

RESEARCH IN THE DEPARTMENT OF SURGERY



SURGERY RESEARCH

DEPARTMENT OF SURGERY 2011 REPORT

2011

UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE

UW Medicine

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

2 0 1 1 R E P O R T

UNIVERSITY OF WASHINGTON
DEPARTMENT OF SURGERY
BOX 356410
SEATTLE, WA 98195-6410

CHAIRMAN: CARLOS A. PELLEGRINI, M.D.

EDITOR: ALEXANDER W. CLOWES, M.D.

MANAGING EDITOR: MARILYN CARLSON

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A Historical Perspective on Research in the Department of Surgery: A Constant During Changing Times

Since 1974, the University of Washington has annually received the highest amount of federal research funding among public universities and the second highest among all public and private universities in the United States. The Department of Surgery is a full participant in this strong scientific culture and considers research to be one of its priorities. Our departmental research enterprise is thriving, in no small part due to the talent, perseverance, and collaborative philosophy of our faculty members.

Our current successes are built on a solid research foundation established since the beginning of our department. The University of Washington (UW) School of Medicine opened in 1946. The first Chairman of the Department of Surgery was Dr. Henry N. Harkins, recruited from Johns Hopkins University. Dr. Harkins brought with him the strong tradition of research from Johns Hopkins, with his own focus on gastrointestinal physiology. New impetus to the department's research activities arrived in the 1970's with Dr. Eugene Strandness, who worked with colleagues in Bioengineering to develop ultrasonic duplex scanning for the diagnosis of vascular disorders. Drs. Alexander Clowes and Ronald Maier in the 1980's expanded the departmental research program with projects in vascular biology and inflammation. Current research interests in the department are broad and include outcomes research, infection, ischemia reperfusion, transplantation, gastrointestinal physiology, vascular biology and imaging, developing, applying and evaluating new advanced laparoscopic techniques, and research on education.

The past decade has produced a substantial growth of research activity in the Department of Surgery, as measured by expenditures from extramural support and dedicated floor space. From 2002 to 2009, total annual expenditures in the Department of Surgery from extramural sources of support grew by 27%, from \$8,124,636 to \$10,335,338. From 2003 to 2010, the amount of designated space for research grew by 32%. The increase during this period came largely from the new multidisciplinary state-of-the-art research space in the Brotman Building at the UW South Lake Union research facility. Aside from simple square footage, the number of sites where Department of Surgery research is now conducted has increased. From a single row of laboratories along the Department of Surgery hallway in the Health Sciences Building, our research units are now found at Harborview Medical Center, the Veterans Affairs Puget Sound Health Care System ("the VA"), the Surgery Pavilion at UW Medical Center, the UW South Lake Union facility, Seattle Children's Hospital, and the University District Building near the UW campus.

Research conducted in the Department of Surgery has also grown in its complexity and collaborative nature. This is demonstrated by the creation of individual research centers that serve not only our own department, but also encompass other departments throughout the UW School of Medicine. In this issue of *Research in the Department of Surgery*, we are introducing a new section to highlight the work of these research centers. This year we feature two of these entities, the Institute for Simulation and Interprofessional Studies (ISIS) and the Surgical Outcomes Research Center (SORCE), both of which have experienced remarkable growth since their inception.

Overseeing research activity in the Department of Surgery has been Dr. Alexander Clowes, who has served as Vice Chairman for Research since 1993. Dr. Clowes has recently relinquished this post, and we give him our utmost thanks for his leadership in raising our research program to its present high level. Dr. David Flum has accepted the task of guiding the Department of Surgery's research activities through the next few years. As Director of SORCE, Dr. Flum is uniquely positioned to expand our program in outcomes research, as well as to help our department meet the challenges and opportunities presented by changes in research funding and regulation. Dr. Flum states, "In the context of this emerging landscape, I look forward to helping the department and its researchers maintain and grow the research portfolio and make the UW Department of Surgery the best in the nation for surgical research."

Changes in the Department of Surgery have come with leadership transitions, medical advances, technological progress, and economic developments. But throughout all these changes, our department's focus on research as part of our three-part mission of patient care, teaching, and research has remained a constant. While this report highlights our research activities, our faculty members are equally dedicated to relaying their knowledge to the next generation of surgeons. Ultimately, our research and teaching serve to provide the best and most up-to-date care possible for our patients. If you would like to support us in our mission, please contact Lynn K. Hogan, Associate Vice President and Chief Advancement Officer, 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 2010

During summer 2010, the laboratory of **ALEXANDER W. CLOWES, M.D.**, PROFESSOR OF SURGERY, was awarded a 4-year grant from the National Heart, Lung and Blood Institute for the lab's project on "Syndecan-1 and the Arterial Response to Injury." This research aims to define the mechanism by which the transmembrane heparan sulfate proteoglycan, syndecan-1, regulates intimal thickening in injured arteries.

HEATHER L. EVANS, M.D., ASSISTANT PROFESSOR, was awarded a K-12 Comparative Effectiveness Research Career Development Award in June 2010. Dr. Evans' research interests are in surgical outcomes and trauma care delivery.

DAVID R. FLUM, M.D., M.P.H., PROFESSOR OF SURGERY AND ADJUNCT PROFESSOR OF HEALTH SERVICES, was appointed to the 15-member Methodology Committee of the federal Patient-Centered Outcome Research Institute (PCORI). The committee will help the institute develop and update methodological standards and guidelines for comparative clinical effectiveness research. Members of this committee were appointed by Gene L. Dodaro, Comptroller General of the United States and head of the U.S. Government Accountability Office.

THE INSTITUTE FOR SIMULATION AND INTERPROFESSIONAL STUDIES (ISIS) received a three-year \$1 million grant from the Josiah Macy Jr. Foundation and additional funding from the Hearst Foundation to develop a simulation-based, team-training program to improve collaborative interprofessional communication. This training program is led by **BRENDA ZIERLER, PH.D., R.N., R.V.T.**, ASSOCIATE PROFESSOR OF NURSING, and **BRIAN K. ROSS, PH.D., M.D.**, PROFESSOR OF ANESTHESIOLOGY AND ISIS EXECUTIVE DIRECTOR.

SARAH HUGHES JAVID, M.D., ASSISTANT PROFESSOR, was awarded \$25,000 for her research on breast surgery by the Seattle Division of Safeway, Inc.

DAVID W. MATHES, M.D., ASSISTANT PROFESSOR, received the Roche Organ Transplantation Research Foundation Award, a \$100,000 award that supports important and innovative research projects relevant to organ transplantation, for his project "Pre-Clinical Models of Tolerance to Composite Tissue Allografts via Mixed Chimerism," which runs 4/1/10 through 3/11/11. In June 2010, Dr. Mathes was awarded a \$50,000 grant by the Plastic Surgery Educational Foundation for his project "Simultaneous Stem Cell and Composite Tissue Transplantation." This is a one-year award that began 7/1/10 and ends 6/30/11.

MICHAEL S. MULLIGAN, M.D., THE UW DISTINGUISHED ENDOWED PROFESSOR IN LUNG TRANSPLANT RESEARCH, received an R-01 award from the National Institutes of Health (NIH) for his project on "The Role of TLR-4 in Lung Reperfusion Injury." The five-year award continues through July 2014.

In October 2010, **DAVID RABKIN, M.D.**, ASSISTANT PROFESSOR, was awarded the American Surgical Foundation's \$75,000 per year Fellowship Award for his research proposal "Cytokine Removal by Hemoadsorption After Brain Death: Effect on Ventricular Function Before and After Orthotopic Transplantation in a Porcine Model." Funding for this fellowship will commence on July 1, 2011 and continue for a second year commencing July 1, 2012.

The **SURGICAL OUTCOMES RESEARCH CENTER (SORCE)** received an \$11.7 million grant award from the Agency for Healthcare Research and Quality (AHRQ) to create a comparative effectiveness research platform across Washington State hospitals. The Principal Investigator is **DAVID FLUM, M.D., M.P.H.**, and the Department collaborators are **ERIK VAN EATON, M.D.**, **MARK MEISSNER, M.D.**, and **ALEXANDER CLOWES, M.D.** SORCE also received a National Research Service Award (NRSA) training grant (T32), funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). This SORCE training program offers one new position to post-doctoral trainees each year, providing training through the UW Masters in Public Health (MPH) program, as well as practical experience in outcomes research activities. In addition, SORCE and its colleagues in the UW Centers for Comparative and Health System Effectiveness (CHASE Alliance) received a K-12 award from the National Cancer Institute, which offers structured training to entry- and mid-level faculty focusing on comparative effectiveness research methodology, practical skill development, and community comparative effectiveness research development. The UW CHASE Alliance brings together researchers from across health science disciplines to create and carry out multidisciplinary research and training for comparative effectiveness research.

PETER C. WU, M.D., ASSISTANT PROFESSOR, received a VA Merit Career Development Award to study "Colorectal Cancer Treatment and Cellular Senescence." The \$176,000 grant provides lab funding for a 3-year period.

PART I: RESEARCH CENTERS

**INSTITUTE FOR SIMULATION AND
INTERPROFESSIONAL STUDIES**

(ISIS)

SURGICAL OUTCOMES RESEARCH CENTER

(SORCE)



ISIS – Educating to Improve Health through Safe Patient Care

Carlos A. Pellegrini, MD, Chair, Board of Directors; Farrah Leland, JD, Administrator; Richard M. Satava, MD, Senior Executive Advisor

Background

In the first four years of its formal existence, ISIS – the Institute for Simulation and Interprofessional Studies – has contributed to UW Medicine’s mission “To Improve Health.” ISIS has supported this mission through the education of medical students, residents, nurses and practicing physicians, through the creation of curricula, and through research in applied engineering and education – all using the platform of simulation and modeling. Much has been accomplished to fulfill these goals as ISIS continues to both mature and expand.

Even in these tumultuous financial times, ISIS has maintained a stable basis to continue both the training and research missions. In addition, by performing rigorous analysis of successes and failures, the initial programs have been strengthened by continuous improvement. A summary list of examples includes:

- Rigorous training to quantitative benchmark levels of competency has improved the clinical outcomes in patients – for example, the Central Venous Catheter (CVC) program has defined a specific protocol which mandates training until a uniformly high level of competence and standard team practice is accomplished between physicians and support staff – the result of which is that complications (including deaths) have been reduced to less than 1 infection per 1000 catheter days across a multihospital enterprise.
- All of the training areas, such as team training, communication skills and psychomotor skills, have begun not only expanding their continuity with other areas to provide a more comprehensive systems-level integrated approach (such as team training that includes hand-off), but have moved toward integration into clinical practice, as manifest by the psychomotor skills component of the CVC program above.

- ISIS has achieved a national leadership position in medical simulation and education through a rigorous training curriculum, innovative research program, and membership in the American College of Surgeons Accredited Education Institutes consortium.
- ISIS has been successful in competing for research funding, competing not only for federal grants and specific congressional special interest programs, but also for civilian opportunities such as the Josiah Macy Jr. Foundation.
- The opening of the new Harborview Medical Center (HMC) expands the ISIS training platform throughout a geographically dispersed simulation system because of the UW Medicine WWAMI regional medical school network (in Washington, Wyoming, Alaska, Montana, Idaho) (Figure 1) and the strong partnership between ISIS and Madigan Army Medical Center (MAMC). UW Medicine’s multi-site facilities at the University of Washington Medical Center (UWMC), Harborview Medical Center (HMC), and soon at Seattle Children’s and the Veterans Affairs Puget Sound Health Care System (VAPSHCS) continue to provide even more capability than ever possible before (Figure 1).
- The ISIS collaboration with MAMC and the Warrior Transition Unit (WTU) demonstrates commitment to our nation through intensive research to improve the health of our returning warriors through innovative application of simulation technologies.

It is patently clear that a complete revolution in medical education is in progress, and that simulation is at the core of this revolution. ISIS has embraced this innovation and is continuing to push the limits of creativity. However, it is ever mindful that, along with innovation and creativity, there must be the stringent evaluation of effectiveness, appropriateness and financial prudence. To address both the creativity and the validation, ISIS will:

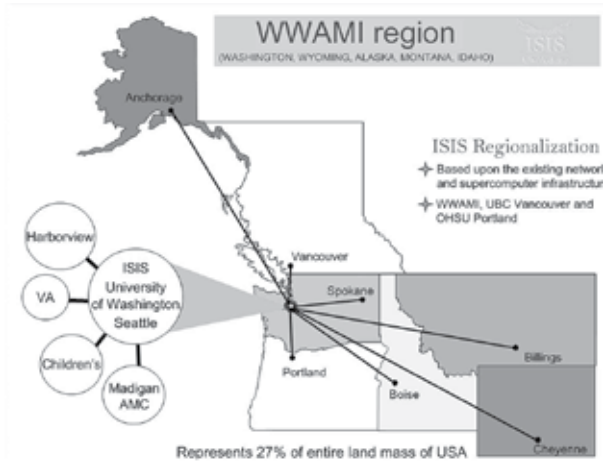


FIGURE 1. The UW Medicine WWAMI region medical school network and multi-site facilities

1. Apply sound principles of learning to design simulation-based curricula for interprofessional learners and then rigorously subject these curricula to validation.
2. Research best methods for, and demonstrate efficacy of, simulation as a platform for educating current and future healthcare professionals.
3. Demonstrate impact of learning through simulated programs of study on the safety and quality of care of patients and families.
4. Create programs that link across geographically dispersed sites through advanced communication and information technology by leveraging the unique advantages of the WWAMI telemedicine network.
5. Expand cooperation with MAMC and the VAPSHCS in contributing to the health and welfare of warriors returning from duty to their country.

Rationale for the Existence and Expansion of UW Medicine ISIS

A. BENEFITS OF SIMULATION AS A TRAINING PLATFORM

From a simplistic perspective, the hypothesis could be stated as follows: creating a learning opportunity through simulation increases the safety and quality of patient care. This occurs because trainees develop and sharpen technical skills under a specific curriculum, using objects and devices to practice on simulators instead of patients until meeting a defined minimum competency. In addition, trainees practice cognitive skills through scenarios in a controlled imitative setting that allows for simulated exposure to significant complications, allowing trainees to make and learn from errors, to hone skills with repetition limited only

by their stamina, and to learn at their own individual pace. Finally, trainees practice the communication skills and coordination of a team in a virtual environment.

The assumption is that patient safety and quality of care are improved by removing the first phase of the trainee's technical learning from the patient care setting. Not only is there scientific evidence that learners who practice in simulated environments improve performance in clinical care, but practitioners who initially train in a virtual setting express a greater degree of confidence in their ability to provide patient care in a safe manner. This is an additional benefit to utilizing this type of training environment. Confidence has been shown to increase the likelihood of appropriate judgment.

B. WHY EXPAND NOW THE USE OF SIMULATION AND INTERPROFESSIONAL STUDIES AS METHODS TO EDUCATE HEALTHCARE PROFESSIONALS?

There are several factors that suggest this is the right time to increase the use of simulation and immersion studies into the changing paradigm of educating medical professionals. First, the health care delivery system has always been searching for ways to **improve patient safety**. This was heightened in the past eight years in the wake of the publication of the Institute of Medicine's landmark book, *Crossing the Quality Chasm* (2001). Aviation and other high-risk industries have embraced education, training and evaluation in a simulated environment; while it is difficult to validate efficacy of these training modalities through double blind trials in high-risk industries, industry leaders fully believe that simulating errors, learning how to solve them, and improving accuracy of operations in all aspects is instrumental in dramatically reducing errors in the field.

Second, **technology and science** are evolving at an ever faster pace. It is increasingly difficult to educate using the apprenticeship education model of the past 100 years as technological and other clinical advances make learning under the "see one, do one, teach one" model untenable. For example, the regulated restriction on residency work hours contributes to the growing inadequacy of the apprenticeship model. Also, the apprenticeship model does not allow for the opportunity of a structured curriculum that ensures the learner will have experience with all the necessary patients or procedures – it is hostage to whatever patient comes through the door. Simulated education on all levels can ensure that in those areas in which patients are not available, there will be the opportunity for simulation to help fill that void.

Third, there is substantiation of opportunities to **reduce cost**. Evidence indicates the time to perform an operation that involves a trainee is shortened if the trainee has completed initial training in basic technical skills in a low tech simulation environment. Other industries have shown evidence that **standardized processes** reduce cost and error (reduction in task saturation contributes to error reduction) through standardized supplies and equipment usage (lower supplies cost, streamlined training and maintenance of equipment), and through time reduction (streamlined processes).

Fourth, residency core competencies (designated by the Accreditation Council for Graduate Medical Education) include systems-based learning and professionalism. The ability to effectively **communicate** is at the fundamental core of both of these competencies. Further, risk management studies have clearly identified that inadequate, absent, or incorrect communication between members of the health care team and with the patient and family are significant causal factors in patient-related errors and in the likelihood that a patient will seek legal retribution. Simulation provides a rich opportunity to learn and practice these vital communication skills.

Fifth, practicing medical professionals should continuously **retrain** throughout their professional lives, embracing the philosophy of lifelong learning. This is evidenced in the requirement for Maintenance of Certification mandated by the American Board of Medical Specialties for all its boards. Furthermore, military deployments for prolonged periods, temporary absence from work for social, personal or family reasons, and the advent of new procedures/technology which keep the health care professional away from the regular practice of medicine for a prolonged period require retraining. Patient safety and quality of care are improved when practicing professionals regularly demonstrate proficiency in technical, cognitive and systems skills in a controlled virtual environment, both for new skills and for select skills they may commonly employ in their practice. Practitioners may also hone technical and cognitive skills for patient **scenarios they rarely encounter in their practice**, but must be able to recognize and respond to appropriately. ISIS will provide educational opportunities for practicing health care providers to acquire new skills and to validate competency in select existing skills.

Sixth, simulation technologies are now moving into the clinical arena through *in situ* training in actual emergency rooms, ICU and operating rooms to improve performance and efficiency in the actual clinical setting. Next generation simulators will permit “surgical rehearsal,” allowing a

physician to practice an operation on the patient-specific image before performing the procedure on a patient. Evidence indicates that “warming up” before a procedure improves performance by decreasing operating time and errors. Data from these and other clinical uses of simulation will feed into quality improvement, risk management, privileging and patient safety committees to improve quality of care while reducing cost.

Research Imperative

As a Research Center in UW Medicine, ISIS is part of one of the top funded academic medical research institutions in the country, with a solid demonstration of collaboration across disciplines and in partnership with other UW world class Colleges and Schools such as the Information School, the College of Engineering and the School of Nursing, to name a few. ISIS is uniquely positioned to capitalize on these strengths to advance the knowledge of simulation as a platform for learning through disciplined research.

JOSIAH MACY JR. AND WILLIAM RANDOLPH
HEARST FOUNDATIONS

Effective communication among health professionals is essential to high quality, patient-centered health care. Yet few training programs exist to impart these interprofessional communication skills, and most existing curricula in this area are directed toward practicing clinicians rather than trainees.

In collaboration with the Schools of Nursing and Pharmacy, ISIS received grants from the Josiah Macy Jr. and William Randolph Hearst Foundations (totaling over \$1 million) to develop an interprofessional curriculum for students based on the Team Strategies and Tools to Enhance Performance and Patient Safety (TeamSTEPPS™) models of effective communication.

TEAMSTEPPS NATIONAL IMPLEMENTATION —
AHRQ/AIR

TeamSTEPPS™, a framework for ISIS training development, is a program developed by the Department of Defense, the Agency for Healthcare Research and Quality (AHRQ, a branch of the U.S Department of Health & Human Services), and the American Institutes for Research (AIR).

This team training framework targets patient outcomes by improving communication and teamwork skills among healthcare professionals (Figure 2). The four dimensions of TeamSTEPPS™ teamwork skills and competencies include: 1) leadership; 2) situation monitoring; 3) mutual support; and 4) communication. By increasing interprofessional

team awareness using the TeamSTEPPS™ tools, the goal is to target students' team performance, knowledge, and attitudes as outcome measures.

The University of Washington was chosen as the fifth TeamSTEPPS™ Training Center of Excellence for national implementation of TeamSTEPPS™. ISIS will hold eight Master Training Implementation training sessions for health organizations around the nation, including internal UW Medicine trainees.

FY10 ISIS-CONGRESSIONAL SPECIAL INTEREST APPROPRIATION FUNDING – DOD

In collaboration with Madigan Army Medical Center and the Boise VA, ISIS received a continuation of directed appropriation with a Congressional Add in the amount of \$4.054 million through the Department of Defense (DoD), United States Army Medical Research and Materiel Command (USAMRMC). The research projects are designed around a series of inter-operable pilot applications within each of the three core projects: 1) Distributed Skills Training and Healthcare Delivery; 2) Individual Healthcare Training; and 3) Team Training with Continuity of Care (TTCC).

ISIS provides an umbrella under which simulation experts combine their skills and develop a foundation for significant growth. ISIS improves resource utilization, provides scalability, reduces duplication of effort, standardizes curricula and provides a unique forum for interdisciplinary training.

By bringing together experts from both the military and public sectors, we have created a five-state simulation consortium that encompasses both the WWAMI region and the Western Regional Medical Command (WRMC).



FIGURE 2. Health professional students work as a team in one of the largest interprofessional training events in UW history.

The strategic goals of the consortium are:

- The advancement of research, development, validation, cost containment, and application of innovative simulation technologies to: enhance best practice and training in the area of medical education; research and then develop innovative distributive information technologies and “Capabilities Toolkits” to facilitate development of educational curricula; use these distributive technologies and toolkits to demonstrate the feasibility of delivering healthcare at a distance.
- To leverage the capabilities and resources of each sector (DoD and UW civilian) to address collaboratively many common regional issues, such that the framework that this collaboration establishes can be migrated as a national infrastructure.
- To conduct pilot research and validation projects which will serve as models for future curriculum development and training projects.
- Create a specific program with MAMC and the WTU to bring remote healthcare to the homes of the returning warriors – to keep them connected to their parent hospital (MAMC) and their military unit.

UW Medicine ISIS Poised to Expand

With all the new opportunities in the world of health service provider education, the challenge is to select those that will provide the greatest benefit. To that end, ISIS leadership has a broad basis of linkages across UW Medicine to help direct the priorities in terms of development of new curricula, creation or acquisition of simulation equipment, and expansion of its research and development initiatives. The Institute's priorities will be guided by the core principle of the Learner-Focused/Patient-Centered Education paradigm. As such, ISIS will prioritize research projects and learner initiatives that contribute to the mission of education to improve health through safe patient care. The learner-focused priorities will be based upon the complementary needs of UW Medicine residents, UW Medicine and affiliate institution interprofessional health care teams (physicians, nurses, and technicians), medical students, graduate medical trainees and WWAMI region community-based health care providers. Other ISIS users are physicians and other health care providers in the WWAMI region, community physicians and health care professionals, and institutions that might benefit from ISIS programs through Continuing Education offerings and other purchased training venues. Additional users would be persons participating in regional, national and international simulation, and interprofessional training programs that collaborate with or purchase access to ISIS programs.

The board of UW Medicine ISIS, comprised of a broad cross section of UW Medicine leadership, will set the strategic priorities that will assist ISIS leadership to critically evaluate the resource cost/benefit of proposed courses, purchases and research projects that utilize simulation and interprofessional studies. ISIS faculty will participate in the research and development of simulators and related equipment that support the educational priorities and in validation studies of simulation as a platform for learning through a series of extramurally and select intramurally funded projects.

As ISIS matures, the opportunity exists to expand its mission to improve health regionally, nationally and globally. As we evaluate this potential, we envision ISIS core trainees to be the students, physicians and interprofessional health care providers of UW Medicine, Seattle Children's and VA. Regionally, ISIS will expand to reach out to practicing health care providers and to affiliate institutions who host UW Medicine trainees, and to State of Washington health care providers and institutions that may find benefit in collaborating with, or purchasing simulation and interprofessional training programs from ISIS. Nationally and internationally, ISIS will improve health as ISIS faculty publish research, offer new curricula, devices, and methods of evaluation and training, and provide regional training across a distributed linked network.

A. ISIS STRUCTURE

The structural design of ISIS is based upon a cross-disciplinary, cross-departmental, cross-school/college model of collaboration in research, curricula design, simulation-based training, and technological development. The governance is centered on an advisory board that includes a broad constituency within UW Medicine, namely, all the owned and operated hospitals, and all affiliated hospitals where UW Medicine practices, and aims to bring aboard faculty from other components of the University of Washington who can expand the interprofessional concept. In order to keep up with the rapid pace of change, the structure continues to evolve as new partners develop and expand their programs and as external collaborations emerge.

Recognizing that such a large board cannot meet with the frequency required for effective management of operations, the Board delegates to an Executive Committee. The Board Executive Committee oversees the ISIS Operations group

which serves as an intermediary between the Executive Committee and three ISIS subcommittees (Patient Safety/Quality; Interprofessional Education/Practice; Research/Development). The ISIS Operations group provides expert advice and counsel to the Executive Committee, and through it, to the ISIS Board on strategies and tactics and leads implementation of the strategic and operational goals recommended to the Dean by the Board.

The Interprofessional Education/Practice committee will oversee medical student, resident and physician/nursing educational initiatives as well as research projects on learning, curricular development, and assessment of practice. The Research/Development Committee will focus specifically on the development of instrumentation, devices, and simulators, and will focus its research on the technical aspects of such developments. In close cooperation with the Interprofessional Education/Practice committee, it will carry out validation of educational methods and new devices that are introduced in the market. The Patient Safety/Quality committee will explore, in close cooperation with hospital centers for clinical excellence and quality improvement, specific areas of patient care that are identified as potential targets for dedicated simulation training for improvement of outcomes. This committee will ensure that UW Medicine quality/safety priorities are clearly reflected in the goals and initiatives of ISIS. In addition, ISIS will provide data and reports back to the respective hospital committees in a manner that will facilitate decisions that improve clinical care, reduce risks, facilitate credentialing, etc. The three committees will collaborate to ensure complementary initiatives and mutual progress, and will be accountable to the Executive Committee, and through it, to the ISIS Board. These committees serve as the interface between the laboratory training of students, residents, nurses and physicians with the continuing effort of improving the clinical practice of medicine.

Operationally, ISIS will have several components in different geographic sites which report to a single core operational umbrella, headed by the Executive Medical Director. The intention of the core oversight is to create a forum to assure cross-site best practices, common policies and procedures, and core expertise to complement site-specific focus and resultant operational variances.

B. ISIS GOVERNANCE



ISIS Leadership

BOARD OF DIRECTORS

Thomas Benedetti, ISIS Chair, Patient Safety and Quality

William Bremner, Chair, Department of Medicine

John Clark, Chair, Department of Biological Structure

TBD, Medical Affairs, Clinical Systems & Community Relations, Vice Dean for Regional Affairs, Rural Health, Graduate Medical Education, Professor of Family Medicine, School of Medicine

Richard Ellenbogen, Chair, Department of Neurological Surgery

David Fisher, ISIS Seattle Children's Representative

Cindy Hecker, ISIS HMC Executive Director

TBD, ISIS Chair, Interprofessional Education and Practice

Tom Norris, Vice Dean Academic Affairs

Carlos Pellegrini, ISIS Board Chair

Paul Ramsey, Dean, UW School of Medicine, CEO, UW Medicine

Larry Robinson, Chair, UW Medicine Safety and Coordination Committee

Brian Ross, ISIS Executive Director

Richard Satava, ISIS Senior Executive Advisor

Debra Schwinn, Chair, Department of Anesthesiology

Mika Sinanan, ISIS Chair, Research and Development

Johnese Spisso, Vice President of Medical Affairs (VPMA) UW, COO, UW Medicine

F. Bruder Stapleton, Chair, Department of Pediatrics

Eileen Whalen, ISIS HMC Executive Representative

Frederick Wolf, Professor and Chair, Medical Education & Biomedical Informatics

Stephen Zieniewicz, ISIS UWMC Executive Representative

Brenda Zierler, Associate Dean of Technology Initiative, Biobehavioral Nursing and Health Systems

EXECUTIVES OF THE BOARD COMMITTEE

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Richard Satava, Senior Executive Advisor

Brian Ross, Executive Director

Mika Sinanan, Chair, R&D Committee

TBD, Chair, Interprofessional education and Practice Committee

Thomas Benedetti, Chair, Patient Safety and Quality Committee

Margaret Gilshannon, Administrative Director

ISIS Faculty

CORE FACULTY MEMBERS

Adedipe, Adeyinka — Emergency Medicine

Davies, Jo — Anesthesiology

Fialkow, Michael — OB/GYN

Hurley, William — Emergency Medicine

Landel, Grace — MedEx

Lin, Simon — Pediatric Dentistry

Lombaard, Stefan — Anesthesiology

McDonough, Karen — Medicine

Metzner, Julia — Anesthesiology

Souter, Karen — Anesthesiology

Taitsman, Lisa — Orthopaedic and Sports Medicine

Varghese, Thomas — Surgery, Cardiothoracic

Vitin, Alexander — Anesthesiology

Wright, Andrew — General Surgery

Wu, Michael — Ophthalmology

Flinger, Corrine — Pathology

Friedrich, Jeff — Surgery

Hagman, Melissa (Moe) — Internal Medicine

Joffe, Aaron — Anesthesiology

Kent, Christopher — Anesthesiology

Martin, Alexa — MEDEX

Morris, Amy — Pulmonary and Critical Care

Neff, Margret — Pulmonary and Critical Care

O'Connell, Kathy — OB/GYN

Payne, John — Surgery (University of Hawaii)

Peterson, Gene — Anesthesiology

Plitt, Ken — Anesthesiology

Reid, Jennifer — Emergency Medicine (Seattle Children's)

Rozet, Irene — Anesthesiology

Sardesai, Maya — Otolaryngology

Sayre, Cindy — Neurological surgery

Sekhar, Laligam — Anesthesiology

Sivarajan, Gouri — Anesthesiology

Sivarajan, Murali — ENT

Souders, Jenny — Anesthesiology

Stanley, Robert — ENT

Starnes, Benjamin — Surgery, Vascular

Strandjord, Tom — Pediatrics (CHRM)

Trescot, Andrea — Anesthesiology

Vater, Youri — Anesthesiology

Von Saint Andre, Amelie — Pediatric ICU

Wolff, Juvann — Nursing

Wong, Karen — Anesthesiology

Zaichkin, Jeanette — Neonatal Outreach

RESEARCH FACULTY MEMBERS

Chen, Frederick — Family Medicine

Gallagher, Thomas — Internal Medicine

Cline, Lauren — Nursing

Jense, Ryan — Anesthesiology

Lendvay, Thomas — Pediatric Urology (Seattle Children's)

Masuda, David — Medical Education

Odegard, Peggy — Pharmacy

Shannon, Sarah — Nursing

Seehan, Florence — Cardiology

Wang, Carolyn — Radiology

Whipple, Mark — Otolaryngology

Zierler, Brenda — Nursing

CVES FACULTY MEMBERS

Oelschlager, Brent — Surgery



FIGURE 3. Mixed Use Cadaver/Dry Lab at ISIS-HMC



FIGURE 4. Orthopaedic surgeons training in the ISIS Cadaver Lab at ISIS-HMC

C. ISIS FACILITY

UW Medicine ISIS is an umbrella organizational structure. ISIS has, or will have, core training facilities at University of Washington Medical Center (UWMC) (opened 2006), Harborview Medical Center (HMC) (opened 2010), Seattle Children's, and the Veterans Administration Puget Sound Health Care System (VAPSHCS). Furthermore, ISIS has close collaborations with several other regional simulation centers, namely, Madigan Army Medical Center, University of British Columbia, and Oregon Health Sciences University. ISIS, along with UW Medicine's Northwest Hospital and Medical Center's Community Health Education & Simulation Center (CHESC), is also a founding member of the Pacific Northwest Healthcare Simulation Collaborative (PNWHSC), a partnership of simulation centers and industry leaders from across the state.

The UW Medicine ISIS facilities and operations are designed to be as flexible as possible to allow for 80% or greater utilization of classroom and training laboratory capacity. While there are similar features among simulation center facilities, each facility may have unique programmatic features that dictate the needs of the physical plant.

ISIS has 2500 square feet of space in the ambulatory Surgery Pavilion on the first floor of UWMC. The facility has a 425 usable square foot "smart" conference room that

holds 40 people, a full-sized equipped operating theater, a large open room (dry lab) holding approximately 12 low-definition virtual equipment stations, and a small administrative suite. The space is adequate to hold 3,680 hours of virtual OR, team training and crisis management courses annually, and can hold at least two multi-trainee courses simultaneously. The space can be made available 24/7 for independent training opportunities.

ISIS has full use of the Center for Videoendoscopic Surgery lab, a Department of Surgery operated facility of approximately 2000 square feet, which has three rooms on the 6th floor of the UW Health Sciences Building: an animal care (cleaning, shaving, etc.) and pre-anesthetic room; a laboratory that has 4 animal operating bays equipped with state-of-the-art videoendoscopic and anesthesia equipment; and a smaller office with two cubicles for computer work. ISIS-HMC has 8,000 square feet of space on the 3rd floor in the new Ninth and Jefferson Building at Harborview Medical Center. It has a 9-bay mixed use cadaver/dry lab (Figure 3); a second dry lab; a cadaver lab (Figure 4); conference facilities; and a small administrative suite. Seattle Children's is considering another option that will be explored in the future: creating an in situ training opportunity in an actual patient care unit by transporting simulators (e.g., mannequins) to an available unit.

The following principles are considered when building simulation training sites for UW Medicine ISIS:

- Public face (reception), administrative suite, and faculty/staff shared work stations
- Conference facility for a minimum of 40 people (classroom)
- State-of-the-art communication software and cabling
- Virtual operating theater with mirrored window to observation station, control room
- Large flexible multi-purpose room with temporary partitions (dry lab)
- Connections to/from live operating room theater(s)
- Storage, supplies and equipment
- Access to a kitchen facility for guests
- Options: Virtual OR, ICU, ER bay, preferably convertible
- Facilities for animal surgery and/or cadaver surgery capability. A number of additional facility factors need to be taken into consideration, such as refrigeration, staging area, ventilation and cleaning requirements. May also require shielding where radiography may be used.

D. ISIS FINANCES

The two most important factors that influence the long-term success of ISIS are a sustainable funding platform for core infrastructure and protected focused faculty effort from a broad representation of UW Medicine and partner colleges and schools, such as Engineering and Nursing.

ISIS facilities are training labs that use inanimate, animal and cadaver models to create training platforms for self-learning and course-based training programs. The core infrastructure is built to encourage use of the facilities through the creation of excellent curricula; coordination and management of self-learning and classroom-based programs; use of the facilities for intra- and extramurally funded research, validation and development projects; and availability of the center for distributed simulation training throughout WWAMI and experimentally, through Second Life virtual worlds. Based on a capacity analysis for UWMC

and the proposed HMC facilities, there are 195,000 total learner hours available; when ISIS is at full capacity (80% utilization), ISIS would provide approximately 160,000 learner hours. The expectation is that intra- and extramurally funded projects will “purchase” facility and core technical faculty and staff resources as appropriate. Additionally, there is a critical basic infrastructure that administers UW Medicine ISIS that cannot rely on project-based funding. It is this infrastructure that requires a form of consistent, reliable funding to ensure the success of UW Medicine ISIS.

UW and UW Medicine leadership have made ISIS a top priority in their conversations with state and federal leaders, key philanthropic and regional and national business partners, and UW Medicine senior leadership. ISIS faculty have submitted competing project proposals to a number of foundations. The ISIS Board and leadership of UW Medicine are confident these efforts will prevail and ISIS will have funding for small and large projects that support its core mission and vision. Intramural funding is also anticipated from multiple UW Medicine constituencies whose core mission aligns with the strategic mission of ISIS and whose constituencies will benefit from the outcomes of ISIS programs and projects, particularly our patients and our trainees.

A second critical factor to ensure the future success of ISIS is to have sufficient protected, focused faculty effort from a broad constituency of UW Medicine and partner colleges and schools. The faculty are the content experts; as such, they are a critical resource to develop curriculum content, provide and evaluate training programs, and develop research and development proposals for future initiatives. It will be incumbent upon the UW Medicine ISIS Board and UW Medicine leadership to build a compelling vision that captures the imagination of faculty leaders and faculty such that there is a groundswell of faculty prepared to include active, focused participation in ISIS into their academic careers. Creative solutions, such as giving credit toward promotion for the development, validation and implementation of specific curriculum, will encourage faculty.

Summary

In six short years, ISIS has gone from a concept to a robust, multi-institutional, interprofessional educational institute that supports the training of the entire UW Medicine enterprise. However, this is a pivotal transition period for UW Medicine ISIS. ISIS-HMC opened in January 2010; discussions are underway for the program and facility at Seattle Children's. ISIS has initiated training of the first phase of a system-wide central venous line placement simulation training module, and even at this early stage, initial data has returned that allows ISIS to modify and improve this course. It is the first system-wide training initiative based on UW Medicine quality and safety priorities. When initial evaluation is completed, this module will likely form the basis for a national model in system-wide training and assessment. ISIS could be the coordinating body for cross-institutional e-learning and simulation-based

initiatives for the health care delivery system. The governance and infrastructure has senior leadership and operational linkages across the broad spectrum of UW Medicine clinical, research and teaching programs, and has close partnerships with key UW schools and colleges. ISIS will be strengthened by a solid foundation of cross-institutional passionate faculty commitment of effort and by a consistent financing of core operating and facility infrastructure. In the years ahead, the current faculty and staff involved in ISIS are excited about these prospects as we look forward to meeting our mission through the support of the broad UW Medicine constituency, our collaborating UW Colleges and Schools, our close ties with the military and veterans medical centers, and our regional and national partner organizations.

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The Surgical Outcomes Research Center (SORCE)

David R. Flum, MD, MPH, Director

The Surgical Outcomes Research Center (SORCE) is a multidisciplinary research center that serves as a home for outcomes, health services, and comparative effectiveness research within the Department of Surgery. Established in 2005, the primary mission of SORCE is to assess the impact of surgical care and interventions on patients, society and the healthcare system in order to improve the field of surgery. SORCE accomplishes this through research, training, outreach, and policy initiatives. SORCE serves several functions: it is an internationally recognized surgical health services research center that houses several large, multi-center prospective studies; it is a leader in the development of community-based prospective surgical registries and community-based participatory research; and it serves the Department of Surgery by offering to faculty and trainees support for research and project development, administration, and management services and by overseeing the general surgical quality improvement initiatives of the Department.

Research Projects

SORCE faculty and affiliated investigators from across the clinical spectrum focus on health services topics, including geographical variation in care, disparities in patient selection and care, surgeon knowledge and behaviors, and influence of policy changes on trends in care. In collaboration with other UW and community investigators, SORCE investigators also study cost-effectiveness, decision analysis, and novel approaches in evaluating return on investment for surgical interventions.

BARIATRIC OUTCOMES AND OBESITY MODELING (BOOM) PROJECT FUNDER: DEPARTMENT OF DEFENSE

The BOOM Project is a collaborative of UW investigators using over 20 data streams and modeling techniques to create a portfolio of research on the economics of obesity and its treatments. BOOM projects include analyses of the U.S. Department of Health & Human Services Centers for Medicare & Medicaid Services (CMS) national decision coverage on patient safety and health outcomes, the role of emerging technology in bariatrics, and quality of life and real world outcomes of patients who have received adjustable gastric bands at most statewide centers.

SURGICAL CARE AND OUTCOMES ASSESSMENT PROGRAM (SCOAP) FUNDERS: LIFE SCIENCES DISCOVERY FUND, AGENCY FOR HEALTHCARE RESEARCH AND QUALITY, SCOAP MEMBER HOSPITALS, AND INDUSTRY

SCOAP is a physician-led collaborative that tracks variance in surgical care across almost all Washington State hospitals and aims at reducing inappropriate care, variation in care, and adverse outcomes while promoting greater cost-effectiveness. SORCE serves as the academic home for SCOAP research and development. Studies are being undertaken to evaluate the use and outcomes of chemoprophylaxis in real world practice, the role of provocative testing in preventing anastomotic failure, and nutritional interventions to avoid surgical complications. SORCE investigators and staff have significantly contributed to the development of existing SCOAP programs (in general surgical care, pediatric surgical care, and vascular medicine) and in new programs including oncologic, urologic, gynecologic, orthopedic spine, and ambulatory care covering clinical care for almost all of the state's surgical patients.

COLLABORATIVE TO IMPROVE NATIVE CANCER OUTCOMES (CINCO) FUNDER: NATIONAL CANCER INSTITUTE

In collaboration with the UW Center for Clinical and Epidemiological Research, this project examines influences ranging from organizational to individual on outcomes among American Indians/Alaska Natives undergoing surgery for the most common cancers. Three distinct studies are being conducted to achieve project aims, including a large-scale retrospective analysis of SEER-Medicare data; development and deployment of lung, prostate, and breast cancer modules within SCOAP; and a patient-reported outcomes study about treatment decision-making and satisfaction using ethnographic and semi-quantitative techniques.

COMMUNICATION TO PREVENT AND RESPOND TO MEDICAL INJURIES: WASHINGTON STATE COLLABORATIVE FUNDER: AGENCY FOR HEALTHCARE RESEARCH AND QUALITY

This project aims to create a statewide initiative to enhance the culture of healthcare communication to improve patient safety, particularly around communication-sensitive events, and to decrease medical malpractice liability. SORCE is partnering with the study Principal Investigator, Dr. Thomas Gallagher, a national leader in patient safety communication, along with stakeholders across Washington State to create a collaborative approach to tracking communication-sensitive events, analyzing adverse events, training healthcare communities, and implementing a novel approach to medical malpractice compensation.

LONGITUDINAL ASSESSMENT OF BARIATRIC SURGERY (LABS) FUNDER: NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

As part of the NIH-funded LABS consortium, this project aims to evaluate short- and long-term outcomes of bariatric surgery through a series of studies following patients before and after bariatric surgery. Overall goals of this study are to analyze the risks and benefits of bariatric surgery and its impact on the health of patients with extreme obesity, and to identify the types of patients who are most likely to benefit from bariatric surgery.

MECHANISMS OF GLYCEMIC IMPROVEMENT AFTER GASTROINTESTINAL SURGERY (RYGB MECHANISMS) FUNDER: NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

This study examines the mechanisms mediating the weight-independent anti-diabetes impact of Roux-en-Y gastric bypass surgery and identifies modifiable components of surgical operations that impact glucose homeostasis.

FEASIBILITY, EFFICACY, AND MECHANISMS OF SURGICAL VS. MEDICAL DIABETES TREATMENT

FUNDER: NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

This study aims to execute a randomized clinical trial comparing the outcomes and effects of gastric bypass surgery versus an optimal medical/lifestyle intervention to treat type 2 diabetes mellitus in patients with a BMI of 30-40 kg/m².

SORCE investigators have several population-based studies in progress evaluating trends in surgical care and nationwide population-based outcomes using secondary analyses of clinical and administrative databases including Surveillance Epidemiology and End Results (SEER), SEER-Medicare, Medicare and the Comprehensive Hospital Abstract Report System (CHARS):

QUALITY AND OUTCOMES OF ESOPHAGEAL CANCER CARE FUNDER: NATIONAL CANCER INSTITUTE**EVALUATING THE USE AND OUTCOMES OF AXILLARY LYMPH NODE DISSECTION (ALND) IN THE TREATMENT OF BREAST CANCER IN ELDERLY PATIENTS** FUNDER: NATIONAL CANCER INSTITUTE**VARIATION AND TRENDS IN UROLOGICAL DISEASES** FUNDER: PRIVATE FOUNDATION**A POPULATION-BASED EVALUATION OF THE USE AND OUTCOMES OF NEOADJUVANT CHEMOTHERAPY** FUNDER: MARSHA RIVKIN CENTER FOR OVARIAN CANCER RESEARCH**TREATMENT TRENDS IN HEPATOCELLULAR CARCINOMA** FUNDER: NATIONAL CANCER INSTITUTE**Department of Surgery Service Activities**

SORCE offers Department of Surgery faculty and trainees support in research and project development. SORCE works with interested faculty investigators by providing expertise in outcomes research design, grant application and budget development, project design and implementation, data management and analysis, and statistical and epidemiological support. Additionally, surgical residents entering into research are advised to meet with SORCE staff for training in and guidance on the grant application process as they work to secure funding for their research years.

SORCE also oversees several Departmental quality improvement activities and manages several surgical clinical data registries, including the Surgical Care and Outcomes Assessment Program (SCOAP), the Vascular and Interventional Radiology-SCOAP (VI-SCOAP), the National Surgical Quality Improvement Project (NSQIP),

the Bariatric Surgery Center Network (BSCN), and a pediatric surgery quality improvement project for gastroesophageal reflux disease. SORCE staff members extract clinical data for registry submission, disseminate reports back to the departmental faculty, and facilitate quality improvement workgroups. Currently, workgroups are addressing preoperative albumin standards, unplanned re-intubations, operating room order set standardization, postoperative transfusion, and perioperative and postoperative glucose control.

Training and Education

SORCE strives to train, educate and build community among health services researchers, clinicians and students.

OUTCOMES RESEARCH COLLABORATIVE (ORC)

SORCE offers multiple educational opportunities for the Department of Surgery as well as the UW community as a whole, including a monthly work-in-progress session called the Outcomes Research Collaborative (ORC). Investigators are able to present their research work and receive valuable feedback and advice from a community of their peers in health services and comparative effectiveness research on project direction, data analysis plans, and areas for future work.

SURGICAL OUTCOMES POST-DOCTORAL FELLOWSHIP

SORCE has a T32 training grant from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) that funds one new fellow per year in health services and outcomes research training. Trainees work with a mentor on a wide variety of projects that expose them to several areas of health services research, and complete coursework to obtain a Masters in Public Health (MPH) degree over the course of the two-year training program.

UNDERGRADUATE PRACTICAL RESEARCH EDUCATION PROGRAM

SORCE offers undergraduate students exposure to clinical disciplines, surgical health services, and research through a supervised work internship program. Students participate in SORCE research projects with opportunities in data entry, medical record review and abstraction, specimen handling and processing, translational

laboratory-based activities, research development and quality improvement activities, and general office operations, as well as having the opportunity to shadow clinicians and conduct their own research. Students have the option to volunteer or participate for course credit.

PARTICIPATING IN THE COMPARATIVE AND HEALTH SYSTEMS EFFECTIVENESS COMMUNITY ACROSS THE UNIVERSITY OF WASHINGTON

SORCE co-founded the UW Centers for Comparative and Health Systems Effectiveness (CHASE Alliance), a multi-disciplinary, collaborative alliance of UW health sciences research groups focused on comparative effectiveness research, technology assessment, and health systems evaluation. The UW CHASE Alliance brings together a community of engaged peers, research teams, and likeminded collaborators to build upon existing and available resources to create economies of scale for high-impact research.

Other founding members of the CHASE include:

- Behavioral Nursing and Health Systems, School of Nursing
- Comparative Effectiveness, Cost and Outcomes Center, Schools of Medicine and Public Health
- Department of Health Services, School of Public Health
- Harborview Injury Prevention and Research Center, Schools of Medicine and Public Health
- Pharmaceutical Outcomes Research and Policy Program, School of Pharmacy
- Seattle Quality of Life Group, School of Public Health
- Veteran's Affairs Health Services Research & Development Center, VA Puget Sound Health Care System

To date, CHASE Alliance member groups have received two major cross-collaborative grants (cancer diagnostics and bariatric surgery outcomes) and three new training grants. In June 2010, the CHASE Alliance also held its first major symposium on using the findings from comparative effectiveness research to inform clinical and reimbursement decisions. SORCE Director David Flum and SORCE Administrator Allison Rhodes currently serve as the Interim Director and Administrator of the CHASE Alliance, offering development and administrative support.

DEPARTMENT CO-INVESTIGATORS

David R. Byrd, MD / Alexander W. Clowes, MD / E. Patchen Dellinger, MD / Farhood Farjah, MD, MPH (Research Fellow) / Adam B. Goldin, MD, MPH / Karen D. Horvath, MD / Sara H. Javid, MD / Saurabh Khandelwal, MD / Steve Kwon, MD (Research Fellow) / Nader N. Massarweh, MD, MPH (Research Fellow) / Mark H. Meissner, MD / Juan Parra, MD / Carlos A. Pellegrini, MD / Venu G. Pillarisetty, MD / David Row, MD / Benjamin Starnes, MD / Erik G. Van Eaton, MD / Thomas K. Varghese, MD, MS / Andrew S. Wright, MD / Raymond S. Yeung, MD

UW CO-INVESTIGATORS

Dedra S. Buchwald, MD; Medicine/Director, Center for Clinical and Epidemiological Research / Jens R. Chapman, MD; Orthopaedics / David E. Cummings, MD; Medicine / Beth Devine, PharmD, MBA, PhD; Pharmacy / Ardith Doorenbos, PhD, RN; Biobehavioral Nursing and Health Systems / Beth Ebel, MD, MPH, MSc; Pediatrics / Todd Edwards, PhD, MA; Health Services / Joann G. Elmore, MD, MPH; Medicine / Michael F. Fialkow, MD, MPH; Obstetrics and Gynecology / Stephan D. Fihn, MD, MPH; Medicine / Karen E. Foster-Schubert, MD; Medicine / Thomas H. Gallagher, MD; Medicine / Louis Garrison, PhD; Pharmacy / Barbara A. Goff, MD; Obstetrics and Gynecology / Jack Goldberg, PhD, MA; Epidemiology/Center for Clinical and Epidemiological Research / John L. Gore, MD, MS; Urology / Jeff Henderson, MD; Center for Clinical and Epidemiological Research / Jeffrey Jarvik, MD, MPH; Radiology/Director, Comparative Effectiveness, Cost, and Outcomes Research Center / Larry Kessler, ScD; Health Services / Mario Kratz, PhD, MSc; Epidemiology / Michael J. Lee, MD; Orthopaedics / Jerry P. Palmer, MD; Medicine / Donald Patrick, PhD; Health Services/Director, Seattle Quality of Life Group / Michael P. Porter, MD; Urology / Lisa Strate, MD, MPH; Gastroenterology / Sean D. Sullivan, PhD; Pharmacy / Peter Tarczy-Hornoch, MD; Pediatrics / Melissa M. Thrall, MD; Research Fellow – Obstetrics and Gynecology / Hunter Wessells, MD; Urology / David Yanez, PhD, MS; Biostatistics / Meliha Yetsigen-Yildiz, PhD, MSc; Biomedical and Health Informatics / Michi Yukawa, MD, MPH; Medicine

COMMUNITY CO-INVESTIGATORS

David Arterburn, MD, MPH; Group Health Research Institute / Ellen Farrokhi, MD; Virginia Mason Medical Center / Rosa Johnson, ARNP, MN, CPHQ; Foundation for Health Care Quality / Eric Larson, MD, MACP; Group Health Research Institute / Justine Norwitz; JSN Consulting / Terry Rogers, MD; Foundation for Health Care Quality / Neal Shonnard, MD; Proliance Surgeons

SORCE STAFF

David R. Flum, MD, MPH ; Director Research Administration, Development, and Project Management /Allison Devlin Rhodes, MS; Administrator / Sarah Lawrence, MA; Program Operations Specialist / Erin Machinchick; Program Operations Specialist / Matania Thoreson;Project Coordinator

DATA ANALYTICS

Rebecca Gaston Symons, MPH; Health Services Research Analyst / Rebecca Donaldson, MBA; Health Services Research Analyst / Hao He; Health Services Research Analyst

QUALITY IMPROVEMENT

Rosemary Grant, RN, BSN, CCRC, CPHQ; Surgical Clinical Nurse Abstractor / Wendy Klamp, MHA; Surgical Quality Improvement Coordinator / Billi Tatum, RN; Nurse Research Coordinator / Halli Olsen; Program Assistant / Tiffany Cleveland; Program Assistant / Anthony Castelli; Student Assistant

TRANSLATIONAL RESEARCH STUDIES

Skye Steptoe, MS, CCRC; Research Coordinator / Katrina Golub, MPH; Research Coordinator / Anne MacDougall; Research Coordinator / Pam Gronbeck; Research Scientist / Traci Weber; Research Scientist / Jen Corpuz; Research Assistant / Hallie Erickson; Student Assistant

TRAINEES; PAST AND CURRENT

Adam Goldin, MD, MPH; 2001-2002 / Diania Sandness, BD; 2001-2002 / Leon Salem, MD; 2002-2003 / Christine Jensen, MD; 2004-2005 / Katie Nason, MD; 2004-2005 / Victor Legner, MD; 2005-2006 / Bahirathan Krishnadasan, MD; 2006-2007 / Farhood Farjah, MD, MPH; 2005-2007 / Nader Massarweh, MD, MPH; 2008-2010 / Steve Kwon, MD; 2009-present

PART II: INDIVIDUAL INVESTIGATORS

CARDIOTHORACIC SURGERY

GABRIEL S. ALDEA, M.D.

GORDON A. COHEN, M.D., PH.D.

D. MICHAEL MCMULLAN, M.D.

MICHAEL S. MULLIGAN, M.D.

Gabriel S. Aldea, M.D.

· Minimizing Morbidity of Cardiopulmonary Bypass, Valve and Aortic Surgery



The William K. Edmark Professor of Cardiovascular Surgery

AWARDS

National Research Service Award in Heart and Vascular Diseases

FUNDING

National Institutes of Health
Edwards Lifesciences
W.L. Gore and Associates

The Medicines Company
Alexion Pharmaceuticals, Inc.

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 cardiopulmonary bypass (CPB), cell salvage and transfusion; and 2) develop alternative therapies for complex aortic and valvular pathologies that do not require CPB.

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 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.

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 with previous reports, the incidence of neurological and persistent neuropsychological deficits following coronary artery bypass grafting (CABG) was markedly reduced to near baseline.

Figure 1 shows a representative scanning electron micrograph 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 compared with conventional non-biocompatible circuits with full anti-coagulation.

Heparin bonded circuits (HBCs) 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 (β -thromboglobulin) and neurological injury (neuron specific enolase, S-100b) are *all* nearly completely blunted when HBC are used and cardiomy suction is eliminated during CPB. Our results suggest that cardiomy 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).

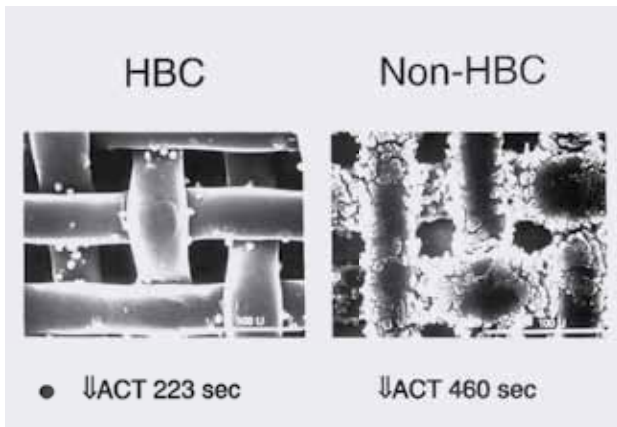


FIGURE 1: Scanning electron micrographs at 200-fold magnification of arterial filter. Lowest activating times (ACT) in seconds are noted. HBC = heparin-bonded circuits. Non-HBC = control non-heparin-bonded circuits.

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 heparin-induced thrombocytopenia thrombosis (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 unfractionated heparin (UFH) is the focus of our future studies.

Our research demonstrates wide differences and individual variability among 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

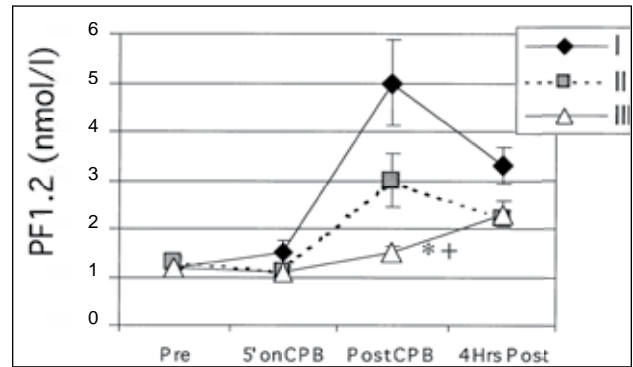


FIGURE 2: PF1.2 for thrombin generation

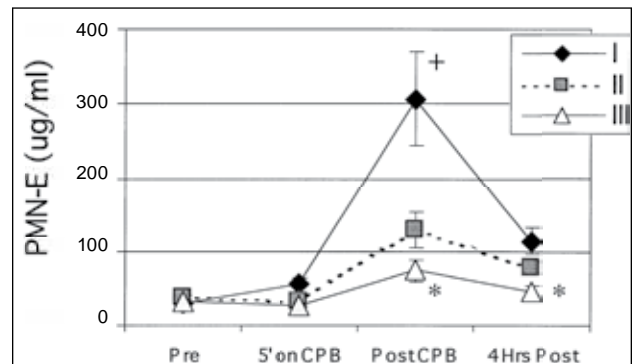


FIGURE 3: PMN-E for elastase

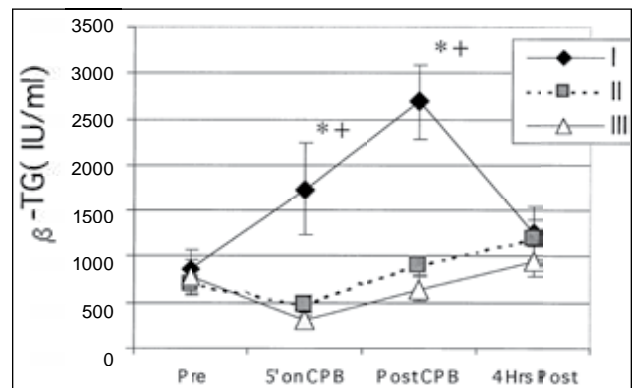


FIGURE 4: beta-Thromboglobulin for platelet activation

of a three-year NIH Specialized Centers of Clinically Orientated Research (SCCOR) grant and in collaboration with Drs. Nelson and Slichter, we are studying these interactions and the effects of the removal of passenger white blood cells from non-autologous blood on clinical and immunological outcomes of patients undergoing cardiac surgery.

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.

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

Traditional open surgical repair of complex descending thoracic pathologies is associated with significant pulmonary and neurological complications. 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 for both thoracic aortic aneurysms and traumatic aortic transections.

Trans-catheter Aortic Valve Therapy

The University of Washington Medical Center has been selected as one of only 25 international sites as part of the international multi-center prospective randomized PARTNER 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 the Division of Cardiology and Drs. Verrier and Aldea from the Division of Cardiothoracic 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

Mark Meissner, M.D. / Benjamin W. Starnes, M.D. / Edward D. Verrier, M.D.

OTHER CO-INVESTIGATORS

Larry S. Dean, M.D.; UW Department of Medicine / **Terry Gernsheimer, M.D.;** UW Department of Medicine / **Karen Nelson, Ph.D.;** Puget Sound Blood Bank / **Mark Reisman, M.D.;** UW Department of Medicine / **Sherrill Slichter Ph.D.;** Puget Sound Blood Bank

Gordon A. Cohen, M.D., Ph.D.

- New Therapeutic Options for Pediatric Patients with Heart Failure



Professor of Surgery
Samuel and Althea Stroum
Endowed Chair in Pediatric
Cardiovascular Surgery

Background and Significance

Despite advances in the treatment of children with acquired and congenital heart disease, many limitations still exist. Even with increased medical management, a proportion of patients will have refractory heart failure. Currently there are only a limited number of options for treating pediatric patients with heart failure. When heart failure reaches end-stage, only transplantation or recovery of failed hearts exist as alternatives. Ultimately our goal is to understand how hearts fail in the pediatric population, especially among children with congenital heart disease, children undergoing cardiac surgery, children experiencing viral infection, or unexplained causes. Often, heart transplantation is the only option for children with heart failure. This is not an ideal solution due to factors such as limited organ availability and uncertain long-term survival rates. Future treatments need to focus on how to recover the failing heart so that a patient can survive without a transplant and go on to live a life of normal quality and duration. Our belief is that we can develop new therapeutic options that can be used to recover a failing heart or improve the outcomes of a transplanted heart. Our current efforts are focused on developing a better understanding of the mechanisms of heart failure. Moreover, we want to develop a multidisciplinary and long-term approach to studying and understanding this complex and diffuse disease process by focusing on ways to treat heart failure through cardiac recovery, rather than transplantation.

Mechanical Support of Children with End-stage Heart Failure

Currently, available modalities to support children at imminent risk of death from heart failure are limited. Our research includes a multifaceted program using different modes of mechanical support including the Berlin Heart EXCOR Pediatric Ventricular Assist Device (VAD), DeBakey Pediatric VAD, Extra-corporeal Membrane Oxygenation (ECMO), and Centrifugal Pump System (CPS). The purpose of our research is to apply new technology to support failing hearts in real clinical situations as a bridge to transplant. We can then evaluate the “remodeling” process that may take place while the child’s heart is being supported.

In 2007 our center was chosen as one of ten centers in North America to participate in the Berlin Heart EXCOR Pediatric VAD trial. This is a prospective, multi-center, single-arm clinical trial to estimate the reasonable safety and probable benefit of the EXCOR® Pediatric VAD in children who are awaiting cardiac transplantation and require mechanical circulatory support. The Berlin Heart, a miniaturized pneumatic paracorporeal VAD, has emerged in the U.S. as a potential alternative to ECMO for infants and small children requiring long-term mechanical circulatory support (MCS). ECMO is the standard of care in the U.S. for children requiring MCS for bridge-to-cardiac transplantation. However ECMO is capable of bridging only half of eligible children to cardiac transplantation. Survival on ECMO often falls short of wait times for a pediatric donor heart in the U.S. Serious complications such as bleeding, infection, stroke, and multi-organ failure predictably develop during the first weeks on ECMO, limiting

Understanding neovascularization could have major and potentially even landmark implications for the treatment of a number of disease processes not necessarily limited to the heart.

the overall effectiveness of ECMO for long-term circulatory support. We have successfully implanted the Berlin Heart EXCOR Pediatric VAD at our center in a child as small as 3 kilograms and provided support for over 200 days. European studies suggest that the Berlin Heart EXCOR Pediatric VAD can provide stable circulatory support for up to 421 days (Dr. Johannes Mueller (Berlin Heart AG), personal communication). Furthermore, our experience has shown that EXCOR® Pediatric patients can be weaned and extubated from mechanical ventilation, discontinued sedation/paralysis, transition from parenteral to enteral nutrition, and in some cases even become ambulatory – clinical benefits considered infeasible with ECMO, but very favorable for continued suitability for transplant. Due to a growing number of emergency-approval implantations in the U.S., the FDA and Berlin Heart, Inc. have recognized the need for a formal clinical trial to assess the overall risk-benefit profile of the Berlin Heart EXCOR® Pediatric VAD in children and provide a procedure for providing the device under the compassionate use regulations. Since the study began, we have successfully bridged multiple patients to transplant with the Berlin Heart EXCOR Pediatric VAD.

Identification of Neovascularization Biomarkers

In collaboration with co-PI Eugene Kolker, Ph.D., and the Bioinformatics and High-Throughput Data Analysis Laboratory at the Seattle Children’s Research Institute, we have been investigating the neovascularization phenomenon in pediatric cardiac patients, with particular focus on understanding the physiologic nature of the development and regression process. Neovascularization is the process by which new blood vessels grow. This process can be a normal compensatory mechanism, or it can be pathologic in nature. Understanding neovascularization could have major and potentially even landmark implications for the treatment of a number of disease processes not necessarily limited to the heart. In some patients with a certain type of congenital heart disease, blood flow is surgically rerouted so that it does not pass through the liver prior to entering the lungs. A commonly observed sequela of this operation is the development of new, pathologic blood vessels known as arterio-venous malformations (AVMs). How and why

this occurs is not entirely clear. But it is known that if blood passing through the liver is brought back to the lungs in patients who have developed arterio-venous malformations, the pathologic neovascularization will regress and even disappear. This observation would suggest that there is some unidentified substance that is being produced by the liver that prevents the growth of new blood vessels; when it is removed from the circulation, new blood vessels will grow. Identifying this substance, the putative “hepatic factor,” would be important in helping us to understand the mechanism by which blood vessel growth is turned on and off. If we understood this mechanism, we could then manipulate it in a variety of clinical situations. For example, in a cardiac patient with a failing heart, we could turn the signal on so that new blood vessels grow within the heart muscle and allow it to recover. Another example would be in cancer patients, in whom we could turn the signal off, which would decrease the blood flow to a tumor, causing it to die. Numerous other possibilities would be plausible. We plan to focus specifically on identifying global protein contents and discovering distinct protein profiles and potential biomarkers to lead us to the identification of the “hepatic factor.” This will allow us to exploit its existence as a tool to understand the mechanism of neovascularization.

Recovering of Damaged Myocardium Using Mechanical Cardiac Assist in Animal Models

In collaboration with Dr. Michael Portman, Director of Pediatric Cardiovascular Research at Seattle Children’s Research Institute, we are evaluating the ability of mechanical cardiac assist in an animal model (pigs) to help recover damaged myocardium. The aim of the study is to determine if metabolic abnormalities, which lead to cardiac dysfunction, atrophy, and ultimately heart failure, can be treated by supplementing the citric acid cycle with pyruvate. Our goal is to determine if pyruvate combined with thyroid hormone supplementation (T3) improves cardiac function and protein synthesis after a prolonged period of ventricular unloading. We are also interested in investigating if supplementation of medium fatty acids with and/or without thyroid hormone promotes protein synthesis during unloading by mechanical circulatory support. We

also want to determine if this targeted strategy improves cardiac function during ventricular reloading or weaning from ECMO. ECMO is used as the model of ventricular assist because it is also used clinically for acute heart failure. The hypothesis is that this study will scientifically

demonstrate that mechanical cardiac assist has a number of beneficial effects that not only support the circulation of a patient, but also aid in the recovery of the heart itself.

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DEPARTMENT CO-INVESTIGATORS

D. Michael McMullan, M.D. / Lester C. Permut, M.D.

OTHER CO-INVESTIGATORS

Harris P. Baden, M.D., UW Department of Pediatrics / **Howard E. Jeffries, M.D.**, UW Department of Pediatrics / **Robert L. Mazor, M.D.**, UW Department of Pediatrics / **James G. Stevenson, M.D.**, UW Department of Pediatrics / **Eugene Kolker, Ph.D.**, Bioinformatics and High-Throughput Data Analysis Laboratory, Seattle Children's Research Institute / **Michael A. Portman, M.D.**, Seattle Children's Research Institute

D. Michael McMullan, M.D.

- Extracorporeal Membrane Oxygenation (ECMO)
- Extracorporeal Cardiopulmonary Resuscitation (ECPR)



Assistant Professor of Surgery
Clinical Director, ECMO Services,
Seattle Children's Hospital

Children and adults with severe, life-threatening cardiopulmonary failure represent some of the most challenging patients in modern medicine. When standard therapeutic interventions are inadequate to support these patients, extracorporeal membrane oxygenation (ECMO) may be used to provide temporary hemodynamic and respiratory stabilization. ECMO involves the use of a specialized external blood pump and oxygenator to either replace or partially support the function of a patient's heart and lungs. Since its introduction three decades ago, over 27,000 patients have been supported by ECMO, with over 1,700 supported in 2009 alone. Although initially developed to treat patients with refractory respiratory failure, ECMO has been increasingly used to provide mechanical support for patients with predominately myocardial failure. Advances in ECMO technology have enabled physicians to expand the role of ECMO in caring for critically ill patients. The ECMO program at Seattle Children's Hospital is one of the busiest in the country.

Integration of Technology

Recent advances in the development of blood pump circuits and improved circuit-blood interface biocompatibility have provided clinicians who treat ECMO patients with the opportunity to offer cardiopulmonary support to a wider range of patients and for longer periods of time. A major focus of my clinical research is examining the interaction of advanced ECMO circuit components in specific clinical settings. We have recently shown that postoperative bleeding can be dramatically reduced by using a specially designed ECMO circuit that requires less systemic anticoagulation (Figure 1). Using this type of ECMO circuit enables pediatric cardiac surgeons to more safely transition to ECMO support in the operating room.

Our team is also examining patients who develop renal failure while supported by ECMO. By incorporating newer chronic renal replacement therapy (CRT) equipment into an ECMO circuit, we hope to achieve safer and more efficient hemodialysis and fluid management.

Patients who require only respiratory assistance may be supported by venovenous ECMO, which involves extracorporeal central venous gas exchange. A significant limitation of this strategy is deformation of the single-lumen venovenous ECMO cannula over time. Seattle Children's Hospital is one of the first American centers to incorporate the new Avalon wire-reinforced venovenous ECMO cannula into our circuit. We are currently examining the safety and efficacy of the new Avalon cannula in neonates with severe respiratory failure.

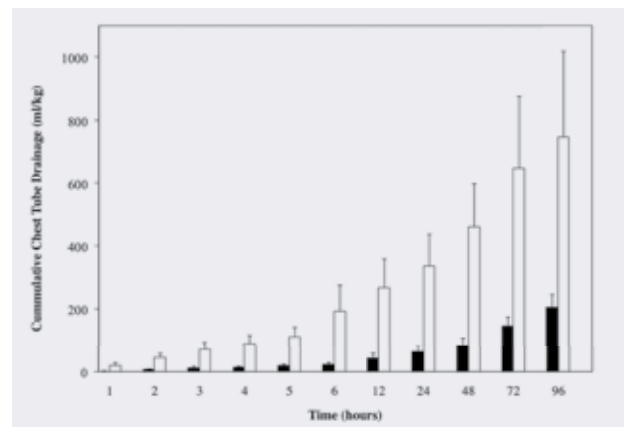


FIGURE 1: Bleeding is reduced when a specialized ECMO circuit is used during the early postoperative period. (Black Bars represent a specialized ECMO circuit; white bars represent a standard ECMO circuit.)

The ECMO program at Seattle Children's Hospital is one of the busiest in the country.

Clinical Outcomes

Using data from several international clinical databases, we are examining differences in clinical outcomes related to ECMO in a variety of patient populations. Specifically, we are evaluating the safety of ECMO when used to support patients at extremely low birth weight and gestational age. We are also evaluating the international trend of using centrifugal ECMO pumps to support neonatal patients. Working with our pediatric cardiology colleagues, we are defining predictors of clinical outcome in patients with life-threatening dysrhythmia who may need ECMO support and patients who require percutaneous cardiac catheterization while receiving ECMO support.

ECPR

Extracorporeal Cardiopulmonary Resuscitation (ECPR) is a novel therapeutic strategy that involves the rapid initiation of ECMO support in children who have experienced cardiac arrest requiring cardiopulmonary resuscitation (CPR). Although ECPR is currently only offered at a limited number of medical centers in the United States, it is emerging as an important method of rescuing critically ill children who would otherwise die. Studies have shown that ECPR improves survival from approximately 1% to 38% in appropriately selected patients. However, neurological injury is a well-recognized complication of ECMO, affecting up to 10% of certain patient populations. Furthermore, long-term neurodevelopmental complications are observed in up to 50% of ECMO survivors. The percentage of ECPR survivors who suffer significant neurologic injury is unknown. We are enrolling patients in a prospective trial designed to determine whether neurodevelopmental outcomes are worse in ECPR patients than in ECMO patients in general.

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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 Transplantation, 2007
Samson Award, Western Thoracic Surgical Association, 2007
Schilling Lecture, University of Washington
Seattle Surgical Society, Best Presentation
Resident Teaching Award 2000

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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 survival rates 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. TNF α , IL-1 β) 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 (NF κ B) 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 multiple stimuli, including inflammatory cytokines (TNF α), 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 ± 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 ± 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

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

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 in 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, implying that SAPK activation in lung ischemia reperfusion injury occurs via a TLR-4 dependent mechanism.

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 phosphate-buffered saline (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 analyses 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 anastomosis using 8-0 suture. The hearts are explanted at various time points. The laboratory is currently gathering data on the role of chemokine blockade on cytokine expression and abrogation of rejection.

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LAB MEMBERS

Junyan Gu M.D. / Mihaela Lie M.D. / Patrick Phelan M.D.

DEPARTMENT CO-INVESTIGATORS

Ronald V. Maier, M.D. / Edward D. Verrier, M.D.

OTHER CO-INVESTIGATORS

Thomas Martin, M.D.; UW Department of Medicine / Peter A. Ward M.D.; University of Michigan

HMC/TRAUMA SURGERY

SAMAN ARBABI, M.D., M.P.H.

EILEEN M. BULGER, M.D.

JOSEPH CUSCHIERI, M.D.

HEATHER L. EVANS, M.D., M.S.

NICOLE S. GIBRAN, M.D.

ANNE M. HOCKING, PH.D.

GREGORY J. JURKOVICH, M.D.

RONALD V. MAIER, M.D.

GRANT E. O'KEEFE, M.D., M.P.H.

TAM N. PHAM, M.D.

Saman Arbabi, M.D., M.P.H.

- Inflammatory Signaling Response to Thermal Injury
- Head Injury in Anticoagulated Patients



Associate Professor of Surgery

AWARDS

Peter C. Canizaro Award
UW Medicine Service Excellence Award
The American Association for the Surgery of Trauma
Faculty Research Award
Surgical Infection Society Faculty Research Award

FUNDING

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 immunomodulators 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.

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.

While chronic anticoagulation may be beneficial in management of some chronic medical conditions, it is a significant liability in trauma.

Head Injury in Anticoagulated Patients

As the U.S. population ages, chronic oral anticoagulation with warfarin is employed with increasing frequency. The number of people using warfarin has progressively increased, with over one million Americans being prescribed warfarin, most of whom are older than 65 years of age. Recent studies have demonstrated that 11-20% of trauma patients 65 or older were being treated with warfarin at the time of injury. While chronic anticoagulation may be beneficial in management of some chronic medical conditions, it is a significant liability in trauma. Due to increased bleeding propensity, intracranial hemorrhage (ICH) such as subdural and subarachnoid hematomas may rapidly increase in size and are associated with a several-fold increase in disability and mortality. However, studies have demonstrated that because of diagnostic and therapeutic difficulties, anticoagulated patients with traumatic ICH are not initiated on reversal agents in a timely fashion. A previous study of a hospital-based protocol demonstrated that rapid confirmation of ICH by expedited head CT scan combined with prompt reversal of warfarin anticoagulation with plasma decreases ICH progression and reduces mortality in patients with traumatic brain injury (TBI). However, no one has implemented and evaluated such protocols in a large regional health care system. In cooperation with the Washington

State Department of Health, the Central Region Trauma Council, which governs the development and operational activities of trauma hospitals in King County (the most populated county in Washington State), will implement a regional protocol, "Head Injury in Anticoagulated Patients" (HIAP). The goal of the HIAP protocol is to reduce the time from presentation to reversal of anticoagulation in patients with ICH who are on chronic warfarin therapy, and thereby reduce morbidity and mortality due to trauma. Since one of the major obstacles in treatment has been rapid availability of plasma, in part due to the time consuming process of thawing fresh frozen plasma (FFP), and a large transfusion volume, this protocol will also use prothrombin complex concentrate (PCC) for the initial reversal of anticoagulation.

In the next few years, we will investigate the population-based prevalence of pre-injury use of warfarin in Washington State trauma patients, time from presentation to diagnosis, and the initial treatment of patients with TBI who are on chronic warfarin therapy. Recently, the Washington State Department of Health has modified the state trauma registry to collect state-wide data on trauma patients on chronic anticoagulation. These additional data will include a requirement to document warfarin use; moreover, in patients who are on warfarin, time to head CT scan and administration of therapy to

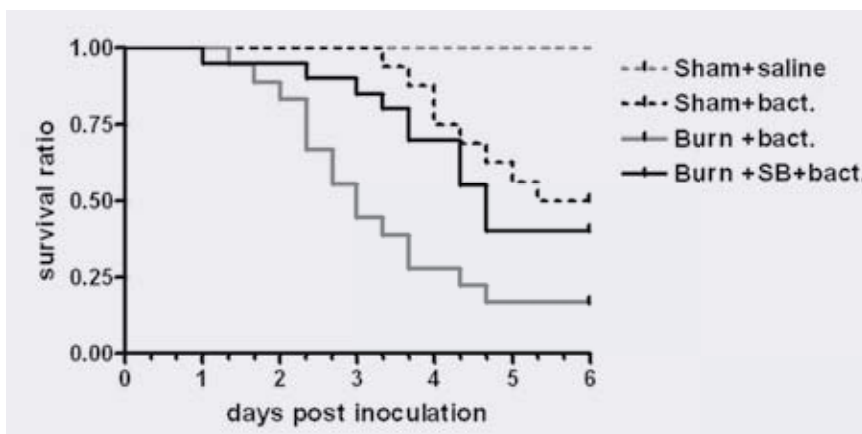


FIGURE 1: Dermal inflammatory source control improves survival in a burn-pneumonia two hit model.

reverse anticoagulation will be documented. Analyses of the Washington State trauma registry over the course of this project will provide state-wide data on pre-injury warfarin use and treatment of trauma patients for the first time.

In the second phase of the study, we will investigate the effectiveness of a regional protocol to rapidly identify ICH in anticoagulated patients and to reverse anticoagulation. We hypothesize that patients in areas where the protocol is in effect will have a shorter time from presentation to both diagnosis of ICH and reversal of anticoagulation.

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DEPARTMENT CO-INVESTIGATORS

Eileen Bulger, M.D. / Joseph Cuschieri, M.D. / Iris Garcia / Gregory J. Jurkovich, M.D. / Ronald V. Maier, M.D. / Grant O'Keefe, M.D., M.P.H.

Eileen M. 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 Infection
- Rib Fracture Management
- Crash Injury Research and Engineering Network (CIREN)
- Massive Transfusion after Severe Injury



Professor of Surgery
Director of Emergency Services
at Harborview Medical Center

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
Department of Defense

Based on a strong interest in trauma and critical care, my research has focused on injury prevention, important clinical questions regarding patient management, and elucidation of 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 Maier and 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 Principal Investigator (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. I am also working with the Department of Defense to define the optimal management strategies for injured patients requiring massive transfusion.

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. In 2005 we 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 included mortality, infectious complications, multiple organ dysfunction, and long-term neurological function for patients with traumatic brain injury. We 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 U.S. and Canada and a data coordinating center based at the University of Washington (PI: Gerald Van Belle; Co-PIs: Graham Nichol, Eileen Bulger). The Seattle and King County Medic One programs, along with Airlift Northwest, represent one 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. With this group, we recently completed two Phase III trials of hypertonic resuscitation in the prehospital environment. One trial focused on patients with hypovolemic shock, and the other on those with severe traumatic brain injury without shock. Both of these trials have closed and do not show any improvement in outcome with this therapeutic strategy. In collaboration with investigators from the University of Toronto and Harvard University, we are continuing to explore the immunologic response in patients from these trials to better understand the influence of hypertonicity on the innate and cellular immune response (PI: Eileen Bulger).

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

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

Prehospital Airway Management and 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,

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 pCO₂ between 30-35mmHg demonstrated improved outcomes, 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 (ED); our current studies are examining the utility of end tidal CO₂ 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 United States 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 emergency medical services (EMS) system design is critical to interpreting the various studies in the literature and to designing future multicenter 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 in a recently completed prospective, randomized trial of high dose enteral vitamins E and C vs. placebo in the surgical intensive care unit (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 developed an animal model of ARDS and 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 (HMC) serves as a regional referral center for patients with severe necrotizing soft tissue infection, and as a result has seen a 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 (Anaya et al., *Surg Infections*, 2009). We have also worked 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.

Massive Transfusion after Severe Injury

With support from the Department of Defense, we are currently participating in a multicenter prospective observational trial of massive transfusion in trauma patients. This dataset will then be used to design an interventional, randomized controlled trial to determine the optimal ratio of packed red blood cells to fresh frozen plasma and platelets for resuscitation of these patients.

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DEPARTMENT CO-INVESTIGATORS

Joseph Cuschieri, M.D. / Hugh Foy, M.D. / Iris Garcia / Gregory J. Jurkovich, M.D. / Robert Kaufman / Ronald V. Maier, M.D. / Sam Mandell, M.D. / Charlie Mock, M.D., Ph.D. / Kier Warner

OTHER CO-INVESTIGATORS

Michael K. Copass, M.D.; HMC Emergency Services / Thomas Edwards, M.D.; UW Department of Anesthesiology / Mickey Eisenberg, M.D.; UW Department of Emergency Medicine / Scott Emerson, Ph.D.; UW Department of Biostatistics / Peter Kudenchuck, M.D.; UW Department of Cardiology / Susanne May, PhD; UW Department of Biostatistics / Graham Nichol, M.D.; UW Department of Medicine / Thomas Rea, M.D.; UW Department of Medicine / Frederick T. Rivara, M.D., M.P.H.; UW Department of Pediatrics

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



Associate Professor
Director, Surgical Critical Care

AWARDS

American College of Surgeons

- Committee on Trauma Region X Competition, Best Basic Science Paper
- Seattle Surgical Society Award

Shock Society Travel Award

- Surgical Infection Society Joseph Susan Memorial Award

FUNDING

National Institutes of Health

- National Institute of General Medical Sciences

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 development of MODS (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.

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-associated 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



FIGURE 1. The development of multiple organ dysfunction syndrome (MODS) after severe injury

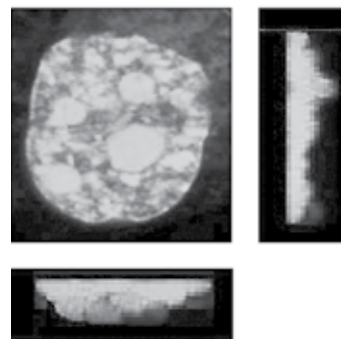


FIGURE 2. Distinct microdomains in the cell membrane that appear to serve as signaling platforms

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

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. Thus, appropriate receptor complex forms to the presenting inciting stimulus resulting in cellular activation.

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, but 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- ζ , is involved. Although the full effects of PKC- ζ 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- ζ 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- κ B and the MAPK. Recently, we have demonstrated that endotoxin tolerance does in fact affect 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

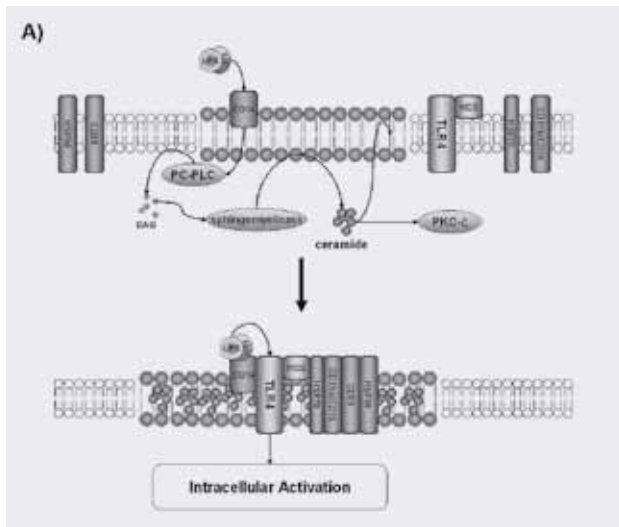


FIGURE 3A. Activation of adapter proteins, leading to cytoskeletal reorganization, protein reorganization, and recruitment of TLR components

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 $\beta 2$ 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 engagement of $\beta 2$ integrin intracytoplasmic tails of paxillin, Pyk2 and other adapter and scaffolding molecules and kinases. As a result,

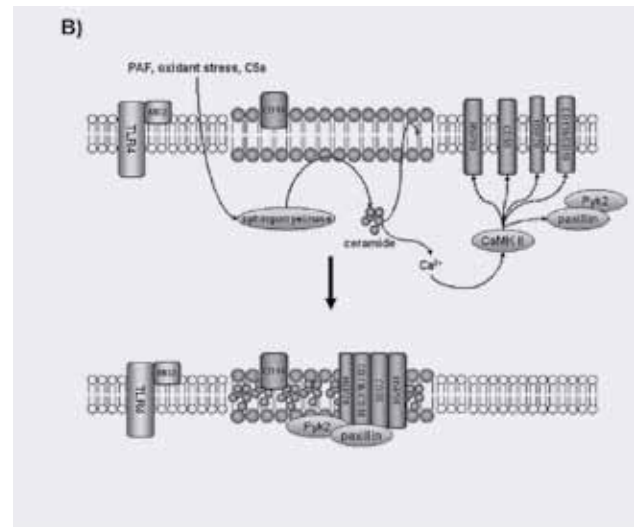


FIGURE 3B. Reprogramming following injury, enabled by clustering and pre-assembly of kinases and scaffolding proteins

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 multicenter 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, intensive care unit length of stay (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 to non-specific disease-based therapy.

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 critically 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's nutritional status. Although the severity of chest injury cannot 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.

	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. Relationship between cytokine (IL-6) expression levels after injury and characteristics of patients' hospital stays

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 methicillin-resistant *Staphylococcus aureus* (MRSA) or *Acinetobacter baumannii* are common and associated with very poor outcomes compared with other infectious organisms. Thus, an attempt to minimize this risk is essential. Recently, we

have begun to use 2% chlorhexidine washes in the ICU. This strategy has led to a significant reduction in the colonization of patients with both MRSA and *Acinetobacter baumannii*. Additionally, initiation of this daily wash has been associated with a reduction in nosocomial infections caused by these organisms.

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DEPARTMENT CO-INVESTIGATORS

Saman Arbabi, M.D. M.P.H. / Eileen Bulger, M.D. / Heather Evans, M.D. / Iris Garcia, B.S. / Megan Knowell, B.S. / Ronald V. Maier, M.D. / Grant O'Keefe, M.D., M.P.H. / Tam Pham, M.D. / Sana Sakr, Ph.D. / Keir Warner, B.S.

Heather Leigh Evans, M.D., M.S.

· Infection Control Measures in the Trauma Setting



Assistant Professor of Surgery

AWARDS / FUNDING

RAS-ACS Leadership Scholarship, American College of Surgeons
Outcomes Research Course, November 2010

UW K12 Comparative Effectiveness Research Training Program,
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Infections that develop in the course of supportive care, such as mechanical ventilation and vascular or urinary catheterization, are a resource-intensive problem associated with considerable morbidity and increased risk of mortality. It has been demonstrated that these health care-associated infections (HAIs) are more often associated with resistant organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA), that are more costly to treat and difficult to eradicate. Accordingly, resistant organism infection is linked to increased use of antibiotic therapy, longer intensive care unit (ICU) stays and longer hospitalizations, which increase the possibility of exposure and risk of transmission to other hospitalized patients. Prevention through multimodality infection control efforts has become the focus of our efforts to decrease the threat of HAIs.

Based on a strong interest in surgical infectious disease fostered during my surgical training at the University of Virginia under the mentorship of Dr. Robert Sawyer, I have focused my research primarily on the evaluation of the effectiveness of a variety of infection control measures. Funded by an individual National Research Service Award as a surgical resident, I earned a Master's of Health Evaluation Science degree, gaining essential methodological training in health services research and applied biostatistical modeling with the guidance of Dr. William Knaus and Frank Harrell. In 2007, I came to the University of Washington to complete my clinical training as a surgical critical care fellow, and I was able to continue to examine the impact of systematic infection control measures with Dr. Joseph Cuschieri in the trauma ICU at Harborview Medical Center. Additionally, building upon the past work of Dr. Eileen Bulger in pre-hospital intubation outcomes, we have begun to look at the key factors of field intubation related to the subsequent development of ventilator-associated pneumonia (VAP). This summer, I was awarded one of

the first UW K12 Fellowships in Comparative Effectiveness Research (CER), funded by the Agency for Healthcare Research and Quality (AHRQ) as a part of the new initiative to train investigators with specific tools to conduct high-quality patient-centered research. With a multidisciplinary group of mentors in surgery, pulmonary medicine, healthcare informatics and pharmacy, I intend to build my early projects at HMC into an independent line of investigation examining the use of information technology to improve compliance with existing diagnostic and therapeutic protocols for VAP, and evaluate the effectiveness of this intervention.

Chlorhexidine Gluconate Decontamination

At Harborview Medical Center, we serve a high volume of critically ill, multiply-injured patients, many of whom require more than a week of mechanical ventilation, as well as urinary and central venous catheterization. In 2006, the baseline rate of MRSA acquisition was 69 per 1000 patient days, despite use of standard infection control methods such as contact isolation. In the face of this strong colonization pressure, a novel infection control measure was initiated to attempt to curb the horizontal transmission of bacteria between patients. After the institution of daily bathing with antiseptic cloths impregnated with chlorhexidine gluconate for all trauma patients admitted to the ICU, we observed a 60% reduction in the rate of MRSA acquisition and significantly fewer catheter-related bloodstream infections and MRSA ventilator-associated pneumonias (*Arch Surg* 2010). Our results were commensurate with data from other investigators demonstrating a decrease in bloodstream infections and gram positive isolates in medical ICU patients. Along with UW Medicine-wide efforts to increase compliance with handwashing, chlorhexidine bathing has since become the standard of care throughout the ICUs at HMC, and we have seen a further decline in the rate of new MRSA acquisition in the past year.

Pre-Hospital Intubation, Aspiration and Ventilator-Associated Pneumonia

Published data from other institutions has suggested that pre-hospital intubation is associated with higher rates of aspiration, pneumonia, and mortality, particularly in head-injured patients. But in a system with an extremely high rate of successful intubation, owing to the rigorous paramedic training program of Seattle Medic One and continuous quality improvement measures, Dr. Bulger had previously observed better outcomes in traumatic brain-injured patients intubated with rapid sequence induction

Comparing the Effectiveness of Automated VAP Screening to Usual Clinical Care

The Institute of Medicine has identified reduction of health care-associated infections (HAIs) as one of the top quartile initial comparative effectiveness research priorities. Ventilator-associated pneumonia (VAP) is the most commonly diagnosed infection in critically ill patients; between 10 percent and 20 percent of patients receiving more than 48 hours of mechanical ventilation develop VAP. Early detection and treatment of this infection is important, as even short-term delays in appropriate antibiotic therapy

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prior to arrival at Harborview. In an effort to define the rates of VAP and associated morbidity and mortality in our emergently intubated trauma population, we conducted a retrospective review of all pre-hospital and emergency department intubations in trauma patients admitted for more than 24 hours over the course of a year. We discovered that there was no significant difference in the rates of VAP, nor the mortality rate, based on the location of intubation (*Arch Surg*, in press).

A subsequent subgroup analysis focusing on specific clinical signs and events immediately before and during intubation suggests that emesis found in the airway is associated with a significantly higher incidence of VAP than any other presentation, including blood present in the oropharynx. This preliminary work to define risk of pre-hospital intubation has laid the foundation to improve and standardize our data collection across pre-hospital provider groups and for the emergent in-hospital intubations throughout the UW Medicine system. In collaboration with Drs. Thomas Rea, Andreas Grabinsky and Sam Sharar, we seek to determine particular factors that may be modified to reduce risk of intubation under challenging conditions. Ultimately, we envision adapting the well-established ICU infection control methods to the pre-hospital setting to prevent VAP from the moment that care of the patient begins.

are associated with higher mortality rates, longer-term mechanical ventilation, and excessive hospital costs.

Early recognition of VAP requires frequent systematic examination of clinical data and an ongoing suspicion about the possibility of respiratory infection. The volume of patient data presented to the clinician in the ICU mandates time consuming, detailed and repetitive screening to identify key clinical events. Extraction and interpretation of meaningful information from the electronic medical record (EMR) at the point of care is complicated by lack of integrated data displays, which may obscure the subtle signs of early infection. One solution has been the implementation of automated screening for syndromes such as acute lung injury and sepsis. Clinicians are already making use of this approach to identify patients at risk for sudden decompensation, and checklists have been employed to minimize the number of days patients are exposed to devices such as central lines, urinary catheters and ventilators. But to date there is no systematic automated support for the diagnostic evaluation of the most common HAIs that arise in critically ill patients.

The next three years will afford me the chance to consider the integration of information technology in medical decision-making and its relation to effective protocol implementation. Recognizing the limitations of observational

studies, my ultimate aim is to acquire the expertise to design and conduct a pragmatic clinical trial to study two strategies of VAP diagnosis: 1) the standard approach in which clinicians use the EMR to look for clinical evidence of early VAP signs, and 2) a novel approach facilitated by an EMR decision aid triggered by automated screening prompts that warn clinicians of possible VAP. We hypothesize that compared to usual clinician-directed

diagnosis, automated screening for clinical features of VAP will reliably identify patients who qualify for bronchoalveolar lavage (BAL) at an earlier stage in their infection. Decision support reminders may facilitate earlier treatment and decrease overall antibiotic usage, length of hospital and ICU stay and costs through better protocol compliance compared to usual care.

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DEPARTMENT MENTORS

Ron Maier, M.D. / David Flum, M.D.

DEPARTMENT CO-INVESTIGATORS

Joe Cuschieri, M.D. / Eileen Bulger, M.D. / Keir Warner

OTHER CO-INVESTIGATORS

Sam Sharar, M.D., UW Department of Anesthesiology / Andreas Grabinsky, M.D., UW Department of Anesthesiology / David Veenstra, Ph.D., UW Department of Pharmacy / Timothy Dellit, M.D., UW Department of Medicine (Infectious Disease) / Catherine Hough, M.D., UW Department of Medicine (Pulmonary) / Thomas Payne, M.D., UW Department of Medicine / Thomas Rea, MD, UW Department of Medicine

Nicole S. 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
- Surgical Wound Repair: Effect of Metabolic Memory on Neuro-Endothelial Responses



Professor of Surgery
Director, Burn Center at Harborview

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Wound repair constitutes an essential component of every surgical subspecialty. The health care system spends millions of dollars annually to apply the latest “*go 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.

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 accentuated 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.

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 determined that activity

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.

levels of neutral endopeptidase, a membrane bound enzyme that degrades substance P, are 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 increase 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.

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

One novel direction in 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 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 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.

Surgical Wound Repair: Effect of Metabolic Memory on Neuro-Endothelial Responses

Type 2 diabetes mellitus, with characteristic hyperlipidemia and hyperglycemia, is common in overweight Americans and is a major risk factor for neurovascular complications. Multiple analyses from the Diabetes Control and Complications Trial demonstrate that patients treated with standard insulin therapy exhibit sustained inflammatory responses and develop vascular complications in spite of subsequent intensive insulin intervention. These findings have generated the widely accepted 'metabolic memory' theory that transient hyperglycemia involves epigenetic modifications that alter gene expression. Epigenetic modifications have been increasingly correlated with human diseases, and such associations are essential for understanding the pathophysiology of chronic diabetic wounds. Our hypothesis for this new project is that: 1) elevated levels of glucose and fatty acids, even transiently, alter paracrine interactions between dermal microvascular endothelial cells and neural progenitor cells in a sustained manner; and 2) antioxidants abrogate the dysfunctional responses to glucose and fatty acids. We have collaborated with Dr. Oleg Denisenko in the Department of Medicine to utilize chromatin immunoprecipitation to identify evidence of DNA or chromatin modulation – evidence that short term hyperglycemia and hyperlipidemia cause epigenetic changes in endothelial cell responses to injury.

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DEPARTMENT CO-INVESTIGATORS

Loren H. Engrav, M.D. / David M. Heimbach, M.D. / Anne M. Hocking, Ph.D. / Matthew B. Klein, M.D. / Ronald V. Maier, M.D. / Grant E. O'Keefe, M.D. / Tam N. Pham, M.D.

OTHER CO-INVESTIGATORS

John E. Olerud, M.D.; UW Department of Medicine / **Oleg Denisenko, M.D.;** UW Department of Medicine

Anne M. Hocking, Ph.D.

• Mesenchymal Stem Cells and Responses to Cutaneous Injury



Research Assistant
Professor of Surgery

FUNDING

National Institutes of Health
National Institute of Diabetes, Digestive and Kidney Disease

Despite significant advances in medical and surgical wound care, treatment of wounds that are slow to heal due to either diabetes mellitus or burn injury remains challenging. Conventional treatments of chronic wounds include topical antibiotics, compression bandages and debridement with or without grafting; advanced therapies include application of bioengineered skin substitutes and growth factors. However, resistance of chronic wounds to these therapies is not uncommon. In the event of burn injury, pressure garments, silicone sheeting and steroid injections have shown only limited success in the reduction of hypertrophic scarring. Clearly, new therapies are urgently needed. The long-term goal of our research is to develop stem cell-based therapies that enhance cutaneous responses to injury while promoting regeneration rather than scar formation. Our current objectives are: 1) to determine the impact of the diabetic metabolic environment of high glucose and fatty acids on adult stem cell regulation of the local cellular responses to injury; and 2) to determine whether therapeutically administered adult stem cells reduce hypertrophic scarring by releasing soluble factors that regulate fibroproliferative responses to cutaneous injury.

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) represent emerging cell-based therapies to ameliorate tissue damage due to injury and disease. These adult stem cells are commonly referred to as mesenchymal stem cells, multipotent mesenchymal stromal cells or stromal progenitor cells. They were first isolated from the bone marrow but have now been found in almost all adult tissues. There is now abundant

evidence demonstrating the therapeutic potential of MSCs for repair and regeneration of damaged tissue in almost all of the major organs of the body including heart, brain, lung, liver, kidney, eye and skin. These studies reporting efficacy and broad applicability have motivated the rapid development of MSC-based therapies as indicated by the ninety clinical trials currently listed in the U.S. National Institutes of Health registry of clinical trials. Differentiation and paracrine signaling have both been implicated as mechanisms by which MSCs improve tissue repair. MSC differentiation contributes by regenerating damaged tissue, whereas MSC paracrine signaling regulates the local cellular responses to injury. Current data suggest that the contribution of MSC differentiation is limited due to poor engraftment and survival of MSCs at the site of injury. Given these limitations, it has been proposed that MSC paracrine signaling is the primary mechanism accounting for the beneficial effects of MSCs on responses to injury such as inflammation, angiogenesis, and fibroproliferation. This hypothesis is further supported by the observation that MSC-conditioned medium also enhances tissue repair.

MSC Paracrine Signaling Regulates Local Cellular Responses to Cutaneous Injury

Cutaneous wounds treated with bone marrow-derived MSCs exhibit enhanced wound repair. Administration of MSCs to either acute or diabetic wounds in rodents accelerates wound closure. Decreased wound size was also observed when autologous MSCs were applied to human chronic wounds. Subsequent focused analyses of wound histology have indicated that treatment with MSCs results in accelerated epithelialization, increased granulation tissue formation and increased angiogenesis *in vivo*. Growing

evidence indicates that MSC paracrine signaling is the predominant mechanism responsible for this enhanced wound repair. MSC-conditioned medium has an effect similar to that of MSCs on wound repair *in vivo*. In addition, we and others have reported that MSC paracrine signaling regulates the responses to injury by dermal fibroblasts, epidermal keratinocytes and endothelial cells. We have recently determined that MSC paracrine signaling induces dermal fibroblasts to proliferate and migrate in response to injury. MSCs also secrete a chemoattractant for dermal fibroblasts. Further investigation showed that MSCs also regulate dermal fibroblast gene expression. Collectively, the combined *in vivo* and *in vitro* data demonstrate that MSC treatment impacts all phases of wound repair including inflammation, epithelialization, granulation tissue formation and tissue remodeling.

Our central hypothesis for this project is that chronic exposure to elevated levels of fatty acids induces MSCs to alter expression of angiogenic and inflammatory mediators, and that these fatty acid-induced changes affect the ability of MSCs to regulate cellular responses to injury. This hypothesis is supported by our recent work showing that exposure to elevated levels of unsaturated fatty acids inhibits MSC proliferation and increases MSC release of both angiogenic and inflammatory mediators. Current aims for this project are: 1) to determine whether exposure to elevated fatty acid levels induces changes in MSC regulation of cellular responses to injury; 2) to determine whether the Toll-like receptor 4 (TLR4) is required for fatty acid-induced changes in MSC paracrine signaling; 3) to determine whether the peroxisome-proliferator-activated receptor γ (PPAR γ) mediates the effects of omega-3 polyunsaturated

The long-term goal of our research is to develop stem cell-based therapies that enhance cutaneous responses to injury while promoting regeneration rather than scar formation.

Mesenchymal Stem Cells and the Diabetic Metabolic Environment

Type 2 diabetes affects almost 200 million people worldwide and increases the risk of heart disease, stroke, kidney failure, limb amputation due to non-healing foot ulcers, and blindness. Currently there is significant scientific and clinical interest in the promise of MSCs to treat diabetes mellitus and diabetic complications. However, critical to the development of MSC-based therapies for patients with type 2 diabetes is an understanding of how their metabolic environment, which consists of high levels of glucose and fatty acids, impacts MSC biology. To date, most studies have investigated the effect of hyperglycemia on MSCs; in contrast, little is known about the impact of elevated plasma fatty acid levels. It remains to be determined whether chronic exposure to elevated levels of fatty acids affects MSCs' ability to: 1) home to sites of tissue injury; 2) release trophic factors that regulate local cellular responses to injury; and 3) differentiate to replace damaged tissue. Our research objective is to address these gaps in the knowledge in order to optimize MSC-based therapies for patients with type 2 diabetes.

fatty acids on MSC paracrine signaling. These studies will provide insight into the efficacy of autologous MSC-based therapies for patients with type 2 diabetes.

MSC-based Therapies to Prevent or Reduce Hypertrophic Scarring

A new direction in our laboratory is to determine the potential of MSC-based therapies for prevention of hypertrophic scar formation after deep dermal injury. Despite studies in the heart, lung and kidney demonstrating that MSCs reduce fibrotic responses to injury, almost nothing is known about the effect of therapeutically administered MSCs on hypertrophic scar formation. In this project, we will determine whether MSC signaling to dermal fibroblasts inhibits TGF- β 1 mediated fibrotic responses to injury such as proliferation, myofibroblast formation, and extracellular matrix homeostasis. We will also determine whether MSC treatment of deep dermal wounds ameliorates fibroproliferative scar formation in the Duroc pig, a validated animal model of hypertrophic scarring. We will assess the effect of MSCs on scar thickness, collagen fiber organization, and numbers of mast cells and myofibroblasts in the healed wound.

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DEPARTMENT CO-INVESTIGATORS

Loren Engrav, M.D. / Nicole Gibran, M.D.

Gregory J. Jurkovich, M.D.

- National Study on Costs and Effectiveness of Trauma Care
- Washington State Trauma Outcomes Project
- Post-Traumatic Stress Disorder in Trauma Patients
- Triage of Trauma Patients from the Field



Professor of Surgery
Chief, Trauma Service at
Harborview Medical Center

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 principal 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 Principal 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 the 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 variations in trauma care between trauma centers and non-trauma centers;
- Examine the relationship between treatment received and mortality, complications, and 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 products from this study have been 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 and payor mix, and how these vary by type of hospital.
- Relationship between cost of trauma care and outcome.
- Recommendations for 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.

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 are most evident in the younger (age < 55) and more severely injured patients (AIS 4-5)—these patients have nearly a 50% survival advantage if treatment is rendered in a trauma center.

- Determination of how transfer status affects outcome.
- Understanding of how trauma systems interact with trauma center status of hospitals to influence outcomes.

The major publication is in the *New England Journal of Medicine* (MacKenzie et al., 2006), 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 are 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 Outcomes Project

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.

Each of the eight emergency medical services (EMS) regions within the state has a quality assurance program, and the overall direction of quality assurance activities within the state trauma system is coordinated via the Outcomes Technical Advisory Committee (Outcomes TAC) of the Governor-appointed Trauma and EMS Steering Committee. Dr. Jurkovich is Chair of the Outcomes TAC. 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 that 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?"

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 among the Departments of 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 the University of California Sacramento, 58% of 269 randomly selected injury survivors who were screened for PTSD, depressive, and peritraumatic dissociative symptoms demonstrated high levels of immediate post-traumatic 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 post-traumatic 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 post-traumatic 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). 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

Dr. Jurkovich has been the Chair of a task force charged with developing national trauma triage guidelines. With support from the CDC and National Highway Traffic Safety Administration (NHTSA), a diverse working group of health care providers from emergency medicine, pre-hospital care, surgical care, and bioengineering has developed, disseminated and implemented 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 CDC has taken the lead with wide dissemination of these guidelines, and the development of a “tool kit” for implementing these guidelines in trauma system design across the country. A summary article in the *Morbidity and Mortality Weekly Report* explains in detail the rationale behind the guidelines.

This working group has now expanded their scope of activities to include developing the methodology and science behind utilizing crash data from the “black box recorders” on newer model automobiles to predict injury severity and telemedicine transfer of this data directly from the scene to care providers.

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DEPARTMENT CO-INVESTIGATORS

Eileen Bulger, M.D. / Lisa McIntyre, M.D. / Other Co-Investigators / Ellen MacKenzie, Ph.D.; Johns Hopkins University / Avery Nathens, M.D.; University of Toronto / Frederick Rivara, M.D., M.P.H.; UW Department of Pediatrics / Doug Zatzick, M.D.; UW Department of Psychiatry / Richard Hunt, M.D.; CDC-Injury Control Center

Ronald V. Maier, M.D.

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



Professor of Surgery
Vice Chairman, Department of Surgery
Surgeon-in-Chief, Harborview Medical Center
Director, Northwest Regional Trauma Center

AWARDS

- American Trauma Society: William S. Stone Award
- Shock Society: Scientific Achievement Award
- University of Washington, Department of Surgery: Distinguished Alumnus Award
- American Heart Association: Lifetime Achievement Award
- American Surgical Association: Flance-Karl Research Award
- Accreditation Council of Graduate Medical Education: Parker J. Palmer Courage To Teach Award

FUNDING

- National Institutes of Health:
- National Institute for General Medical Sciences
- University of Washington: Jane & Donald D. Trunkey Chair in Trauma Surgery
- National Science Foundation: Engineering Research Center

The Global Burden of Surgical Disease is increasingly recognized as a major impact on the economic health of any country. Within surgical disease, trauma is a major contributor, costing the United States over \$250 billion per year. However, since trauma overall consists primarily of minor injuries, and patients at any given institution mostly do well, a sense of accomplishment and under-recognition of the true impact of major trauma is frequently assumed by physicians, the public and our legislators. This, combined with the great challenges involved in developing a high quality detailed physiologic dataset of the impact of severe trauma, has led the non-combatant into believing the war has been won.

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 and minimize ultimate morbidity. Trauma system developments and improvements in acute care, including optimal resuscitation, will reduce early deaths during the resuscitation phase and minimize subsequent morbidity. Finally, elucidation of the genomic and molecular responses to severe injury will identify treatment targets to prevent the dysregulated autodestructive inflammatory response causing organ dysfunction and death following trauma.

Harborview Injury Prevention and Research Center

I am Senior Advisor to the Harborview Injury Prevention and Research Center (HIPRC). The HIPRC is linked closely with the Northwest Regional Trauma Center at Harborview Medical Center. The goal of the HIPRC is to diminish the impact of trauma on people's lives and to draw analyses based on the effectiveness of the Northwest Regional Trauma Center's injury prevention and trauma treatment programs. Established at HMC in 1985, the 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 pre-hospital to rehabilitation, is ongoing.

The goal of the Harborview Injury Prevention and Research Center is to diminish the impact of trauma on people's lives and to draw analyses based on the effectiveness of the Northwest Regional Trauma Center's injury prevention and trauma treatment programs.

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 were evaluated, including penetrating abdominal injury and multisystem blunt trauma with, at 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 that participate in the University HealthSystem Consortium. Results indicate that 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. These benefits were only evident in patients at the highest risk for adverse outcomes, and not in the vast majority of lesser-injured patients.

Splenic Injury

An ongoing study at the HIPRC investigates geographic variation in the management, outcome and costs of splenic injury. Reducing the variation that exists in health care practice is necessary for both improving health care quality and reducing unnecessary costs. Splenic injury is

an appropriate model to study variation in trauma care, as it is a common injury that has undergone a recent evolution in management. The Healthcare Cost and Utilization Project (HCUP) State Inpatient Database (SID) for 2001, 2004, and 2007 was used to detect significant variation in a state-to-state comparison of management and outcomes for 33,131 hospitalized patients with splenic injury. After adjusting for patient, injury, and hospital factors, multivariate regression demonstrated up to a 1.7-fold difference in the use of splenectomy between states, a 2.4-fold variation in mortality, and a 1.7-fold variation in costs. Reducing this variation through the development of and adherence to management guidelines should be a priority for the delivery of cost effective care.

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:

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 multiorgan failure (MOF) and to ultimately affect patient outcome. Work at Harborview Medical Center (HMC) has shown a high incidence of vitamin C and vitamin E deficiency in trauma patients admitted to the HMC intensive care unit (ICU). Reports from other institutions document a low plasma vitamin C concentration in 28–83% of hospitalized ICU patient populations and 12–21% 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. 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 hypothesized that routine supplementation of vitamins C and E would protect against oxidant-induced organ injury in severely injured and stressed patients, and avoid the diffuse insult predisposing to ARDS and other organ dysfunction, along with 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 the intrapulmonary inflammatory response, there was a decrease by 50% in the incidence of ARDS and a significant decrease in the 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 Trauma-Related Macrophage Inflammatory Response to Prevent ARDS, MOFS and Death

A 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 associated with 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, in part, 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 p38. 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.

A major focus is on the ability of antioxidants, 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 r38. This was correlated with an inhibition of stress fiber formation in the macrophages and appears to link to 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

Lastly, to better understand the pathophysiologic phenotype in the severely injured patient, a collaborative study has been developed with the Stanford Genome Institute and Harvard Bio-Statistics Department, and funded by the National Institutes of Health National Institute of General Medical Sciences (NIH-NIGMS) for a consortium and large-scale project grant or “Glue Grant.” The intent is to study the entire human genomic response serially across time to the stress of severe injury, resuscitation and subsequent MOF or nosocomial infection. To enable this, the technological developments necessary for reproducible, high quality isolation of RNA, including microfluidics, 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 tools of accepted methodologies for the analyses of total RNA expression data.

Whole blood obtained from healthy subjects or trauma patients had total RNA isolated from the circulating leukocyte “Buffy coat.” cRNA was hybridized to Affymetrix GeneChips, and unsupervised analyses, including hierarchical cluster analysis, were used to measure RNA expression. Subjects for severe blunt trauma and hypotension or acidosis, requiring resuscitation with blood products, were studied at 7 participating institutions with blood sampling at the initial 12 hours and on days 1, 4, 7, 14, 21 and 28 days after injury. Whole blood leukocytes were isolated, and genome-wide expression analyses were performed. Severe trauma alters the expression of >75% of the leukocyte genome over 28 days post injury. The response is highly coordinated and reproducible. Variations in the quantity of blood transfused contribute only modestly to the changes in gene expression. Individuals with a complicated clinical recovery in organ failure demonstrate selective prolonged increases and decreases in the expression of genes involved, primarily in the innate and adaptive immune response, respectively. There is no genomic evidence of either multiple inflammatory events consistent with the second hit, or the delayed expression of adaptive immunity genes associated with a compensatory anti-inflammatory response, which has been commonly portrayed as dogma in this field of investigation. The functional genomic units affected primarily by severe injury are now being identified (Figure 1), and can be utilized as a focus for potential therapeutic intervention to confirm both causal relationship of alteration to subsequent phenotype, and also as a much greater opportunity to identify therapeutic interventions that are likely to succeed in modifying patients’ phenotypic response and ultimate clinical outcome.

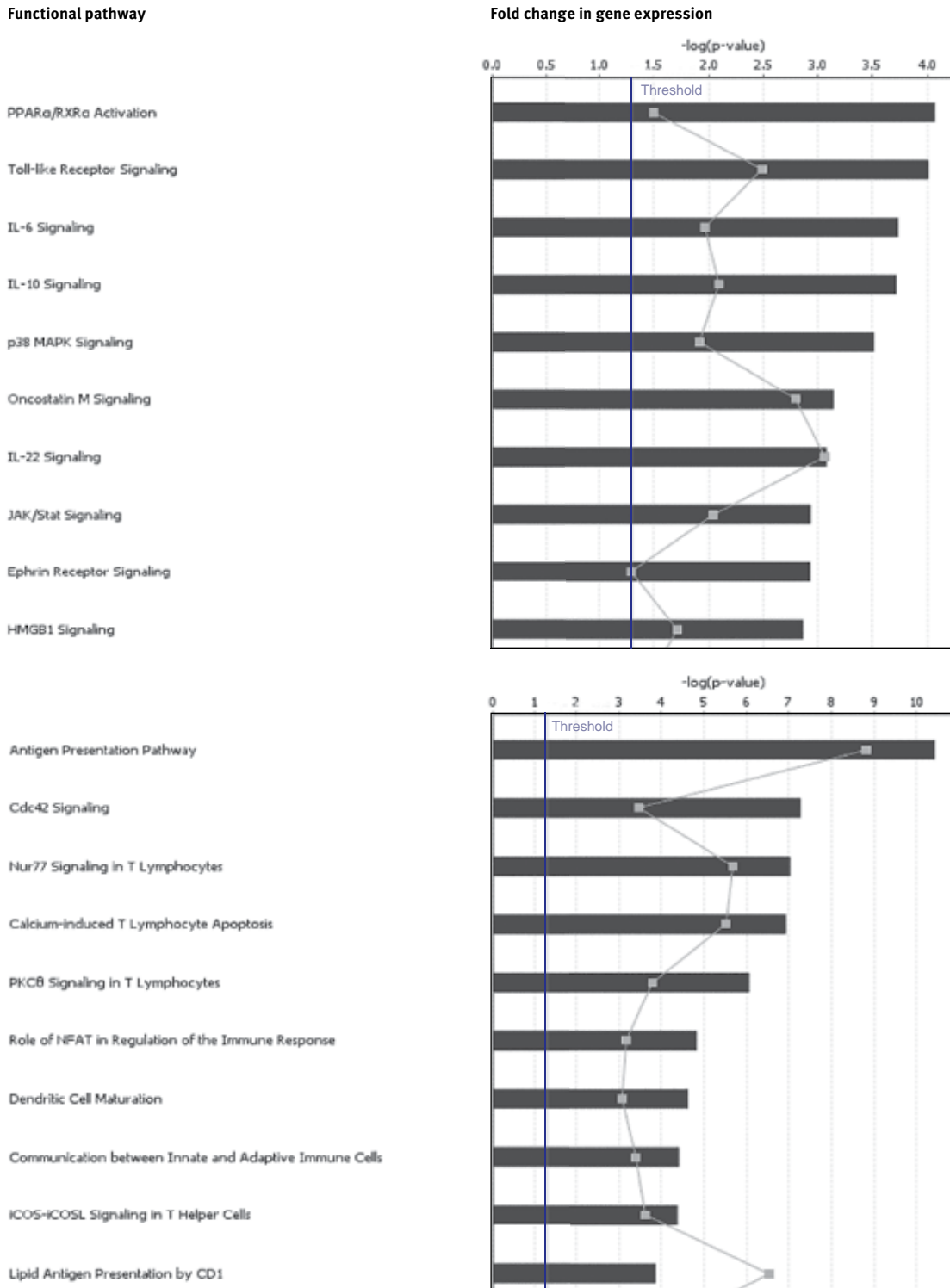


FIGURE 1. The functional genomic pathways that are primarily affected by severe injury

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DEPARTMENT CO-INVESTIGATORS

Eileen M. Bulger, M.D. / Michael K. Copass, M.D. / Joseph Cuschieri, M.D. / Heather L. Evans, M.D. / Iris Garcia / Nicole Gibran, M.D. / Laura V. Hennessy / Gregory Jurkovich, M.D. / Lisa McIntyre, M.D. / Grant E. O'Keefe, M.D., M.P.H. / Tam N. Pham, M.D. / Erik Van Eaton, M.D.

OTHER CO-INVESTIGATORS

J. Perren Cobb, M.D.; Massachusetts General Hospital, Harvard University / Ronald W. Davis, Ph.D.; Stanford University / Tom Koepsell, Ph.D.; UW Department of Epidemiology / Michael Mindrinos, Ph.D.; Stanford University / Joseph Minei, M.D.; University of Texas, Southwestern Medical School / Linc Moldawer, Ph.D.; University of Florida / E. Eugene Moore, Jr., M.D.; Denver Health Medical Center, University of Colorado / Avery Nathens, M.D.; University of Toronto / Margaret Neff, M.D.; UW Department of Pulmonary and Critical Care Medicine / Graham Nichol, M.D.; UW Center for Prehospital Emergency Care / Frederick T. Rivara, M.D., M.P.H.; UW Department of Pediatrics & HIPRC Senior Director / Patrick S. Stayton, Ph.D.; UW Department of Bioengineering / John D. Story, Ph.D.; Princeton University / Ronald G. Tompkins, M.D.; Massachusetts General Hospital, Harvard University / Timothy R. Watkins, M.D.; UW Department of Pulmonary and Critical Care Medicine / Michael West, M.D.; University of California San Francisco / Wenzhong Xiao, Ph.D.; Stanford University

Grant E. O’Keefe, M.D., M.P.H.

- Pathophysiology of Post-Injury Infection and Organ Failure
- Risk for and Consequences of ICU-related Infections



Professor of Surgery

FUNDING

National Institute of General Medical Sciences

Severe traumatic injury results in biochemical and physiological changes that can lead to nosocomial infection (pneumonia, wound infections, etc.) and remote organ (lung, kidney, liver) failure. In patients who survive beyond the initial few hours after injury, infection and organ failure (MODS; multiple organ dysfunction syndrome) are leading causes of death and contribute to prolonged and resource-intensive hospital stays. Our understanding of the biology of these complications is incomplete. We understand many of the clinical factors that predict who will develop post-traumatic infection and organ failure and know that a number of inflammatory markers are increased after injury and that many of these identify patients who progress to organ failure. For example, and as shown in Figure 1, severely injured patients who develop

organ failure (predominantly lung and kidney failure) have greater elevations in circulating interleukin (IL)-6 in the first 24 hours after injury. This is one circulating marker that reflects the systemic physiological response that sets the stage for remote organ failure and infections.

However, there are many gaps in our understanding of how early changes after injury contribute to later complications. Furthermore, other factors, independent of the injuries, doubtless contribute to these complications. Delays and other variation in treatment are likely detrimental, pre-existing health status can increase the risk for infections and organ failure, and individual genetic differences might influence a person’s risk for complications and ability to recover from them once they develop.

Sequencing the human genome has led to a greater understanding of its structure (DNA sequence variation) and function (mRNA expression and protein translation) in new and comprehensive, “genome-wide” ways. Our program incorporates new methods and technology into understanding post-traumatic infection. First, we are studying the genetic basis for variation (single nucleotide polymorphisms; SNPs) in inflammatory responses and how these differences influence the clinical course of sepsis. Second, and based upon our clinical and experimental observations, we are studying pathways that have traditionally been not considered “inflammation-related”, but appear to affect the human inflammatory and innate immune responses. Finally, we are also studying how established treatments might influence outcome in ways that have previously been overlooked. For example, we have examined the role of blood transfusion in the development of post-traumatic infection and are also re-examining infectious complications after injury to try to understand their connection to altered biology.

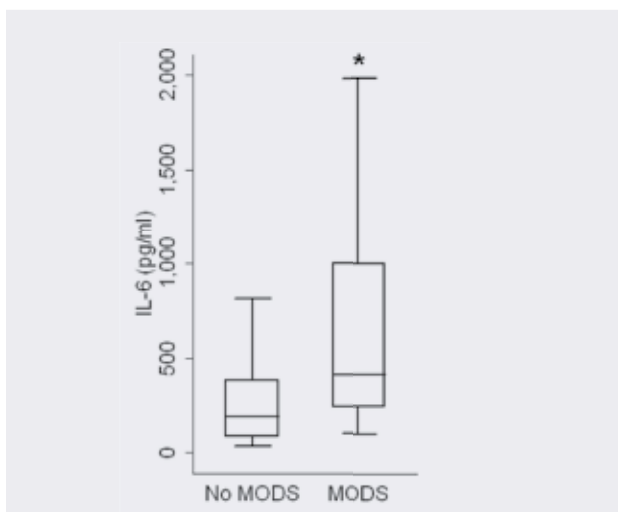


FIGURE 1: Plasma IL-6 and multiple organ dysfunction
Plasma IL-6, measured within 24 hours of injury, is associated with an increased risk for subsequent multiple organ dysfunction syndrome (MODS). This finding was validated in a second cohort of patients. An IL-6 concentration > 350 pg/ml was highly predictive of MODS and prolonged hospitalization. These data are from reference 7.

Identification of genetic variation associated with post-traumatic ventilator-associated pneumonia (VAP)

Genetic variation in the innate immune response contributes to the marked variation seen in the risk of and outcome from a number of infectious diseases. 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- α (TNF- α), toll-like receptor 4 (TLR4) lipopolysaccharide binding protein (LBP), and others.

Genome-wide approaches to identifying associations with common diseases (diabetes, hyperlipidemia, etc) have generally replaced studies focused on small numbers of candidate polymorphisms. We have developed a large DNA bank that is linked to clinical data for over 3500 injured patients and have studied specific SNPs and inflammation-related genes, and have now begun to identify genome-wide associations (a genome-wide association study, GWAS) with infectious outcomes after injury. We have recently completed the first phase of a GWAS aimed to identify genetic variation associated with VAP after injury.

Understanding the role 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 cells expressing the β -adrenergic receptor. Data indicate that stimulation of β_2 adrenergic receptors (β_2 AR) increases intracellular cAMP and decreases production of pro-inflammatory 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

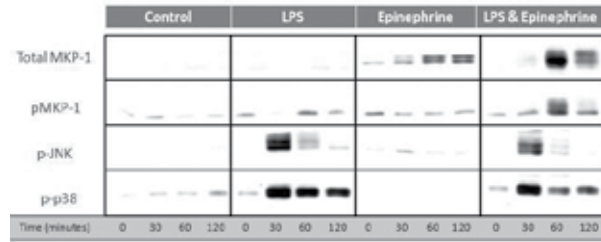


FIGURE 2: Effects of epinephrine on endotoxin-induced kinase activation
LEGEND: This figure is an image of an immunoblot for protein, and demonstrates that monocytes exposed to epinephrine have higher levels of total MKP-1 and phosphorylated MKP-1 beginning at 30 minutes after stimulation compared with endotoxin alone. This elevation in MKP-1 corresponds to a more rapid decrease in phosphorylated p38 and JNK.

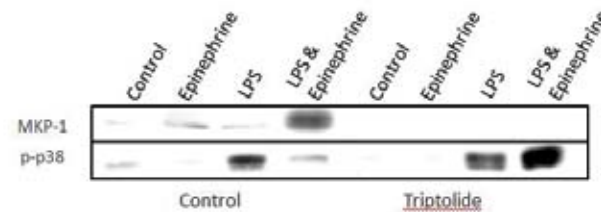


FIGURE 3: Inhibition of MKP-1 restores MAPK phosphorylation
 This figure is an immunoblot for protein after exposure of monocytes to an MKP-1 inhibitor (triptolide). It demonstrates that complete blocking (removal) of MKP-1 protein restores p38 phosphorylation; eliminating the suppression due to epinephrine.

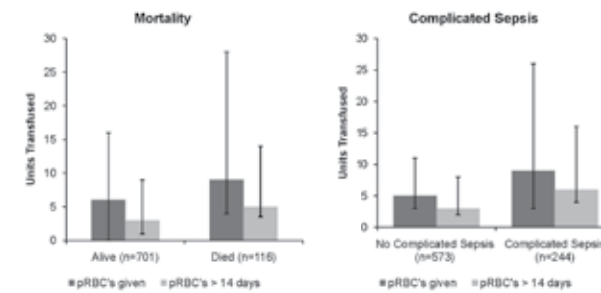


FIGURE 4: Mortality and complicated sepsis after blood transfusion
 This figure illustrates the results of our study examining the relationship between the transfusion of packed red blood cells and outcomes after injury. As shown on the left, the patients who died received more units of blood in total than the patients who lived. The amount of older blood (storage > 14 days) transfused was somewhat higher in the patients who died. The observed association of the amount of older blood transfused and the development of severe infections (complicated sepsis) was strong. Even after adjusting for the total amount of blood transfused, patients who developed complicated sepsis received significantly more units of older blood. These data are from reference 6.

Our program is focused on developing new knowledge in the field of innate immunity and inflammation in the context of severe traumatic injury. We also aim to learn how we can better apply existing therapies to critically ill patients. We use these objectives and research areas as the foundation on which to educate and train future investigators in biological sciences.

for an individual's ability to respond to 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 Network Analysis, we have identified a potential role for the MAP kinase phosphatase MKP-1 (also known as DUSP1). In a human monocyte cell line, we have also observed total MKP-1 protein and phosphorylated MKP-1 to increase rapidly in response to LPS stimulation, epinephrine stimulation and, most strikingly, combined stimulation to both agents. Additional experiments have examined both phosphorylation and subsequent dephosphorylation of p38 and JNK, intracellular signaling molecules important to inflammatory activity. We observed that initial MAPK activation was not influenced by β -adrenergic stimulation but that MAPK **deactivation or dephosphorylation** was. Results of these experiments are shown in Figure 2. Our most recent observations seem to confirm a role for MKP-1 in β -adrenergic mediated immune suppression. By pharmacological inhibition of MKP-1 we have shown a restoration of p38 and JNK phosphorylation (Figure 3).

Critically ill patients have early elevations in circulating epinephrine and are often treated with adrenergic and anti-adrenergic agents. Our data suggest that these agents, whether endogenous or administered, have important effects on innate immune function. This may represent an opportunity to manipulate the inflammatory response and reduce the incidence of post-traumatic infection.

Measuring the influence of duration of blood storage on infectious complications in injured patients receiving transfusions

The transfusion of allogeneic red blood cells (PRBCs) is life-saving but also known to suppress immunity and influence outcomes in critically ill and injured patients. The influence of blood on the risk of infection and death may, in part, be related to the duration of storage. We investigated

whether there was a relationship between the duration of blood storage and outcomes in trauma patients who received transfusions.

We studied 820 patients who were transfused at least 1 unit of blood within 24 hours of injury. Patients who died ($n = 117$) received more units of older blood than those who lived (5 units [interquartile range, IQR 2-9] versus 3 units [IQR 2-6], $p < 0.001$). Patients with complicated sepsis ($n = 244$) received a greater volume of older blood than those without complicated sepsis (6 units [IQR 2-10] versus 3 units [IQR 1-5], $p < 0.001$). These data are shown graphically in the right pane of Figure 4. After adjusting for clinical factors, including the total amount of blood transfused, patients receiving ≥ 7 units of older blood had a higher risk of complicated sepsis than patients receiving 1 or fewer units (odds ratio, OR = 1.9, $p = 0.03$).

Our observations have demonstrated that the risk for complicated sepsis and death in trauma victims who are transfused blood is high and that the amount of older blood transfused is associated with complicated sepsis. In addition to avoiding unnecessary transfusions, our data suggest it is important to avoid transfusing multiple units of older blood.

Understanding the potential manifestations of alterations in innate immunity after traumatic injury

In conjunction with our studies of the influences of genetic differences on innate immune responses and clinical outcomes of critically ill patients, we have examined the responses to and outcomes from severe nosocomial infections. Ventilator-associated pneumonia (VAP) is the most common nosocomial infection in critically ill patients, and is considered an important cause of prolonged hospitalization and mortality. With timely diagnosis and appropriate antimicrobial treatment, patients with VAP typically survive. This is particularly true in trauma victims who are generally young and have few co-morbid conditions. However, bacteremia in conjunction with pneumonia may

reflect a failure of the host's innate immune response, and may be a harbinger for death. To examine this possibility, we have studied the clinical course of 544 trauma patients who developed VAP.

Of 554 patients with VAP, 74 (14%) patients had associated bacteremia, and 480 (86%) had VAP alone. Nineteen of 74 patients (26%) with bacteremia died. Patients with VAP alone had a much lower case-fatality rate of 12% (56/480 patients). The unadjusted relative risk for death associated with bacteremia was 2.2 (95% confidence interval, CI = 1.4 – 3.5).

Patients with bacteremia also spent a longer period of time in the intensive care unit (IUC) than patients with VAP alone. The median ICU length of stay for patients with bacteremia was 25 (16 – 41) days compared to 17 (12 – 25) days for patients with VAP without bacteremia. We also observed that patients with bacteremia who died were more likely to die as a consequence of infection and remote organ failure, and they had a more protracted stay in the ICU before dying (median stay of 26 versus 15 days, $p = 0.005$). As a cause of death, multiple organ failure secondary to infection was more common in patients with bacteremia than in patients without bacteremia (13/18, [72%] versus 15/48 [31%], $p = 0.003$).

Our observations suggest that bacteremia in association with VAP identifies a sub-group of patients with a substantial risk of dying in the ICU. In most cases, death occurs after a lengthy ICU stay that is punctuated by repeated infections and culminates in progressive organ failure. There appears to be a window of time where we may be able to intervene and prevent this progressive deterioration and death.

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. In order to find better ways to treat critically ill patients, we must understand both the clinical risks and the pathophysiological basis of the important complications.

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DEPARTMENT CO-INVESTIGATORS

Ronald V. Maier, M.D. / Joseph Cuschieri, M.D. / Tam N. Pham, M.D.

OTHER CO-INVESTIGATOR

Mark M. Wurfel, M.D., Ph.D.

Tam N. Pham, M.D.

- Older Adults' Responses to Traumatic Injury
- Infections in Critically Ill Burn and Trauma Patients



Assistant Professor of Surgery

AWARDS

American Burn Association

- NBR Best Paper by a Physician, 2008

FUNDING

American Burn Association / Department of Defense

With the aging of the U.S. 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 \geq 55 years did not similarly benefit. Thus, better stratifications of outcomes and improved understanding of aged responses to injury are necessary to develop effective treatment strategies. We have analyzed the National Burn Repository (NBR) to assess risks for complications and excess resource utilization in older adults with burns. Our most recent study highlighted the importance of co-morbidities over chronological age in pneumonia development in older adults with burns. Our ongoing projects focus on practice variations in the care of older patients and 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 Harborview Medical Center (HMC). In this study, peripheral blood mononuclear cells (PBMC) from enrolled subjects are isolated, and activation assays performed with and without exposure to the non-selective beta antagonist propranolol. We are also performing *in vitro* studies to characterize adrenergic effects on monocyte gene expression.

Infections in Critically Ill Burn and Trauma Patients

Lung and bloodstream infections represent common nosocomial infections in mechanically ventilated patients in the Burn and Surgical Intensive Care Unit. Many tools and guidelines developed for the general ICU population remain to be studied and validated in the injured patient population. Our burn center is a participating site in the American Burn Association/ Department of Defense-funded study on early detection of *Staphylococcus* sepsis in burn patients using the polymerase chain-reaction technique (PCR). This study

Our recent study highlighted the importance of comorbidities over chronological age in pneumonia development in older adults with burn injuries.

technique (PCR). This study will enroll subjects over the next three years. Pneumonia is the most common nosocomial infection in mechanically ventilated patients. Prompt diagnosis of ventilator-associated pneumonia (VAP) constitutes a high priority in surgical critical care. We have evaluated the limitations of a standard scoring system (Clinical Pulmonary Infection Score) compared with bronchoscopy for VAP detection in burn

patients. We have authored the most recent American Burn Association guideline on the prevention, diagnosis and treatment of VAP in burn patients. Our recently completed project compared vancomycin vs. linezolid in treating methicillin-resistant *Staphylococcus aureus* (MRSA) VAP in critically ill patients. Our ongoing project aims to correlate bronchoscopic findings of inhalation injury and subsequent risks of lung infection.

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DEPARTMENT CO-INVESTIGATORS

Nicole S. Gibran, M.D. / Matthew B. Klein, M.D. / Grant O'Keefe, M.D.

OTHER CO-INVESTIGATORS

May J. Reed, M.D.; UW Department of Medicine

PEDIATRIC SURGERY

KENNETH W. GOW, M.D.

PATRICK J. JAVID, M.D.

JOHN H. T. WALDHAUSEN, M.D.

Kenneth W. Gow, M.D.

• Pediatric Surgical Oncology



Associate Professor of Surgery

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 complications or recurrence. We are striving to study each of these areas of contribution.

Central Venous Catheters

Insertion of a central venous catheter (CVC) is one of the most commonly performed procedures in children. For a child with cancer, the CVC is key to effective administration of chemotherapy. The CVC was developed at the Fred Hutchinson Cancer Research Center in Seattle. We are currently reviewing the 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 standardizing other aspects of catheter insertion and management to try to reduce the rates of thrombosis and infections. Ultimately, this process will be the basis for further study that should improve patient care and reduce costs.

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 collaborated with our Nuclear Medicine Department to establish 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 [1, 2]. Further, we use 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 that of deep-seated lung lesions, for which we have pioneered the application of minimally invasive thoracoscopic ultrasound (MITUS) [3]. 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 [4]. Our center has been prominent in advocating the role of minimally invasive surgery (MIS) for biopsy of masses in children as well as resection [5].

Resection

As discussed above, the role of MIS in resection of childhood solid tumors is a new area that we are helping to advance [5], 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 positron emission tomography (PET) probe and beta probes that optimize real-time intra-operative localizations. Finally, robotically assisted methods have been used to help surgeons to resect lesions in difficult to reach locations such as the chest and pelvis [6].

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.

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 (ECLS) Registry to review the use of ECLS for children and adults with neoplasms or HSCT [7-9].

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. Seattle Children's Hospital 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.

Education

As an institution, one of our tasks is to disseminate current and new techniques to the families and to the next generation of surgeons. To do this, we have utilized novel methods that leverage the new learners and their unique needs for visual education [10].

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Patrick J. Javid, M.D.

- A Pilot Study of the Safety and Immunogenicity of the Rotavirus Vaccine in Infants with Intestinal Failure
- Assessment of Hepatic Function in Short Bowel Syndrome Using the Novel 13C-Methionine Breath Test
- Neurodevelopmental Outcomes of Children with Intestinal Failure
- Pediatric Intestinal Failure Consortium



Assistant Professor of Surgery

AWARDS

American Academy of Pediatrics Rosenkrantz Resident Research Award, 2003

John A. Mannick Research Award, Department of Surgery, Brigham and Women's Hospital, 2003

Best Clinical Paper, Canadian Association of Pediatric Surgeons Annual Meeting, 2003

FUNDING

Thrasher Research Fund Early Career Award
University of Washington Institute of Translational Health Science (ITHS) Small Pilot Grant

Seattle Children's Hospital Academic Enrichment Fund

Pediatric intestinal failure, of which short bowel syndrome is the most common etiology, has been transformed into a chronic disease. Many infants with intestinal failure are surviving to school age and beyond. The evolution of pediatric intestinal failure is directly attributable to advances in medical, nutritional, and surgical treatments of this disease. As such, there are now multiple opportunities for clinically relevant and effective research into different aspects of pediatric intestinal failure.

My research efforts focus on the clinical and translational study of young children with intestinal failure. As a pediatric surgeon with both clinical and academic interests in pediatric intestinal failure, I work with children with intestinal failure on a daily basis and am positioned to study different ways to optimize short- and long-term outcomes. I am an active member of the Intestinal Failure Program at Seattle Children's Hospital, which has both outpatient and inpatient components. Through this program, the pediatric surgeons at our institution work closely with pediatric gastroenterologists and pediatric transplant specialists to tailor the care of each individual child with intestinal failure. The Intestinal Failure Program emphasizes the multidisciplinary aspect of treatment for the child with intestinal failure, and recent data show a distinct mortality benefit from this type of structured care.

The multidisciplinary nature of our clinical program translates nicely into collaborative partnerships for research. I work closely with members of our Divisions of Gastroenterology, Transplantation, and Infectious Disease on multiple research initiatives. In addition, we have full-time support from dedicated pediatric nutritionists, pharmacists, and social workers. My current laboratory includes a surgical resident research fellow, a medical student, and a part-time undergraduate student.

My research investigates a diverse array of issues that pertain to the child with intestinal failure. My current active projects include the following:

A Pilot Study of the Safety and Immunogenicity of the Rotavirus Vaccine in Infants with Intestinal Failure

Infection with rotavirus is the most common etiology of severe diarrhea in the pediatric population. Despite recent attempts at improving prevention, over 600,000 infants worldwide die each year from rotavirus-related gastroenteritis. In the United States alone, rotavirus results in 27,000 hospitalizations, 360,000 emergency room visits, and 374,000 clinic visits each year. In 2006, an oral rotavirus vaccine was approved for use by the Food and Drug Administration, and routine immunization of infants was recommended by the Centers for Disease Control and Prevention and the American Academy of Pediatrics.

Rotavirus is known to cause severe clinical manifestations in infants with intestinal failure, including electrolyte imbalance, dehydration, and prolonged hospitalization. Such patients appear to be more susceptible than healthy children to the infection. Importantly, rotavirus has been shown to delay intestinal adaptation and tolerance of enteral nutrition in children with intestinal failure.

To date, there are no published data as to the utility of rotavirus vaccination in the pediatric intestinal failure population. Given this group's differences in intestinal motility, bowel surface area, and gut-associated immune system, the effect of the vaccine in this population remains unknown. This active pilot study is measuring the safety and immunogenicity of the rotavirus vaccine in pediatric intestinal failure patients. The hypothesis of the study is that a currently approved rotavirus vaccine will be safe and will induce serum immunogenicity to rotavirus in children

The Intestinal Failure Program emphasizes the multidisciplinary aspect of treatment for the child with intestinal failure, and recent data show a distinct mortality benefit from this type of structured care.

with intestinal failure. The study is designed as a prospective pilot study that will focus on the evaluation of adverse events unique to this population after the administration of the vaccine. In addition, the study will incorporate bench laboratory methodology to measure stool shedding of the virus and systemic absorption of the vaccine, variables which can affect vaccine safety in this cohort. The study will assess seroconversion by measuring immunoglobulin specific to rotavirus.

Ultimately, if the pilot data demonstrate that the rotavirus vaccine is safe in this cohort, the study will serve as the foundation for a multi-center prospective study throughout the Pediatric Intestinal Failure Consortium (PIFCon).

Assessment of Hepatic Function in Short Bowel Syndrome Using the Novel ¹³C-Methionine Breath Test

Infants with short bowel syndrome are at risk for liver disease associated with the use of parenteral nutrition (PN-associated liver disease, or PNALD). This carries a high mortality rate. Indeed, the long-term survival of children with short bowel syndrome is limited primarily by the progression of liver disease. Current tests used to evaluate PNALD, such as hepatic transaminases and bilirubin, may not accurately assess hepatic function, and the prothrombin time is generally not elevated until late in the course of liver injury. Moreover, histopathology evaluation is not routinely available given the morbidity associated with liver biopsy. It has also been demonstrated that prolonged administration of PN can impair the function of hepatic mitochondria, similar to the liver injury induced by hepatic cirrhosis and fatty liver. These data suggest that assessment of hepatic mitochondrial function may be an appropriate marker for liver injury in patients dependent upon PN. This study proposes to validate an innovative measure of mitochondrial liver function in children with short bowel syndrome.

Non-radioactive, stable isotopes have been used safely and effectively for decades to obtain functional assessments of several organ systems. The present study proposes to use a stable (i.e., non-radioactive) isotope breath test to

measure synthetic liver function in conjunction with the liver biochemical profile (bilirubin, transaminases and prothrombin time). The test involves administering a substrate that is metabolized primarily by the liver (the stable isotope L-[1-¹³C]-methionine) and collecting byproducts of liver metabolism in expired breath (¹³CO₂). We hypothesize that the ¹³C-methionine breath test (¹³C-MBT) will provide a quantitative measure of synthetic hepatic function that can be used in conjunction with current biochemical liver tests to better evaluate the degree and progression of PNALD in patients with short bowel syndrome.

Methionine is an essential amino acid metabolized by the liver through the primary pathway of transmethylolation. The transmethylolation of methionine by adenosyltransferase into homocysteine occurs only in the liver, as other tissues lack the required enzymes. S-adenosyl-L-methionine is converted into S-adenosyl-homocysteine by the hepatic enzyme N-methyltransferase. This enzyme functions to remove methyl groups, leading to excretable byproducts; the major pathway to remove excess methionine methyl groups is via sarcosine production. Sarcosine is oxidized by sarcosine dehydrogenase to produce a one-carbon fragment that can be subsequently converted to CO₂. Sarcosine dehydrogenase is oxidized by the mitochondrial oxidation system, and the sarcosine oxidase system is present exclusively in hepatic mitochondria. Therefore, measuring the labeled CO₂ byproduct of ¹³C-labelled methionine in samples of expired breath represents a unique and promising technique to evaluate the oxidative capacity of liver mitochondria.

This study is active and funded by the Seattle Children's Hospital Academic Enrichment Fund. We are performing this study in close collaboration with pediatric surgical investigators at Children's Hospital Boston, where this study was first trialed. Our ultimate goal is to combine our data into a multi-institutional, federally-funded study to study this innovative technique in a broad cohort of children with intestinal failure.

Neurodevelopmental Outcomes of Children with Intestinal Failure

Children with intestinal failure are at high risk for poor neurologic and cognitive development. These patients often undergo multiple complex operations under general anesthesia, which has previously been shown to be a risk factor for impaired cognitive development. Indeed, recent data have shown that premature neonates who require surgery for necrotizing enterocolitis (NEC) have worse neurodevelopmental outcomes than neonates with non-operative NEC. Children with intestinal failure require multiple prolonged hospital stays, another factor demonstrated to negatively affect cognitive development in children. In addition, dependence on parenteral nutrition, which is common in these patients, can lead to a high incidence of indwelling central venous lines and subsequent blood stream infections that can predispose to sepsis. Systemic infections have been correlated with neurodevelopmental impairment in several cohorts of children. Finally, these children are often hemodynamically unstable during the neonatal period due to their primary diagnosis, thereby increasing the risk of neurologic injury early in life.

Despite these risk factors for impaired cognition, there is scant data in the literature focusing on the neurodevelopmental outcomes of these patients. Given their risk factors for neurodevelopmental impairment and the fact that these children can expect to reach school age and beyond, effective study of neurodevelopmental outcomes in this population would contribute valuable data that may impact their clinical care. This research will allow for the identification of patients at high risk for poor neurocognitive development, and may lead to the implementation of early intervention programs to improve development and quality of life.

This pilot study aims to measure the neurodevelopmental status of young children with intestinal failure using validated tools and to compare these to well-accepted normative values. The hypothesis of this study is that children diagnosed with intestinal failure will have substandard neurodevelopmental outcomes due to their unique and multiple co-morbidities when compared with well-accepted normative values. The study focuses on children aged 1 to 3 years, as this serves as an ideal time to implement early intervention programs in

those children at highest risk for cognitive impairment. A secondary aim of this study is to identify potential predictive risk factors for neurodevelopmental impairment in this group.

This study has been designed to maximize the resources available at Seattle Children's Hospital (SCH) and the University of Washington Medical Center (UWMC). All patients will be recruited from the active census at the SCH Intestinal Failure Program. Subjects will undergo a focused neurodevelopmental evaluation at the University of Washington High-Risk Infant Follow-Up Program (HRIF), which has specialized in the measurement of neurologic impairment of premature infants and children for over 30 years. Neurodevelopmental indices in this cohort will be evaluated through validated and well-established tools that have been utilized for decades to assess the neurodevelopmental status of numerous cohorts of young children. These tests will include a focused physical examination to document hearing and visual impairment, and cerebral palsy, as well as a neurocognitive evaluation using the Bayley Scales of Development or Stanford-Binet Intelligence Scales. These neurocognitive tests incorporate well-defined control data that will allow comparative analysis of our chosen study cohort with healthy, aged-matched children.

This study has the potential to serve as a preliminary benchmark for the neurological, cognitive, and developmental status of long-term survivors of pediatric intestinal failure. As survival continues to increase in children with this disease, the information gained from the study may be invaluable in the future care of these patients. For example, early identification of children in this cohort at risk for long-term neurodevelopmental impairment may allow for the implementation of early intervention services to improve overall outcome. In addition, the study has been constructed to allow for future long-term follow-up in a well-established facility with a consistent team of experienced investigators. Finally, the pilot data obtained from this study may serve as a foundation for a future multi-institutional study within the existing Pediatric Intestinal Failure Consortium (PIFCon), thereby increasing the size of the subject cohort and the power of the study.

It is anticipated that this study will start enrolling subjects in late 2010.

Pediatric Intestinal Failure Consortium

The Pediatric Intestinal Failure Consortium (PIFCon) is a multidisciplinary collaborative network constructed to effectively study the management of pediatric patients with intestinal failure. Its research efforts include basic science as well as clinical and translational techniques. The collaborative is composed of pediatric practitioners from 18 academic centers throughout the US and Canada; in fact, SCH was an original member of the consortium. The group has active funding through the NIH (1R21DK081059-01), and PIFCon is currently applying for additional grant support through the NIH U01 mechanism.

Seattle Children's Hospital was a founding member of PIFCon, and members of the Intestinal Failure Program play an active role in its leadership. PIFCon has collected retrospective data on children with intestinal failure from all 18 centers, and additional federal funding will allow for the collection of prospective data. This data will be available to analyze with the support of PIFCon statisticians. Our laboratory will take an active role in acquiring and analyzing this data to help define the clinical parameters and treatments that optimize long-term outcomes in children with intestinal failure.

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DEPARTMENT CO-INVESTIGATOR

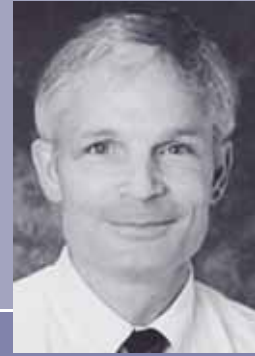
Patrick J. Healey, M.D.; Division of Pediatric General and Thoracic Surgery, Division of Transplantation, Seattle Children's Hospital

OTHER CO-INVESTIGATORS

Simon P. Horslen, M.B., Ch.B.; Division of Gastroenterology, Seattle Children's Hospital / **Janet Englund, M.D.;** Division of Infectious Disease, Seattle Children's Hospital / **F. Curt Bennett, M.D.;** Department of Pediatrics, Seattle Children's Hospital

John H. T. Waldhausen, M.D.

· Surgical Treatment Review Improves Children's Healing Process



Professor of Surgery
Chief of Pediatric Surgery,
Seattle Children's Hospital

Pediatric surgery is in general a very clinically oriented field, although there is an increasing emphasis on research in our division. Most of our division's research activity in the past has been oriented toward outcomes and what we do in the operating room and on the hospital ward. 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. While retrospective clinical reviews may be useful, much of our current outcomes research is now driven by examination of large patient databases that cover large populations. We are using these databases in a rigorous academic manner to critically evaluate how various treatments affect an entire population rather than just the smaller number that we may have cared for. We are also looking at quality of life studies to better evaluate how what we do affects our patients in the long run. Studies of this sort have been sorely lacking in our field.

In addition to outcomes, we have several faculty members focusing on quality and patient safety. The methodology being used in this area derives from the Toyota Production System and its use of lean methodology. While it may seem odd that automobile manufacture and health care could use the same basic tools as a model for success, we have found this to be quite true. Lean methodology uses a plan/do/check/act cycle (PDCA) to make improvements to a system, whether it be the production of cars or how patients move through the hospital. This PDCA cycle is nothing more than the scientific method that was learned in medical school or the science laboratory, where a series of experiments are designed to evaluate, define and improve a process. In the business world of Toyota and in health care, these experiments are called rapid process improvement workshops (RPIW), and they are nothing more than experiments

meant to define and refine a process. All of these RPIWs are data driven. Anecdote and conjecture have little place in them. Just as with any scientific experiment, data must be collected prior to performing the RPIW. These data are then used to help refine a process and make improvements, just the way one would in a science experiment. These improvements cover a broad range of issues, from moving a child expeditiously through the operating room, to improving patient flow through the clinic, or to making sure the child receives the correct medication. The goal is to cut out wasted time and effort in patient care, not just for the patient but for the providers as well. Each RPIW attempts to add standardization to a process. This cuts down on variability and thus should improve safety for the patient. When a process is standardized, it is more likely that one will recognize deviation from what is expected. Problems can then be caught before they reach the patient. In addition, by standardizing a process so that we do it the same way each time, we cut out wasted effort because we do not have to reinvent the wheel every time we do something. One of the critical points in the entire process is that it is not static, but rather, is a dynamic cycle. Each cycle of PDCA repeats itself so that if something does not work, there is the opportunity to keep refining the process as one would with any scientific experiment. At first blush, faculty members worried that standardization would stymie creativity, but because of the PDCA cycle and decisions based on data collection, this is actually the ultimate scientific means of delivering health care to our pediatric patients.

Minimally invasive surgery (MIS) continues to play an important role in pediatric surgery. Many MIS operations have become the standard procedure for many conditions. Laparoscopic and thoracoscopic surgery both have steep learning curves, especially for the more advanced cases. For some pediatric surgeons, it may take years to acquire these

The scope of research in the Division of Pediatric Surgery at Seattle Children's Hospital has significantly increased in the past several years. Quality, outcome and innovative techniques are the main areas of focus in our division.

skills because of the limited number of patients with a specific diagnosis, such as gallbladder disease, compared with adults. Several of our faculty are involved with pediatric robotic surgery and are international leaders in this field. While the exact place of the robot is still being defined in pediatrics, it has been put to innovative use by our faculty and has broadened the potential scope for MIS in children. Other researchers in our division are focusing on intestinal failure and the physiology of short bowel syndrome. The survival of infants and children with short bowel syndrome has markedly improved; with the addition of intestinal transplantation, many of these children now have the opportunity to live healthy lives.

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 the Departments of Orthopedics and Pulmonary Medicine has allowed us to be part of a national collaborative study on the use of the vertical expandable prosthetic titanium rib (VEPTR), which has become a standard modality 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. The use of the

expandable rib allows 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. Outcomes for some of these children are promising and suggest that this technique can successfully improve or stabilize these children's pulmonary function. Seattle Children's Hospital was an FDA study center for the evaluation of this device, and we are continuing to take a leading role in determining the efficacy of this treatment. In addition, we have expanded the use of the VEPTR for chest wall reconstruction, a novel use of this device, to bridge large gaps in the rib cage due to tumor excision. We have made special modifications to the VEPTR to allow its use in small infants and children, as well as place the VEPTR in an intrathoracic position to treat children with a kyphotic component to their skeletal deformity.

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. All of the research in our division is directed toward bettering the quality, safety and outcome of our patient care, and improving the lives of our children.

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DEPARTMENT CO-INVESTIGATORS

Jeffrey Avansino, M.D. / **George Drugas, M.D.** / **Adam Goldin, M.D.** / **Kenneth Gow, M.D.** / **Patrick Healey, M.D.** / **Patrick Javid, M.D.** / **John Meehan, M.D.** / **Cabrini La Riveria** / **Daniel Ledbetter, M.D.** / **Robert Ricca, M.D.** / **Sabrina Sanchez, M.D.** / **Robert Sawin, M.D.** / **Jacob Stephenson, M.D.**

OTHER CO-INVESTIGATORS

Arlene Libby, Seattle Children's Hospital / **Howard Jeffries, M.D.**, UW Department of Pediatrics / **Tom Lendvay, M.D.**, UW Department of Urology / **Kit Song, M.D.**, UW Department of Orthopaedics and Sports Medicine / **Greg Redding, M.D.**, UW Department of Medicine

PLASTIC & RECONSTRUCTIVE
SURGERY

CRAIG B. BIRGFELD, M.D.

JEFFREY B. FRIEDRICH, M.D.

MATTHEW B. KLEIN, M.D.

DAVID W. MATHES, M.D.

Craig B. Birgfeld, M.D.

Maximizing Outcomes of Pediatric Craniofacial Surgery

- Craniosynostosis
- Craniofacial Microsomia



Assistant Professor
of Surgery

FUNDING NIH

Craniofacial surgery involves the treatment of congenital deformities of the head and face, such as craniosynostosis, cleft lip and palate, and craniofacial microsomia as well as acquired deformities of the face through trauma or tumor extirpation. Our research focuses on the outcomes of our treatments for patients with craniosynostosis, cleft lip and palate, craniofacial microsomia, and facial trauma.

Craniosynostosis

Craniosynostosis is the premature fusion of one or more cranial sutures. These sutures are vital for normal development of the rapidly growing infant brain. Premature fusion can occur sporadically, or as part of an inherited syndrome and carries with it the risk of elevated intracranial pressure, developmental delay and blindness. Treatment options vary, but generally involve expansion of the cranium. Our research focuses on the outcomes of these treatments.

SAFETY

We are currently analyzing the safety of the surgical approaches offered at Seattle Children's Hospital. Factors such as blood loss, intraoperative events and post-operative complications are analyzed along with anesthesia protocols to establish the safest practice. We have found that early transfusion of blood and plasma have improved the safety of these procedures and shortened the length of hospital stay.

NEURODEVELOPMENT

An important outcome measure in craniosynostosis surgery is the child's neurocognitive development. Coupled with our research into the safety of our procedures is development of the patients after surgery. This project seeks to determine the effects of anesthesia, surgery,

and post-operative complications on the development of children during the first decade after birth. Neurocognitive tests are administered at various time points during childhood and compared to controls to determine the effects of craniosynostosis and its surgical treatment.

MORPHOLOGY

One component of the surgical treatment of craniosynostosis is alteration of cranial morphology. Abnormal skull shape is a hallmark of craniosynostosis, and altering the shape and establishing a normal morphology is one goal of our surgical correction. Our outcomes research includes analysis of 3-dimensional CT scan data before and after surgery as well as long term follow up (Figure 1). These measurements are used to predict severity of phenotype, success of surgery, and stability of our reconstruction.

Craniofacial microsomia

Craniofacial microsomia (CFM) is one of the most common congenital deformities of the face treated in the Craniofacial Center at Seattle Children's Hospital. This condition affects 1 in 3,000 live births. It presents a wide spectrum of phenotypic severity, as it is known to affect development of the orbits, the jaws, the facial musculature and soft tissue, the ears, and the facial nerve in varying degrees. Because of this wide spectrum, craniofacial microsomia is difficult to treat and requires a team approach to care, similar to that in cleft lip and palate. Surgical treatments for CFM vary and are constantly evolving. Newer techniques, such as distraction osteogenesis and facial reanimation, improve options for these patients and likely improve their outcomes.

“As surgeons, we assume that improvements we make on patients’ medical conditions will improve the quality of their lives. We are administering a quality of life tool (the Y-QOL), initially developed at the University of Washington and modified for online use, to assess the changes in life quality seen in our patients before and after surgery.”

PHENOTYPE

Classification of patients with CFM is challenging due to the wide spectrum of phenotype. This, historically, has made research and treatment planning difficult. A multi-specialty group at Seattle Children’s Hospital, including pediatricians, geneticists, psychiatrists, surgeons, speech pathologists, and epidemiologists, are currently working on improving the care provided for these patients. Our research has created a phenotyping tool, the PAT-CFM (Phenotypic Assessment Tool for Craniofacial Microsomia) that utilizes a pictorial to classify patients of varying severity in an effort to streamline research and treatment (Figure 2). This tool is currently undergoing inter- and intra-rater reliability studies and is being used in an NIH-funded multi-center study (FACIAL network). Additionally, we are using this tool to assess patients with CFM using a 2-dimensional photographic protocol as well as 3-dimensional photos using the 3dMD camera system (Figure 3). With this tool, we hope to quantify severity of phenotype, group similar phenotypes to facilitate multi-center research, and analyze outcomes of our surgical interventions.

QUALITY OF LIFE

As surgeons, we assume that improvements we make on patients’ medical conditions will improve the quality of their lives. Yet, for conditions such as CFM, multiple surgeries through sensitive developmental periods of life are necessary to approach normal form and function. It is possible that the surgeries themselves, along with the child’s fear and pain during recovery, have negative effects on their quality of life which may supersede any improvements our surgeries make. Our research is, therefore, including a component to measure quality of life. We are administering a quality of life tool (the Y-QOL), initially developed at the University of Washington and modified for online use, to assess the changes in life quality seen in our patients before and after surgery.

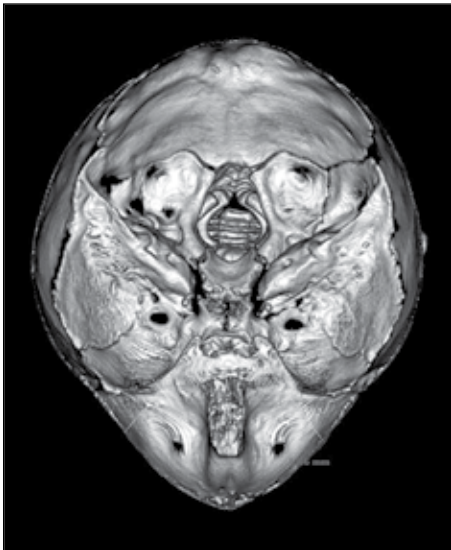


FIGURE 1. Top down view of the skull base in a patient with metopic craniosynostosis. Analytic software is used to measure angles and distances from the skull base to the frontal bones and orbital bandeau. These measurements are used to document phenotypic severity, measure degree of surgical improvement, and monitor amount of relapse when applied to pre-op, post-op and 2-year follow-up CT scans.

Date: / /
 Rater: _____
 Study ID: _____

		RIGHT					LEFT					
ORBIT		O0	O1	O2	O3	O4		O0	O1	O2	O3	O4
S/P SURGERY												
UNABLE		NORMAL	Abnormal size	Inferior orbital displacement	Superior orbital displacement	Abnormal orbital size and position		NORMAL	Abnormal size	Inferior orbital displacement	Superior orbital displacement	Abnormal orbital size and position
OCCLUSAL PLANE		OP0	OP1	OP2	OP3			OP0	OP1	OP2	OP3	
S/P SURGERY												
UNABLE		NO CANT	1-5 degrees	6-15 degrees	> 15 degrees			NO CANT	1-5 degrees	6-15 degrees	> 15 degrees	
MANDIBLE		M0	M1	M2A	M2B	M3		M0	M1	M2A	M2B	M3
S/P SURGERY												
UNABLE		NORMAL	Mild asx	Moderate asx	Mod-Severe asx	Severe asx		NORMAL	Mild asx	Moderate asx	Mod-Severe asx	Severe asx
EAR		E0	E1	E2	E3	E4		E0	E1	E2	E3	E4
S/P SURGERY												
UNABLE		NORMAL	All parts present, mild deformity	Auricle 1/2-2/3 of predicted size, not all parts present	Severely malformed, often peanut shaped	ANOTIA		NORMAL	All parts present, mild deformity	Auricle 1/2-2/3 of predicted size, not all parts present	Severely malformed, often peanut shaped	ANOTIA
NERVE		N0	N1	N2	N3	N4		N0	N1	N2	N3	N4
S/P SURGERY												
UNABLE		ALL NORMAL	Brow palsy Normal brow	Orbic palsy Normal orbic	Smile palsy Normal smile	Lower lip palsy Normal lip		ALL NORMAL	Brow palsy Normal brow	Orbic palsy Normal orbic	Smile palsy Normal smile	Lower lip palsy Normal lip
SOFT TISSUE		S0	S1	S2	S3			S0	S1	S2	S3	
S/P SURGERY												
UNABLE		NORMAL	Minimal soft tissue deficiency	Moderate soft tissue deficiency	Severe soft tissue deficiency			NORMAL	Minimal soft tissue deficiency	Moderate soft tissue deficiency	Severe soft tissue deficiency	
CLEFTING		C0	C1	C2				C0	C1	C2		
S/P SURGERY												
UNABLE		NO CLEFT	Cleft terminates medial to anterior border of masseter	Cleft terminates lateral to anterior border of masseter				NO CLEFT	Cleft terminates medial to anterior border of masseter	Cleft terminates lateral to anterior border of masseter		

NOTES: _____



FIGURE 2. ABOVE Page 1 of the Phenotypic Assessment Tool for Craniofacial Microsomnia (PAT-CFM); Global Assessment. This tool was created to document phenotypic severity in patients with craniofacial microsomnia and was modified to improve ease of use in the clinic and with both 2D and 3D photographs. Severity of Phenotype can then be compared to post-surgical results to monitor quality of care and can be compared to quality of life scores to help predict outcomes.

FIGURE 3. LEFT Example of a 3D photo taken using the 3dMD camera system. This camera system is being used to document patients with various craniofacial anomalies, especially craniofacial microsomnia both to document phenotypic severity and to track and measure surgical outcomes.

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3. Birgfeld CB, Neligan PC. Treatment of facial nerve disorders. *Skull Base: an Interdisciplinary Approach*. Accepted for publication, October 2010
4. Birgfeld CB, Gruss JS. The importance of early, accurate bony reconstruction in orbital injuries with globe loss. *Craniofacial Trauma and Reconstruction*. Submitted for publication October 2010.

Jeffrey B. Friedrich, M.D.

• Traumatic Injury to the Upper Extremity



Assistant Professor
of Surgery
Adjunct Assistant Professor
of Orthopaedics

FUNDING

2010 Plastic Surgery Educational Foundation
Pilot Grant

Traumatic injury to the upper extremity is one of the most common reasons to seek emergency medical care. These injuries can occur in all segments of the population, including working patients. Attempts to correlate outcome with various injury and patient characteristics have not yielded consistent results. However, it has become more apparent that patient-reported outcomes often correlate well with functional outcome and return to work. Valid patient-reported outcome instruments (PROs) that allow outcome study standardization and decrease the respondent burden are required.

The purpose of my research is to initiate the development of an upper extremity-specific PRO instrument that correlates with outcome following traumatic injury. Currently, we are using expert panels to elucidate physical function items

from existing upper extremity functional outcome metrics to assemble a comprehensive set of outcome questions. Subsequently, we will conduct cognitive interviews with patients who have sustained upper extremity injuries to test the items. The information assimilated in this current qualitative study will be used to develop a standardized PRO for upper extremity injury that will then be suitable for administration in future qualitative studies.

This work is necessarily collaborative in nature, and we are working with Dagmar Amtmann, Ph.D. in the Department of Rehabilitation on this effort. Dr. Amtmann is the director of the University of Washington's participation in the NIH's Patient-Reported Outcomes Measurement Information System, and has extensive experience with psychometric outcomes testing.

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OTHER CO-INVESTIGATORS

Dagmar Amtmann, Ph.D.; UW Department of Rehabilitation Medicine

Matthew B. Klein, M.D., M.S.

· The UW Regional Burn Center Burn Outcomes Research Program



Associate Professor of Surgery
David and Nancy Auth-Washington
Research Foundation Endowed
Chair for Restorative Burn Surgery

The mission of the UW Regional Burn Center burn outcomes research program is to improve the lives of those who survive burn injury by optimizing acute care treatment and long-term psychosocial and functional outcomes. Our research program has been supported over the years by funds from the National Institutes of Health (NIH), Washington State Council of Firefighters Foundation, the International Association of Firefighters, the National Institute on Disability and Rehabilitation Research, and now enjoys continued funding through the David and Nancy Auth-Washington Research Foundation Endowment. Our outcome research program includes collaborators from the Departments of Pediatrics, Public Health, Health Services, Rehabilitation Medicine, and Geriatric Medicine.

A summary of ongoing projects is listed below.

1. Development of a Burn-Specific Quality of Life Measurement Instrument

One of our top research priorities is to develop a burn-specific quality of life instrument. This would be a tremendous benefit to the burn community, as it would provide a valid and useful tool to evaluate patient outcomes as well as to assess the effectiveness of new technologies and innovations. There are few existing patient-reported outcome instruments that have been validated in burn survivors, and each has significant limitations. We are in the process of conducting pilot focus groups and individual interviews as part of the planning of a larger instrument development. This research is being carried out in conjunction with the Seattle Quality of Life Group.

2. Long-term Cost Outcomes of Pediatric Burn Injury

This will be the first study to longitudinally follow a cohort of severely burned children over time to ascertain the costs associated with burn injury. While much has been written about the intensive resources required for acute burn care, little is known about the long-term costs associated with severe burn injury.

3. Functional and Psychosocial Outcomes of Older Adults

We have conducted and published a number of studies over the past few years examining the long-term outcomes of older adults following burn injury. We have focused on the patient (i.e., age, comorbidities), injury and treatment factors that impact mortality, and in-hospital complications as well as long-term outcome. We recently submitted for publication the first ever multicenter prospective study of psychosocial and functional outcomes of burn injury in older adults. The ultimate goal of this research is to develop a clinical intervention that will improve the functional and psychosocial results of older adults who survive burn injury.

4. Organization and Delivery of Burn Care

The delivery of optimal burn care is a resource-intensive endeavor requiring specialized equipment and experienced personnel that are typically available only at dedicated burn centers. Currently in the United States there are 128 self-identified burn care facilities, only 56 of which have been verified by the American Burn Association (ABA). Over the past few years, we have conducted a number of studies examining the organization and delivery of burn

*While much has been written about the intensive resources required for acute burn care,
little is known about the long-term costs associated with severe burn injury.*

care in the United States, with the ultimate goal of defining standards to ensure that all persons who sustain burn injury can have access to quality burn care. These research projects have been conducted in close collaboration with the Harborview Injury Prevention and Research Center (HIPRC). We have examined the impact of transfer status on patient outcome, the safety of long-distance transport of burn patients, the patient and injury characteristics associated with definitive burn treatment in Washington and in the United States and, most recently, examined population-based access to burn centers by air and ground transport.

5. Development of an International Burn Registry

We have begun the early stages of developing an international burn registry. With assistance from international burn leaders and the World Health Organization, we have organized the first-ever international collaborative database of burn injury. Seven countries from around the world have contributed data in the pilot/proof-of-concept phase.

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 2. Lundgren RS, Kramer CB, Rivara FP, Wang J, Heimbach DM, Gibran NS, Klein MB. Influence of comorbidities and age on outcome following burn injury in older adults. *J Burn Care Res* 30:307-314, 2009.
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 9. Mosier MJ, Pham TN, Klein MB, Gibran NS, et al. Early acute kidney injury predicts progressive renal dysfunction and higher mortality in severely burned adults. *J Burn Care Res* 31:83-92, 2010.
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-

David W. Mathes, M.D.

· Induction of Immunologic Tolerance and Long-Term Survival without Chronic Immunosuppression



Assistant Professor
of Surgery

To date, 13 face and 54 hand transplants have been performed across the world, demonstrating the clinical feasibility of composite tissue allotransplantation (CTA). Thus far, the clinical results have been promising, with recipients demonstrating varying degrees of return of function and the restoration of not only their outward appearance but also, more importantly, the return of a sense of well-being. Many of the recipients have even been able to return to work, a fact that may improve the cost-benefit analysis of these non-life-saving transplants. Despite these tangible benefits, the survival of the transplant is dependent on the use of chronic non-specific immunosuppressive medications. The medications are costly and associated with drug toxicities, opportunistic infections and malignancy. Even with the application of these modern immunosuppressive regimens, all of the patients have experienced episodes of acute rejection. More recently, reports have surfaced of patients exhibiting signs of chronic rejection after hand transplantation. Thus, the further advancement of the field of reconstructive transplantation is dependent on the reduction or elimination of the need for chronic immunosuppression.

Immunological tolerance is a natural process by which the immune system does not mount an immune response to a specific foreign or self-antigen. Theoretically, this process can be harnessed to allow a transplant recipient to undergo an organ transplant without the need for immunosuppression while still maintaining immunocompetence. While immune tolerance has been demonstrated to various solid organ transplants in

small animal models, the translation to pre-clinical large animal models has proven to be much more difficult. This is especially true in the field of CTA, where there have been only sporadic reports of tolerance to CTA in large animal models. The challenge of CTA is likely from the need to transplant skin, which has long been thought of as the most difficult organ to transplant due to its highly immunogenic structure.

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, but also to the 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.

1. Establishment of stable immunologic chimerism in a canine model

We have established a stable dog leukocyte antigen (DLA)-identical marrow graft. 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-myeloablative 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

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.

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.

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 extended this work, showing that this donor-specific tolerance can be induced in our CTA model (1). 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.

In our first series of experiments, mixed chimerism was achieved via non-myeloablative conditioning with 2 Gy total body irradiation (TBI) and bone marrow transplantation. After the injection of the bone marrow, a short course of immunosuppression with MMF and CSP was given. The animals were then followed for the presence of donor cells by virus neutralization test–polymerase chain reaction (VNT-PCR). After stable mixed chimerism was established for greater than three months post-transplant, the animals were ready for composite tissue transplantation. We performed 5 transplants, all of which demonstrated long-term tolerance for greater than one year without any further need for immunosuppression. The allografts were followed both clinically for evidence of rejection and through protocol biopsies. The allografts appear normal with excellent hair growth. In addition, the biopsies have not demonstrated any evidence of rejection. 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. Histologic analysis has demonstrated minor but stable perivascular infiltrate but no signs of acute rejection (Figures 1 and 2).

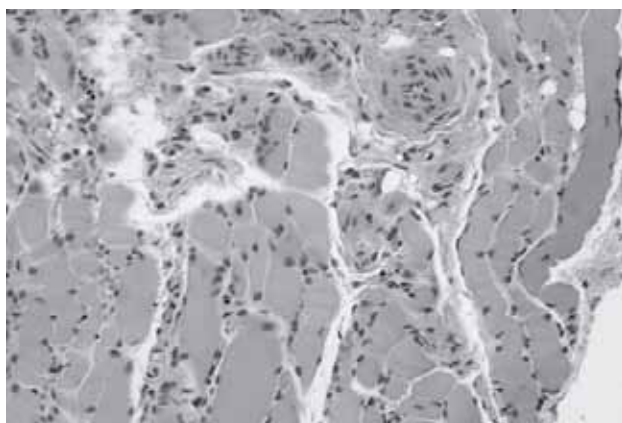


FIGURE 1: G244 Muscle bx Pod 41 days

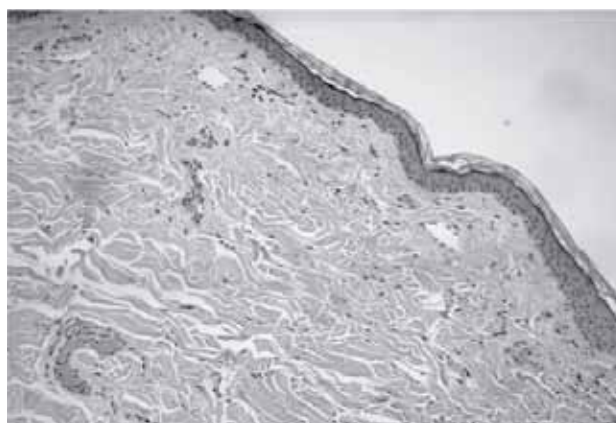


FIGURE 2: G767 Skin bx Pod 56 days

The purpose of our next series of experiments was to develop a large animal model for the simultaneous transplantation of hematopoietic stem cells (HSC) and CTA using our established mixed chimerism protocol. This would be more clinically relevant and could be applied to deceased donor transplants as well as living donors. All of the experimental animals have demonstrated long-term tolerance to their CTA (497, 467, 465, and 400 days after transplant). However, only three of the four dogs had detectable donor chimerism. One dog lost its chimerism at 10 weeks post-transplant but remained tolerant to the allograft. The expression of foxP3 decreased in the peripheral blood but remained stable in the transplanted muscle and skin. The two dogs that underwent the regimen without any HSC

rejected their transplants at 40 to 45 days after the cessation of the post-grafting immunosuppression. All of the tolerant dogs accepted the donor skin graft and promptly rejected the third-party skin graft. This study demonstrated that simultaneous transplantation of HSC and CTA is feasible and leads to tolerance to both the skin and muscle of the transplant. This tolerance induction appears to be dependent on the administration of HSC but not on the long-term engraftment of the HSC and the persistence of donor cell chimerism.

Our most recent focus has been to extend these findings to greater genetic disparities. We are also looking into the role of T regulatory cells and the thymus in the development and maintenance of tolerance in this model.

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TRANSPLANT SURGERY

JAMES D. PERKINS, M.D.

JORGE D. REYES, M.D.

James D. Perkins, M.D.

- Clinical Outcomes Research in Transplantation
- Quality Performance Measures in Surgery



Professor of Surgery
Vice Chair for Quality,
Department of Surgery

Clinical Outcomes Research in Transplantation

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 immunosuppressive therapy, or shorter hospital stay.

Several of our recent projects have now been completed, producing useful data and publishable results, as shown in our list of Related Publications. Building on our discoveries of the clinical factors that predict the recurrence of hepatocellular carcinoma (HCC) after liver transplantation (references #4 and #5 below), we are extending these discoveries to the genomic level with the following study:

- **Identifying HCC Genomic Patterns that Predict Recurrence Following Liver Transplant** (James Perkins, M.D.; Matthew Yeh, M.D., Ph.D.; Michael Katze, Ph.D.; Cosette LeCiel, B.S., M.S.; Angela Rasmussen, Ph.D., Robert Carithers, M.D.)

Quality Performance Measures in Surgery

Related to clinical outcomes research, but at a broader perspective, is the development of relevant and accurate quality performance measures in surgery. Driven not only by the nationwide economic need to contain costs,

but also by the pursuit of excellence and the emphasis on patient safety, the University of Washington Medical Center has been building computerized tracking systems to monitor events such as postoperative infections or bleeding that may signal areas in need of further attention. Such tracking systems are not simple: a complex code for illnesses, procedures, and complications, plus the training and discipline to use that code, are needed to make a tracking system work. Much time and effort could be saved if a computer could be “taught” to recognize certain phrases and record pertinent information. That is the goal of this new study:

- **Natural Language Processing to Determine Clinical Events for Quality Performance Measures** (Thomas Payne, M.D., Meliha Yetisgen-Yildiz, Ph.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 available resources and an environment conducive to investigation. Our statistical expertise, together with our custom-designed clinical transplantation database, allows us to perform multiple clinical outcomes research projects as ideas are developed. Our transplant fellows are afforded an excellent opportunity to learn research methods and receive guidance and encouragement through faculty mentorship.

In the area of health care quality, the current economic and social environment provides the sense of urgency and the motivation needed to find better ways of doing things, ultimately leading to higher proficiency in the performance of surgery. Patients will be the beneficiaries of improvements we make relative to safety, performance measures, and optimum outcomes.

Our most valuable 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 and from the surgical care that we deliver.

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DEPARTMENT CO-INVESTIGATORS

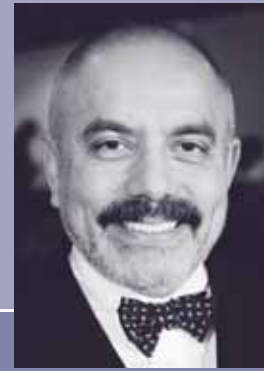
Ramasamy Bakthavatsalam, M.D. / Andre Dick, M.D. / Adam Goldin, M.D. / Jeffrey Halldorson, M.D. / Patrick Healey, M.D. / Oliver Lao, M.D. / Marie Peccoud, Ph.D. / Jorge Reyes, M.D.

OTHER CO-INVESTIGATORS

Robert Carithers, M.D., UW Department of Medicine / **Julie Duncan**, UW Medical Center / **Michael Katze, Ph.D.**, UW Department of Microbiology / **Thomas Payne, M.D.**, UW Department of Medicine / **Angela Rasmussen, Ph.D.**, UW Department of Microbiology / **Kay Wicks**, UW Medical Center / **Matthew Yeh, M.D., Ph.D.**, UW Department of Pathology / **Meliha Yetisgen-Yildiz, Ph.D.**, UW Department of Medical Education and Biomedical Informatics

Jorge D. Reyes, M.D.

· Translational Studies and Clinical Outcomes
Investigations in Transplantation



Professor of Surgery
Adjunct Professor of Pathology
Chief, Division of
Transplantation

FUNDING

National Institutes of Health (NIH)
National Institute of Diabetes and
Digestive and Kidney Diseases (NIDDK)
Astellas

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. Translational studies

Translational studies seek to apply the principles that are learned from basic science investigations to patient care.

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.”

The University of Washington Medical Center is involved in a Phase II trial sponsored by the National Institutes of Health (NIH) that may give us insights into the mechanisms for the achievement of tolerance, as well as when to withdraw immunosuppression.

Over the last few years, Dr. Frances Malone, working in the Transplant Research Laboratory, has collaborated with Dr. Wei Li of the University of Washington to elucidate the roles of specific surface proteins and regulatory cells on the development of tolerance in murine liver transplantation. Dr. Malone has published two manuscripts on the roles of PD-L1 signaling and gamma delta lymphocytes in transplantation and was invited to present other work on the inverse expression of gamma delta T cells and Foxp3CD25CD4 T regulatory cells after murine liver transplant at the American Association of Immunologists Meeting (May, 2009). She has also designed a prospective clinical trial evaluating the changes in lymphocyte subsets between two standard immunosuppression protocols at the University of Washington Medical Center. This work is funded as an investigator-initiated proposal through Astellas Pharma; she is currently analyzing samples.

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 medication 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

The University of Washington Medical Center is involved in a Phase II trial sponsored by the National Institutes of Health (NIH) that may give us insights into the mechanisms for the achievement of tolerance, as well as when to withdraw immunosuppression.

rejection, levels of HCV in the blood and liver, and for the response of the immune system to the withdrawal of immunosuppression.

Being able to wean patients from their immunosuppressive medications would truly be an achievement, giving transplant recipients improved outcomes following transplantation and improve their health and quality of life.

2. Clinical outcomes investigations

A multitude of factors influence the outcome of each transplant procedure, including physiologic factors of the organ donor and recipient, surgical technique, logistics factors in transporting the donor organ for the recipient operation, the type of immunosuppressive medication, and geographic and sociological factors. Our clinical outcomes studies have been correspondingly broad in an effort to address several different ways in which transplant outcomes can be improved:

- **Eliminating health disparities for minorities undergoing solid organ transplantation** (Andre Dick, M.D.)
- **Weaning liver transplant recipients from immunosuppressive drug therapy in order to avoid the side effects of this therapy** (Jorge Reyes, M.D.)
- **Biliary Atresia and Intestinal Failure** (Frances Malone, Ph.D., A.R.N.P.). Two different mechanistic studies are underway to accompany two U01 proposals (Biliary Atresia and Intestinal Failure) one of which has been funded (U01-ChildREN NIDDK/U01 DK084575-01). Other current projects include finalizing manuscripts for three retrospective studies evaluating the clinical outcomes related to children with intestinal failure or requiring small bowel transplant, as well as a manuscript detailing the results of the Intestinal Care Program's use of omega-3 fatty acid supplementation in children with intestinal failure and liver disease. The data from each of these manuscripts have been presented at the 2009 International Small Bowel Transplant Symposium, the National Association of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN), and other regional surgical/academic meetings.
- **Pediatric Intestinal Failure Consortium (PIFCON)** (Frances Malone, Ph.D., A.R.N.P.). This national research consortium has been established to study children with intestinal failure. The group has received R21 funding and submitted a U01 proposal based on retrospective data (NIDDK/ 1R21DK081059-01).
- **Factors influencing liver transplant survival rates, including recommendations on optimum MELD scores for liver transplantation** (James Perkins, M.D.)
- **Improving the quality of donor/recipient matching in liver transplantation** (Jeffrey Halldorson, M.D.)
- **Maximizing the experience of surgical residents for optimum learning of the principles and techniques of abdominal organ transplantation** (Ramasamy Bakthavatsalam, M.D.)

RELATED PUBLICATIONS

1. Reyes J, Zeevi A, Ramos H, Tzakis A, Todo S, Demetris AJ, Nour B, Nalesnik M, Trucco M, Abu-Elmagd K, Fung JJ, Starzl TE: The frequent achievement of a drug free state after orthotopic liver transplantation. *Transplant Proc* 25:3315-3319, 1993.
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10. Jarvid PJ, Malone FR, Reyes J, Healey PJ, Horslen SP. The experience of a regional pediatric intestinal failure program: successful outcomes from intestinal rehabilitation. *Am J Surg* 199:676-679, 2010.

DEPARTMENT CO-INVESTIGATORS

Ramasamy Bakthavatsalam, M.D. / Andre A. S. Dick, M.D. / Jeffrey B. Halldorson, M.D. / Frances Malone, Ph.D., A.R.N.P. / James D. Perkins, M.D.

UWMC/ GENERAL SURGERY

BENJAMIN O. ANDERSON, M.D.

DAVID R. FLUM, M.D., M.P.H.

SAURABH KHANDELWAL, M.D.

BRANT K. OELSCHLAGER, M.D.

JAMES O. PARK, M.D.

CARLOS A. PELLEGRINI, M.D.

ROGER P. TATUM, M.D.

ANDREW S. WRIGHT, M.D.

RAYMOND S. YEUNG, M.D.

Benjamin O. Anderson, M.D.



The Breast Health Global Initiative



Professor of Surgery and Global Health – Medicine, University of Washington
Chair and Director, Breast Health Global Initiative (BHGI)
Full Member, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center
Director, Breast Health Clinic, University of Washington/Seattle Cancer Care Alliance

INTRODUCTION

The global breast cancer problem cannot be solved in a single large step. It takes small incremental steps in different parts of the world at different times that allow advances to be made.

In 2002, with the co-sponsorship of the Fred Hutchinson Cancer Research Center and Susan G. Komen for the Cure®, we established the **Breast Health Global Initiative (BHGI)** in Seattle as an ongoing public-private global health alliance devoted to medically underserved women with a mission to develop, implement and study evidence-based, economically feasible, and culturally appropriate guidelines for low- and middle-income countries (LMCs) to improve breast health outcomes of women around the world.

BHGI Background and Significance

Over the past nine years and led by Dr. Anderson, the Breast Health Global Initiative has become a world leader in women's health initiatives through its pioneering development of the first comprehensive, resource-sensitive, evidence-based clinical *Guidelines for International Breast Health and Cancer Control* ("Guidelines") for breast health care delivery and cancer control in LMCs, intended to assist ministers of health, policy makers, health care institutions and others to prioritize resource allocation in breast care programs in LMCs.

The BHGI began with a basic premise of defining guidelines for "best practices with limited resources" for breast health and cancer control to improve breast cancer outcomes in LMCs, through the successful dissemination and implementation of BHGI Guidelines. The principal target is LMCs, although the same guidelines apply to underserved populations in high-income countries.

Through strategic global alliances with governmental, NGO, non-profit and for-profit health organizations (please refer to the last page of this report), the BHGI has been able to accomplish its innovative work through international collaboration and a biennial series of Global Summits.

The BHGI process of collaborative consensus guideline development created an international "hub" for **organizational linkages** and alliances, providing a common network for key groups to work together to advance systematic change, through interdisciplinary communication, cooperation and alliance-building via the BHGI Global Summits, the BHGI web-based portal (www.bhgi.info), and pilot research and demonstration projects between **three key groups**:

- Clinicians and governmental health care agencies
- Public health researchers
- Advocacy, non-governmental, non-profit and for-profit organizations.

From 2002-2008, through the BHGI biennial series of *Global Summit(s) on International Breast Health* (2002, 2005, 2007), the organization was able to develop a model approach for Guidelines to effectively detect, diagnose and treat breast cancer LMCs, producing three successful Global Summits on International Breast Health that produced three consecutive published editions of the Guidelines. The fourth *Global Summit* was held in June, 2010. Dr. Anderson was the scientific course chair for the series of Global Summits.

In 2009, and following the BHGI resource-stratified guideline approach (see below), the Asian Oncology

Summit held in April 2009 in Singapore developed 6 consensus statements on 6 different cancers commonly seen in Asia, publishing these results in *The Lancet Oncology* later that year. Dr. Anderson served as consultant to that international conference and served as second author on each consensus publication.

The Global Summit Series

SEATTLE, 2002 GLOBAL SUMMIT: Health Care Disparities

BETHESDA, 2005 GLOBAL SUMMIT: Resource Stratification

BUDAPEST, 2007 GLOBAL SUMMIT: Guideline Implementation

CHICAGO, 2010 GLOBAL SUMMIT: Optimizing Healthcare Delivery

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) These guidelines have been made freely available in an unrestricted fashion on the internet for world-wide access at <http://www.fhrcr.org/science/phs/bhgi/>

GLOBAL SUMMIT 2005 (BETHESDA): RESOURCE STRATIFICATION

(“Guidelines for International Breast Health and Cancer Control,” *Breast J* 2006;12, Suppl 1)

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.

GLOBAL SUMMIT 2007 (BUDAPEST): GUIDELINE IMPLEMENTATION

(“Guidelines for International Breast Health and Cancer Control—Implementation” *Cancer* 2008;113, Suppl 8)

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

GLOBAL SUMMIT 2010 (CHICAGO): OPTIMIZING HEALTHCARE DELIVERY

The systematic analyses performed during this 3-day Summit were guided by the previously published BHGI resource-sensitive comprehensive breast care guidelines for early detection, diagnosis and treatment (*The Breast*, April, 2011 supplement).

The 2010 summit, the fourth in the biennial series, held through the support of the BHGI strategic global alliance of organizations, co-convened in association with the Latin American and Caribbean Society of Medical Oncology SLACOM (Sociedad Latino Americana y del Caribe de Oncologia), was led by Dr. Anderson and co-chair Eduardo

Cazap, SLACOM president also president of the UICC-International Union against Cancer. As a result of the 2010 Global Summit, three consensus recommendations and 11 special reports addressing key issues of *Optimizing Healthcare Delivery* were developed and published in a supplement in the European journal *The Breast* (Global Breast Health Care: Optimizing Delivery in Low- and Middle-resource Countries, A Supplement of Consensus Statements & Special Reports to The Breast), in April 2011. This anthology is an important medical tool with practical, corresponding, evidence-based information, a rare resource for LMCs. Also, as a result of the 2010 Global Summit, an article was developed for publication in *The Lancet Oncology*, *Optimizing Breast Cancer Management in Low and Middle Resource Countries: Executive Summary of the Breast Health Global Initiative Consensus Recommendations 2010*, published in April 2011.

UNIVERSITY OF WASHINGTON FACULTY PRESENTERS AND PANELISTS AT THE GLOBAL SUMMIT:

David B. Thomas, MD, DrPH, Senior International Research Advisor, Breast Health Global Initiative, Division of Public Health Sciences, Program in Epidemiology, Fred Hutchinson Cancer Research Center; Department of Epidemiology, School of Public Health and Community Medicine, University of Washington

Gabrielle M. Kane, MB EdD FRCPC, Senior Education Advisor, Breast Health Global Initiative, Fred Hutchinson Cancer Research Center; Associate Professor, Department of Radiation Oncology, Department of Medical Education and Biomedical Informatics, University of Washington School of Medicine; Medical Director, Residency Program Director, University of Washington Medical Center

Julie R. Gralow, MD, Professor, Medical Oncology, University of Washington School of Medicine, Director, Breast Medical Oncology, Seattle Cancer Care Alliance / University of Washington School of Medicine, Associate Member, Clinical Research Division, Fred Hutchinson Cancer Research Institute

Engelberta (Beti) Thompson, PhD, Public Health Sciences, Full Member, Cancer Prevention Research Program, Fred Hutchinson Cancer Research Center; Professor, Health Services, School of Public Health, University of Washington

Peggy Porter, MD, Member, Divisions of Human Biology and Public Health Sciences, Fred Hutchinson Cancer Research Center; Head, Women’s Cancer Research Program: Breast Section, Fred Hutchinson/University of Washington Cancer Consortium; Professor of Pathology, University of Washington

BHGI Vision for Implementation: Strategic Implementation Science Projects

In 2009, with a shifting of focus from development and expansion of the *Guidelines for International Breast Health and Cancer Control* to address effective implementation and integration of breast health care interventions described in the Guidelines, the BHGI began its next phase of innovation with a multi-year plan for Guidelines implementation, collaborating with global, regional and local organizations to pioneer the development of **Learning Laboratories (LL)** to define critical in-country methodology for establishing or expanding breast cancer care programs in low- and middle-resource countries (LMCs) **through a diverse portfolio of implementation science projects.**

The purpose of BHGI Learning Laboratories is to establish educational and research pilot projects that will form the basis for breast health program expansion in LMCs with a clear, compelling global vision to fulfill global health needs while creating opportunities for international collaboration. The first BHGI Learning Laboratory was established in Kumasi, Ghana, West Africa in 2009 with a program focus on **development and testing of education and training modules** that will serve as **core training curricula.**

An integral component of the BHGI implementation mission is to provide transformational collaborative opportunities and innovative ‘system-based’ solutions, designed to address specific issues of optimizing breast health care and cancer treatment in LMCs. Thus, in 2009, BHGI developed a technical foundation for international collaboration, dialoguing and information dissemination through www.bhgi.info, an interactive web-based portal. This virtual environment includes a “Breast Health-Common Interest Group” and an ‘open access’ searchable Literature Library populated with thousands of citations relevant to breast cancer in LMCs. As a knowledge broker, the BHGI virtual environment is a core resource for our work, enabling international research collaborations with colleagues around the globe.

Through a diverse portfolio of **implementation science projects**, a process of effective knowledge transfer to successfully achieve improved health outcomes, BHGI is working with international partners, clinical communities, public health researchers and advocacy groups, with a clear, compelling vision of global health improvement to take theory into practice.

The BHGI strategic **Global Portfolio of Implementation Science Projects** is outlined in an abbreviated list format below. The projects provide opportunities for collaboration with international partners with shared interests.

For detailed information about the BHGI and BHGI Global Portfolio of Implementation Science Projects, please visit www.bhgi.info.

Breast Health Global Initiative

GLOBAL PORTFOLIO OF IMPLEMENTATION SCIENCE PROJECTS

AFRICA

EDUCATION AND TRAINING:

BHGI LEARNING LABORATORY IN GHANA

Ghana Breast Cancer Alliance (GBCA) and BHGI medical training course through HopeXchange and HopeXchange Ghana Health Project

Initial funding for this project via HopeXchange, Susan G. Komen for the Cure® sub-award

Project Director: Benjamin O. Anderson, MD

Research Advisor: David B. Thomas, MD, DrPH, BHGI Senior International Research Advisor, Breast Health Global Initiative, Division of Public Health Sciences, Program in Epidemiology, Fred Hutchinson Cancer Research Center, Department of Epidemiology, School of Public Health and Community Medicine, University of Washington

Education Advisor: Gabrielle M. Kane, MB EdD FRCPC, Senior Education Advisor, Breast Health Global Initiative, Fred Hutchinson Cancer Research Center; Associate Professor, Department of Radiation Oncology, Department of Medical Education and Biomedical Informatics, University of Washington School of Medicine; Medical Director, Residency Program Director, University of Washington Medical Center – **PROJECT IN**

PROGRESS

Guideline implementation requires targeted physician education to address key care delivery issues in their specific practice environment. To develop and test education and training modules based on the BHGI clinical breast health and cancer control Guidelines that can be adapted and used by diverse communities, and to build medical capacity to address breast health and cancer control, the BHGI endeavors to develop comprehensive, context appropriate and Guideline-based postgraduate education modules on public health and patient education, early detection, pathology, radiology, surgery, radiation oncology and medical oncology, tailored to the resources available in low- and middle-resource countries. Through an international

collaborative effort, the **Ghana Breast Cancer Specialty Training Course** is the first educational course of its kind developed by the BHGI through establishment of the first BHGI Learning Laboratory (LL), in Kumasi, Ghana.

GHANA BREAST CANCER SPECIALTY TRAINING COURSE

- Initial Site assessment: Kumasi, Ghana, August 2009
- BHGI Learning Lab Delivery of Breast Cancer Course #1: Kumasi, Ghana, January 2010
- BHGI Learning Lab Delivery of Breast Cancer Course #2: Accra, Ghana, August 2010

VIRTUAL UNIVERSITY FOR CANCER CONTROL AND REGIONAL TRAINING NETWORK (VUCCNET)

International Atomic Energy Association's Programme of Action for Cancer Therapy and BHGI partnership announced in 2010 – **PROJECT IN PROGRESS**

The International Atomic Energy Association's Programme of Action for Cancer Therapy (IAEA-PACT) and the BHGI agreed to join forces in June of 2010 to advance comprehensive cancer control in low-resource and middle-resource countries, a goal shared by both organizations. Through partnership, PACT and BHGI are now working together to curb the number of breast cancer deaths occurring in Africa through collaboration on several projects, including PACT's new **Virtual University for Cancer Control and Regional Training Network** (VUCCnet), developed with the aim of helping to fill the human resources gap in Africa, by means including expanding the reach, progress and outcomes of the BHGI Learning Labs through detailed training on the applications of breast cancer medicine.

The VUCCnet, a distance-learning apparatus developed by PACT, will now incorporate the breast cancer curriculum jointly created by BHGI, HopeXchange and the Ghana Breast Cancer Alliance for Ghana. With the BHGI incorporation into the VUCCnet, the curriculum currently in use on a pilot project basis in Ghana will soon be accessible in three additional African countries, now in pilot phase for VUCCnet: Tanzania, Uganda and Zambia.

ASIA

PILOT PROJECT: EARLY BREAST CANCER DETECTION THROUGH CLINICAL BREAST EXAMINATION TRAINING FOR MIDWIVES, IN RURAL JAKARTA, INDONESIA

Funding for this project via Komen for the Cure® sub-award;

Principal Investigator: Kardinah, MD, Principal Investigator, Dharmais Cancer Hospital, Jakarta Research Advisors: Benjamin O. Anderson, MD; David B. Thomas, MD, DrPH, Senior International Research Advisor, Breast Health Global Initiative, Division of Public Health Sciences, Program in Epidemiology, Fred Hutchinson Cancer Research Center, Department of Epidemiology, School of Public Health and Community Medicine, University of Washington – **PROJECT IN PROGRESS**

TRANSLATION: CHINESE TRANSLATION: Overview Article of the "Guidelines for International Breast Health and Cancer Control" (CANCER Vol 113/8 Oct 15-08) *Developed through BHGI collaboration with Shanghai Center for Disease Control*

Translation Available www.bhgi.info

EASTERN EUROPE

PILOT PROJECT: SURVEY ON A PILOT MAMMOGRAPHIC SCREENING PROGRAM, TURKEY

Pilot Research Project in Bahcesehir Istanbul, Turkey

Funding for this project via Komen for the Cure® sub-award;

Principal Investigator: Vahit Ozmen, MD, Istanbul Faculty of Medicine, Istanbul University

Research Advisors: Benjamin O. Anderson, MD; David B. Thomas, MD, DrPH, Senior International Research Advisor, Breast Health Global Initiative, Division of Public Health Sciences, Program in Epidemiology, Fred Hutchinson Cancer Research Center, Department of Epidemiology, School of Public Health and Community Medicine, University of Washington – **PROJECT COMPLETED**

TRANSLATION: RUSSIAN TRANSLATION:

Overview Article of the "Guidelines for International Breast Health and Cancer Control" (CANCER Vol 113/8 Oct 15-08) *Developed through BHGI collaboration with Dr. Soldak, Director of Programs, Resource & Policy Exchange, Inc. with The Blokchin Institute in Moscow and Novartis*

Translation Available www.bhgi.info

LATIN AMERICA**PILOT PROJECT: PILOT INTRODUCTION OF BREAST CANCER EARLY DETECTION PROGRAMS**

(*opportunistic screening*), in Bogotá, Colombia

Funding for this project via Komen for the Cure® sub-award;

Principal Investigator: Raul Murillo, MD, National Cancer Institute, Colombia

Research Advisors: Benjamin O. Anderson, M.D.; David B. Thomas, MD, DrPH, Senior International Research Advisor, Breast Health Global Initiative, Division of Public Health Sciences, Program in Epidemiology, Fred Hutchinson Cancer Research Center, Department of Epidemiology, School of Public Health and Community Medicine, University of Washington – **PROJECT ONGOING**

PILOT PROJECT: READINESS ASSESSMENT FOR IMPLEMENTATION OF BREAST CANCER TREATMENT GUIDELINES,

in Bogotá, Colombia

Funding for this project via grant from Puget Sound Partners for Global Health;

Principal Investigator: Benjamin O. Anderson, MD of the University of Washington, coordinated through Raul Murillo, MD, National Cancer Institute, Colombia, in Bogotá, Colombia,

Research Advisor: David B. Thomas, MD, DrPH, BHGI Senior International Research Advisor, Breast Health Global Initiative, Division of Public Health Sciences, Program in Epidemiology, Fred Hutchinson Cancer Research Center, Department of Epidemiology, School of Public Health and Community Medicine, University of Washington – **PROJECT ONGOING**

PILOT PROJECT: LAEDI BRACA – LATIN AMERICAN EARLY DETECTION INITIATIVE FOR BREAST CANCER

Implementation of science research programs aimed at both the public sector and medical establishment to achieve down-staging of breast cancer; initial Pilot Project in Mexico in collaboration with the Mexico National Cancer Institute and the Seattle Cancer Care Alliance.

Principal Investigator: Gabriela Torres Mejia, Directora de Diabetes Mellitus y Cáncer
Dirección de Investigación en Enfermedades Cardiovasculares y Cáncer Mexico

Director of Diabetes Mellitus and Cáncer, Direction of Investigation in Cardiovascular Diseases and Cancer; Co-PI: Benjamin O. Anderson, MD– **PROJECT IN DEVELOPMENT**

TRANSLATION: SPANISH, PORTUGUESE DUAL

TRANSLATION: Overview Article of the “Guidelines for International Breast Health and Cancer Control” (*Cancer* Vol 113/8 Oct 15-08)

Developed through BHGI collaboration with PAHO-Pan American Health Organization

Translation Available www.bhgi.info

GLOBAL PROGRAMS**SCIENTIFIC GRANT REVIEWS**

Co-chaired by Benjamin O. Anderson, MD and Peggy Porter, MD, Member, Divisions of Human Biology and Public Health Sciences, Fred Hutchinson Cancer Research Center; Head, Women’s Cancer Research Program: Breast Section, Fred Hutchinson/University of Washington Cancer Consortium; Professor of Pathology, University of Washington

Reviews completed and pending, to date

Grant reviews (3): January 10-11, 2009; November 15-16, 2009; October 8-9, 2010 – **COMPLETED**

Grant review (1): Fall 2011 – **UPCOMING**

Funding provided for reviews completed and pending, to date, by the Glaxo SmithKline Oncology-Ethnic Research Initiative

External scientific grant reviews provide an essential service to organizations that fund research. Consequently, it is an aim of the Breast Health Global Initiative to be part of the process that encourages funding of international research. Through the Glaxo SmithKline Oncology (GSK) Ethnic Research Initiative (ERI), BHGI has organized and managed scientific reviews of ERI grant applications to identify viable international pilot projects for funding through the GSK/ERI funding mechanism.

Dr. Peggy Porter and I co-chair the BHGI Scientific Review Committee of the GSK ERI, which meets at the Fred Hutchinson Cancer Research Center in Seattle to review the grant applications. Dr. Porter is Full Member in the Divisions of Human Biology and Public Health Sciences, and Head of the Breast Cancer Research Program at the Fred Hutchinson Cancer Research Center and Professor in the Department of Pathology, University of Washington.

**BHGI INFORMATICS PORTAL (WWW.BHGI.INFO):
BREAST HEALTH-COMMON INTEREST GROUP**

Funding via grant from U.S. National Cancer Institute –

PROJECT ONGOING

An integral aspect of the BHGI's implementation mission and implementation plan focusing on breast cancer early detection in low- and middle-resource countries (LMCs) is development of a virtual environment to facilitate transformational collaborative opportunities and innovative 'system-based' solutions to address specific issues of optimizing breast health care and cancer treatment in LMCs.

In collaboration with the National Cancer Institute (NCI), the Breast Health Global Initiative (BHGI) developed a technical foundation (www.bhgi.info), and launched the first "**Breast Health – Common Interest Group**" (BH-CIG), endorsed by the International Union Against Cancer (UICC). The CIG concept was introduced by the UICC Board of Directors to create a common venue for individuals and organizations dedicated to finding innovative solutions to critical cancer control issues. The aims of the BH-CIG are to support the global breast health community, facilitate collaboration on breast healthcare and cancer control in LMCs, and increase the UICC global network. Now functional and online, the BH-CIG provides a platform for information dissemination to researchers, healthcare providers, NGOs and healthcare ministries working in LMCs. The BH-CIG is a technical framework providing interactive modules:

- Member directory
- Searchable literature library
- Focus groups
- Secure member discussion
- Key links
- Information sharing areas
- Secure project workspaces.

Professionals from around the world can utilize this intuitive resource by logging onto www.bhgi.info and signing into the **Common Interest Group**. Registered users having an interest in breast cancer in LMCs will not only have access to resources on this topic, but will be able to interact with colleagues from around the world with similar interests.

MAKING LITERATURE ACCESSIBLE TO LOW- AND MIDDLE-INCOME COUNTRIES

The BHGI Literature Library has a free searchable literature database of articles relevant to breast healthcare and cancer control oriented to low- and middle-resource countries. The original library database of articles was assembled by the **U.S. Centers for Disease Control & Prevention (CDC)** through a generous grant provided by the **Susan G. Komen for the Cure®**. In addition to the CDC contribution, the International Network for Cancer Treatment and Research contributed articles to the literature database.

BHGI and the Office of International Affairs of the U.S. National Cancer Institute (NCI) jointly coordinate the use of the portal and the Breast Health-Common Interest Group, with oversight from the (UICC).

Currently, the BHGI is consulting with the Department of Global Health Distance Learning Unit of the I-Tech-International Training and Education Center on HIV, at the University of Washington, to develop a platform for Learning Management System (LMS) on www.bhgi.info.

FINANCIAL BENEFACTOR ORGANIZATIONS OF THE BHGI

Breast Health Global Initiative, 2010, 2011 strategic global alliance of governmental, NGO, non-profit and for-profit health organizations

FOUNDING ORGANIZATIONS

Fred Hutchinson Cancer Research Center / Susan G. Komen for the Cure®

2010 GLOBAL SUMMIT ON INTERNATIONAL BREAST HEALTH

“Optimizing Healthcare Delivery” was convened in association with the Latin American and Caribbean Society of Medical Oncology-SLACOM

SUSTAINING SCIENTIFIC ORGANIZATION PARTNER

U.S. National Cancer Institute, Office of International Affairs / Scientific Partners / American Society of Clinical Oncology / European School of Oncology / Pan American Health Organization / Sheikh Mohammed Hussein Al-Amoudi Center of Excellence in Breast Cancer / The Lancet Oncology, Elsevier / Corporate Partners / GE Healthcare / Sanofi Aventis

COLLABORATING ORGANIZATIONS

LiveSTRONG / U.S. Centers for Disease Control and Prevention / U.S. Department of Health and Human Services, Office on Women’s Health / U.S. National Cancer Institute, Office of Science Planning and Assessment / U.S. National Institutes of Health, Office of Research on Women’s Health

PARTICIPATING ORGANIZATIONS PROVIDING IN-KIND SUPPORT TO BHGI

American Society of Breast Disease / World Health Organization / International Union Against Cancer / International Network for Cancer Treatment and Research / International Atomic Energy Agency of the United Nations / International Society of Nurses in Cancer Care / Middle East Cancer Consortium / Oncology Nursing Society / Breast Surgery International / Woman’s Cancer Foundation

RELATED PUBLICATIONS

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David R. Flum, M.D., M.P.H.

• Outcomes Research



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Professor of Surgery
Adjunct Professor, Department
of Health Services
Director, Surgical Outcomes
Research Center (SORCE)

Over the last decade, “outcomes” research became a catch phrase for healthcare administrators, providers and researchers, but outcomes research means different things to different people. For some, it is viewed as a way to provide more services for fewer dollars. For others, it means finding ways to regulate physician variability to improve care. Neither of these definitions fully describes the potential of this form of research. I believe outcomes research means moving beyond a research culture that shows us what can be done by surgeons, to one that emphasizes what should be done by surgeons. The “should” in that statement indicates a balance of the feasibility of an operative procedure with an assessment of the burden of that operation on the patient and society. Only by determining the impact of procedures in their totality can we understand what should be done rather than simply what can be done.

To do this, we have to consider the impact of the operation on the patient’s life, both in the context of life expectancy and quality of life, while assessing the burden of that intervention for the patient and society. Since the publication of the Institute of Medicine report, *To Err is Human*, the public has focused on the “burden” of the healthcare system as it refers to adverse outcomes and medical errors. Answering the question, “What should we be doing?” requires that we address these adverse clinical outcomes in the context of system-level quality improvement.

To do this, outcomes researchers use a set of tools borrowed from health economics, decision analysis, epidemiology and biostatistics. To address this goal of system-level quality improvement for all areas of clinical interest, we use these tools to answer four necessary questions.

Can we determine the way surgical procedures impact the average patient?

Risk of adverse outcome is a component of all surgical procedures. While the informed consent process tries to address this by providing the patient with a summary of the expected risk, in fact what we really offer in the consent process are the results found in the published case series of the best practitioners in the field. For the vast majority of general surgical procedures, we simply don’t know the community-level risk of adverse outcome. As such, we are unable to determine what should be considered the standard, who are the outliers (both good and bad), and what techniques work outside of the research environment. In the absence of a tracking system for outcomes, we often rely on estimates derived from randomized trials (which for most general surgical procedures have not been completed) or administrative data. Only by understanding the real level of risk can we determine the opportunities for improvement in the system.

The research I have been involved with has addressed this issue of community-level risk in commonly performed general surgical procedures by using administrative data. Determining population-level risk requires the analysis of large databases. For example, in evaluating rates of misdiagnosis in appendectomy, we studied 80,000 patient records and found that the rate of misdiagnosis in appendicitis has not improved in the past 13 years (~15% overall and ~25% in women of reproductive age) despite the growing availability of CT scanning. We studied over 30,000 patients undergoing cholecystectomy to describe the rates of major common bile duct (CBD) injury over time and found that rates of this outcome (0.025%) have not significantly improved with time.

I believe outcomes research means moving beyond a research culture that shows us what can be done by surgeons, to one that emphasizes what should be done by surgeons.

To study outcomes from antireflux procedures, we studied over 86,000 patients and found that while the rates of splenectomy have decreased significantly with time, the rates of in-hospital mortality and esophageal injury have not. Furthermore, while the rates of adverse outcome identified were low (~2% chance of splenectomy, <1% likelihood of death, ~1% chance of esophageal injury), these rates were between 2 and 20 times higher than results published in large case series.

This illustrates the importance of population-level results in estimating risk for the average patient. This research technique is also helpful in checking conventional wisdom about the benefits of new technology. For example, of ~10,000 patients undergoing incisional hernia repair, we quantified the rate of reoperative repair and found no improvement in this measure of recurrence in the era of laparoscopy. It is also important in addressing two important forms of bias in published estimates of outcome. Cholecystectomy-related bile duct injury is the leading source of surgical malpractice claims. Determining outcome after bile duct injury is challenging because the results of surgical experts are excellent (publication bias) while reports of cases that progress to litigation (selection bias) detail dismal outcomes.

We recently evaluated the risk of death after bile duct injury among all Medicare beneficiaries nationwide, and found they were 2.5 times more likely to die within the

first few years after an injury compared to uninjured patients (Figure 1). Another way to assess the impact of care is to quantify patient-described outcomes as they relate to quality of life, function and well-being. Standard quality-of-life instruments measure chronic health states and do not adequately capture the dynamic process of pre-operative states, anticipatory stress, post-operative morbidity and then evolution to either recovery or chronic states. Working with industry, we are developing an internet-based interactive survey instrument aimed at capturing, quantifying and validating changes in Quality Adjusted Days (QAD) “lost” over the relevant time course of a patient. We hope that “lost” QADs will be an important outcome measurement tool that captures the patient-level burden of surgical procedures. By quantifying outcomes both on an individual and community level, we can then move on to the next step in improving clinical outcomes.

What are the avoidable factors associated with these adverse outcomes?

Health services researchers believe that most adverse outcomes have a system-level component. While all individuals make mistakes, it is a flawed system that allows these mistakes to adversely impact the patient. To that end, there are almost always avoidable factors that are associated with adverse outcomes. Understanding those associations and quantifying their impact is an important step in the quality improvement process. For example, using administrative data, we have quantified the degree to which both surgical inexperience and the failure to use a cholangiogram are associated with CBD injury. Surgical inexperience (a surgeon’s 1st through 19th cholecystectomies) and failure to use a cholangiogram result in a 60-70% increase in the likelihood of CBD injury. When combined, these factors have even greater impact. Surgeons are 2.2 times more likely to have a CBD injury during their first 20 operations if they do not use a cholangiogram compared to procedures performed at later points in the experience

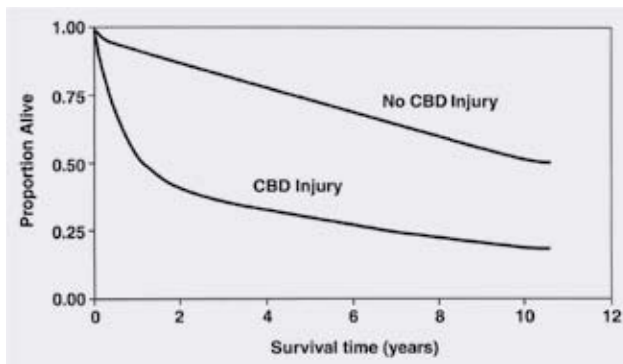


FIGURE 1. Survival after bile duct injury in Medicare beneficiaries (n = 1.57 million)

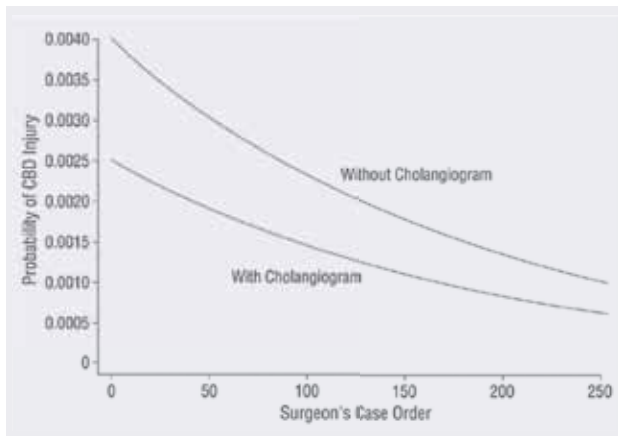


FIGURE 2. Probability of bile duct injury with and without cholangiogram, by case-order of surgeon (n = 36,000)

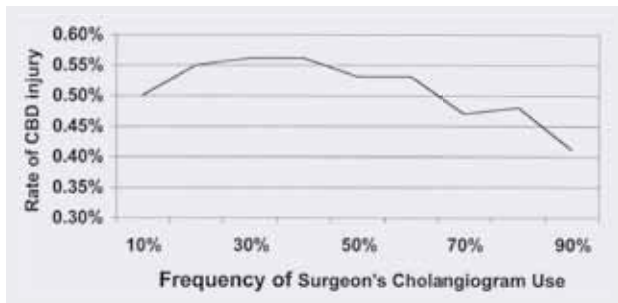


FIGURE 3. The effect of increasing the surgeon's frequency of cholangiogram use on the rate of common bile duct (CBD) injury

curve. Defining the risk relationship associated with CBD injury is also important in informing patients and surgeons of the predicted probability of this adverse outcome (Figure 2). This may be a more effective way of “informing” the informed consent process. This work was reinforced by a study of all Medicare beneficiaries undergoing cholecystectomy. In that study, we found that patients who did not have a cholangiogram were approximately 70% more likely to have had a CBD injury. We also determined that this “protective” effect of cholangiography was noted whether or not the surgeon was a routine or infrequent cholangiographer. The lowest rates of injuries were found among routine cholangiographers (Figure 3).

What are the implications (using cost/decision analysis and randomized trials) of avoiding those factors?

Once we have quantified the problem and determined the avoidable factors that influence these outcomes, we can try to imagine what the practice of clinical surgery would be like with these factors controlled. For example, a recently completed cost and decision analysis demonstrated that if routine cholangiograms were required, the cost per CBD injury avoided would range between \$50-86,000. The incremental cost per operation of adding the cholangiogram would be \$100. When considering the overwhelming costs (both system wide and medico-legal) of a CBD injury, this may be considered a cost effective intervention. Another example is a cost analysis showing that nationwide, nearly \$740 million is spent each year on misdiagnosed appendicitis. Modeling potential ways to improve care is also being applied in a theoretical decision and cost analysis for routine CT scanning of patients with presumed appendicitis and teleproctoring in antireflux surgery. These models are often helpful when the practical barriers of a randomized trial are significant. With colleagues in the Division of General Surgery, we are hoping to develop and obtain funding for randomized trials in the management of appendicitis (routine versus selective CT scan use), for incisional hernia (laparoscopic versus open), and for the optimal management of patients with diverticulitis.

How can we make system level changes and monitor the impact of those changes?

The ultimate goal of this work is to improve surgical care for the average patient in the average hospital. The first steps are detailed above and involve getting good data and performing effective analyses. The next step is system-level change either on the local, professional organization, or statewide level. Another opportunity for system-level change is found in working with the main financial stakeholders. For example, in coordination with administrators from the Healthcare Financing Administration (Medicare) we are helping to determine the mechanisms that could be used to increase the number of cholangiograms performed nationwide. Similarly, administrators at Group Health Cooperative are interested in optimizing the care of patients with presumed appendicitis, and look to our analysis of their CT scan use as an opportunity to determine future care pathways.

In collaboration with the Washington State Health Care Authority, the Center for Medicare Services, the Foundation for Healthcare Quality, Medicaid and Qualis, our group is developing a statewide system for helping hospitals identify adverse outcome outlier status and use the techniques of the quality improvement community to address outliers. This Surgical Clinical Outcomes Assessment Project (SCOAP) is part of a 5-year project to create a surgical quality infrastructure in the state that will assure the incorporation of evidence-based approaches to surgical care in common practice. (<http://depts.washington.edu/sorce/>).

Involving the financial stakeholders may be the most effective way to improve system level care, but it may not be the best way. Over the last century, the surgical community has shown real leadership in addressing adverse outcomes and taking responsibility for them. The morbidity and mortality conference, so long a part of the surgical culture, was ahead of its time in trying to improve the results of future interventions by avoiding past mistakes. Unfortunately, it has become apparent that conferences alone cannot deal with system-level factors involved in adverse outcome. Outcomes researchers are doing just that, and the surgical community has an opportunity to use this research in leading the way towards quality improvement.

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DEPARTMENT CO-INVESTIGATORS

Alexander W. Clowes, MD / E. Patchen Dellinger, MD / Farhood Farjah, MD, MPH, Research Fellow / Karen D. Horvath, MD / Sara H. Javid, MD / Steve Kwon, MD, Research Fellow / Nader N. Massarweh, MD, MPH, Research Fellow / Mark H. Meissner, MD / Carlos A. Pellegrini, MD / Benjamin Starnes, MD / Erik G. Van Eaton, MD / Thomas K. Varghese, MD, MS / Andrew S. Wright, MD / Raymond S. Yeung, MD

OTHER UW CO-INVESTIGATORS

Dedra S. Buchwald, MD, Medicine/Director, Center for Clinical and Epidemiological Research / Jens R. Chapman, MD, Orthopaedics / David E. Cummings, MD, Medicine / Beth Devine, PharmD, MBA, PhD, Pharmacy / Ardith Doorenbos, PhD, RN, Biobehavioral Nursing and Health Systems / Todd Edwards, PhD, MA, Health Services / Joann G. Elmore, MD, MPH, Medicine / Karen E. Foster-Schubert, MD, Medicine / Thomas Gallagher, MD, Medicine / Louis Garrison, PhD, Pharmacy / Barbara A. Goff, MD, Obstetrics and Gynecology / Jack Goldberg, PhD, MA, Epidemiology/Center for Clinical and Epidemiological Research / John L. Gore, MD, MS, Urology / Jeff Henderson, MD, Center for Clinical and Epidemiological Research / Jeffrey Jarvik, MD, MPH, Radiology/Director, Comparative Effectiveness, Cost, and Outcomes Research Center / Larry Kessler, ScD, Health Services / Mario Kratz, PhD, MSc, Epidemiology / Michael J. Lee, MD, Orthopaedics / Jerry P. Palmer, MD, Medicine / Donald Patrick, PhD, Health Services/Director, Seattle Quality of Life Group / Michael P. Porter, MD, Urology / Lisa Strate, MD, MPH, Gastroenterology / Sean D. Sullivan, PhD, Pharmacy / Peter Tarczy-Hornoch, MD, Pediatrics / Melissa M. Thrall, MD, Research Fellow – Obstetrics and Gynecology / Hunter Wessells, MD, Urology / David Yanez, PhD, MS, Biostatistics / Meliha Yetsigen-Yildiz, PhD, MSc, Biomedical and Health Informatics / Michi Yukawa, MD, MPH, Medicine

COMMUNITY CO-INVESTIGATORS

David Arterburn, MD, MPH, Group Health Research Institute / Ellen Farrokhi, MD, Virginia Mason Medical Center / Rosa Johnson, ARNP, MN, CPHQ, Foundation for Health Care Quality / Eric Larson, MD, MACP, Group Health Research Institute / Justine Norwitz, JSN Consulting / Terry Rogers, MD, Foundation for Health Care Quality / Neal Shonnard, MD, Proliance Surgeons



LEFT TO RIGHT: Oelschlager, Pellegrini, Tatum, Wright, Khandelwal

Brant K. Oelschlager, M.D.; Professor of Surgery; Byers Endowed Professor in Esophageal Research

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

Roger P. Tatum, M.D.; Assistant Professor of Surgery; Director, Student Education

Andrew S. Wright, M.D.; Assistant Professor of Surgery

Saurabh Khandelwal, M.D.; Acting Assistant Professor of Surgery

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I. GASTROESOPHAGEAL REFLUX DISEASE (GERD)

I.A. BROAD ASSESSMENT OF CLINICAL OUTCOMES AFTER LAPAROSCOPIC ANTIREFLUX SURGERY (LARS)

There is considerable discussion regarding “success” rates for laparoscopic anti-reflux surgery (LARS). Our experience with the long-term follow-up of these patients taught us that the success or failure of this operation is much more difficult to define. For example, there are patients who are extremely satisfied with the degree to which their symptoms have been controlled even though the esophageal acid exposure has not completely returned to normal. On the other hand, there are patients whose presenting symptoms have essentially disappeared but who have developed substantial side effects of the operation and for whom the quality of life has deteriorated. As our experience grew, we realized that instead of talking about “success” or “failure” of the operation, we ought to analyze the multiple domains affected by the operation and try to define the results in the context of each domain. We felt this type of comprehensive assessment would bring clarity that would allow physicians to

understand how to best describe the expectations to patients in the different domains – the ultimate way to define a true informed consent.

Toward this end, we analyzed our extensive experience over a substantial number of years taking a broad view, attempting to document outcomes in what we thought were the major and most important domains, thereby allowing for a more thorough assessment of LARS. Thus, our work is not necessarily an endorsement of LARS, but instead a thorough analysis of all areas affected, positively and negatively, by the operation. This should provide the kind of data that would allow patients and physicians to decide for themselves whether the operation fits their needs.

We hypothesized that, in part, this was a reflection of the outcome variables used. We therefore defined 8 specific variables (within 3 categories) and assessed outcomes for each in a large cohort of patients (Table 1).

METHODS: Four hundred patients (208 females; median age 52y/o) who underwent LARS at the University of Washington from 1993 to 2008 were given a comprehensive questionnaire to assess various aspects of their outcomes from LARS. In addition, we analyzed all functional studies and all endoscopies performed in these patients in our institution, whether the patients had symptoms or not, and compared the findings to all available preoperative values.

RESULTS: The median follow-up was 92 (6–175) months.

A. SYMPTOMS:

- Effect on presenting symptoms:* Heartburn (n = 376) improved in 326 (87%), regurgitation (n = 365) improved in 331 (91%), and chest pain (n = 265) improved in 207 (78%), measured by patient's perception.
- Durability:* The percentage of patients with successful control of symptoms of gastroesophageal reflux disease (GERD): 88% at 1 year, 83% at 2 years, 77% at 5 years, and 74% after 10 years.

- Development of new symptoms (side effects):* The following side effects developed (were new) or worsened (present before surgery): dysphagia in 72 (18%) patients; bloating in 96 (24%) patients; diarrhea in 61 (15%) patients. The severity (0–10 scale) of these symptoms were: dysphagia 5.1 ± 2.6 , bloating 6.5 ± 2.2 , diarrhea 6.5 ± 2.9 .

- Patient perception of overall success:* Currently, 279 (70%) patients rate their operation as a complete success, 86 (22%) as partially successful and 35 (8%) as unsuccessful. Those with partial or no success cited recurrent reflux (n = 70), a side-effect (n = 37), or both (n = 14) as the reason.

B. EFFECTS ON ESOPHAGEAL FUNCTION AND MUCOSAL INTEGRITY:

- Esophageal Acid Exposure:* The average pre-op DeMeester (DM) score was 56.8 ± 48.1 (n = 320), which decreased to 16.0 ± 31 in patients who underwent post-op testing between 1 month and 1 year (n = 149). Among 129 patients who had both pre-op and post-op values available, 92 (71%) had normalization of a previous abnormal DM score, while 114 (88%) had at least some improvement.

TABLE 1: Outcome domains of LARS

DEFINITIONS OF SUCCESS	SUCCESS RATE (%)
A. Symptoms (subjective)	
Improvement of the primary presenting symptom.	89
Full resolution of a GERD symptom (heartburn, regurgitation or chest pain)	54-76
Improvement of <i>any</i> GERD symptom.	87-91
Complete success of LARS by the patient's perception	70
Complete or partial success of LARS by the patient's perception	92
Complete or partial success of LARS by the patient's perception due solely to poor control of reflux symptoms	79
Absence of any side effect after LARS (dysphagia, bloating, diarrhea).	34
No newly developed or worsening side effects (dysphagia, bloating, and diarrhea) after LARS	62
B. Acid exposure and mucosal integrity	
Normalization of DeMeester score at post-op follow-up	71
Prevention of development of Barrett's esophagus	97%
Prevention of development of high grade dysplasia or cancer in patients with previous diagnosis of Barrett's esophagus	97%
C. Need for additional therapy	
Taking a lower dosage of anti-acid medication after LARS	77
Taking no anti-acid medication after LARS	59
Improvement of GERD symptoms accepting the use of anti-acid medication after LARS	98
No need for revisional operation	96.3

2. Mucosal integrity: Progression of Barrett's esophagus (BE):

Of the 58 patients with BE before LARS, 2 developed high grade dysplasia (HGD)/cancer (or 1 per 258 patient years). Out of 342 patients in our study without BE prior to LARS, 9 developed BE at a rate of 1 per 275 patient years (0.36% per year).

C. NEED FOR ADDITIONAL THERAPY:

1. Medication use: 236 (59%) patients remain completely off medications for GERD; 164 (41%) are using antireflux medications. Of these, 73 (45%) patients are taking less medication than before LARS. The most common reasons for continuing GERD medication after LARS were: heartburn (n = 100) 60%, regurgitation (n = 13) 8%, Barrett's esophagus (n = 13) 8%.

2. Reoperations: Fifteen (3.7%) patients required reoperations, 9 for recurrent reflux and 6 for side-effects.

CONCLUSION: The success or failure of LARS cannot be defined in a single domain. A comprehensive analysis of outcomes requires categorization that includes symptom response, side effects, patient's perception and objective measurement of acid exposure, mucosal integrity, and the need for additional medical or surgical treatment. Only then can patients and physicians better understand the role of LARS and make informed decisions.

I.B. Pepsin

I.B.i. IT IS NOT ABOUT ACID ANYMORE: THE ROLE OF WEAK ACID, IMPEDANCE, AND PEPSIN IN THE PATHOGENESIS OF GERD-RELATED LARYNGITIS

Gastroesophageal reflux disease (GERD) is the most common gastrointestinal disease in the United States. While most patients suffer from typical esophageal manifestations (e.g., heartburn and regurgitation), it is recognized that many patients experience laryngeal manifestations (e.g., cough, hoarseness, globus sensation) that are increasingly being linked to GERD. It is assumed that a common pathway for these manifestations is aspiration, whereby gastric material travels proximally and traverses the upper esophageal sphincter (UES) to enter the laryngopharynx. As a result, many now refer to this as laryngopharyngeal reflux or LPR. The clinical spectrum of LPR is wide and includes laryngeal injury (cough, hoarseness, subglottic stenosis, laryngeal cancer), dental erosion, asthma, and other respiratory tract disorders (e.g., chronic cough, sinusitis, and recurrent pneumonia).

It is estimated that 3–10% of the US population has significant LPR symptoms for which treatment is sought, 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, potentially containing acid, pepsin and bile acids. To date, diagnosis and treatment have 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 (PPIs) 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) has demonstrated a strong association of non-acidic reflux with laryngeal symptoms and injury. Three, we have also demonstrated in prior work a neutralizing effect on refluxate as it extends up the esophagus, so that by the time the refluxate can be aspirated, its pH is usually > 4. Four, 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.

Medical therapy provides relief to some patients, but with less consistency than for those with typical symptoms of GERD. This may be due to persistent aspiration and injury components of the refluxate. Surgical therapy has been more successful in treating some of these disorders, although its effectiveness is inferior in this group of patients when compared with patients who have typical symptoms. Therefore, while patient selection is key in directing the treatment of GERD, patient selection is even more necessary for reflux-associated respiratory disorders such as LPR.

The problem is partly due to lack of a good diagnostic test. Typically, the diagnosis of GERD is made by a combination of thorough patient history plus several imaging and physiologic studies, with 24-hour ambulatory pH monitoring considered the "gold standard" for the diagnosis of GERD. While 24-hr pH monitoring can diagnose GERD, there is no specific test in patients with airway disease for linking respiratory symptoms with GERD. As a result, even effective treatment and resolution of GERD does not always result in the abatement of symptoms. There are two shortcomings of pH monitoring, especially in the pharynx. One is the sole reliance on pH, even though reflux that travels from

Natural Orifice Transluminal Endoscopic Surgery (NOTES) represents a paradigm shift that may significantly change the management of gastrointestinal and intra-abdominal diseases. It is an exciting time. Not since the adoption of laparoscopic surgery 20 years ago has there been such a radical change in the way surgery is viewed.

the stomach to the pharynx may no longer have a pH<4, and injury from aspiration may not be dependent on acid. The other is that the pharynx, unlike the esophagus, is a larger cavernous cavity that does not collapse. It constantly contains both liquid and gas and makes catheter-based methods of detecting reflux problematic. There are two new technologies that may combat these shortcomings.

The first is a newly designed pH probe. The Restech probe (24-hour pharyngeal pH measurement) is a new minimally invasive device used to measure acid exposure in the posterior oropharynx. This probe is able to detect aerosolized acid and does not require endoscopy or manometry for proper placement. Recently, a set of normal values and discriminating thresholds for pharyngeal acid exposure using the Restech probe was described in the literature. The 95th percentile values (normal) for the components and the composite score of pharyngeal Ph exposure at the discriminating pH thresholds are analyzed separately because the mean pharyngeal pH is lower during the supine period than in the upright period. This is because salivary flow is reduced during the night, resulting in a lower pharyngeal pH. The calculated threshold for the upright period is 5.5 and for the supine period 5.0. The components with normal values established are: % time pH below the threshold: 0.13 and 5.15 minutes; number of episodes: 1 and 4; longest episode: 0.71 and 18.97 minutes; and RYAN Score (composite pH score for pharyngeal acid exposure): 9.41 and 6.79 for upright and supine period, respectively.

The second technology is multichannel intraluminal impedance (MII), which has been introduced to measure bolus presence and transit and to detect reflux independent of its pH. MII permits not only identification of liquid, gaseous, or mixed intraesophageal/intrapharyngeal materials, but also the direction in which the elements travel. MII technology in conjunction with a pH sensor allows discrimination of acid (pH < 4.0) from non-acid (pH 4 and above) reflux. Our group has recently demonstrated that the majority of the reflux episodes into the pharynx are

in fact non-acidic. In addition, we have also shown that in patients with reflux-related laryngitis, the amount of non-acidic reflux in the distal esophagus, as well as in the pharynx, is greater than in controls. Thus, the traditional 24-hour pH monitoring system may underestimate the extent of reflux that may be playing a significant role in the pathogenesis of LPR.

Laryngoscopy is a common screening tool for patients with symptoms such as hoarseness, cough, and laryngitis. This often reveals erythema, nodularity, ulceration, granuloma, or leukoplakia, but to date no single finding seems to be pathognomonic of reflux-induced laryngeal reflux. We note that laryngoscopic findings have strengthened the case for a positive response to laparoscopic Nissen fundoplication (LNF), but have not been seen to have a strong predictive value.

Still, MII and Restech, even at the level of the pharynx, are indirect measurements. Pepsin may represent a more direct and accurate tool in the work-up of patients thought to have reflux-related laryngitis. We have reported the presence of pepsin in laryngeal biopsy specimens taken from patients with clinically diagnosed LPR, not detected in "normal" control subjects. Furthermore, we have recently discovered that pepsin is taken up by laryngeal epithelial cells by receptor mediated endocytosis, irrespective of its proteolytic activity. It was originally thought that pepsin would only cause injury in acidic refluxate. However, we have shown that pepsin, at pH 7, causes intracellular (mitochondrial) damage in laryngeal epithelial cells (unpublished data). Using electron microscopy, we have demonstrated the presence of pepsin in late endosomes and in the trans-reticular Golgi (TRG), which is approximately pH 5 (unpublished data). Thus, it is possible that inactive pepsin (in non-acidic reflux) is taken up by laryngeal epithelial cells and reactivated in either late endosomes or in the TRG, due to their lower pH, causing intracellular damage. Alternatively, binding/activation of the cell surface receptor may cause a cell

signaling event, ultimately having a detrimental effect on the cell. This novel mechanism of peptic injury could explain why many patients with reflux-attributed laryngeal injury have persistent symptoms despite acid suppression therapy. Ongoing studies to identify the receptor will help delineate the role of pepsin in reflux-attributed laryngeal injury. Pepsin inhibitors and receptor antagonists are being tested *in vitro* to investigate whether they prevent peptic uptake/injury and thus have therapeutic potential.

Currently, it is well known that LNF results in good short- and long-term relief of airway symptoms, even for patients with poor responses to medical therapy. However, the reported efficacy of LNF for airway symptoms (65-75%) is inferior to that reported for heartburn and regurgitation (90%). Failure to correctly identify the patients who will respond to LNF is the most likely reason for not reaching 90% to 100% success. This highlights the lack of a diagnostic test or algorithm with the necessary accuracy to link GERD and airway disease in all patients.

Therefore, we propose a prospective study of 20 patients with clinical LPR who are being considered for LNF, whereby each patient would have reflux measurements with Restech and MII, as well as have laryngeal pepsin checked before and after LNF. Our hypothesis is that LNF will result in the elimination of reflux from the esophagus and pharynx, and pepsin from the laryngeal epithelium. If this

is confirmed with testing after LNF has been performed, we should be able to more fully understand the pathophysiology of LPR and assess the relative value of these tests to predict the response of LPR to LNF.

I.C. LINX™ Reflux Management System Clinical Study

Since there are so many patients with GERD and no perfect therapy, we are constantly looking for the perfect solution, or more likely, solutions to fit a subset of the GERD population that will address their specific problems. A possible solution for some patients is the LINX™ Reflux Management System. This device is designed to help people with GERD by reinforcing or strengthening the esophageal sphincter function.

Torax™ Medical, Inc. has designed a device to help facilitate the function of the lower esophageal sphincter (LES). The LES is a ring of muscle that forms a valve at the lower end of the esophagus, where it joins the stomach. A healthy LES opens when food is swallowed, allowing food to pass into the stomach. The LES then closes after the food has passed to prevent the stomach contents from going back into the esophagus (Figure 1).

The LES is considered an important part of preventing reflux, and can sometimes become weak, allowing gastric contents to “reflux” into the esophagus (Figure 2).

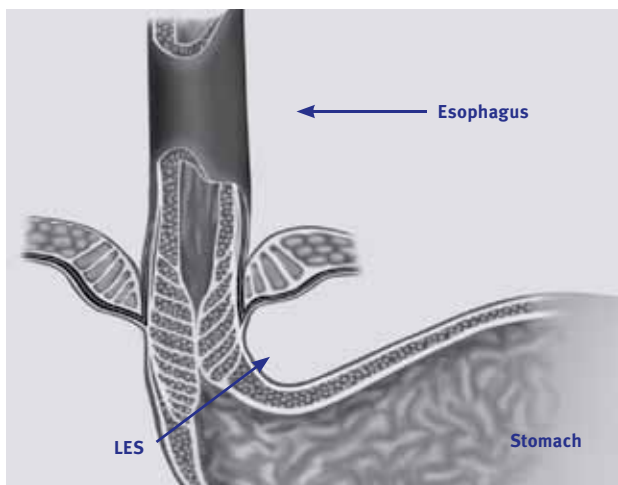


FIGURE 1. Normal, healthy lower esophageal sphincter

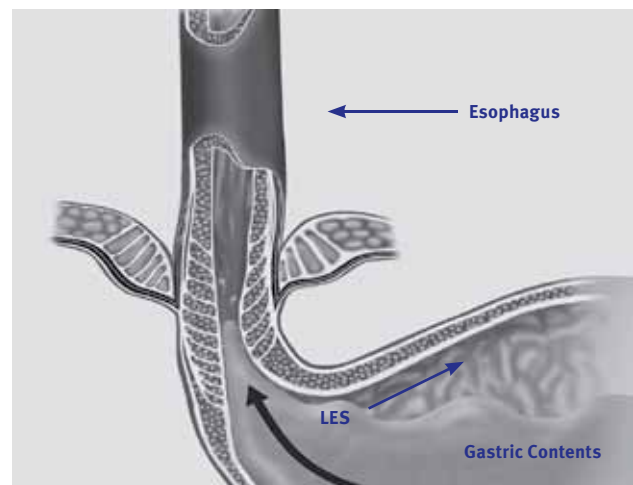


FIGURE 2. Weak LES allowing gastric contents to reflux into esophagus

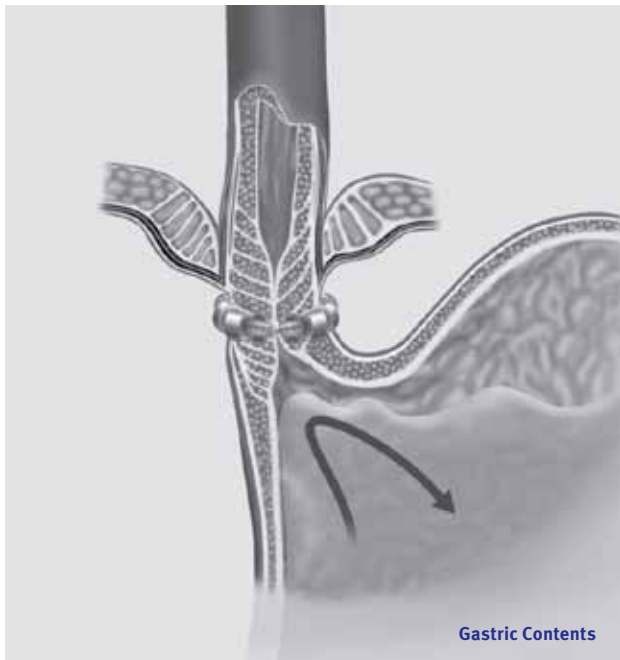


FIGURE 3. LINX™ device in place on esophagus

An implantable device consists of a series of magnetic beads that are attached with wires to create a ring shape. The device is placed on the external esophagus in the region of the LES. The attractive force of the magnetic beads provides additional strength to keep a weak LES closed (Figure 3).

As food is swallowed, the magnetic force decreases as the magnetic beads move away from each other on the wires. This allows the esophagus to stretch open, much like a good working esophagus, and allows the swallowed food to pass into the stomach (Figure 4).

The device is a permanent implant for the treatment of GERD. The procedure is reversible and can be surgically removed if necessary. Removal of the device does not likely have any harmful effects on other treatments for GERD.

The University of Washington, together with nine other clinical sites in the United States, participated in a multi-centered prospective trial to evaluate the safety and efficacy of this device in patients with moderate GERD. One hundred patients were implanted with the device. This fall we hope to complete the one-year follow-up on the first 100 patients and be able to evaluate what role this may play in the large population of patients with GERD.

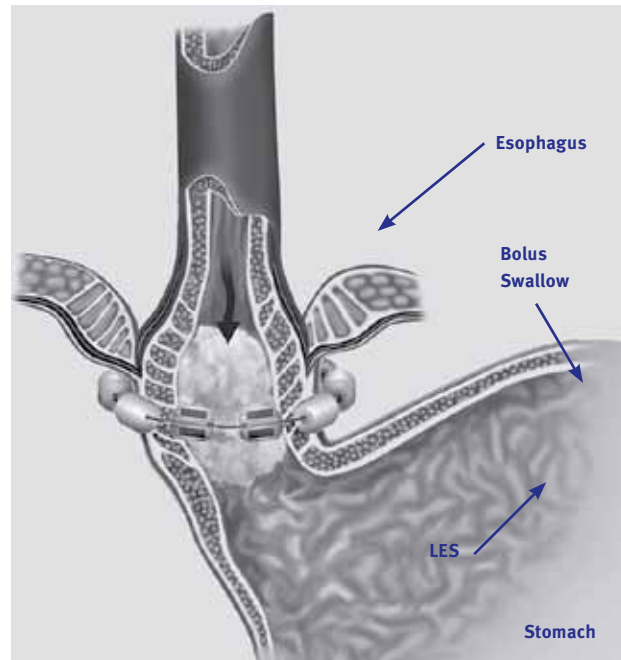


FIGURE 4. LINX™ device actuation during swallow

II. Paraesophageal Hernia

II.A. LONG-TERM OUTCOMES OF BIOLOGIC PROSTHESIS TO PREVENT RECURRENCE AFTER LAPAROSCOPIC PARAESOPHAGEAL HERNIA REPAIR

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. In 2006 we reported at the American Surgical Association the results of a randomized trial showing that the use of a biologic prosthesis (small intestinal submucosa, SIS) decreased hiatal hernia recurrence from 24% (with primary repair, PR) to 9%, 6 months after LPEHR. Recently, we have completed the second phase of this trial, which was designed to determine the long-term durability of biologic mesh-reinforced repair at 5 years. We systematically searched for each of the 108 patients reported in the Phase I trial to assess clinical symptoms and quality of life (QOL) and to determine anatomy by follow-up upper gastrointestinal series (UGI) read by 2 radiologists blinded to the treatment received. From the original multi-center randomized cohort of 108 patients, 72 had completed clinical follow-up and 60 patients underwent a UGI to assess for the presence of recurrence.

TABLE 2. Change in symptom severity, QOL, hernia size, and recurrence from pre-op to long-term follow-up

	Heartburn*	Dysphagia*	QOL (SF-36/PCS)	Hiatal Hernia Vertical Ht (mm)	Recurrent Hernia (>2cm)
PR	1.5	1.7	43	21 ± 21	59%
SIS	2.0	1.3	44	24 ± 20	54%
p-value	0.44	0.5	0.8	0.9	0.7

*Symptoms score: visual analog scale (0-10); PR: primary repair; SIS: small intestinal submucosa; PCS: physical component summary

Primary Outcome: At a median follow-up of 58 months, there was no significant difference in radiographic hiatal hernia recurrence between the PR group and the SIS group (Table 2). The greatest rate of recurrence in the SIS cohort was identified in the patients with at least 5 years elapsed since their surgery.

Secondary Outcomes: Clinical symptoms and QOL remained improved from the baseline and were largely similar to the results observed at 6 months. There were no significant differences between the PR and SIS cohorts with respect to severity of clinical symptoms or QOL (Table 2).

This trial has demonstrated that LPEHR produces long and durable relief of clinical symptoms and improvement in QOL. In addition, the benefit of biologic mesh seems to diminish after 5 years.

As part of this larger randomized trial, we also plan to gain more insight into the natural history of laparoscopic hernia repair. A subsequent study will be performed where we plan to determine the long-term anatomic results, QOL, and symptom severity outcomes for all patients undergoing LPEHR. In addition, we will determine whether the presence of a recurrent hiatal hernia detected on UGI correlates with worsening QOL and clinical symptoms. These are two big questions which remain yet to be answered in the surgical community. A well-designed study with long-term follow-up is expected to provide some answers.

II. B. SAFETY OF THE USE OF BIOLOGIC MESH IN THE REPAIR OF LARGE, COMPLICATED HIATAL HERNIA

Although it has been shown in the past that the use of biologic mesh can reduce the risk of recurrence at 6 months, there have been several case series reporting on complications associated with its use, such as erosion into the esophagus and dysphagia.

We therefore decided to analyze the safety of the use of biologic mesh for large, complicated hiatal hernia in our patients. All patients at the University of Washington who had an operation with the use of biologic mesh at the hiatus with a minimal follow-up of 1 year were contacted. The patients were asked to fill out a questionnaire on symptoms related to gastroesophageal reflux, hiatal hernia and complications of the mesh. When available, the post-operative upper-GI study and endoscopy reports were analyzed. The primary outcome measure was complication due to the mesh leading to intervention. Secondary outcome measures were post-operative dysphagia and satisfaction with the operation.

Outcomes: Of the 126 patients that were found to be eligible for the study, 71 (56%) patients returned the questionnaire. Four patients had died to causes unrelated to the operation, four refused to participate, and 47 were not able to be contacted. The majority of patients were female (71%) and obese (mean BMI 30.6 kg/m²). There were no peri-operative complications directly related to the use of mesh. Median follow-up for patients who returned the questionnaire was 45 months. One patient had a re-operation due to recurrent hernia and reflux. No complication directly related to the mesh was found. Post-operative dysphagia that was worse than before the operation was seen in six patients. In four of these patients, causes other than the mesh could explain their swallowing difficulty. In the other two patients, the cause of their dysphagia was unclear, and it therefore could be related to the use of mesh. Patients rated the overall result of the operation as good or excellent in 89% of cases.

This study shows that our use of biologic mesh for repair of large, complicated hiatal hernia is safe.

III. Esophageal Cancer And Diverticulum

III.A. ESOPHAGEAL CANCER

III.A.i. OUTCOMES OF LAPAROSCOPIC ASSISTED ESOPHAGECTOMY FOR ADENOCARCINOMA OF THE ESOPHAGUS

In the last decade, the incidence of esophageal adenocarcinoma has increased more rapidly than any other gastrointestinal malignancy. The prognosis of patients with esophageal cancer remains poor. Only 56% of 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; and 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) and long-term (cancer free survival, 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 (range, 1-35), whereas the hospital stay was nine days (range, 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 required 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 the performance of laparoscopic-assisted transhiatal esophagectomy as 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.

III.B. EPIPHRENIC DIVERTICULUM

III.B.i. 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. In the majority of cases an underlying neuromuscular disorder is present, causing 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 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 gastroesophageal 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 with treating 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 our high volume of patients, we are reviewing our treatment outcomes with minimally invasive surgery for epiphrenic diverticula.

Material and Methods: From 1997 to 2008, 23 patients underwent surgery for epiphrenic diverticula at the University of Washington. Our initial approach was laparoscopy in 19 patients, video-assisted thoracic surgery (VATS) in two and open thoracotomy in two. Details of the operation and postoperative course were recorded in our database. In June 2008 the patients were contacted by one of the investigators regarding current symptoms.

Results: The median age was 57 years (range, 23-83). The median follow-up was 45 months. Eighteen patients had esophageal manometry in our institution; 12 of them were abnormal (66.67%). The median diameter of diverticula was 4 cm (range, 2-10). From the 19 patients approached by laparoscopy, there was one conversion to open thoracotomy, in a patient with an associated leiomyoma. Both patients approached by VATS were converted to thoracotomy. Considering just the patients approached by laparoscopy, the median length of stay was 3 days. There was one contained esophageal leak. The 30-day mortality was 5% (n = 1), from a port site hernia leading to small bowel obstruction and sepsis. Of the contacted patients, 92% had improvement of their dysphagia, and 77% obtained relief from regurgitation. None of the patients developed recurrent diverticula. Eighty-five percent of the patients rated the results of the operation as good or excellent.

Conclusion: Most epiphrenic diverticula can be treated successfully using a laparoscopic approach with low morbidity, low conversion rates and good symptom control. As a result of this work, we are now approaching nearly all of these diverticulae laparoscopically, resulting in a positive impact on patient recovery and outcomes.

IV. Surgical Treatment Of Achalasia

IV.A. IMPROVEMENT OF RESPIRATORY SYMPTOMS FOLLOWING HELLER MYOTOMY FOR ACHALASIA

Recently, our group reported the high prevalence of respiratory symptoms in patients who suffer from achalasia, a motor disorder of the esophagus. The University of Washington's long-term experience with the diagnosis and surgical management of this disease led to the clinical question of what happens to these respiratory symptoms in such patients after they undergo surgical treatment with Heller myotomy.

To address this question, we studied the course of 111 patients who underwent Heller myotomy at the University of Washington between 1994-2008. Study participants were given questionnaires post-operatively that assessed their preoperative and postoperative symptoms. The median follow-up time after surgical myotomy was 71 months.

Patients were asked to indicate both preoperative and postoperative frequency and severity of respiratory symptoms, including dyspnea (shortness of breath), hoarseness, cough, wheezing, pneumonia, and/or sore throat, as well as more typical esophageal symptoms such as dysphagia, regurgitation, chest pain, and heartburn on a 5-point scale (0 = never, 1 = once a month, 2 = once a week, 3 = once a day, 4 = several times daily). Severity of symptoms was rated on a 10-point visual analog scale ranging from 0 (absent) to 10 (worst). Patients reporting respiratory symptoms (dyspnea, hoarseness, cough, wheezing, or sore throat) occurring at least once per week prior to myotomy and/or a history of asthma or pneumonia were considered to have respiratory symptoms or diseases and included in our analysis.

Our results were compelling. The high prevalence of respiratory symptoms was again confirmed. Of the 111 patients who participated in this study, 63 (57%) reported at least one clinically significant baseline respiratory symptom or respiratory disease prior to undergoing Heller myotomy. There were no significant demographic or clinical differences between those patients with and those without respiratory manifestations. Of the sixty-three patients who did report respiratory symptoms, 55 (87%) patients experienced

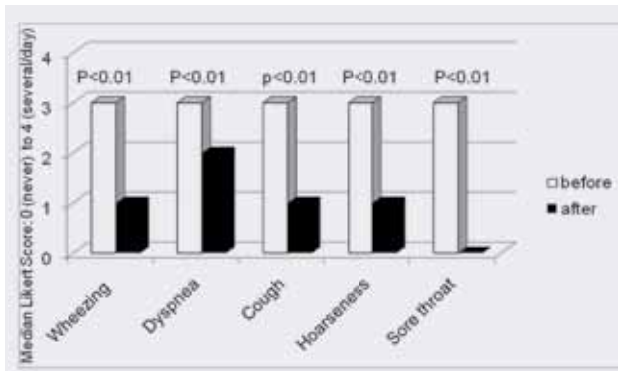


FIGURE 5. Comparison of preoperative and postoperative frequency (0-4) of respiratory symptoms

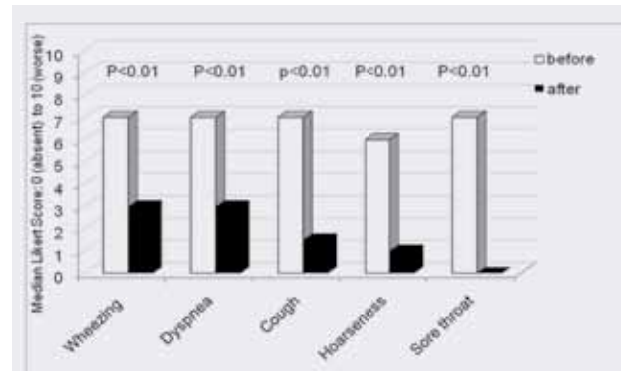


FIGURE 6. Comparison of preoperative and postoperative severity (0-10) of respiratory symptoms

urable improvement in their dysphagia, confirming the efficacy of surgical myotomy. The frequency and severity of all respiratory symptoms decreased significantly following surgery (Figures 5 and 6). Twenty-four of the 29 patients (82%) who reported a history of pneumonia prior to surgery did not experience recurrent episodes for up to 5 years following Heller myotomy.

In our group's previous study, we found a high prevalence of pulmonary symptoms/disease in patients with achalasia. In this study we demonstrated significant improvement in these symptoms following successful surgical treatment of achalasia with Heller myotomy, and these improvements parallel improvements in dysphagia. The most logical causal link between esophageal obstruction and the presence of respiratory symptoms in the setting of achalasia is esophageal non-emptying and aspiration of retained food and secretions from the esophagus into the upper and lower respiratory tracts.

These findings present a strong case that highlights the presence of and explains the pathophysiology of respiratory disease in patients with achalasia. Delayed esophageal emptying is certainly the most likely reason for these respiratory symptoms, and the improvement after performance of Heller myotomy strengthens the likelihood of this association. Moreover, the substantial improvement in respiratory symptoms and disease after Heller myotomy, which was heretofore not appreciated, is yet another benefit of surgical therapy for this disease.

IV.B. DOR VS. TOUPEL FUNDOPPLICATION: A 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.

IV. C. LONG-TERM OUTCOMES OF HELLER MYOTOMY FOR ACHALASIA

At the University of Washington from 1994 to 2010, we have performed over 400 Heller myotomies for the treatment of achalasia. This year we plan to complete our long-term assessment of both postoperative clinical symptoms and physiologic outcomes, including manometry and 24 hour pH study results. We have been prospectively collecting a standardized questionnaire using visual analog scales to determine the frequency and severity of a wide range of clinical symptoms both before and after surgery. In addition, we have been collecting manometry and 24 hour pH study data prospectively on all patients at 6 months following surgery since 1997. The primary objective of this study will be to determine our overall success rate in alleviating dysphagia. We also plan to identify preoperative predictors of favorable long term outcome to further guide optimization of patient selection criteria.

V. Esophageal Motility

V.A. FACTORS RESPONSIBLE FOR FUNDOPLICATION FAILURE AS ASSESSED BY HIGH-RESOLUTION ESOPHAGEAL MANOMETRY (HRM)

HRM is a recently developed tool in the evaluation of esophageal motility. It 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 a 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. We have been using HRM in the evaluation of all patients referred to the University of Washington Center for Esophageal and Gastric Surgery for esophageal motility testing in the last 2 years; to date, we have performed more than 1,200 HRM studies.

With this new technology, we suspected that we would be able to identify detailed physiologic abnormalities in patients with esophageal symptoms. One group of particular interest to our center is that of patients who

present with recurrent symptoms after antireflux surgery. It has been estimated that 10 to 25% of patients undergoing antireflux procedures eventually redevelop symptoms resulting from anatomic failure of the hiatal repair or the fundoplication. Because HRM allows for reliable evaluation of the lower esophageal sphincter (LES) in detail, including subtle evidence of a hiatus hernia that may be difficult to detect using other methods, we sought to characterize the dynamics and function of the LES postoperatively using this technique in order to determine which elements may contribute to recurrent symptoms after antireflux surgery.

Thirty-four patients who had a Nissen fundoplication – 23 with recurrent symptoms and/or abnormal 24h pH monitoring (Unsuccessful group) and 11 asymptomatic patients who were tested as part of routine follow-up (Successful group) – were evaluated using HRM. HRM tracings were analyzed for percentage of peristaltic contractions, LES pressure (LESP), length of the high pressure zone (HPZ), residual pressure during LES relaxation, and the presence of a dual HPZ (indicating a recurrent hiatus hernia – see Figure 7). Results were compared between the two groups, and HRM findings in the symptomatic patients were also compared to findings on upper GI and endoscopic examinations.

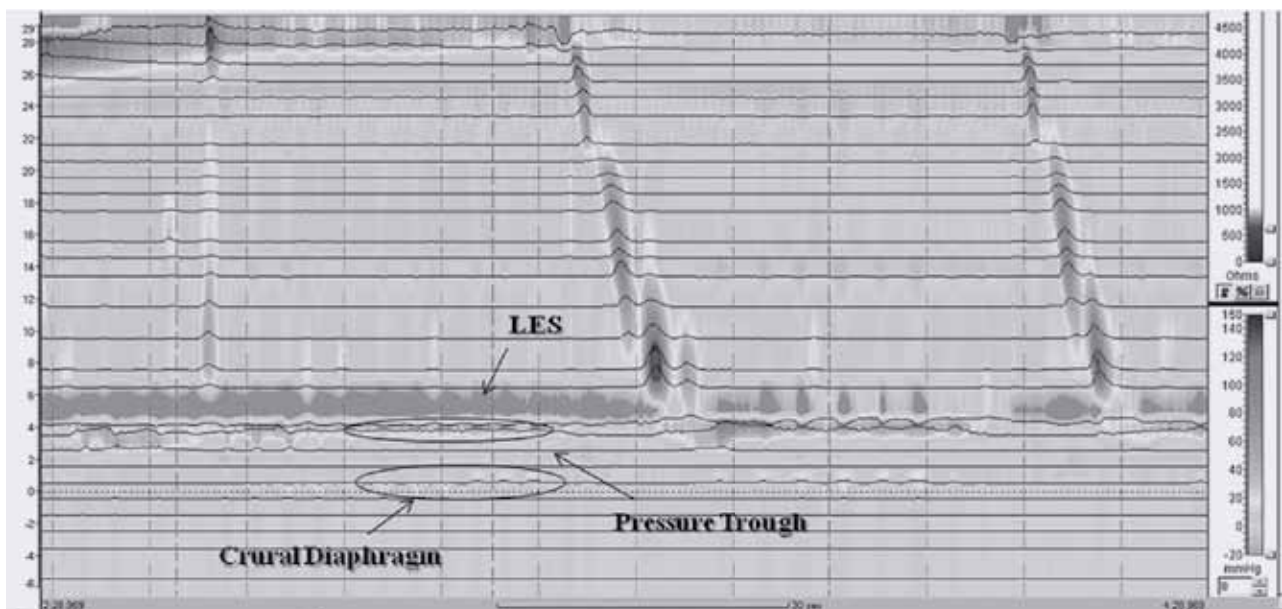


FIGURE 7. HRM tracing in a patient with recurrent hiatus hernia demonstrating a dual HPZ

Mean LESP tended to be greater in the Successful group compared with the Unsuccessful group ($p = 0.068$). There were no differences when comparing both groups on length of the HPZ, pressure profiles, residual pressures, and rates of peristalsis. A dual HPZ was identified in 13 patients in the Unsuccessful group (56%), and 1 (9%) of the Successful patients ($p < 0.05$). An abnormal DeMeester score was observed in 79% of patients with a dual HPZ, compared with only 35% of patients without a dual HPZ ($p < 0.05$). In contrast, the DeMeester score was abnormal in 70% of patients with a recurrent hiatus hernia on either UGI or esophagogastroduodenoscopy (EGD) ($p = 0.16$).

From these results, we concluded that the presence of a dual HPZ on HRM in patients who have had a fundoplication appears to be a strong predictor of recurrent GERD, and it may be useful as an initial test to guide further workup. In patients with recurrent symptoms after antireflux surgery, HRM also provides valuable information regarding peristalsis and LES characteristics that help plan appropriate patient management.

The results of this study were presented in December 2009 at the Annual Meeting of the Southern Surgical Association and published in the *Journal of the American College of Surgeons* in May 2010.

V.B. THE PREVALENCE OF RESPIRATORY SYMPTOMS IN PATIENTS WITH ACHALASIA

Achalasia is a primary esophageal motor disorder that results in poor clearance of the esophagus. Although an esophagus filled with debris and undigested food should put these patients at risk for aspiration, the frequency with which the latter occurs has never been documented. Therefore, we sought to determine the incidence of respiratory symptoms and complaints in patients with achalasia.

A comprehensive symptom questionnaire was administered to 110 patients with achalasia presenting to the Swallowing Center at the University of Washington between 1994–2008 as part of their preoperative work-up. These questionnaires were analyzed for the frequency of respiratory complaints in addition to the more typical symptoms of dysphagia, regurgitation, and chest pain.

Ninety-five patients (86%) complained of at least daily dysphagia. Fifty-one patients (40%) reported the occurrence of at least one respiratory symptom daily, including cough in 41 patients (37%), aspiration (the sensation

of inhaling regurgitated esophago-gastric material) in 34 patients (31%), hoarseness in 23 patients (21%), wheezing in 17 patients (15%), shortness of breath in 11 patients (10%) and sore throat in 13 patients (12%). Neither age, gender, nor manometric findings differed between those with and those without respiratory symptoms.

We concluded that patients with achalasia experience respiratory symptoms with much greater frequency than the approximately 10% which was previously believed. Awareness of this association may be important in the workup and ultimate treatment of patients with this uncommon esophageal disorder. The results of this work have recently been accepted for publication in the journal *Diseases of the Esophagus*.

V.C. SUBTYPES OF INEFFECTIVE ESOPHAGEAL MOTILITY—IMPLICATIONS FOR DIAGNOSIS AND MANAGEMENT

Ineffective Esophageal Motility (IEM) is a motility pattern diagnosed on esophageal manometry which is characterized by >30% of swallows being other than normal peristaltic sequences. Subgroups within this classification can be identified based on the predominant type of failed swallowing sequence, including hypocontractile swallows, those that fail to propagate in the distal esophagus, and simultaneous, low amplitude contractions. The clinical significance of IEM subgroups remains unclear, however. We sought to combine high resolution esophageal manometry (HRM) data in patients experiencing IEM with information obtained from ambulatory pH monitoring and patient symptom questionnaires in order to investigate the correlation between IEM subtype and esophageal acid exposure, bolus transit, and patient symptoms, and to determine if this might have an impact on further workup and management in these patients.

HRM tracings, pH data, clinic notes and symptom questionnaires in 84 patients with IEM and 50 control subjects with normal esophageal manometry and a diagnosis of GERD are being reviewed. We will compare esophageal acid exposure (% time pH <4 in the distal esophagus, DeMeester scores), bolus transit and patient symptoms between IEM subtypes and control subjects using analysis of variance. Through this study, we expect to be able to better determine which patients with IEM are more likely to have either increased acid exposure, more severe symptoms, or both, and to make suggestions as to how patients with such findings might warrant more aggressive treatment for associated problems, in particular GERD.

VI. Natural Orifice Transluminal Endoscopic Surgery

Natural Orifice Transluminal Endoscopic Surgery (NOTES) represents a paradigm shift that may significantly change the management of gastrointestinal and intra-abdominal 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 peroral 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 currently being done via traditional surgical methods, but instead using a transluminal approach. We have developed relationships with our 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. Not since the adoption of laparoscopic surgery 20 years ago has there been such a radical change in the way surgery is viewed.

VI.A. 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 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.

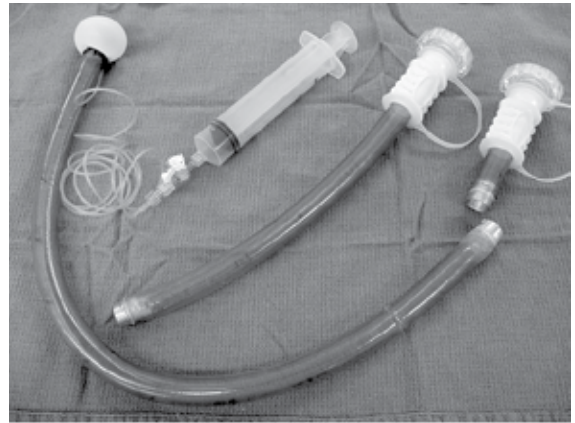


FIGURE 8. Overtube used in NOTES

The overtube (Figure 8) 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 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.

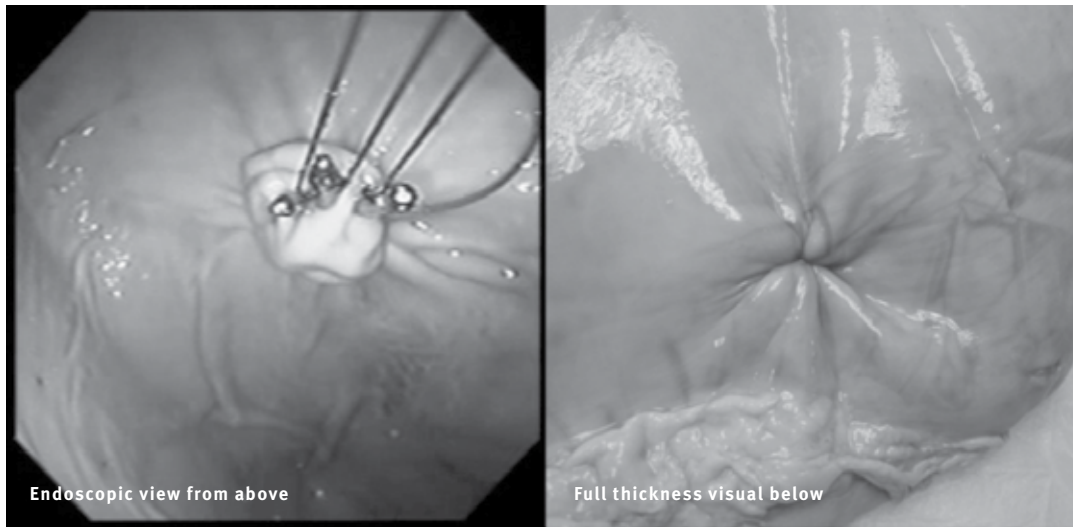


FIGURE 9. Image of closure in NOTES gastrotomy

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.

VI.B. 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 (Figure 9, left image). On visual inspection, 2/6 appeared to achieve a full thickness closure (Figure 9, right 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.

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DEPARTMENT CO-INVESTIGATORS

Laura Filippa, M.D. / Yong S. Kwon, M.D. / Ana Valeria Martin, M.D. / Rebecca P. Petersen, M.D., MSc. / Eelco B. Wassenaar, M.D., PhD.

OTHER CO-INVESTIGATORS

Joo Ha Hwang, M.D., Ph.D.; UW Departments of Medicine and Bioengineering / Albert L. Merati, M.D.; UW Department of Otolaryngology / Tanya K. Myer, M.D.; UW Department of Otolaryngology / Nikki Johnston, Ph.D.; Medical College of Wisconsin

James O. Park, M.D.

· Application of Nanotechnology in the Diagnosis and Treatment of Hepatocellular Carcinoma



Assistant Professor
of Surgery

AWARDS

American Surgical Association Foundation
Research Fellowship Award

FUNDING

American Surgical Association

With over 660,000 new cases and 630,000 resultant deaths estimated in 2009, primary liver cancer, or hepatocellular carcinoma (HCC), is the fifth most prevalent malignancy and the third leading cause of cancer-related deaths worldwide. Although 80% of cases occur in developing regions where hepatitis B infection is endemic, the incidence of HCC in the United States is rising at epidemic proportions as a result of the rampant spread of hepatitis C four decades ago. The incidence of hepatitis C cirrhosis is projected to peak in 2015. However, a sharp decline in HCC is not anticipated for several decades due to the current epidemic of obesity which will in part replace hepatitis C as the etiology of HCC. Obese patients with metabolic syndrome can develop non-alcoholic steatohepatitis (NASH) with progression to cirrhosis and resultant HCC. The lethality of HCC is demonstrated by its equal annual incidence and mortality, and the dismal 8-month median survival without treatment. However, when HCC is detected at an early stage, curative treatments such as surgical resection, liver transplantation, and ablative therapies can be implemented, achieving 5-year survival rates of up to 75%, highlighting the importance of early detection.

Challenge #1: Accurate Radiographic Diagnosis

The diagnosis of early stage HCC is heavily reliant on quality multiphase, contrast-enhanced computed tomography (CT) and magnetic resonance (MR) imaging, the current gold standards. While characteristic arterial enhancement with portal venous washout of a liver lesion on CT or MR is diagnostic of HCC, indeterminate lesions are frequently detected. This is especially true in cases where the tumor is small (< 1cm), the tumor exhibits atypical contrast enhancement characteristics, and when concomitant regenerative and dysplastic nodules from cirrhosis are

present. As the sensitivity of CT and MR continues to improve, and surveillance imaging for HCC is adopted, more such indeterminate lesions will be detected. This uncertainty translates into costly repeat imaging or unnecessary biopsy with its inherent risks of bleeding or tumor seeding, all resulting in delay of treatment and potential complications. Furthermore, eligibility for liver resection or transplantation based on suboptimal scans either results in early recurrences and poor outcomes, or missed treatment opportunities. Studies comparing tumor extent observed on preoperative imaging to pathologic evaluation of the liver explant demonstrate troubling rates of discordance. The false positive rate for CT or MR in the setting of cirrhosis is estimated to be as high as 15%. As a result, roughly 7% of donor livers in the U.S. are transplanted into patients who do not have cancer. These are unacceptable figures, especially given the shortage of donor organs. Imaging that definitively captures small volume, atypical disease, and distinguishes from benign processes would be invaluable in improving our ability to stage and risk-stratify these patients for potentially curative therapies.

Challenge #2: More Effective Treatment

Treatment of HCC remains a formidable challenge. While surgical resection, liver transplantation, and radiofrequency ablation are the mainstay of potentially curative therapy, and can provide 5 year-survival figures of up to 75%, only 10-15% of patients present with disease that is amenable to these modalities. Trans-arterial chemoembolization (TACE), a catheter-based therapy, can be employed as an effective bridging therapy or for symptom control, but is largely palliative. Numerous systemic chemotherapy regimens have been explored for HCC treatment with marginal results. Based on a large multicenter trial, sorafenib, a multi-kinase inhibitor, has emerged as the current standard of care

The long-term goal of this research is to develop a multifunctional HCC-specific magnetic nanovector that would enable cancer-specific targeting and effective delivery of a sufficient dose of siRNA to target cells to induce gene silencing, while providing the capability of carrier monitoring through MRI and bioluminescence imaging.

for patients who are not surgical candidates. However, the objective response rate for sorafenib is a meager 2%, and the resulting improvement in median survival is a modest 3 months. Novel therapies are urgently needed for this lethal global health crisis.

Research Objective

To overcome the above mentioned challenges and improve the diagnosis and treatment of HCC, a collaborative effort has been forged with Professor Miqin Zhang's group in the UW Department of Materials Sciences and Engineering. The long-term goal of this research is to develop a multifunctional HCC-specific magnetic nanovector that would enable cancer-specific targeting and effective delivery of a sufficient dose of siRNA to target cells to induce gene silencing, while providing the capability of carrier monitoring through MRI and bioluminescence imaging. This biocompatible and biodegradable nanovector will target ligands highly expressed by most HCC cells (e.g., glypican-3) and deliver siRNA that inhibits components within the Wnt/ β -Catenin pathway that are implicated in the modulation of HCC tumorigenesis, cell proliferation, survival, and cell fate. Our research aims are illustrated in Figure 1.

Nanotechnology

A magnetic nanoparticle (NP) platform, consisting of an iron oxide core coated with a cationic copolymer of chitosan-grafted-polyethylene glycol (PEG), is utilized. The superparamagnetic core enables real-time monitoring by MR imaging. The nanopolymer substratum consists of a chitosan polymer backbone grafted with a low molecular weight, heterobifunctional polyethyleneglycol (PEG). This platform is not only biocompatible and biodegradable, rendering it safe for human use, but the anti-fouling properties of PEG prevents agglomeration and uptake by macrophages, prolonging blood circulation time and bioavailability. The PEG grafting not only improves the

aqueous solubility of chitosan, adding to its biostability, but also provides terminal functional groups for covalent conjugation of targeting and signaling components. The hydrodynamic size (50nm) is large enough (> 5nm) to circumvent prompt renal clearance, while small enough (< 200nm) to evade sequestration and elimination by the reticuloendothelial system. The negative zeta potential minimizes non-specific binding and uptake by surrounding tissues, allowing for deeper tissue penetration toward the target. Moreover, the higher binding capacity of a NP with more functional amine groups is carefully balanced against the steric hindrance created by its bulk. Near-infrared fluorescent (NIRF) fluorophores make up the second signaling component of the NP, which are detectable by laser confocal microscopy and the Xenogen IVIS fluorescence imaging system.

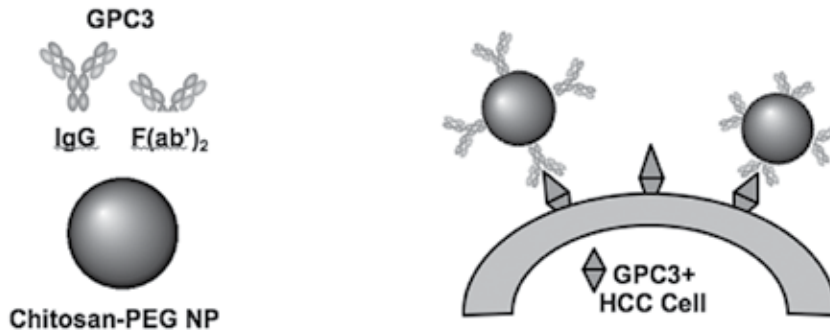
HCC Targeting

Glypican-3 (GPC3) was selected as a novel molecular target for HCC based on qualities crucial to successful targeting and promising for future applications. GPC3 is a heparan sulfate proteoglycan essential in regulating embryonal cell growth, as evidenced by its mutation causing the Simpson-Golabi-Behmel overgrowth syndrome. While its expression is absent in normal adult tissues, GPC3 is significantly over-expressed in up to 80% of human HCCs. Attached to the cell membrane via a glycosyl-phosphatidylinositol anchor, GPC3 is readily accessible for antibody-mediated targeting and binding. Moreover, GPC3 has been shown to promote HCC growth by stimulating the canonical Wnt signaling pathway, exhibiting potential as an important therapeutic target. These auspicious attributes make GPC3 an ideal biomarker for HCC targeting. We have successfully demonstrated specific targeting of GPC3 expressing HCC cells using biotin conjugated GPC3 antibody with subsequent detection of the NP via fluorescence microscopy and MR. GPC3 antibody production has been conducted by Dr. Elizabeth Wayner's group at the Fred Hutchinson Cancer Research Center.

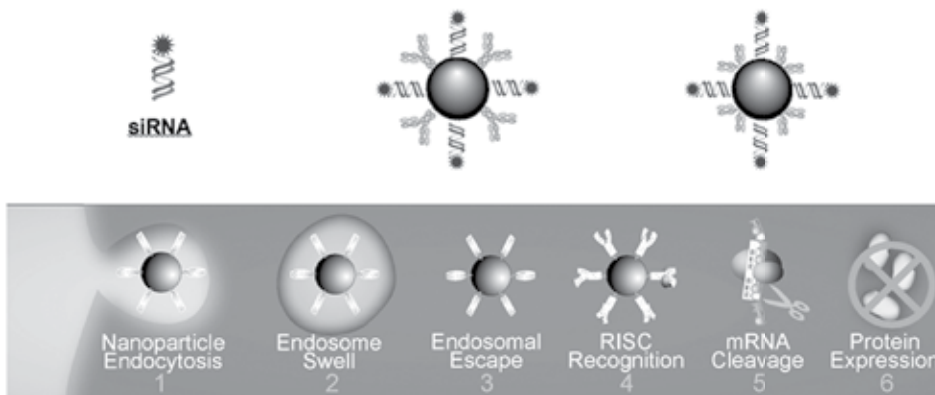
Therapeutics

Various therapeutic payloads can be delivered to tumor cells using the nanocarrier construct. One such payload is siRNA for RNAi. Ribonucleic acid interference (RNAi) is an endogenous mechanism by which cells regulate gene expression using small RNA molecules. Short interfering

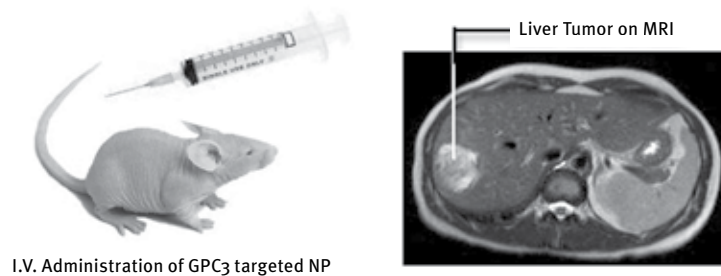
RNA (siRNA), a non-coding 21 base-pair RNA duplex, binds complementary messenger RNA to direct gene silencing via endonuclease degradation within the RNA-induced silencing complex. siRNA-based RNAi is a rapidly developing gene therapy frontier with immense potential



Aim 1: Anti-glypican-3 (GPC3) immunoglobulin (IgG) and its antigen-binding fragment (F(ab')₂) are generated and subsequently conjugated to magnetic chitosan-polyethylene glycol (PEG) iron-oxide nanoparticles (NP). The binding of nanoparticles to GPC3 expressing hepatocellular carcinoma (HCC) cells is tested *in vitro*.



Aim 2: Gene silencing using an established green fluorescent protein (GFP) short-interfering ribonucleic acid (siRNA)-nanoparticle system is tested in HCC cells, and then siRNA targeting β -Catenin is optimized for the NP *in vitro*.



Aim 3: The GPC3-specific β -Catenin silencing NP is tested *in vivo*.

FIGURE 1. Research Aims

application in cancer therapy. However, effective delivery of the siRNA to the target cancer cell is a major hurdle to *in vivo* applications. The anionic, hydrophilic siRNA exhibits limited internalization by cell diffusion, and ineffective intracellular trafficking in cancer cells hinders potency. Poor site specificity, low gene silencing efficacy, and lack of non-invasive delivery monitoring are additional challenges.

The pathogenesis and progression of HCC is a complex multi-step process involving several signal transduction pathways (e.g. Ras/Raf/MEK/ERK, PI3K/Akt/mTOR, and Wnt/ β -Catenin). Aberrant activation of the canonical Wnt/ β -Catenin signaling pathway, resulting in cytoplasmic stabilization and nuclear accumulation of β -Catenin with consequent induction of downstream regulators of cell proliferation (e.g. c-Myc, glutamine synthetase, and cyclin D1), is a frequently observed, important event implicated in HCC tumorigenesis, making this an attractive molecular treatment target for HCC. Over-expression of the Wnt3 glycoprotein ligand and its activation of the Wnt/ β -Catenin signal transduction cascade through interaction with the

Frizzled7 receptor have been previously demonstrated. Hence, siRNA-mediated inhibition of these components and β -Catenin has been chosen for further study.

Our NP platform is designed to address the limitations of siRNA delivery. The siRNA are covalently attached to the NPs to prevent degradation by extracellular or intracellular enzymes, enabling proper intracellular trafficking and thus improving the efficacy in gene silencing. The targeting of GPC3 adds to improve the efficacy of tumor-specific delivery of the siRNA.

Future Clinical Applications

The NP construct has the unique advantages of dual modality imaging; targeted MR imaging, which can be used for pretreatment staging/planning; and targeted NIRF imaging which can be used in the operating theater to ensure adequate surgical margins during tumor resection.

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OTHER CO-INVESTIGATORS

From UW Department of Materials Science and Engineering: Miqin Zhang, PhD, Professor / Omid Veiseh, PhD, Conroy Sun, PhD, Hyejung Mok, PhD, Post-doctorates / Forrest M. Kievit, Zachary Stephen, Chen Fang, Matthew Leung, Graduate students

Raymond S. Yeung, M.D.

· The Role of the TSC1/2 Complex in Tumor Development



Professor of Surgery

AWARDS

LAM Foundation Senior Investigator Award

FUNDING

National Institutes of Health
LAM Foundation
Tuberous Sclerosis Alliance
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.

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

The current model suggests that tuberlin 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 tuberlin 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 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 the 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.

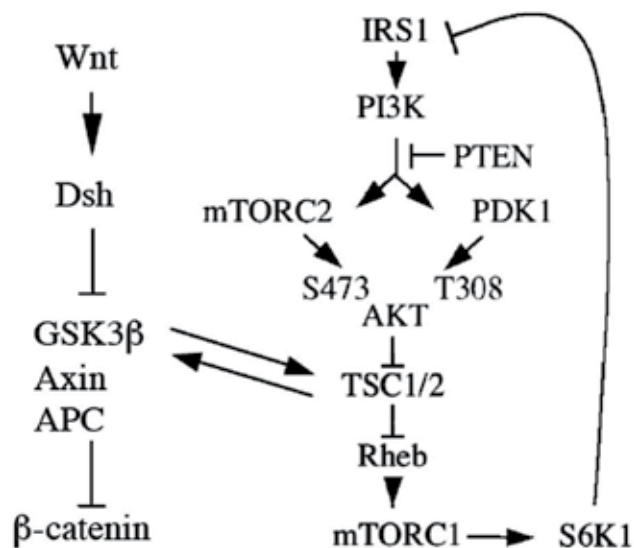


FIGURE 1: Model of TSC1/TSC2 pathway

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 tuberlin 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 depends 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 tuberlin, we found that they indeed reside in multiple compartments (i.e., cytosol, microsome, cytoskeleton). Of particular interest is the vesicular component in which tuberlin 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 tuberlin have mis-localized caveolin-1 and reduced formation of caveolae at the plasma membrane.

Recent studies point to a role of tuberlin in regulating the transport of proteins such as caveolin-1 from the Golgi apparatus to the membrane. The molecular mechanism mediating this function of tuberlin 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 burdens. 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.

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VAPSHCS/GENERAL SURGERY

LORRIE A. LANGDALE, M.D.

MICHAEL SOBEL, M.D.

PETER C. WU, M.D.

Lorrie A. Langdale, M.D.

• Mechanisms of Injury Control after Hepatic Ischemia-Reperfusion



Associate Professor of Surgery
Director, Veterans Affairs
PSHCS Surgical ICU
Chief of General Surgery,
Veterans Affairs PSHCS

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 $TNF\alpha$, $IL-1\beta$, 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 complement, 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 (Figure 1).

While the progression of injury following liver ischemia-reperfusion (IR) 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

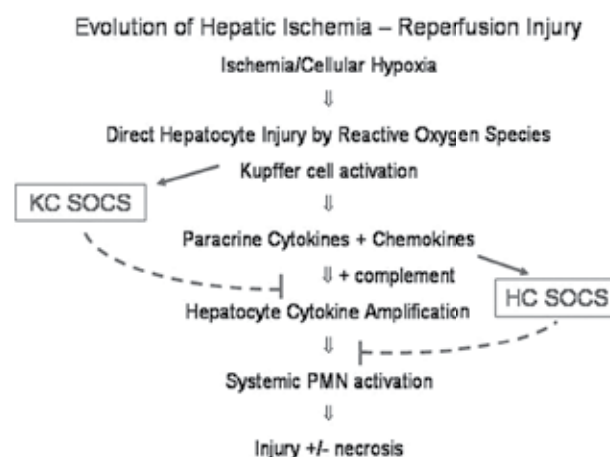


FIGURE 1: Evolution of Liver Injury and Resolution

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 $\text{TNF}\alpha$, $\text{IFN}\gamma$, IL-1, IL-6, IL-10 and erythropoietin. These mediators are, in turn, controlled, at least in part, by SOCS proteins.

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 a 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 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 inhibit 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 effect 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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-

OTHER CO-INVESTIGATORS

Jean Campbell, Ph.D.; UW Department of Pathology / **Nelson Fausto, M.D.;** UW Department of Pathology

Michael Sobel, M.D.

• The Vascular Research Program at the VA Puget Sound Health Care System



Professor of Surgery
Vice Chairman,
Department of Surgery
Chief of Surgery, Veterans Affairs
Puget Sound Health Care System

The VA Puget Sound has a robust research infrastructure that supports two principal investigators in the Division of Vascular Surgery: Dr. Michael Sobel, Professor of Vascular Surgery, and Errol Wijelath, Research Associate Professor.

Current projects include the following:

1. Preventing Vein Graft Stenosis in Peripheral Vascular Surgery.

Dr. Sobel and Dr. Katie Moreno, a University of Washington surgery resident on a research fellowship, are conducting this study. This is a prospective, longitudinal observational study of patients undergoing infrainguinal bypass surgery. The leading hypothesis is that derangements in a patient's thrombo-inflammatory responses are associated with pathological vascular healing and clinical events like vein graft stenosis and graft failure. We are developing novel methods to measure the co-activation of platelets and monocytes in the circulating blood, and trying to define phenotypic and clinical subgroups. The long-term goal is to identify the thrombo-inflammatory pathways associated with vein graft failure, for drug targeting.

2. Oncostatin M in Atherosclerosis and Vascular Disease.

Dr. Wijelath has identified this little known cytokine as a key player in the pathological proliferation and migration of vascular smooth muscle cells during the evolution of atherosclerosis and the response to injury. Through the study of atherosclerotic plaques and vascular lesions, as well as advanced molecular biological manipulations of oncostatin M (OSM) receptors *in vitro*, Dr. Wijelath is mapping the pathways of OSM action, and defining its roles in vascular disease.

3. Modulating Endothelialization of Cardiovascular Grafts.

As its funding ends, this joint project involving several Division of Vascular Surgery faculty members is winding down. In this project we discovered and refined a family of novel angiogenic proteins that enhance the effects of vascular endothelial growth factor, and can be used to promote natural endothelialization of prosthetic grafts.

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DEPARTMENT CO-INVESTIGATORS

Errol Wijelath, Ph.D. / Katie Moreno, M.D.

Peter C. Wu, M.D.

• Gastrointestinal Cancer Treatment and Cellular Senescence



AWARDS

Society of Surgical Oncology
• James Ewing Research Award

FUNDING

American Cancer Society
Progenics Pharmaceuticals, Inc.
VA Merit Career Development Award

Assistant Professor
Director, VA Cancer
Telemedicine Program
Chair, VA Cancer Committee
SWOG PI, VA Puget Sound

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).

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.

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, β -galactosidase (SA- β -gal; Figure 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.

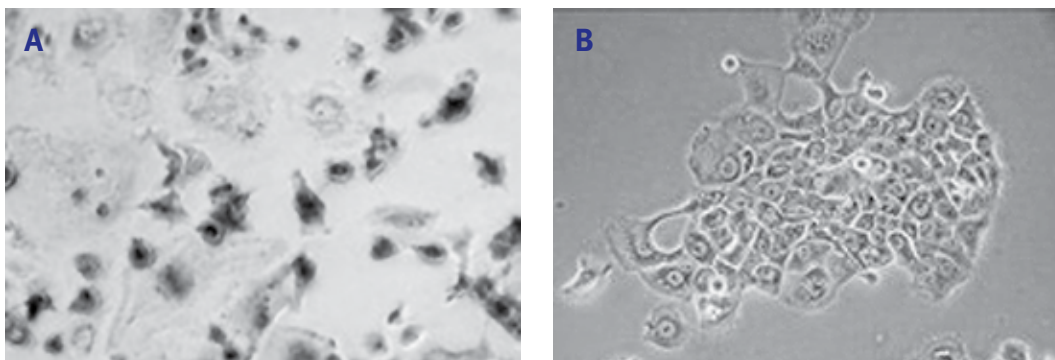


FIGURE 1. Light microscopy of blue-appearing tumor cells in therapy-induced senescence stained with X-gal. (A) H1299 lung carcinoma cells. (B) Bx-PC3 pancreatic carcinoma cells.

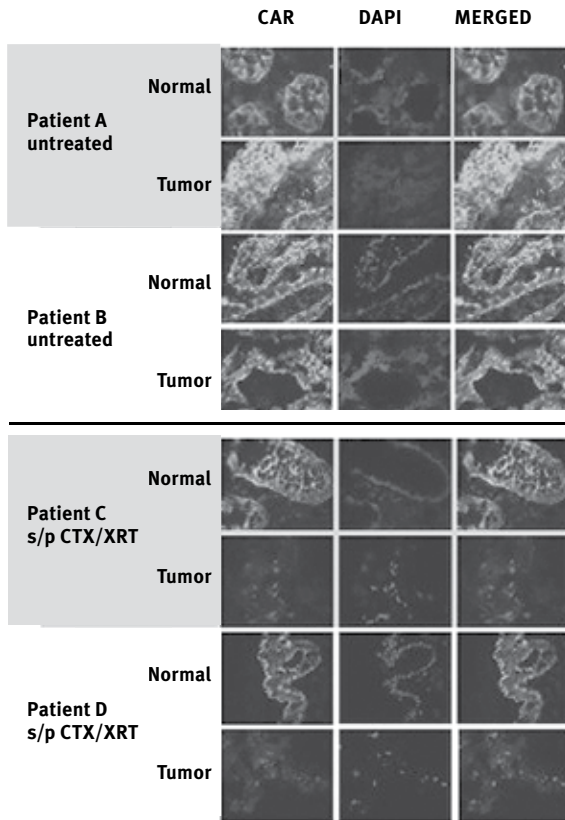


FIGURE 2. CAR expression and senescence in colorectal cancer. Human tumor and adjacent normal tissue specimens were obtained from colorectal cancer patients undergoing curative resection treated with or without preoperative chemoradiotherapy. Freshly frozen specimens were stained with CAR primary antibody followed with FITC-conjugated secondary antibody or with DAPI. Immunofluorescence micrographs (40x) of CAR, DAPI and merged images are shown.

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 colorectal cancer patients treated at the VA. Using coxsackie-adenovirus receptor (CAR) as a novel biomarker for senescence, we have shown that senescence response can be demonstrated in rectal cancer patients treated with neoadjuvant chemoradiation (Figure 2). Tumor specific down-regulation of CAR expression is observed in treated tumors compared to normal adjacent mucosa and a control DAPI nuclear stain.

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 repopulate, 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 that 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.

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. Although 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 is to ask: "Does telomerase regulate senescence status during colorectal cancer treatment?"

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DEPARTMENT CO-INVESTIGATORS

Michael Sobel, M.D.

OTHER CO-INVESTIGATORS

Daniel Y. Wu, M.D., Ph.D.; UW Department of Medicine / William M. Grady, M.D.; UW Department of Medicine

VASCULAR SURGERY

KIRK W. BEACH, PH.D., M.D.

ALEXANDER W. CLOWES, M.D.

GÜNTER DAUM, PH.D.

THOMAS S. HATSUKAMI, M.D.

BENJAMIN W. STARNES, M.D.

GALE L. TANG, M.D.

Kirk W. Beach, Ph.D, M.D.

- Arterial Vibrometry
- Tissue Pulsatility Imaging of Brain
- Tissue Pulsatility Imaging of Placenta
- Multi-dimensional Doppler and Computational Hemodynamics
- Carotid Duplex Ultrasound Reading Center



Research Professor Emeritus

Half a century ago: when D. Eugene Strandness joined the University of Washington (UW) Department of Surgery as the Vascular Surgeon, Robert Rushmer, founder of Bioengineering at UW, was exploring cardiovascular physiology and Don Baker, using a newly available transistor, had just developed a portable ultrasonic Doppler for studies of blood flow at UW. Strandness collaborated with Rushmer and Baker to develop noninvasive diagnostic methods for arterial and venous diseases, leading the worldwide revolution in noninvasive

In 1985, David Phillips demonstrated that tiny tissue motions ($0.04 \text{ mm} = 40 \text{ }\mu\text{m}$) could be monitored with ultrasound through the skin. Phillips measured the motions of arterial walls during the heart cycle. Since that time, ultrasound instruments have evolved, changing from analog systems using electronic circuitry to process signals into digital “PC” computer-based systems using computer programs to process signals. We can now easily modify the way ultrasound instruments acquire, process and display signals just by changing the computer

The University of Washington Ultrasound Reading Center (UWURC) in the Strandness Vascular Laboratory has provided quality control for eight carotid stent studies including 15,182 examinations on 5,319 patients performed by more than 300 field centers.

diagnostic methods. To document these audio signals on paper for publication and teaching purposes, Strandness pioneered the use of spectrum analysis waveform display Doppler signals. By 1985, the Strandness team had established the guidelines for the modern duplex/Doppler spectrum analysis methods, which are still the standard. In addition to Doppler ultrasound, other diagnostic methods were used in the Strandness laboratory including: 1) plethysmography, measuring the normal 1% inflation and deflation of arterioles and venules in tissue with blood due to the cardiac cycle and respiratory cycle, and 2) phonoangiography, measuring the intensity and frequency of “bruits” (sounds generated by blood flow through arterial stenoses). These methods all depend on precise measurements of tissue motion.

program that operates the instrument. This has allowed us to develop and test new methods for examining tissue motions; we can now resolve motions as small as $0.1 \text{ }\mu\text{m}$.

Both plethysmography and phonoangiography are based on studying tissue motion: plethysmography studies tissue expansion with the cardiac and respiratory cycle; phonoangiography studies tissue vibration in the frequency range of hearing between 20 Hz and 1000 Hz. Traditionally, these methods are applied to a large body part such as arm, leg, finger, eye, skin and neck. However, by programming new methods of signal analysis into a conventional ultrasound instrument, these motions (expansion or vibration) can be measured inside the body, allowing the detection and characterization of vascular disease in organs and/or small regions of tissue.

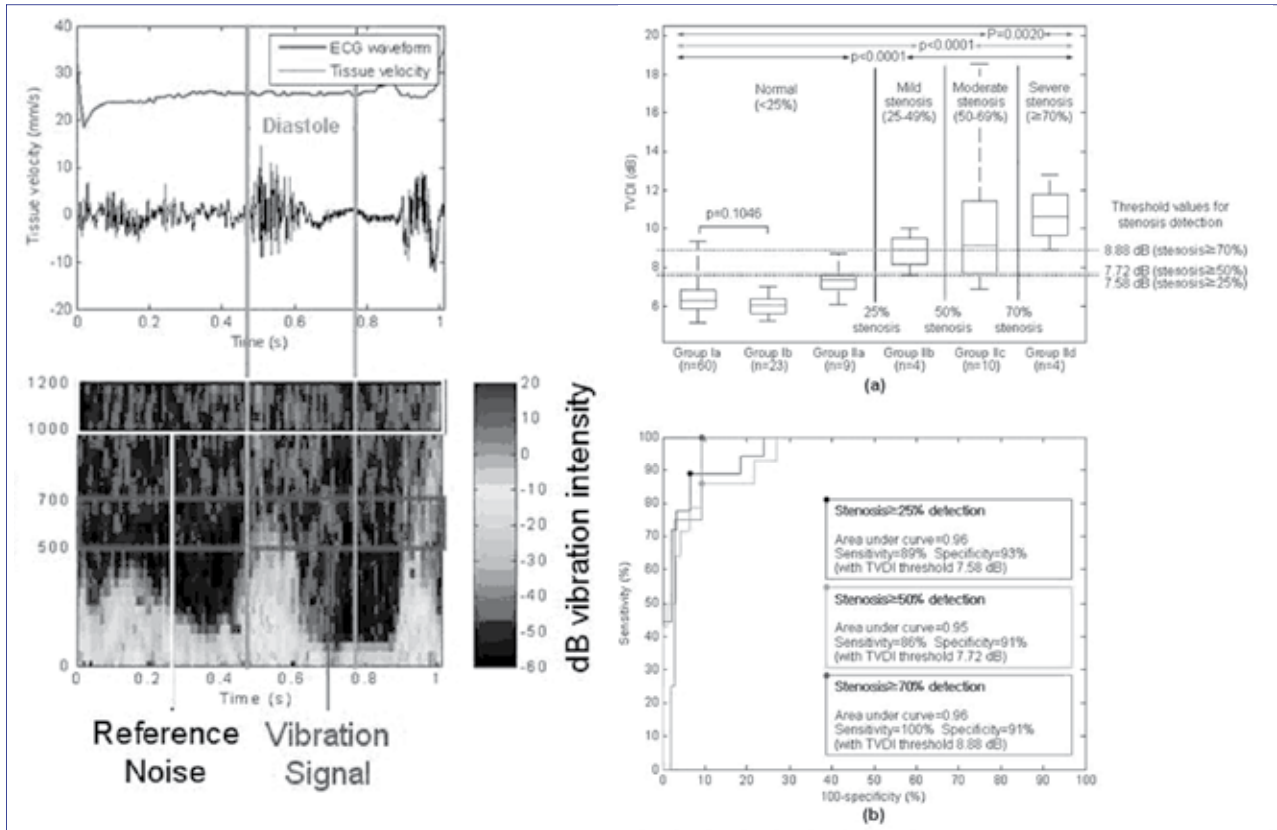


FIGURE 1 Left Anterior Descending Coronary Artery (LAD) Vibrometry

LEFT UPPER: Velocity of heart muscle (vertical) near the LAD during the cardiac cycle showing the diastolic time window (between brown lines) used for analysis. Left coronary artery flow is high in diastole. The vibration at the beginning of diastole mark the stenosis.

LEFT LOWER: Frequency spectrum of vibrations showing significant frequency band (red box) which is compared to a higher frequency noise band (black box). The vibration frequency in early diastole reaches 600 Hz.

RIGHT UPPER: Box and whisker plot of diastolic bruit (500 Hz to 700 Hz) intensity (vertical) vs coronary artery angiographic classification (horizontal)

RIGHT LOWER: Sensitivity and Specificity for different diagnostic bruit intensity thresholds.

Arterial Vibrometry

A stenosis in a major arterial pathway, which limits the blood pressure supplied to distal tissues, often causes a bruit (murmur) that can be heard through the skin with a stethoscope. These sounds are most easily heard if the stenosis is close to the skin. Bruits are often heard in the carotid arteries in the neck and femoral arteries in the leg. Although theoretically, a stenosis in a smaller, deeper artery will also emit a bruit, such bruits are rarely heard with a stethoscope because of the low intensity of the sound source and the attenuation through the tissue between the skin and the stenosis. However, an ultrasound system can detect small tissue motions at depths of 15 cm resolving vibrations with frequencies as high as 1200 Hz.

One important application is the detection of coronary artery stenosis. In collaboration with Professor Yongmin Kim in Bioengineering and a startup company who licensed the technology from the UW Center for

Commercialization, the ability of vibrometry to detect coronary artery stenosis with vibrometry was tested by cardiologist Dr. Keith Comess (Figure 1).

In 16 of 18 cases with left anterior descending coronary artery stenosis, diastolic bruits were detected with frequencies between 500 and 700 Hz, but only 5 of 92 non-stenotic cases had similar bruits.

Although a variety of methods to detect coronary artery stenosis are already commonly used in clinical practice, non are used in front line primary care due to combinations of cost, risk, and lack of specificity. We think that coronary artery vibrometry can be implemented in an application-specific low-cost automatic ultrasonic instrument that will noninvasively identify the location of each coronary artery stenosis and grade the severity to provide a guide to the primary care physician for remedial therapy or referral.

Tissue Pulsatility Imaging of Brain

A significant arterial stenosis, in addition to causing a bruit, will also delay the transmission of the cardiac pulse to distal tissues by more than 50 milliseconds. This delay is large enough to be detected by pulse palpation on physical examination. Pulse delay can be measured with plethysmography between left and right legs, eyes or other body parts. Pulse delay can also be measured in deep tissues using a specially programmed ultrasound system.

Pulsations in brain tissues can be detected with ultrasound, and pulse delays can be measured separately in brain tissues supplied by the anterior, middle or posterior cerebral arteries. We believe that by detecting pulse delays in regions of the cerebral cortex, significant stenoses in the cerebral branch arteries above the circle of Willis can be identified. Preliminary studies with primitive signal processing show that pulsations can be measured; improvements in signal processing are needed to resolve significant pulse delay in the far hemisphere where data from larger regions of the cerebral cortex can be conveniently acquired.

Stenoses in the cerebral arteries can be detected by conventional MR and X-ray angiographic methods, but the cost and risk are prohibitive for screening purposes. Brain tissue pulsatility imaging can be implemented in an application-specific low-cost automatic ultrasonic instrument that noninvasively identifies the location of each hypotensive region of the brain distal to a stenosis and grades the severity to provide a guide to the primary care physician for remedial therapy or referral.

Tissue Pulsatility Imaging of Placenta

Placenta is a unique organ perfused by both maternal (low pulse rate) and fetal (high pulse rate) circulations (Figure 2). For nutrition to pass through the placenta to the fetal blood, each segment of the placenta must be perfused by both fetal and maternal circulations. By using tissue pulsatility imaging, both the maternal and fetal pulses can be detected. Graduate student Asanka Dewaraja, working with Tom Easterling and Mark Moehring, is testing regions of the placenta to measure both maternal and fetal pulsations.

If the fetal pulse is absent in a placental region, then nutrition from that maternal blood is not supplied to the fetus, and the vascular resistance of the placenta to fetal blood is increased. If the maternal pulse is absent in a placental

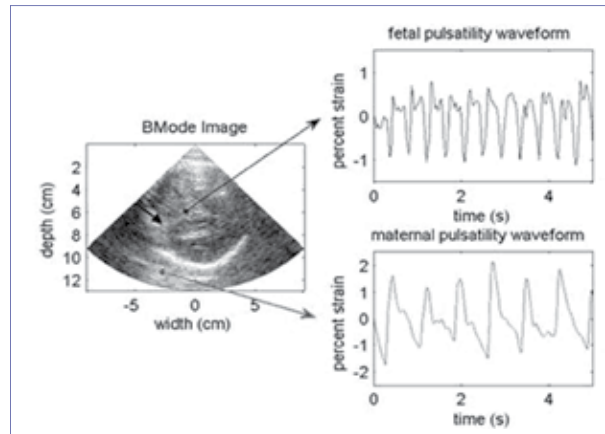


FIGURE 2 Tissue Pulsatility Image of Fetus, Image by John Kucewicz

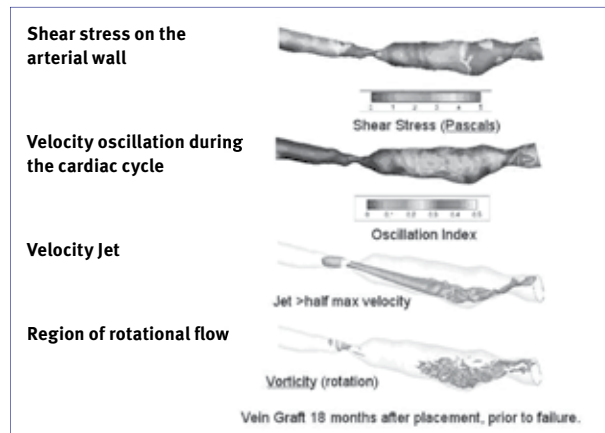


FIGURE 3 Computational Hemodynamics in Stenotic Vein Graft

region, then the fetal blood is not receiving nutrition from the maternal blood. In both cases, nutrition to the fetus is reduced. A map of the fetal and maternal pulsations in the placenta may provide early warning of placental dysfunction so that therapy can be instituted.

Multi-dimensional Doppler and Computational Hemodynamics

Programmable ultrasound instruments also allow the detailed study of blood velocity vectors in three dimensions using an ultrasound system developed by Dan Leotta. This system provides new insight into the rapid fluctuations in complicated blood flow patterns. Now we are combining these anatomic and Doppler velocity details with fluid mechanical simulations developed by Alberto Aliseda in the Department of Mechanical Engineering to predict the forces applied to vascular walls in patients.

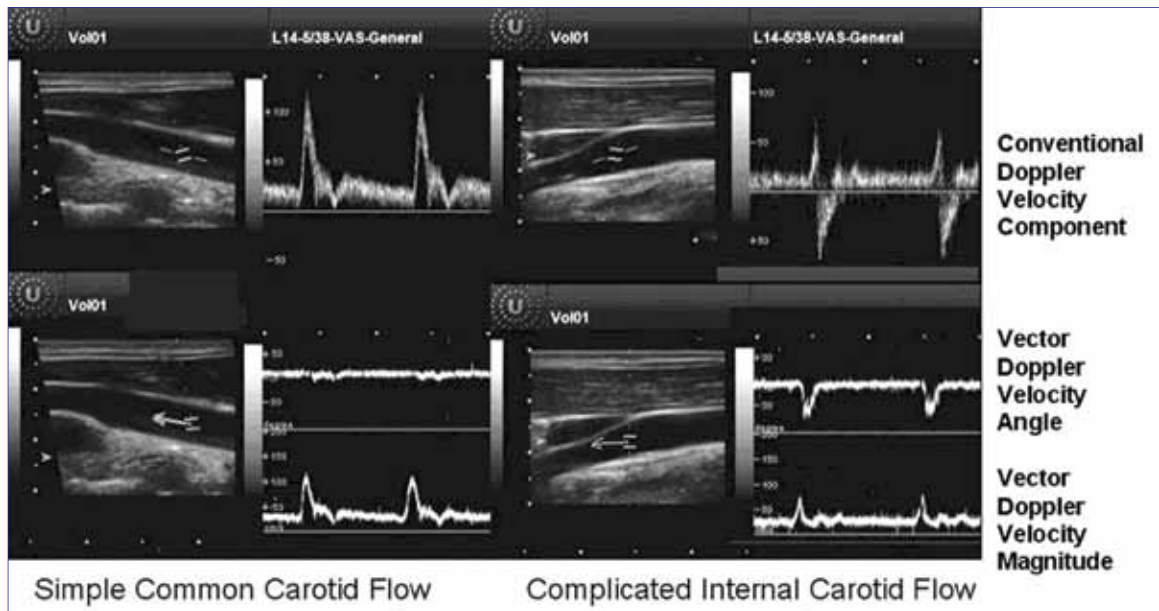


FIGURE 4 Conventional Doppler waveform compared to Vector Doppler waveform.

LEFT UPPER: Conventional Doppler Common Carotid Artery waveform showing a 60 degree Doppler angle component of simple flow parallel to the artery axis.

LEFT LOWER: Vector Doppler Common Carotid Artery waveform showing the blood velocity angle waveform and the velocity magnitude waveform.

RIGHT UPPER: Conventional Doppler Internal Carotid Artery waveform showing a 60 degree Doppler angle component of flow with a deceleration vortex, appearing as an apparent flow reversal.

RIGHT LOWER: Vector Doppler Internal Carotid Artery waveform showing the blood velocity angle waveform which changes during deceleration and the velocity magnitude waveform.

The 3-dimensional ultrasound images are used to provide a computational model of the shape vascular conduit. The conduit shape combined with the blood flow waveform, is processed with a computational fluid mechanics computer program to provide a detailed map of the flow including the impact of the blood on the wall (Figure 3).

According to published literature, either the hemodynamic shear on the vascular wall or the velocity oscillations stimulate remodeling of the arterial conduit wall. This remodeling will either lead to accommodation of the flow or to stenosis and occlusion. With serial 3-dimensional ultrasound studies correlating the hemodynamics computed at the wall with the remodeling of the wall, we hope to discern the effect of hemodynamic parameters on cellular proliferation, plaque formation and other remodeling factors.

At each examination, the computational hemodynamic results are correlated with detailed ultrasonic Doppler studies to assure that the computations correctly predict the flows that are actually present in the artery.

Although conventional ultrasonic Doppler measurements provide a component of the blood velocity that can be compared to the computations, more detailed Doppler data is now available using Vector Doppler (Figure 4).

Because of the high time resolution of the vector Doppler analysis, details of turbulence and actual wall shear stress can be measured for comparison with computations.

Carotid Duplex Ultrasound Reading Center

Over the last decade, a series of studies have been initiated to evaluate the treatment of carotid stenosis with stents. The University of Washington Ultrasound Reading Center (UWURC) in the Strandness Vascular Laboratory has provided quality control for eight carotid stent studies including 15,182 examinations on 5,319 patients performed by more than 300 field centers. The UWURC reviews all ultrasound images and waveforms, verifying locations, measurements and classifications from the source images.

Although carotid Doppler ultrasound has been widely used for over 25 years, questions still remain about exactly how the examinations should be performed and interpreted. Examiners disagree about whether Doppler signals should be acquired at a fixed Doppler angle of 60 degrees to the vessel axis or whether a variety of Doppler angles less than 60 degrees is preferable. A second question is how systolic velocity should be measured in waveforms with end-systolic deceleration turbulence.

Doppler velocity measurements are easily acquired at different angles from the same location in the carotid artery. This experiment shows that use of a higher Doppler angle results in a higher velocity value. Although this finding has stimulated discussion about which is the “correct” Doppler angle to get the “correct” velocity, none of the alternatives has resulted in superior correlation with carotid angiographic measurements. By exploring changes in velocity measurements within each patient over time, the UWURC will compute the variance of serial measurements for estimates of surveillance precision to determine how the selection of Doppler examination angle affects Doppler studies.

The interpretation of carotid Doppler waveforms has evolved empirically, adopting a logical policy so long as it appears to work. As a result, there is disagreement

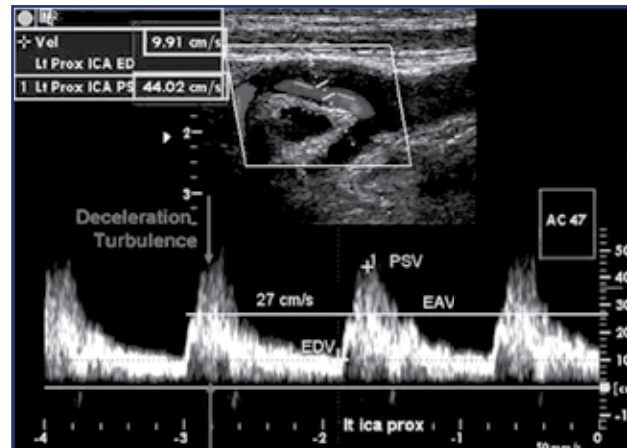


FIGURE 5 Doppler Waveform with End Acceleration Velocity

about exactly how to place the measurement cursors on some waveforms. When faced with turbulence during end-systolic deceleration, most examiners choose the highest value “peak systolic velocity (PSV) (Figure 5), but when deceleration turbulence is present, a lower end acceleration velocity (EAV) might be more appropriate. No comprehensive study has explored whether one choice should be preferred. By exploring changes in velocity measurements within each patient over time, the UWURC will tabulate whether deceleration turbulence is present in the same patient on repeat studies, and if so, whether choice of PSV or EAV provides the lowest variance between examinations.

Conclusion

The noninvasive vascular laboratory in the Department of Surgery has contributed to the long history of ultrasonic Doppler methods for vascular diagnosis. The close relationship between clinical practice and engineering innovation, pioneered by Gene Strandness when he founded the laboratory, is continuing to provide leadership in the development of instruments and methods that improve patient outcome.

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DEPARTMENT CO-INVESTIGATORS

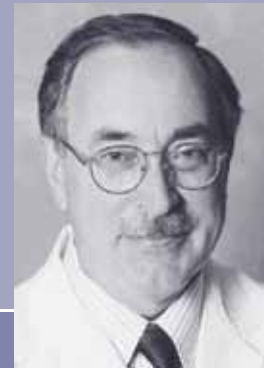
Robert Bergelin, M.S./ Edward Stutzman, B.S., R.V.T. / R. Eugene Zierler, M.D.

OTHER CO-INVESTIGATORS

Alberto Aliseda, Ph.D., UW Mechanical Engineering / **Keith Comess, M.D.**, CorazonX / **Asanka Dewaraja, M.S.**; Department of Bioengineering / **Tom Easterling, M.D.**; UW Department of Obstetrics and Gynecology/ **Yongmin Kim, Ph.D.**; UW Department of Bioengineering / **Dan Leotta, Ph.D.**; Center for Industrial & Medical Ultrasound, UW APL / **Hai-Dong Liang, Ph.D.**, Department of Medical Physics and Bioengineering, University Hospitals Bristol NHS Foundation Trust, Bristol, UK / **Mark Moehring, Ph.D.**; Spencer Technologies / **Siddhartha Skidar, Ph.D.**; George Mason University / **Peter NT Wells, Ph.D.**, School of Engineering, Cardiff University, Cardiff, UK.

Alexander W. Clowes, M.D.

• Regulation of Vascular Smooth Muscle Cell Growth



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

AWARDS

National Heart, Lung and Blood
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.

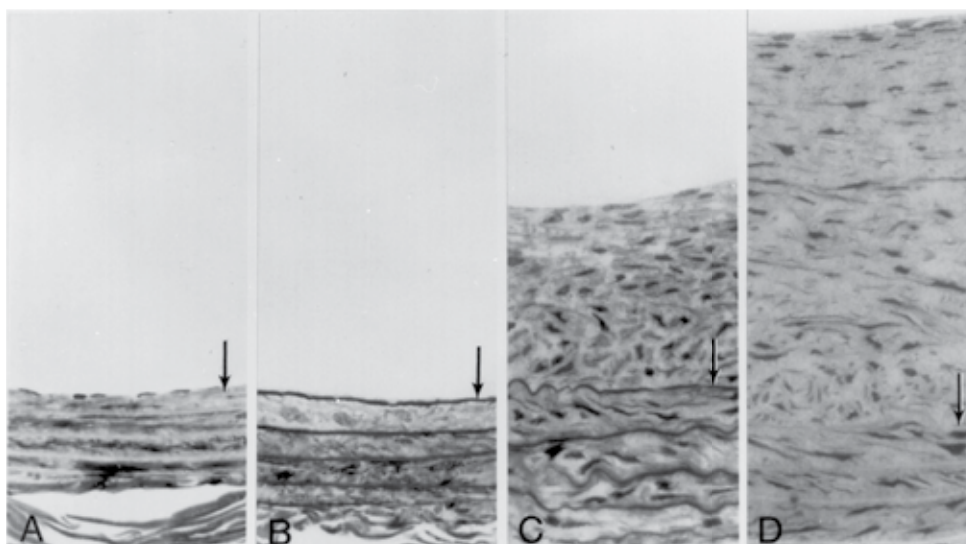


FIGURE 1: This series of photographs shows how a normal rat carotid artery (panel A-histologic cross-section) responds to injury. Angioplasty of the artery removes the surface endothelium (panel B). By two weeks (panel C), smooth muscle cells have migrated from the media into the intima (region above 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).

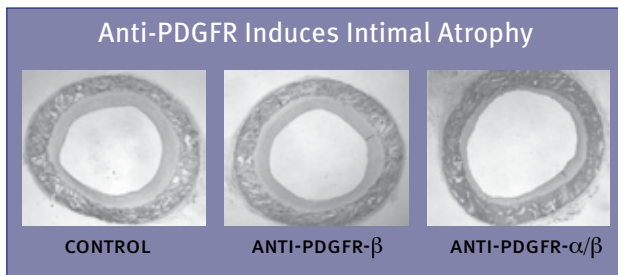


FIGURE 2: Histological cross-sections of normal flow PTFE grafts at 2 weeks following initiation of treatment with vehicle control, blocking antibodies to PDGFR- β , or blocking antibodies to both PDGFR- α and PDGFR- β . (H&E staining, 16X).

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.

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. In recent experiments, we have shown that syndecan-1, like heparin, is an inhibitor of smooth muscle cell growth, since genetic deletion of syndecan-1 in mice is associated with marked thickening in their carotid arteries in response to injury. We are currently investigating the underlying mechanism for this activity.

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.

Recent experiments performed in our lab using a mouse monoclonal antibody developed jointly with ZymoGenetics, Inc. 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. We licensed the patent to Celltech, Ltd. to “humanize” the antibody; it was then tested in a human trial for the prevention of restenosis after coronary stent

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.

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.

Human Vein Graft Failure

In collaboration with many centers throughout the country, we have conducted a pharmacological trial (PREVENT III; 1400 patients) designed to determine whether a drug (edifoligide; an E2F decoy) administered locally to vein bypass grafts could suppress intimal hyperplasia and prevent stenosis. Although there was no

difference between the drug-treated and control groups, we did show in this large cohort of patients that 25% of vein grafts develop significant stenosis during the first year after surgery. Furthermore, conventional cardiovascular risk factors are of no value in predicting outcome. Thus, there is a very pressing need to understand which patient is at risk for graft failure and to develop novel pharmacology to prevent it.

Our recent studies have begun to investigate the variability in vein graft outcome. We are testing the hypothesis that the smooth muscle cells in the wall of vein grafts that develop stenosis are different. The preliminary experiments indeed do define a difference in growth, at least in cell culture; cells from grafts that develop stenosis proliferate more than cells from grafts that heal normally. We are now pursuing the genetic basis for this observation.

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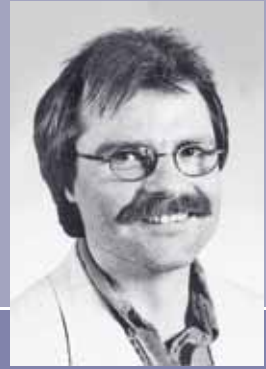
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DEPARTMENT CO-INVESTIGATORS

Lihua Chen, Ph.D. / Guenter Daum, Ph.D. / Jessie Deou / Lu Gao, M.D., Ph.D. / Richard Kenagy, Ph.D. / Ted Kohler, M.D. / Michael Sobel, M.D. / Gale Tang, M.D.

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

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.

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

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

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

FIGURE 1

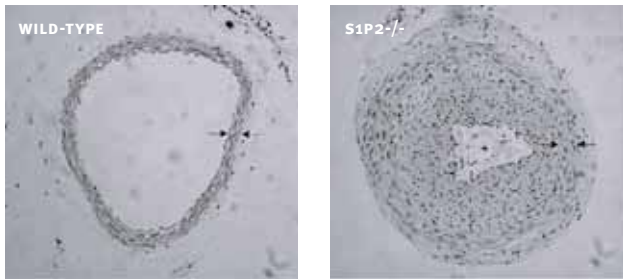


FIGURE 2

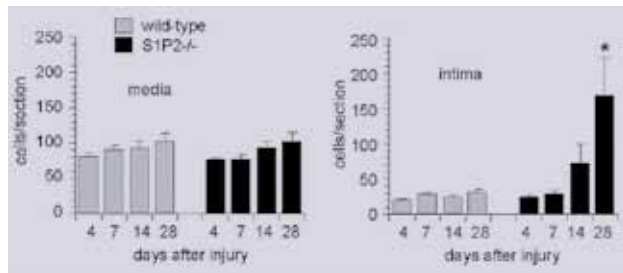


FIGURE 3

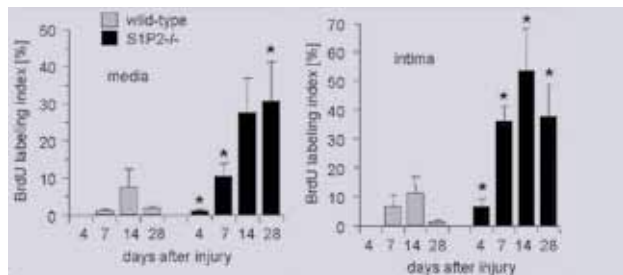


FIGURE LEGEND: Littermates of wild-type and S1P2-null mice underwent carotid ligation injury (4-6 animals/data point). Mice were injected intraperitoneally with bromodeoxyuridine (BrdU, 30 µg/g body weight) at 1, 9 and 17 hours before sacrifice. Mice were perfusion-fixed with 4% paraformaldehyde in PBS, 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) **FIGURE 1:** A typical cross section at 28 days after injury is presented. Arrows mark external and internal lamina, which define the media boundaries. Data for **FIGURE 2:** medial and intimal cell number, and **FIGURE 3:** BrdU index (labeled nuclei/total nuclei x 100) 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.

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 (Figure 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 (Figures 2 and 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 an approach should have few side effects as it lacks general cytotoxicity.

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DEPARTMENT CO-INVESTIGATORS

Alexander W. Clowes, M.D.

OTHER CO-INVESTIGATORS

William M. Mahoney, Jr., Ph.D.; UW Department of Pathology / **Michael A. Reidy, Ph.D.**; UW Department of Pathology / **Renee C. LeBoeuf, Ph.D.**; UW Department of Medicine / **Brian R. Wamhoff, Ph.D.**; University of Virginia / **Ulrich Walter, MD.**; University of Wuerzburg, Germany

Thomas S. Hatsukami, M.D.

· Magnetic Resonance Imaging of the High-Risk Atherosclerotic Plaque



Professor of Surgery

FUNDING

National Institutes of Health
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AstraZeneca
Pfizer
KOWA

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 United States 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.



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.

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 1), 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 of lumen narrowing and overall plaque burden (*Circulation* 1998; 98:2666 and *Magnetic Resonance in Medicine* 2000; 44:968), fibrous cap thinning and rupture (Figure 2; *Circulation* 2000; 102:959),

Over 16.7 million people die of cardiovascular disease (CVD) each year – one person every 2 seconds. Our primary goal is to develop and validate high-resolution imaging methods that will improve our ability to identify individuals at highest risk. Furthermore, by allowing us to non-invasively visualize the diseased vessel wall, these imaging tools will enable us to assess the effectiveness of novel therapies for CVD.

the lipid-rich necrotic core and intraplaque hemorrhage (Figure 3; *Arteriosclerosis, Thrombosis and Vascular Biology* 2005; 25:234), and the degree of neovasculture and inflammatory cellular infiltration of the plaque (Figure 4; *Circulation* 2003; 107:851 and *Radiology* 2006; 241:459).

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% among those with intraplaque hemorrhage, compared with -0.15% for those without hemorrhage ($p =$

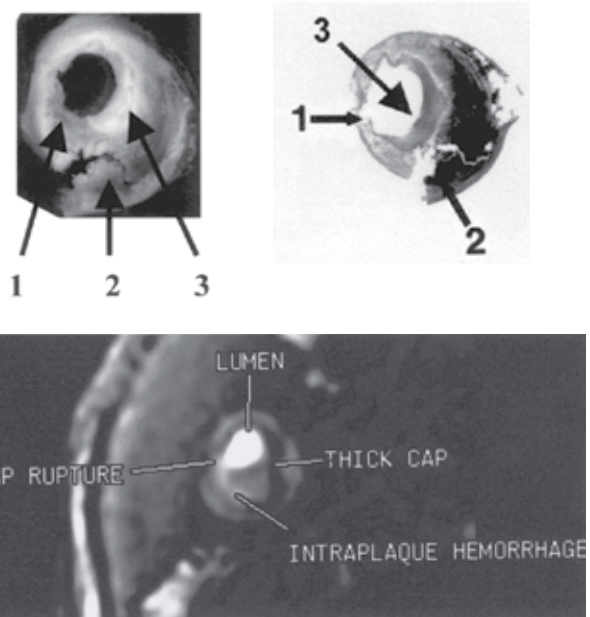


FIGURE 2: Example of a common carotid plaque with fibrous cap rupture and intraplaque hemorrhage. Photo of gross section of common carotid artery (left panel), trichrome stained histological section (right panel), and corresponding TOF MR image (bottom panel). Arrow 1 indicates an area of cap rupture, 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 1: Modified American Heart Association (AHA) classification scheme for describing atherosclerosis lesion types.

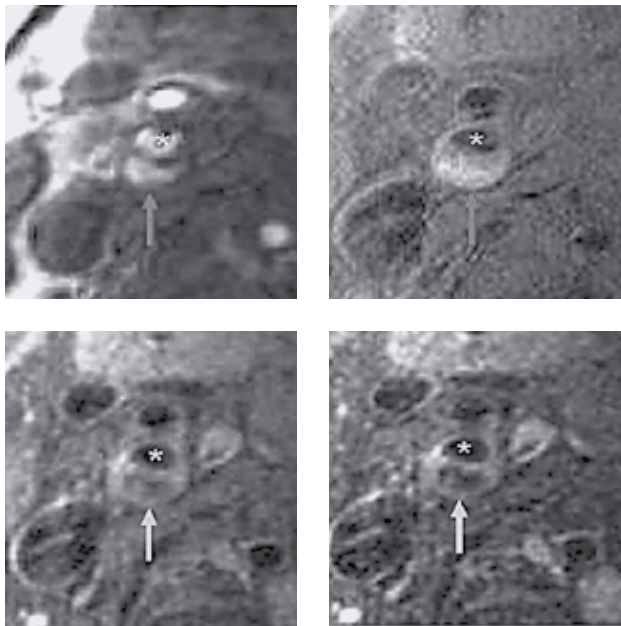


FIGURE 3: Example of an AHA Type VI (complicated) lesion with acute hemorrhage into the lipid-rich necrotic core. The asterisks indicate the lumen of the internal carotid artery. Early intraplaque hemorrhage, seen on the corresponding histological cross-section on the right, is identified by a hyperintense (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.

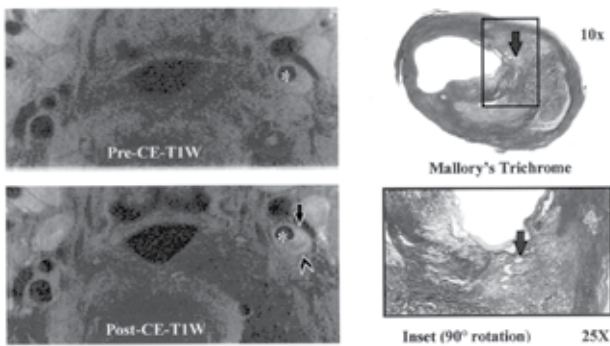


FIGURE 4: Pre-gadolinium contrast enhanced T1-weighted image of common carotid artery in left upper panel, post-contrast enhanced T1W image in left lower panel, and corresponding 10X and 25X trichrome stained histological sections. Note the enhancement seen in the shoulder region (arrow) in the post-contrast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

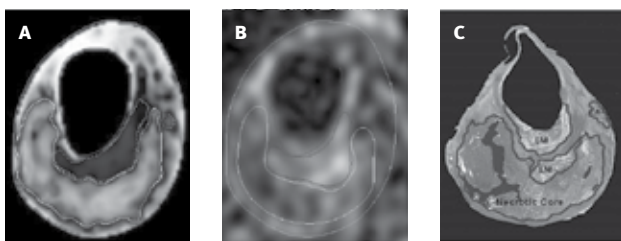


FIGURE 5: Segmentation results showing (a) automated quantitative image analysis tool; (b) manual outline by expert reviewer; and (c) corresponding histology section demonstrating a large necrotic core, loose matrix (LM) and a small area of calcification (CA). The dark regions within the necrotic core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

0.009). The lipid-rich necrotic core increased by 28.4% in plaques with hemorrhage, compared with -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 with 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 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 mm² 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).

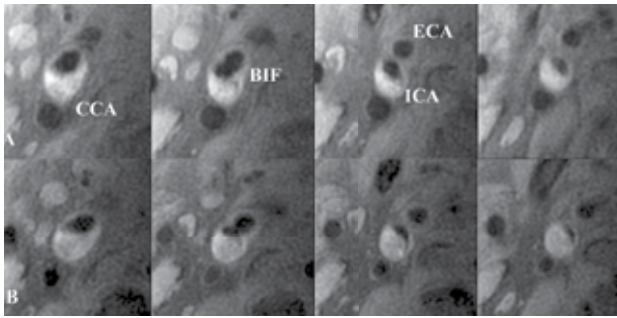


FIGURE 6: T1-weighted images of progression of atherosclerosis associated with intraplaque hemorrhage in the right carotid artery. Each column presents matched cross-sectional locations in carotid artery from baseline MRI (A) and MRI obtained 18 months later (B). Lumen area was decreased, and wall area 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.

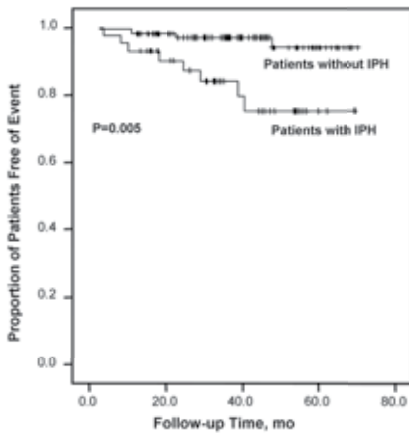


FIGURE 7: Kaplan-Meier survival estimates of the proportion of subjects remaining free of ipsilateral TIA or stroke with (lower curve) and without (upper curve) IPH. IPH = intraplaque hemorrhage.

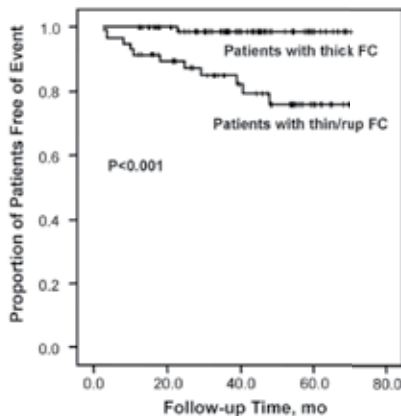


FIGURE 8: Kaplan-Meier survival estimates of the proportion of subjects remaining free of ipsilateral TIA or stroke with (lower curve) and without (upper curve) thin/ruptured fibrous cap. FC = fibrous cap.

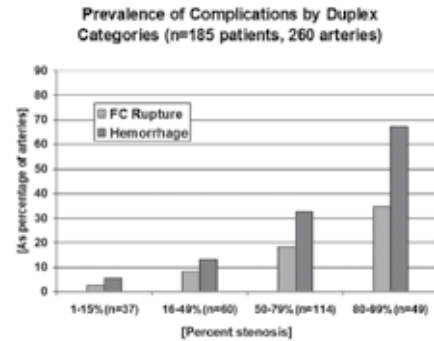


FIGURE 9: Prevalence of MRI-identified fibrous cap (FC) rupture and intraplaque hemorrhage by degree of stenosis in carotid plaques (n = 260) of asymptomatic volunteers. The degree of stenosis was determined by duplex ultrasound.

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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DEPARTMENT CO-INVESTIGATORS

Co-Principal Investigator and Co-Director, Vascular Imaging Laboratory / Chun Yuan, Ph.D.; UW Department of Radiology

OTHER CO-INVESTIGATORS

Gwenn Garden, M.D.; UW Department of Neurology / **Gail Jarvik, M.D., Ph.D.;** UW Division of Medical Genetics / **Jerry Jarvik, M.D., M.P.H.;** UW Department of Radiology / **Bill Kerwin, Ph.D.;** UW Department of Radiology / **Ted Kohler, M.D.;** Division of Vascular Surgery / **Kenneth Maravilla, M.D.;** UW Department of Radiology / **Steve Schwartz, M.D., Ph.D.;** UW Department of Pathology / **Vasily Yarnykh, Ph.D.;** UW Department of Radiology / **Xue-Qiao Zhao, M.D.;** UW Department of Medicine, Division of Cardiology

FELLOWS, STUDENTS, AND RESEARCH STAFF

Marina Ferguson, M.T. / Hunter Underhill, M.D. / Dongxiang Xu, Ph.D. / Baocheng Chu, M.D., Ph.D. / Anna Naumova, Ph.D. / Naranjan Balu, Ph.D. / Gador Canton, Ph.D. / Jie Sun, M.D., Ph.D. / Yunjing Xue, MD, PhD / Huijun Chen, Ph.D. / Bernard Chiu, Ph.D. / Rui Li, PhD / Florence Baluyot / Tong Zhu / Wendy Hamar / Dan Hippe / Jennifer Totman / Zach Miller / Juan Pablo Paredes / Randy Small

Benjamin W. Starnes, M.D.

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



Associate Professor
of Surgery
Chief, Division of
Vascular Surgery

The Harborview Experience

Harborview Medical Center is the only Level 1 Trauma Center in the Pacific Northwest and covers 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. Abdominal Aortic Aneurysm (AAA) disease is the 12th leading cause of death in the United States; 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 over the past two decades. Harborview treats between 30 and 50 patients with ruptured abdominal aortic aneurysm (rAAA) per year, with a historical 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 2).

New Technology

In 1991, Juan Carlos Parodi performed the first human endovascular AAA repair 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.¹ These procedures can often be performed using a totally percutaneous approach under local anesthesia.² 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.^{3,4}

A Modified Approach

Intrigued by the opportunity to offer these patients a minimally-invasive alternative to major surgery—which many of these patients cannot tolerate—our group implemented a new protocol in September 2007 designed to treat rAAA patients with a preference for EVAR under local anesthesia when feasible (Figure 3).

Before now, few studies existed 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%.^{5,6,7} In a recent meta-analysis of published series of rEVAR, mortality rates for people who underwent rEVAR were found to be lower than

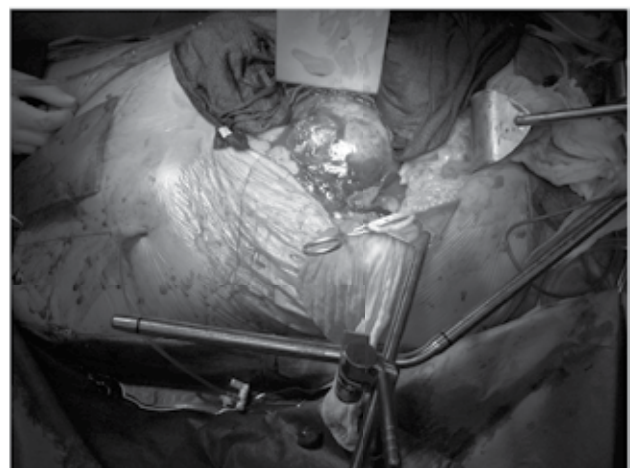


FIGURE 1. Intra-operative photo of patient with a ruptured AAA undergoing open repair. The head of the patient is to the right and an aortic cross clamp is in place.

in historical reports of unselected people undergoing open repair.⁸ At Harborview we use the rAAA protocol with continuing success. Currently, 80–90% of patients presenting at Harborview with rAAA are treated using an EVAR approach. We foresee the gradual diminishment in the use of open rAAA surgical repair except when patients present with anatomical features ruling out the rEVAR option.

During our study period, 187 patients with rAAA presented to our institution. Before implementation of the algorithm, 131 patients with rAAA presented and 128 were treated. The 30-day mortality rate was 57.8%. After implementation of the protocol, 56 patients with rAAA were managed. Twenty-seven patients (48%) underwent successful EVAR, and 24 patients (43%) underwent open repair. Five patients (9%) underwent comfort care only. In the post-protocol period, 5 patients in the EVAR group (18.5%) and 13 patients in the open group (54.2%) died during the follow-up period, for an overall 30-day mortality rate of 35.3% ($P = .008$ vs 57.8% pre-protocol). After implementation of a structured protocol for managing rAAA, there was a relative risk reduction in 30-day mortality of 35% compared to the time before implementation of the protocol (95% confidence interval [CI], 14%–51%) corresponding to an absolute risk reduction of 22.5% (95% CI, 6.8%–38.2%)(Figure 4).⁹

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 persons 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. Given the non-trivial size of the US population currently defined by these known risk factors, and who may be harboring asymptomatic aneurysmal disease, efforts to diagnose and treat this patient population early in the course of their disease have recently become a high priority public health issue.

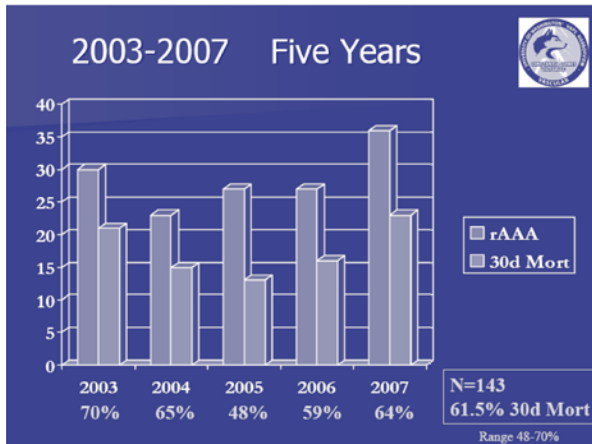


FIGURE 2: Mortality rates for 143 patients presenting with ruptured AAA at Harborview Medical Center between 2003 and 2007.

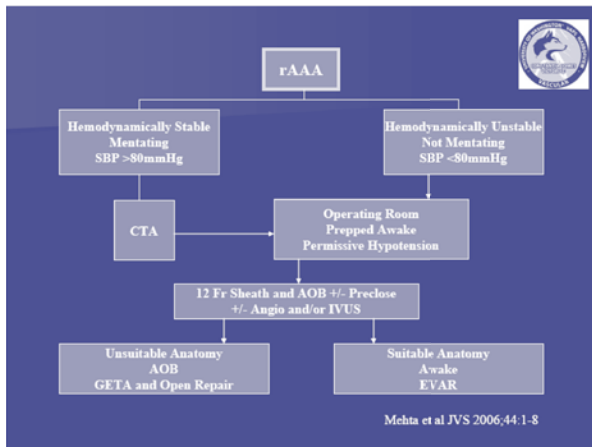


FIGURE 3: Protocol for management of patients with ruptured AAA. rAAA= ruptured abdominal aortic aneurysm, SBP= systolic blood pressure, CTA= computed tomographic angiography, AOB= aortic occlusion balloon, IVUS= intravascular ultrasound, GETA = general endotracheal anesthesia, and EVAR = endovascular aneurysm repair.

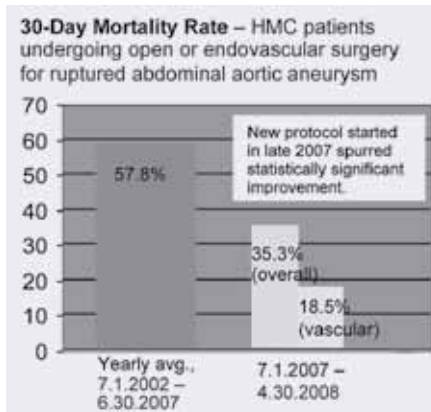


FIGURE 4: 30-Day Mortality Rate

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

Summary

A 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 at Harborview, we have reduced mortality for patients with ruptured aortic aneurysms by roughly 50% for the first time in over twenty years. Our group has begun to define variables affecting outcome for an endovascular approach, and we have partnered with several manufacturers to define those aortic stent grafts that prove most beneficial.

Future Directions

Our clinical research focuses on identifying those underserved patients who lack sufficient screening to detect aneurysms prior to progression to rupture. There is also a large subset of AAA patients with asymptomatic disease (and who come from all walks of life) who may be eligible for elective EVAR procedures, thereby avoiding the stresses of an emergency open operation. Modification of stent grafts for unique patients, in order to preserve critical branch vessels, is now under study and may become an important new direction as EVAR therapy becomes more widespread. Finally, we are also interested in defining those variables that are predictive of a 100% mortality rate—including, for example, the effect of hypothermia on the patient's chances for survival—thereby improving the efficiency with which we manage these desperately ill patients.

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Gale L. Tang, M.D.

• Mechanisms of Collateral Artery Development in Small Animal Hindlimb Ischemia



Assistant Professor
of Surgery

FUNDING

Division of Vascular Surgery start-up funds
SIBCR start-up funds

Arteriogenesis, or growth and development of pre-existing collateral arteries, is an important compensatory mechanism to restore blood flow after stenosis or occlusion of major conduit arteries. This process is frustratingly incomplete in many patients with peripheral vascular disease, leading to clinical manifestations of intermittent claudication, rest pain, and gangrene. The known mechanisms stimulating arteriogenesis include activation of shear-stress responsive genes as well as cytokines released from monocytes and progenitor cells. We are interested in further exploring cellular and molecular mechanisms that either stimulate or stop arteriogenesis using rodent models of hindlimb ischemia (Figures 1 and 2).

Pericytes are a progenitor cell population that has not been explored for a role in stimulating arteriogenesis. These mesodermal progenitor cells reside in the perivascular tissue and can both differentiate into multiple cell lineages and secrete paracrine growth factors and cytokines to influence neighboring cell behavior. The protein regulator of G protein signaling-5 (RGS5) has previously been demonstrated to be up-regulated in pericytes involved in angiogenesis and neovascularization. In collaboration with William Mahoney, PhD in the Department of Pathology, we have demonstrated that RGS5 null mice have a deficit both in native collateral function (as determined by an exaggerated decrease in perfusion following femoral artery ligation) as well as impaired recovery in hindlimb perfusion over time. We plan future experiments to further characterize both the deficit in the native collateral circulation as well as in their collateral artery development.

Small Animal Hindlimb Ischemia Models

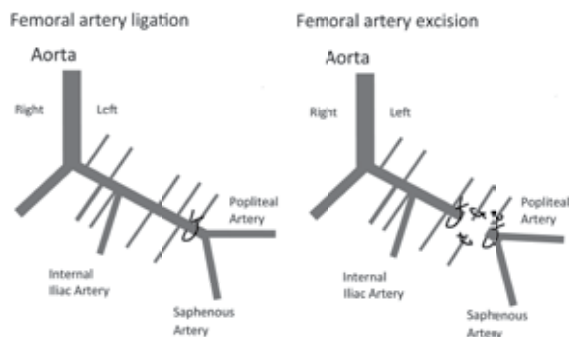


FIGURE 1. Hindlimb ischemia models. Left panel shows left femoral artery ligation. Right panel shows left femoral artery excision.

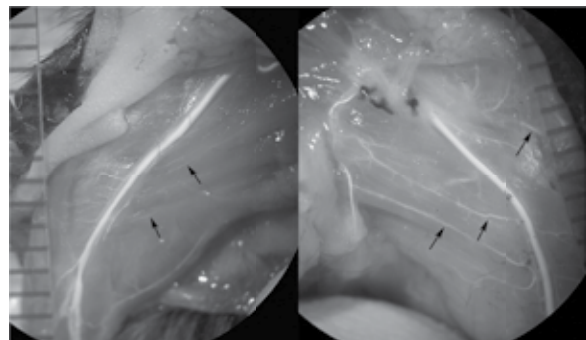


FIGURE 2. Collateral development in C57Bl/6 mouse 28 days after left femoral artery excision. Black arrows indicate collateral vessels. Left panel shows small native adductor collaterals on the unoperated control side. Right panel shows developed adductor collaterals after hindlimb ischemia.

We are ... exploring cellular and molecular mechanisms that either stimulate or stop arteriogenesis using rodent models of hindlimb ischemia.

It is thought that once shear stress has returned to physiologic levels, collaterals mature and stop developing further, but the molecular nature of this stop signal remains unknown. The syndecans are molecules that might be involved in translating such a stop signal, as both syndecan-1 and syndecan-4 have been shown to be regulated in vascular smooth muscle cells by mechanical stretch. Syndecans act as receptors for extracellular matrix proteins and for growth factors such as VEGF, FGF-2, and PDGF, which have all been shown to stimulate arteriogenesis in animal models. They cooperate with integrins to control cell adhesion to the extracellular matrix and cell migration. They are also expressed on monocytes. Their role in arteriogenesis has not previously been studied, and manipulation of the syndecans would be a novel approach to therapeutic arteriogenesis.

In collaboration with Dr. Alexander Clowes in the Division of Vascular Surgery, we have recently determined that syndecan-1 null mice revascularize poorly after hindlimb ischemia. Interestingly, the syndecan-1 null mice appear to have increased vascular smooth muscle cells (by nuclear counts and by measurement of medial layer thickness) in the collateral vessels. Although their native collateral diameter is slightly larger than wildtype mice, their density appears to be less than wildtype mice, suggesting an additional role for syndecan-1 in native collateral development. We are currently determining whether syndecan-4 null mice have a similar or opposite phenotype. It is unclear whether the effect of syndecan-1 on arteriogenesis is due to increased smooth muscle cell proliferation or effects on monocyte recruitment. Future experiments will focus on measuring smooth muscle cell and monocyte proliferation within the developing collateral arteries in syndecan-1 null and wildtype mice.

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DEPARTMENT CO-INVESTIGATOR

Alexander W. Clowes, M.D.

OTHER CO-INVESTIGATORS

William Mahoney, Ph.D., UW Department of Pathology / **Kevin Weitz, B.S.**, UW Department of Pathology

For copies or more information,
please contact:

MARILYN CARLSON
MANAGING EDITOR

UNIVERSITY OF WASHINGTON
DEPARTMENT OF SURGERY
BOX 356410
SEATTLE, WA 98195-6410

(206) 548-7797

MARCARL@UW.EDU