-vein fistula beyond the graft. We have also found that special coating of the graft surface with phospholipids can stop this thickening process.

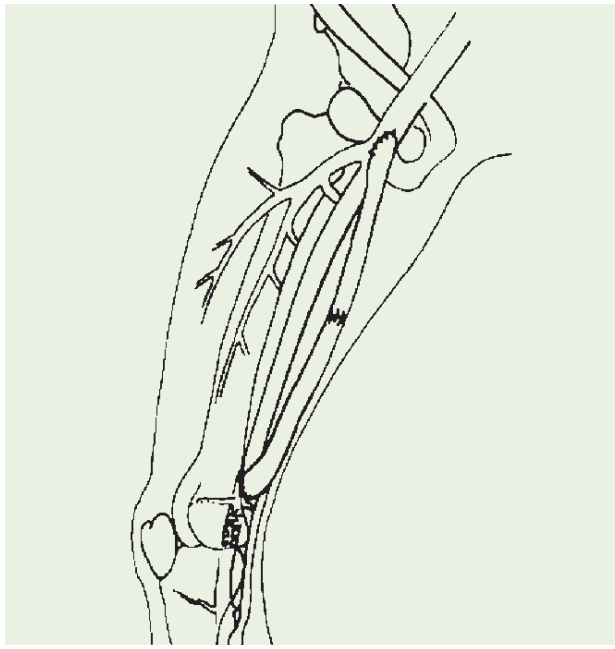


FIGURE 1: PTFE in the femoropopliteal position

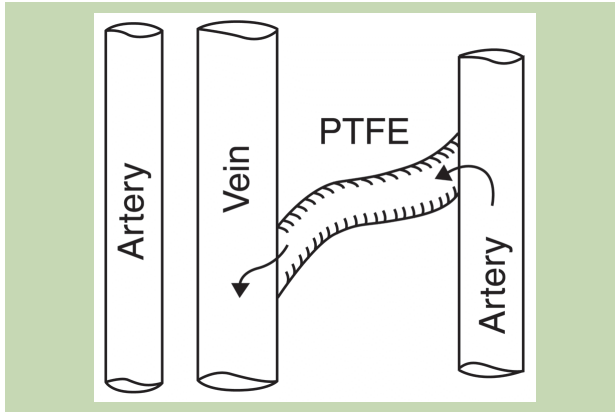


FIGURE 2: Fistula created between artery and vein to provide high-flow conduit

The three principle components of graft healing and lumen narrowing are endothelial ingrowth, smooth muscle cell proliferation, and thrombosis. These are evaluated using scanning electron microscopy, morphometry, and immunohistochemistry. We can also use simulated dialysis to assess the potential role in graft failure of the various components of the dialysis procedure.

Like the clinical specimens, the sheep lesions have focal regions of prominent cellular proliferation, often adjacent to thrombus and in granulation tissue surrounding the graft. This can be seen in Figure 3, showing a proliferating cell nuclear protein (PCNA)-positive nucleus marked by an arrow.

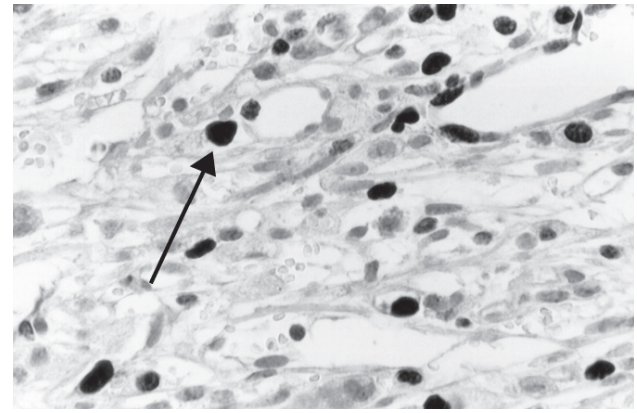


FIGURE 3: PCNA-positive nucleus

Organizing thrombus contributes significantly to luminal narrowing. The continued presence of thrombus and high rates of cellular proliferation suggest ongoing injury as an important cause of vessel narrowing. Rapid development of lesions morphologically similar to those found in clinical stenoses makes this model uniquely suited for study of the cellular mechanisms of dialysis failure

Paclitaxel is an effective drug that prevents smooth muscle cell growth and migration and inhibits inflammation. It is one of the drugs that are effective in preventing restenosis in drug-eluting coronary artery stents. We have now demonstrated that a bioabsorbable mesh containing paclitaxel can prevent intimal hyperplasia and narrowing of the venous end of PTFE access grafts in sheep (Figure 4 shows the venous end of these grafts at eight weeks in control and drug-treated animals). We are now consulting with AngioTech Pharmaceuticals, Inc. on a clinical trial now underway with this promising new approach.

Factors Involved in Restenosis

Restenosis following revascularization or dialysis access is a major cause of morbidity, prolonged hospital stay, and increased cost. Intimal hyperplasia causes failure of almost one-third of all vascular reconstructions. This process results from wall thickening due to smooth muscle cell proliferation narrowing the lumen. Inflammation caused by local trauma at the site of reconstruction (stent, atherectomy, angioplasty, or bypass) causes proliferation and migration of myofibroblasts and constriction of the vessel lumen by remodeling of the surrounding tissue. Endothelial damage causes local thrombosis that enhances this process and contributes scaffolding for further neointimal formation.

Thickening is greatly reduced if the grafts are sewn into an artery instead of a vein, even if blood flow is increased by creation of an artery-to-vein fistula beyond the graft.

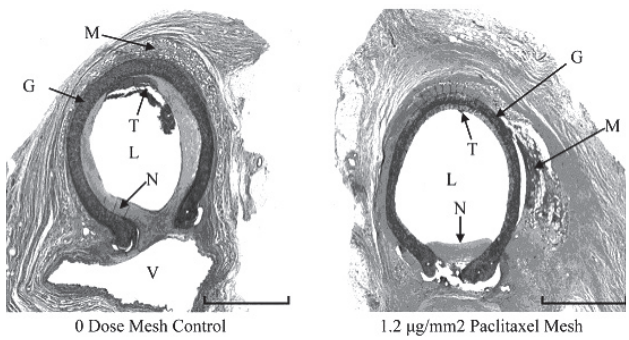


Figure 4: **Example of a section through the toe of the graft at low power showing where capillary density in the neointima is greatest (bar represents 4 mm). The inset is a high power from this region demonstrating multiple capillaries (arrow).**

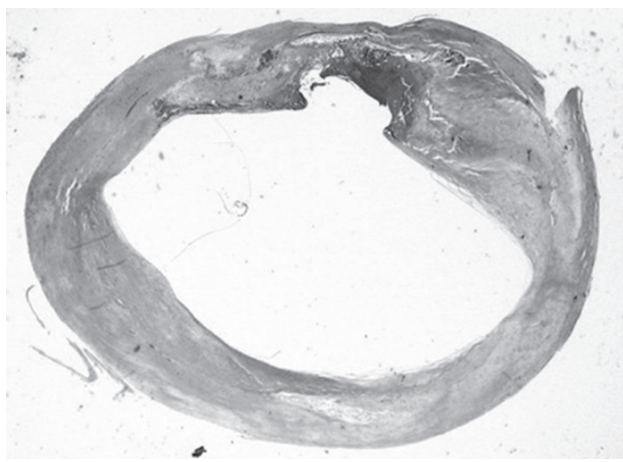


Figure 5: **An ulceration in the common carotid artery. Conventional wisdom is that embolization is a result of plaque in the internal carotid artery. Our histology review suggests that erosive lesions may occur at more remote sites, such as this common carotid artery with a large ulcer, and may place patients equally at risk for stroke.**

Much research has been devoted to understanding the cellular pathology of this process and to developing ways to combat it with drugs (like paclitaxel and heparin), new devices, and genetic modification of the cells involved. In collaboration with Drs. Sobel and Clowes, we are prospectively studying patients who are undergoing vein graft bypasses. We will compare inflammatory and thrombotic responses and the propensity of explanted smooth muscle cells to proliferate in patients who do and do not develop restenosis. We hope to find ways to predict which patient will develop this problem and to gain a better understanding of the process so we can target patients at high risk for failure with better preventive therapies.

Endovascular Repair of Abdominal Aortic Aneurysms

Endovascular therapy uses a catheter-based delivery system rather than conventional open techniques. Patient morbidity and hospital stay are dramatically decreased. Endovascular grafts are held open and in proper position by attached metallic stents and are placed by a simple arterial cutdown or, in some cases, percutaneously. These devices have been very successful in mid-term clinical trials yet it remains to be seen if they will perform as well over the long term (decades) as conventional grafts. The primary concern is whether or not the devices will remain well attached to the native artery at either end despite the native vessel's tendency to dilate over time. Dr. Kohler and Dr. David Glickerman, from interventional radiology, began the endovascular therapy program at the Seattle VA in 1999. We are a site for the VA Cooperative Trial of Open versus Endovascular Repair of Abdominal Aortic Aneurysms (Dr. Kohler is on the Executive Board). This trial has now recruited nearly 900 patients randomized to one or the other approach. Five-year follow-up will include analysis of cost as well as morbidity, mortality, and effectiveness in preventing death from aneurysm rupture.

Histologic Feature of Carotid Plaque Instability

Currently 80% of patients who undergo carotid endarterectomy do not benefit from the procedure because their plaque would not have caused a stroke. Dr. Hatsukami's laboratory has been developing high-resolution MRI techniques to study the composition of carotid plaques in order to better define which patients are at high risk for stroke and therefore which patients will benefit from intervention. In the course of this work hundreds of intact carotid endarterectomy specimens have been collected and analyzed histologically, yielding the largest existent database of carotid plaque morphology. We are collating information in this dataset to define the characteristics of the advanced carotid plaque and associations of histologic features with surface instability. This information will help define which regions are at highest risk for embolization and which features are most important to define on MRI imaging.

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· Morbidity and Mortality in Patients with Ruptured Abdominal Aortic Aneurysms, Improving Outcomes with a Modified Approach

Associate Professor and Chief
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Abdominal Aortic Aneurysmal disease is the 12th leading cause of death in the United States and more than 15,000 Americans die from ruptured abdominal aortic aneurysms each year (Figure 1). Furthermore, mortality from open repair of this disease process has not significantly changed in over the past two decades.

Scope of the Problem

An abdominal aortic aneurysm (AAA) is defined as a >50% dilation or widening of the normal aorta over time. There are defined risk factors associated with the development of AAA and those at risk are typically male with a history of hypertension and tobacco abuse. Aortic aneurysms are most often asymptomatic and are typically only incidentally detected when an individual has an imaging study of the abdomen for some other clinically indicated reason. Aortic aneurysms can often progress to rupture without elective surgical or endovascular management. The overall mortality of ruptured AAA is noteworthy at 85% (95% CI 80-91) with 66% of patients dying without operation or before reaching the hospital.[1] Peri-operative mortality after *open repair* is between 41 and 48% in selected series. [2,3]

New Technology

In 1991, Juan Carlos Parodi performed the first human endovascular AAA repair in Argentina using a covered stent and ushered in an era of minimally invasive "Endovascular Aneurysm Repair" or EVAR which has revolutionized the care of patients with aortic aneurysms.[4] These procedures can often be performed using a totally percutaneous approach under local anesthesia.[5] For patients with *non-ruptured* aortic aneurysms, two prospective, randomized controlled trials exist showing superiority in 30 day mortality rates of EVAR over conventional open repair. [6,7]

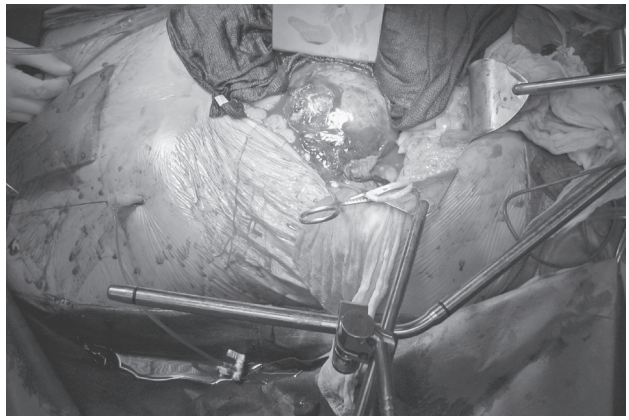

Figure 1: Intra-operative photo of patient with a ruptured AAA undergoing open repair. The head of the patient is to the right and an aortic cross clamp is in place.

Few studies exist evaluating the use of EVAR for the management of *ruptured* aortic aneurysms (rEVAR). This is mostly because there are few institutions with a robust experience in managing these patients. Published mortality rates for rEVAR are between 24 and 46%. [8,9,10] In a recent meta-analysis of published series of rEVAR, mortality rates for people who underwent rEVAR were found to be lower than in historical reports of unselected people undergoing open repair.[11] Pooled mortality after rEVAR was 21% (95% CI 13-29) but it is unclear whether this was due to chance, selection bias or benefit of the technique. These authors did determine that a structured algorithm served as a surrogate for an organized approach to patients with ruptured AAA's and as an overall marker for good quality care. Mortality

With this protocol in place, we have reduced mortality for patients with ruptured aortic aneurysms by 50% for the first time in over twenty years.

rates were 18% (95%CI 10 to 26; I^2 86.9%) for studies in which a structured algorithm was instituted versus 32% (95%CI 20-44; I^2 90.2%) where no formal protocol existed.[11] One must be careful in interpreting these data as the confidence intervals overlap.

Our group recently published an analysis of the Nationwide Inpatient Sample (NIS) for ruptured AAA's between 2001 and 2004. [12] The NIS is a random sample of 20% of hospital discharges nationwide. During this four year period, 28,123 patients were admitted with rAAA. The use of EVAR for this time period increased from 6 to 11% and mortality declined from 43 to 29%. Mortality for open repair showed no change (40-43%) (Figure 2). Patients who underwent EVAR had lower mortality, shorter hospital stays and were more likely to be discharged home. Interestingly, mortality associated with rEVAR was significantly less in teaching hospitals (21%) when compared to non-teaching hospitals (55%) ($p<0.01$). In fact, mortality in non-teaching hospitals was no different between open and endovascular repair. (Figure 3)

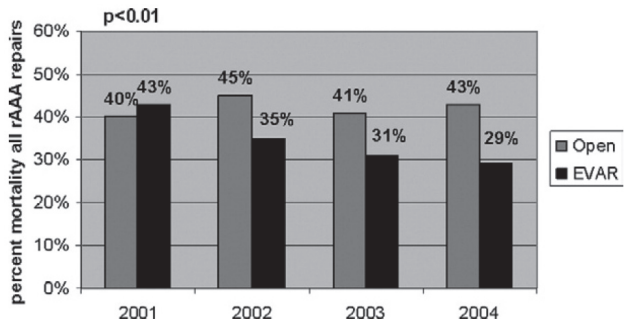

Figure 2: Mortality rate of open (gray bars) vs endovascular (*EVAR, black bars*) repair of ruptured abdominal aortic aneurysms (*rAAA*).

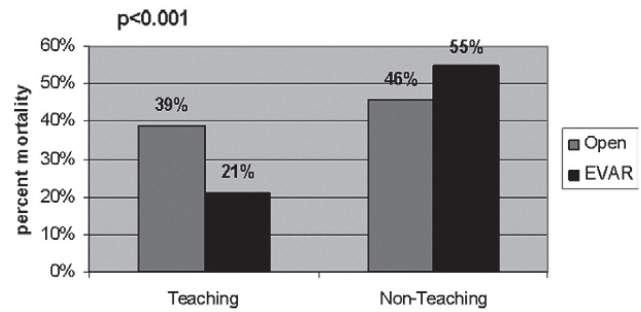

Figure 3: Mortality rate of open (*gray bars*) vs endovascular repair (*EVAR, black bars*) of ruptured abdominal aortic aneurysms in teaching and nonteaching hospitals.

The Harborview Experience

Harborview Medical Center is the only Level 1 Designated Trauma Center in the Pacific Northwest covering a five state region (Washington, Wyoming, Alaska, Montana and Idaho). This wide geographic area represents 25% of the land mass of the United States and roughly 15 million people. Harborview treats between 30 and 50 patients harboring ruptured abdominal aortic aneurysms per year with a mortality rate between 48 and 70%. In a review of the five year period spanning 2003 to 2007, the average mortality rate for all patients with rAAA was 61.5% (Figure 4).

A Modified Approach

In September, 2007, our group implemented a protocol to treat patients with ruptured AAA's with a preference for EVAR under local anesthesia when feasible (Figure 5). Over a 10 month period, 34 patients with rAAA have been treated. 14 underwent successful EVAR (41%) and 19 underwent open repair (56%). One patient elected comfort care only (3%). After implementation of this protocol, overall mortality declined significantly from a historic 61.5% to 35% ($p<0.01$). Patients undergoing purely EVAR experienced a 21% mortality whereas those undergoing traditional open repair experienced a 42% mortality.

Summary

Routine endovascular approach for ALL ruptured infrarenal abdominal aortic aneurysms is feasible. Streamlined protocols improve outcomes for patients presenting with rAAA. With this protocol in place, we have reduced mortality for patients with ruptured aortic aneurysmsby roughly 50% for the first time in over twenty years. Our group hopes to define variables affecting outcome for an endovascular approach. We have partnered with several industry stent graft manufacturers to define those aortic stent grafts that prove most beneficial for the management of patients presenting with ruptured abdominal aortic aneurysms.

Future Directions

Our clinical research focuses around identifying those underserved patients who lack sufficient screening to detect aneurysms prior to progression to rupture. We are also interested in defining those variables that are predictive of a 100% mortality rate, thereby improving the efficiency with which we manage these desperately ill patients.

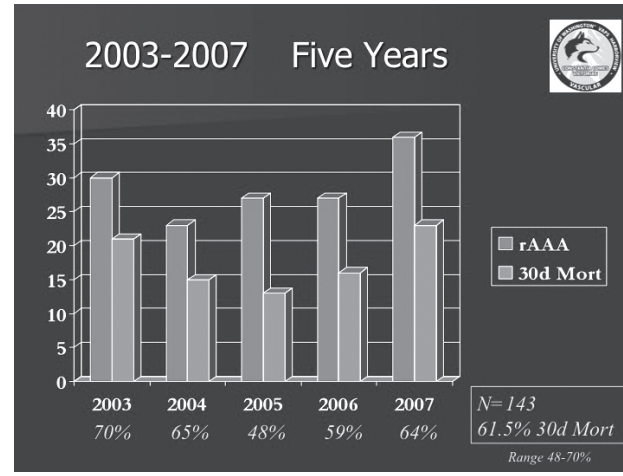

Figure 4: Mortality rates for 143 patients presenting with ruptured AAA at Harborview Medical Center between 2003 and 2007.

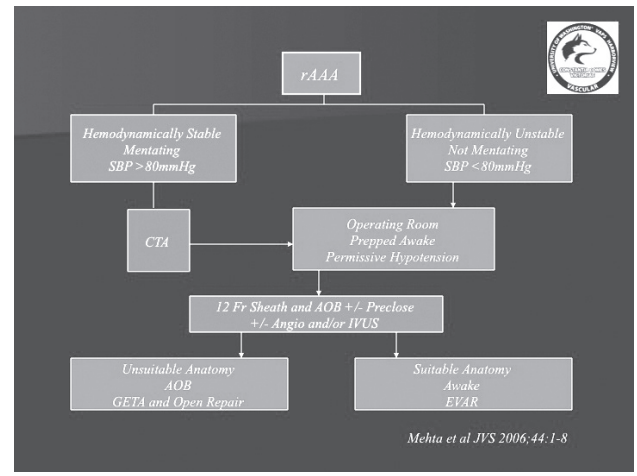

Figure 5: Protocol for management of patients with ruptured AAA. rAAA= ruptured abdominal aortic aneurysm, SBP= systolic blood pressure, CTA= computed tomographic angiography, AOB= aortic occlusion balloon, IVUS= intravascular ultrasound, GETA = general endotracheal anesthesia, and EVAR = endovascular aneurysm repair.

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