



SURGERY RESEARCH

DEPARTMENT OF SURGERY 2002 REPORT

UNIVERSITY
OF WASHINGTON
SCHOOL OF
MEDICINE



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

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Building a Solid Foundation

Academic surgery today requires skills in at least four interdependent areas: patient care, teaching, research and administration. Successes and challenges in one area directly affect the other three. To thrive, each must also have a firm foundation and strong leadership. Our department has done more than withstand the economic, political, and social upheavals of the past year — we have prospered. Our continued success is directly related to the foundation laid down over 55 years ago by **Dr. Henry N. Harkins** and reinforced by his successors. The national stature we enjoy today in these three areas did not just happen; it took hard work. This report focuses on our research endeavors and how they enhance our patient care and teaching activities.

The excellence of our department research is acknowledged in several ways: Most of our investigators receive funding from the National Institutes of Health (NIH), National Science Foundation, Centers for Disease Control, U.S. Army and Navy, and other federal & private sources. In terms of dollars funded, the NIH again ranked our department number 7 out of 86 for departments of surgery in fiscal year 2000. It is well recognized that competition for these dollars is intense. Although our overall funding is down 6% from last year, our NIH funding actually increased by \$533,546. The overall decrease is due to a combination of certain grants completing their multi-year awards and faculty attrition. However, one of our recent faculty recruitments, **Dr. Michael Sobel**, brings with him a \$1 million NIH grant, parts of which will be reflected in this current fiscal year's reconciliation.

The University of Washington selected **Dr. Alexander Clowes** for its Science in Medicine series last year (2000-01). This year (2001-02), **Drs. Nicole Gibran, Tom Hatsukami, and Matthias Stelzner** were selected as Science in Medicine "New Investigator" Lecturers, and **Dr. Ronald V. Maier** was also invited to be a Science in Medicine lecturer. Since the entire current lecture series encompasses 12 events, members of the Department of Surgery will have contributed 33% of these lectures. Considering the total number of

investigators in the School, and the large number of departments, this high representation is a testimony to the quality of the research performed in the department of Surgery and, indeed, an honor.

The American College of Surgeons honored **Dr. Matthias Stelzner** with the *George H.A. Clowes, Jr., M.D. Memorial Research Career Development Award*. The award — an incredible five year one — will advance Dr. Stelzner's novel technique of transplanting stem cells from the mucosa of the distal small bowel to that of the proximal small bowel in rodents. Development of this transplantation methodology forms the foundation for new clinical therapies. If, for example, important segments of the ileum were lost due to the ravages of Crohn's disease or during cancer surgery, we could replace its function by populating the proximal small bowel with a new type of functional environment. This work also has significant potential to benefit children with short bowel syndrome.

The prestigious American Surgical Association awarded one of its two research fellowships to **Dr. Michael Mulligan** to support his work in acute inflammatory lung injury and graft dysfunction. He has, at both the cellular and molecular levels, expertise to undertake *in vivo* blocking of mediator pathways and determine those that mediate acute inflammatory lung injury. Continued development of this work holds great promise for increased longevity of the lung transplant patient among other things.

Trailblazing work in cellular and molecular biologic investigations continues to emerge from the Harborview Research & Training Building under the guidance of **Dr. Ronald V. Maier**. Current awards amount to \$6.3 million in direct support for Department of Surgery PIs, and pending grant applications total an additional \$3 million. Recently, Harborview Medical Center became a major component of a national consortium of leading trauma centers awarded a "Glue Grant" by the NIH for \$37 million to elucidate the genomic and proteomic patterns predictive of outcome and morbidity following major injury and

infection. Dr. Maier has been charged with the development of a new institute, the Trauma Research Institute at the R&T Building, and we are recruiting a director for this important leadership position.

Our recruitment efforts focus on attracting the best creative minds to join us in improving patient care through teaching and research. Please join me in welcoming our newest faculty members:

Dr. Michael Sobel is the new Chief of Surgery at the Veterans Affairs Medical Center and Vice Chairman of the Department of Surgery at the University of Washington. Dr. Sobel was most recently Professor and Chief of the Vascular Surgery Section at Upstate Medical University, State University of New York, and Chief of Surgery at the Syracuse Veterans Affairs Medical Center, Syracuse, NY. He describes the Surgery Service at the VAMC as "one of the great sanctuaries for academic surgery." His stated goal is to "nurture this unique environment by attracting and retaining outstanding academic surgeons & scientists; to help focus their talents as they grow in their own careers; and to lead and shape their collective contributions towards a healthy, vibrant organization that provides outstanding surgical care." Dr. Sobel's research focuses on platelet-heparin interactions in cardiovascular surgery. His Vascular Research Laboratories at the Seattle VA are trying to determine the factors that cause abnormal clotting and bleeding, especially at sites of vascular surgery or where blood vessels have been injured. Through a better understanding of how blood cells and platelets contribute to the clotting process, and through the design of new heparin-like blood thinners with refined structures and activities, he hopes to improve outcomes for patients undergoing cardiovascular surgery.

Dr. Nicholas B. Vedder assumed the position of Chief of the Division of Plastic and Reconstructive Surgery and Department Vice Chairman, succeeding **Dr. Loren Enggrav** who founded the Division and acted as chief for 23 years. Dr. Vedder joined the faculty in 1991 at the rank of assistant professor of surgery in a joint appointment with the Department of Ortho-

paedics and was promoted in 1995 to associate professor. During his career at the University of Washington, he has overseen an extremely productive research laboratory at Harborview. His research and bibliography attest to his expertise in the field of reconstructive microsurgery.

Dr. Ramasamy Bakthavatsalam accepted a position of acting assistant professor in the Division of Transplantation. Dr. "Baktha" is no stranger to the Division or to the University of Washington Medical Center, having just completed his two-year fellowship here in multi-organ transplantation. Dr. Baktha graduated from Madras Medical College in Madras, India, following which he completed residencies in general surgery in India and England. From 1994-1999 Dr. Baktha trained in urology and renal/pancreas transplantation at Beaumont Hospital in Dublin, Ireland.

Dr. Jana Cole accepted a position as acting assistant professor with the Division of Plastic Surgery, based at UWMC, where she works with **Dr. Frank Isik**. Dr. Cole will also assist in the support of the Division of Plastic Surgery service at the VAPSHCS hospital. Dr. Cole was an active and productive researcher during her residency years. She was presented with the Snyder Award for best resident paper at the 2000 Plastic Surgery Research Council meeting.

Dr. Richard A. Hopper, assistant professor, comes to the UW Division of Plastic Surgery from the Institute of Reconstructive Plastic Surgery in New York where he completed the A. Ross Tilley Fellowship for Plastic and Reconstructive Surgery. Dr. Hopper joins **Drs. Joseph Gruss** and **Michael Whelan** at Children's Hospital & Regional Medical Center. He will also support the traumatic craniofacial practice at Harborview Medical Center. He has been active in research and was a recipient of the Plastic Surgery Research Council Snyder Award in 1997.

Dr. Lillian Kao joined the General Surgery Division as an acting instructor. Dr. Kao received her B.S. in Biomedical Sciences from the University of Michigan, where she graduated with Highest Distinction, and her M.D. from the same institution in 1996.

Dr. Kao plans to focus on advanced gastrointestinal surgical procedures and will work with **Dr. Mika Sinanan** in extending the existing laparoscopic resident laboratory education program to open procedures and techniques.

Dr. Daniel Leotta accepted an appointment at the rank of research assistant professor in the Division of Vascular Surgery. Dr. Leotta received his M.S. degree in Electrical Engineering at Massachusetts Institute of Technology in 1985 and his Ph.D. in Bioengineering at the UW in 1998. Joining our Department as a research engineer in 1999, Dr. Leotta has been instrumental in furthering the development of three-dimensional medical ultrasound imaging. His continued collaboration with **Drs. Kirk Beach** and **D. Eugene Strandness, Jr.** forms an essential part of our NIH grant on research of vein graft failure.

Dr. Mark Mattos joined the Division of Vascular Surgery as an associate professor to establish a state-of-the-art endovascular surgical program at the University of Washington. During the last decade, he has been at the forefront of endovascular surgery and has played a major role in the development of endovascular training programs for vascular surgeons. With Dr. Mattos' leadership and in partnership with the Divisions of Cardiothoracic Surgery and Trauma Surgery, we plan to provide opportunities for training not only for our fellows in vascular surgery but also for other surgeons in our department so that we may make full use of this new and exciting technology.

Dr. Brant K. Oelschlager joined the General Surgery Division faculty as an acting assistant professor. Dr. Oelschlager was born in Lenoir, North Carolina, and also received his degrees in that state: his B.A. from Davidson College, majoring in Economics, and his M.D. in 1995 from the University of North Carolina,

following which he completed a Holderness fellowship at the same institution. Dr. Oelschlager works in the Swallowing Center with me and will continue the program development of laparoscopic gastric bypass with **Dr. E. Patchen Dellinger**, as well as performing the full range of general surgery gastrointestinal procedures.

On February 1, we are honored to welcome **Dr. Ori David Rotstein** as the 8th Annual Resident Research Symposium and Helen & John Schilling Lecturer. Dr. Rotstein is the Peter A. Crossgrove Chair in General Surgery from the University of Toronto. We extend our deepest appreciation to Mrs. Schilling for her continued support of our residents. For further information about the lecture, call 206-543-9890.

We have redesigned our web site to make it easier to learn about our research endeavors, patient care, teaching activities, and other departmental events. Please visit us at <http://depts.washington.edu/surgery/>. In addition, our quarterly department newsletter, *Surgery Synopsis*, focuses on various aspects of our department's activities. To receive a complimentary copy, please call 206-543-7625.

On behalf of the Department of Surgery, please accept my most sincere thanks to all those who support and contribute to our various activities. Your generosity provides crucial support for our research, clinical, and training programs in these challenging times.



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

CARDIOTHORACIC SURGERY

GABRIEL S. ALDEA, M.D.

BRIAN W. DUNCAN, M.D.

MICHAEL S. MULLIGAN, M.D.

TIMOTHY H. POHLMAN, M.D. / EDWARD D. VERRIER, M.D.

Gabriel S. Aldea, M.D.



• MINIMIZING MORBIDITY OF CARDIOPULMONARY BYPASS

FUNDING:

Baxter Cardiovascular

Medtronic, Inc.

National Institutes of Health

National Research Service Award in Heart and vascular Diseases
PhramaSpec Corporation

Despite advances in traditional techniques, coronary artery bypass graft (CABG) 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 neurologic and neuropsychological function. Alternatives to traditional cardiac surgical methods, including “minimally invasive” techniques, are being developed to address the concerns and problems associated with conventional CABG.

Many of the complications of CABG are related to the biologic response of the body to artificial perfusion and gas exchange through the non-endothelialized cardiopulmonary bypass (CPB) circuit. Within seconds

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.

The use of these circuits has 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%.

Alternatives to traditional cardiac surgical methods, including “minimally invasive” techniques, are being developed to address the concerns and problems associated with conventional CABG.

of CPB, formed and unformed blood elements come into contact with the large surface area of the CPB circuit. Despite anticoagulation with heparin, this interaction results in extensive activation of platelets, neutrophils, complement, cytokines and the fibrinolytic system, producing a complex and intense “inflammatory” response. Although these responses are usually short lived and leave no residual deficits, they can lead to long-lasting cardiac, pulmonary, renal, and neurologic dysfunction in a subset of patients.

Using recent advances in perfusion technology and research in biomaterial sciences we have developed specific surgical techniques which have resulted in the routine application of more biocompatible circuits, such as heparin-bonded cardiopulmonary bypass

Compared to previous reports, the incidence of neurological and persistent neuropsychological deficits following CABG were markedly reduced.

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

We are involved in several ongoing investigations. First, we are studying ways to disassociate the contribu-

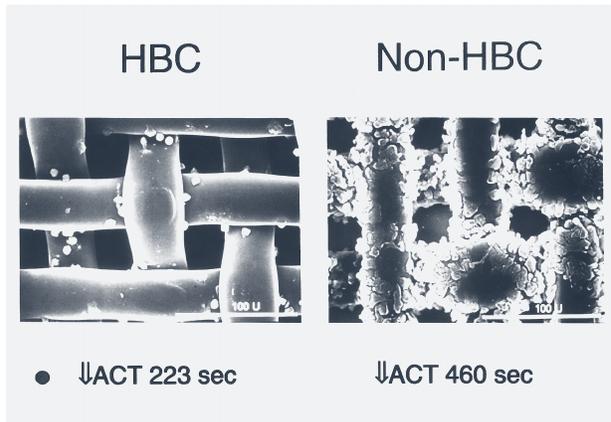


figure 1: Scanning electron micrographs at 200 fold magnification of arterial filter. Lowest activating times (ACT) in seconds are noted. HBC= heparin-bonded circuits. Non-HBC- control non-heparin-bonded circuits.

tion of biocompatible circuits from the specific surgical techniques (the effects of cardiomy suction vs. use of cell saver technology) on markers of hemostasis, inflammation and neurologic and neuropsychological deficits. Although both result in blood conservation, one (cardiotomy suction) reinfuses blood directly into the arterial side of the CPB machine and the other (cell saver technology) washes the cells prior to intravenous reinfusion. These different approaches may result in markedly different effects on inflammation and thrombin generation during artificial perfusion. Markers of thrombin generation (TAT, F1.2), inflammation (IL-6, IL-8, TNF, elastase, complement), hemostasis and platelet function, and cellular signaling of systemic inflammation (NF kappa B) are being evaluated.

Second, drawing on the divisional interest and expertise in evaluating the cellular aspects of ischemia-reperfusion, we will also investigate potential strategies to blunt the inflammatory response to cardiopulmonary bypass and open (intra) cardiac cases when cardiomy suction and open systems are required (such as in aortic and mitral valve surgery). Third, we are investigating targeted pharmacological interventions as well as further biomaterial modifications of the perfusion surface to further attenuate platelet, neutrophil, and complement activation, and cytokine release. Fourth, alternative surgical approaches which avoid cardiopulmonary bypass all together or through smaller incisions are also being assessed.

Coronary Sinus Retroperfusion: Delivery of blood with modified retroperfusate to ischemic myocardium is limited by antegrade flow of blood beyond coronary artery occlusion. Therapeutic intervention to limit ischemic damage has been limited to systemic

therapy. We have developed a simplified method of blood delivery to ischemic myocardium beyond such occlusion by reversing regional flow in the coronary venous circulation (simplified coronary sinus retroperfusion or SRP, Figure 2).

We were able to demonstrate in a porcine model that even after 90 minutes of ischemia, delayed therapy with SRP resulted in a >50% reduction in the ratio of area of risk to area of necrosis. Furthermore, using SRP, we were able to demonstrate selective drug delivery to the area at risk (with acute imposition of occlusion of antegrade blood flow) in a 10:1 therapeutic ratio compared to adjacent myocardial regions.

Current research in collaboration with Dr. Edward Verrier's lab will focus on whether targeted pharmacological therapy aimed at limiting ischemia-reperfusion injury delivered selectively with SRP can further reduce resulting damage. In addition, the possible use of SRP for selective delivery of gene therapy to promote angiogenesis will also be investigated.

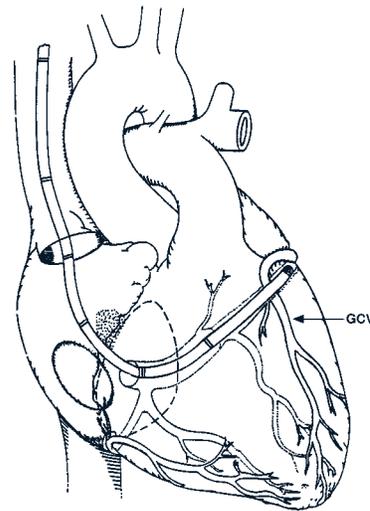


figure 2: A catheter inserted percutaneously is inserted under echocardiographic or fluoroscopic guidance through the coronary sinus and positioned at the level of the great cardiac vein (GCV).

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DEPARTMENT CO-INVESTIGATORS:

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OTHER CO-INVESTIGATORS:

Wayne Chandler, M.D.; UW Department of Laboratory Medicine

Brian W. Duncan, M.D.

• ANGIOGENESIS IN CHILDREN WITH CONGENITAL HEART DISEASE

Children with cyanotic congenital heart disease often demonstrate abnormal angiogenesis resulting in pathologic blood vessel proliferation. This proliferation may take several forms: arteriovenous malformations may develop in the lungs of children after palliative cardiac operations, or widespread systemic collateral vessels may develop in any child who is cyanotic. This abnormal vascular proliferation causes significant morbidity and mortality in these patients. Currently the only treatment options available are invasive, either in the cardiac catheterization laboratory or in the operating room. Our research is directed at understanding the mechanisms leading to abnormal angiogenesis in children with cyanotic congenital heart disease.

density has been used as a marker of angiogenesis in tumors. Increased microvessel density is associated with early metastasis and poor prognosis. Clinically symptomatic patients with PAVM had an increased microvessel density count compared to control lung. Asymptomatic patients after cavopulmonary anastomosis demonstrated increased numbers of microvessels compared to control lung as well. The number of microvessels in these asymptomatic patients was intermediate to the levels demonstrated by normal controls and patients with clinically symptomatic PAVM (4).

Animal Model of PAVM after Cavopulmonary Anastomosis: We developed a small animal model of pulmonary arteriovenous malformations by performing a classic Glenn shunt in rats. Male Sprague-Dawley rats under-

Microvessel density has been used as a marker of angiogenesis in tumors. Increased microvessel density is associated with early metastasis and poor prognosis.

Pulmonary Arteriovenous Malformations after Cavopulmonary Anastomosis

In-depth Histologic Analysis of PAVM in Humans: We have previously reported the histologic analysis of tissue from children after cavopulmonary anastomosis (1). We have subsequently performed a detailed immunohistochemical analysis of lung specimens in children after cavopulmonary anastomosis. Vascular endothelial growth factor (VEGF), a potent stimulator of angiogenesis, and its receptor (FLK-1) were increased in the lungs of children after cavopulmonary anastomosis. These children also demonstrated increased numbers of actin-positive vessels and decreased CD-31 staining. We are currently investigating the same battery of factors used in the human study in our animal model.

Human Microvessel Density: We performed microvessel density determinations on lung biopsy tissue from patients after cavopulmonary anastomosis. Microvessel

went a right cavopulmonary anastomosis using microsurgical techniques (Figure 1). Animals were sacrificed between 2 and 12 months. At the time of sacrifice an angiography was performed with subsequent removal of the lungs. The lungs were then prepared for molecular and histologic studies to characterize the animal model.

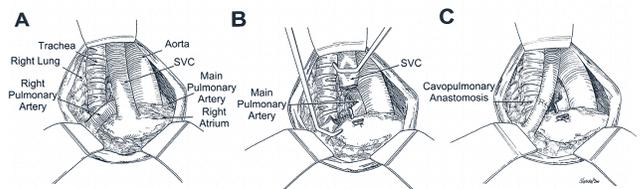


figure 1: **Schematic of the classic Glenn shunt performed in our rat model. Illustrations: (A) demonstrates initial view after performing the thoracotomy. (B) Shows the transected superior vena cava and right pulmonary artery. (C) Depicts the completed anastomosis and ligated main pulmonary artery and superior vena cava.**

Angiography

Angiography was performed on all animals for both the right (shunted) lung and the left (control non-shunted) lung immediately before the animals were sacrificed. Pulmonary angiography at one year demonstrated findings typical of arteriovenous malformations (abnormal capillary phase and early pulmonary venous opacification) while the control lungs were normal (Figure 2). Animals from earlier time points did not demonstrate these angiographic features. The time dependent changes reflected angiography results which closely resemble those of the human condition.

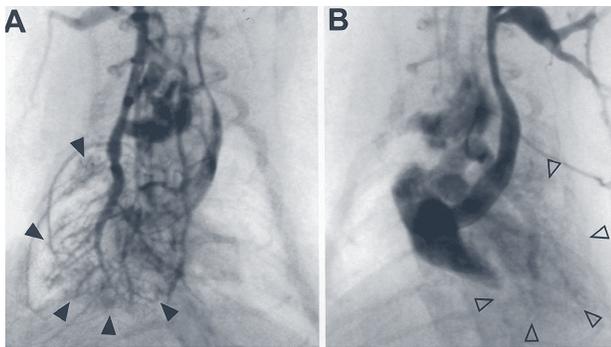


figure 2: Positive angiography and negative control from an animal one year post cavopulmonary anastomosis. Note: (arrows) the extensive collateral vessels in the shunted right lung (A) compared to the non-shunted control left lung (B).

Animal Model Microvessel Density: Microvessel density was determined by counting vessels staining positively for Von Willebrand Factor. Results for the shunted side were compared to the non-shunted (control) side. These animals demonstrated histologic features (Figure 3) identical to pulmonary arteriovenous malformations developing in humans after cavopulmonary anastomosis. These findings suggest that this is a valid model that may be used to further study the development of pulmonary arteriovenous malformations after cavopulmonary anastomosis.

Molecular Studies

We are currently completing molecular studies evaluating the level of expression of VEGF in our animal model. At this time, relative quantitative (RQ) reverse transcriptase polymerase chain reaction (RT-

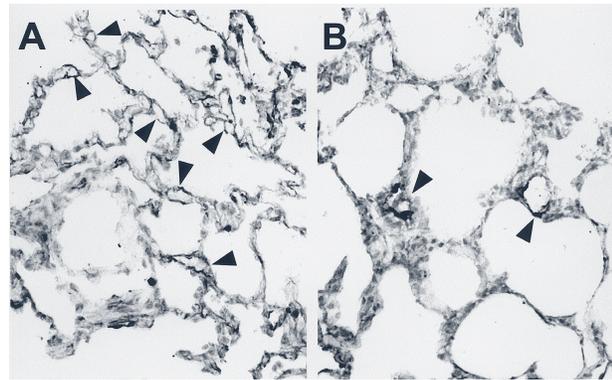


figure 3: Immunocytochemical staining ipsilateral to the shunt demonstrated increased numbers of vessels positive for Von Willebrand factor extending into the lung periphery. Microvessel density was greatly increased in the lung ipsilateral (A) to the shunt compared to the contralateral control lung (B) (33.8 ± 2.1 versus 18.2 ± 2.3 , $p < 0.01$).

PCR) studies are concluding for VEGF in the animal model. Preliminary findings indicate the presence of two alternate splice variants in rat lung. These findings correlate with information from the literature that reports VEGF₁₆₅ and VEGF₁₈₉ as the principal VEGF isoforms present in lung tissue (Figure 4).

Future Directions

Future molecular studies will include the analysis of VEGF receptor (FLK-1), i Nos and e Nos in children after cavopulmonary anastomosis as well as in the animal model. Previously reported findings on the decrease in CD-31 will be investigated for possible mechanistic implications in the formation of PAVMs. Finally, we intend to evaluate the possible localization of Angiopoietin I and II in these lesions.



figure 4: RT-PCR of control rat total RNA with target specific primers for VEGF yielded two primary reaction products. Band A, approximately 520-bp and band B approximately 450 bp. Band A corresponds to VEGF₁₈₉ and band B to the VEGF₁₆₅ variant. The marker (left) is a 100-bp molecular ladder; the gel is a 2% Agarose visualized with ethidium bromide.

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DEPARTMENT CO-INVESTIGATORS:

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OTHER CO-INVESTIGATORS:

Rolf A. Brekken, Ph.D.; Hope Heart Institute



Michael S. Mulligan, M.D.

• CYTOKINES AND CHEMOKINES IN DIRECT ISCHEMIA REPERFUSION INJURY OF LUNG AND CARDIOTHORACIC TRANSPLANT REJECTION

AWARDS:

Schilling Lecture, University of Washington, Prize, 2000
Seattle Surgical Society, Best Presentation, 1999
Resident Teaching Award 2000

FUNDING:

Bayer Corporation
Novartis
PrimeSource Surgical
Thoracic Society Directors Association

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

Attempts to alleviate immediate reperfusion injury in the grafted lung have focused on improving preservation techniques, minimizing ischemic times and modifying preservation solutions. More recently a number of studies investigated the role of cytokines and inflammatory peptides in the pathophysiology of reperfusion injury. Roles for several cytokines in reperfusion injury in clinical lung transplantation have been postulated for some time and animal studies suggest that these mediators may play a critical role. Chemokines of the IL-8 family have been isolated in various models of inflammation and may be involved in mediating reperfusion injury.

The chemokines are a family of chemotactic cytokines with a high degree of specificity for subpopulations of leukocytes. Four groups of chemokines have been characterized based on the structure of the peptides, CC, CXC, CX₃C, and C. The CC chemokines or the b chemokines have two adjacent cysteine residues, and function primarily as monocyte and lymphocyte chemotactic agents. Members of this family include MCP-1, RANTES and MIP-1a, MIP-1b, to name just a few. The second group, the CXC chemokines, are also referred to as the a chemokines. This group is character-

ized by the presence of an amino acid between the two cysteine residues, and includes powerful neutrophil chemoattractants, such as IL-8, MIP-2, and CINC. Two recently discovered groups of chemokines include the C and CX₃C families. These chemokines are chemotactic for T lymphocytes and monocytes and include lymphotactin (C) and fractalkine, also known as neurotactin (CX₃C).

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. This is strikingly similar to other models of acute lung injury such as immune-complex alveolitis, anti-basement membrane associated injury and secondary lung injury after remote tissue ischemia. A number of cytokines have been identified (i.e. TNF α , IL-1b, PAF) as important mediators in these models and to a lesser degree, in lung reperfusion injury.

Likewise three C-C chemokines, MCP-1, MIP-1a, and RANTES, have been shown to play roles in the development of several of these models, but only IL-8 has been investigated for any potential role in lung ischemia reperfusion injury. MIP-1a is upregulated *in vitro* following hypoxic stress and increased MIP1a messenger RNA is found in liver allografts shortly after reperfusion. Secondary lung injury develops following reperfusion of ischemic limbs, and liver that is at least partially regulated by C-C and potentially C-X-C chemokines. These findings would suggest that chemokines are likely to play some role in regulating direct ischemia reperfusion injury of the lung.

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 mRNA expression for MIP-1a in reperfusion

injury has been suggested by preliminary findings. Unmanipulated control lungs and those from animals undergoing ischemia plus 0.5, 1, 2, 3 and 4 hours of reperfusion were extracted for MIP-1 α mRNA. Message was not detectable in the unmanipulated lung but appeared at 30 minutes of reperfusion and was present throughout the reperfusion period. Using ELISA technology developed in our laboratory, we have also demonstrated increased protein expression MCP-1 (C-C), and CINC (C-X-C) content in BAL fluid from reperfused lungs (data not shown).

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 blocking antibody to MIP 1 α , experienced a mean 35% reduction in permeability compared to injured controls ($p < .001$). The lungs were also analyzed for myeloperoxidase (MPO) content as a measure of tissue neutrophil accumulation.

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 patients 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

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.

Increased tissue neutrophil content is detectable after two hours of reperfusion, is significant by three hours and is marked by four hours. In contrast, lungs from animals treated with anti-MIP-1 α demonstrated a 42% reduction in MPO content compared to four hours reperfused controls ($p = .02$). Ongoing studies are also investigating the mechanisms of chemokine regulation of reperfusion injury. 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. We have also developed strategies for blocking multiple chemokine receptors and interfere with common second messenger pathways. These studies should reveal the maximal effectiveness of chemokine blockade at numerous points in the inflammatory cascade.

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 beta chemokines in the development of experimental OB.

In addition to a variety of other mediators, two of the β -chemokines, MCP-1 and RANTES, were studied for their potential role in the development of obliterative bronchiolitis. Rat tracheas and main stem bronchi were heterotopically transplanted into the subcutaneous tissue of allogeneically mismatched (BN-LEW) or syngeneically matched (LEW-LEW) recipients. Control animals received daily injections of PBS or non-immune rabbit serum; additional animals were treated with polyclonal blocking antibodies against MCP-1 or RANTES. Tissue was explanted at two weeks and

examined histologically to quantify change in airway cross sectional diameter and loss of epithelium. Northern and Western blot analysis were performed to measure up-regulation 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 IOx operating microscope followed by a hand sewn anastomosis using 8-0 suture. The hearts are explanted at various time points and the laboratory is currently gathering data on the role of chemokine blockade on cytokine expression and abrogation of rejection.

In addition to the *in vivo* work done in the Mulligan lab, there is significant complementary *in vitro* work. All of the chemokines and cytokines discussed previously will be investigated in tissue sample using ELISA and Western Blot for protein analysis and Northern and RPA blots for mRNA analysis. The *in vivo* work is therefore complemented by sophisticated molecular techniques. With this in mind, the lab has embarked on a project to reconstitute reperfusion injury using cell culture. Specifically culture of type II pneumocytes, alveolar macrophages, pulmonary artery endothelial cells and neutrophils will be undertaken separately and in combination to elucidate the specific response of these cells to hypoxia and reoxygenation.

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• CELLULAR AND MOLECULAR MECHANISMS OF ISCHEMIA-REPERFUSION INJURY

AWARDS:

American College of Surgeons

- National Committee on Trauma Competition, First Place
- Region X Committee on Trauma Competition, Finalist
- Washington State Chapter Henry Harkins Resident Research Award, First Place
- Washington State Chapter Henry Harkins Scientific Presentation Award

American Heart Association

- Vivian Thomas Young Investigators Award in Cardiothoracic Surgery, Finalist

Helen and John Schilling Resident Research Symposium Awards

Thoracic Surgery Foundation For Research and Education Fellowships (2).

Western Thoracic Surgery Association Sampson Resident Research Award

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ZymoGenetics, Inc.

The vascular endothelium has a principal role in several of the biologic events that affect the preoperative, operative, and postoperative course of nearly all surgical patients. In response to injury, endothelial cells become activated, releasing or expressing a number of inflammatory mediators that enhance leukocyte adhesion, promote coagulation and induce vasoconstriction. These responses to injury are beneficial to the patient when limited to localized areas of infection or tissue disruption. During severe systemic illness (for example, cardiopulmonary bypass, sepsis, or shock), inflammatory reactions may become generalized, however, initiating a distinct pathologic state called the "Systemic Inflammatory Response Syndrome" (SIRS). Systemic inflammatory reactions in general cause damage to tissue, which leads to organ dysfunction.

Ischemia-reperfusion injury: Ischemia/reperfusion (I/R) injury contributes significantly to morbidity and mortality in surgical patients. I/R injury

inciting a deleterious inflammatory reaction in and around reperfused tissue. Because restoration of oxygen delivery to ischemic tissue is critical to survival, a substantial amount of research in the last decade has focused on treating or preventing this important consequence of reperfusion.

In our laboratory, we have examined the molecular mechanisms of *regional* I/R injury that complicates cardiothoracic surgical procedures, and *systemic* I/R injury that is the result of resuscitation in the trauma patient with hemorrhagic shock. The scope of our research includes the study of basic biologic processes at molecular, cellular and physiologic levels, and the examination of the pathophysiologic mechanisms of I/R injury. Our goal is to translate an understanding of the molecular mechanisms of I/R injury into applications for clinical practice.

The cellular and molecular mechanisms of endothelial cell activation during I/R injury are complex. These mechanisms result in tissue factor expression

Although cell culture is a highly artificial system, it allows us to examine in precisely controlled conditions specific questions about the effects of hypoxia and reoxygenation on molecular pathways in human cells.

is the principal pathogenetic event in stroke, complications of peripheral vascular disease, hemorrhagic shock, and early transplant graft dysfunction. Paradoxically, reperfusion of oxygen-deprived tissue, the mainstay of therapy for ischemia, causes further tissue injury by

(leading to microvascular thrombosis and disseminated intravascular coagulation [DIC]); neutrophil adhesion secondary to upregulation of neutrophil adhesion molecules on activated endothelium (for example, E-selectin); and leukocyte activation and chemotaxis

caused by the release from I/R injured endothelium of chemokines (for example, Interleukin-8) and growth factors. One component of our research is based on the transcription factor, NF- κ B, that regulates transcription and expression of the genes that encode these proteins.

NF- κ B is composed of subunits from the NF- κ B/Rel family of proteins. Five distinct DNA-binding proteins of the family, p50, p52, p65 (also known as RelA), c-Rel, and RelB, are involved in mammalian transcription. Members of this family are defined by the presence of a highly conserved region of approximately 300 amino acids called the "Rel homology domain," which bears the DNA binding site, located in the amino-terminus half of the domain.

During I/R injury, NF- κ B activation may be preceded by signal transduction through a cytoplasmic molecule, p38, of the MAP kinase family of signal transduction proteins. Once activated in response to environmental changes surrounding a cell, p38 in turn activates transcription factors leading to changes in gene expression in cardiac cells, endothelial cells of coronary vessels, or inflammatory cells such as macrophages.

Thrombin is generated during reperfusion and may mediate reperfusion injury. Thrombin interacts with a specific cell receptor, protease-activated receptor-1 (PAR-1), present on endothelial cells, cardiac myocytes, and macrophages, signaling changes in gene expression in these cells. Complement chemotactic fragments, C3a and C5a, are also generated during reperfusion of ischemic tissue and, with thrombin, may be the initiating signals of I/R injury.

The specific aims of our research are: (1) Determine the molecular pathways that lead to NF- κ B activation during ischemia and reperfusion; (2) determine the role of NF- κ B-mediated gene transcription in regional and systemic I/R injuries; and (3) identify novel therapies that block NF- κ B activation only during I/R injury, preserving the capacity of the cell, and the patient within whom it resides, to respond to other injuries (e.g., sepsis).

Experimental techniques: We utilize cultured cells to examine molecular mechanisms that are involved in the response to I/R injury. Although cell culture is a highly artificial system, it allows us to examine in precisely controlled conditions specific questions about the effects of hypoxia and reoxygenation on molecular pathways in human cells.

In addition, cell culture gives us the capability to move DNA sequences into human cells in a controlled

fashion to deduce cellular mechanisms of activation based on the effect of the protein encoded by the transfected DNA on cellular function. Finally, by employing differential array and DNA microchip technology, we can identify and characterize novel protein kinases or transcription factors that, in concert with NF- κ B, regulate the cellular response to hypoxia and reoxygenation. Interpretations of findings *in vitro* are provisional, however, until they can be confirmed *in vivo*.

We have developed several animal models of regional and systemic inflammatory responses induced by I/R injury. We have also included in our experimental repertoire *ex vivo* perfusion of hearts by the method of Lagendorf. Recently we have found that rabbit hearts made ischemic by transient coronary artery ligation, express large amounts of tissue factor after release of the ligature and reperfusion of the ischemic segment. Furthermore, we have recently reported that inhibition of IL-8 significantly blocks myocardial I/R injury.

We have developed and utilize a mouse model of myocardial I/R injury. A well-defined I/R injury is induced in mouse hearts by transient occlusion of the left anterior descending coronary artery. Following reperfusion we determine the size of the infarcted region to quantify the magnitude of cardiac I/R injury. Although the mouse myocardial I/R injury model is technically challenging and is performed in only two other laboratories in the U.S., use of transgenic or gene knockout strains allows us to examine the effect of specific genotypic changes (and thus phenotypic changes) on myocardial I/R injury.

For example we have examined mice that have been genetically engineered to lack PAR-1 (PAR-1 knockouts; or PAR-1 $-/-$). Compared to wild-types, PAR-1 knockouts develop a significantly smaller infarct after myocardial I/R injury — confirming, as we have postulated, that thrombin (through its interaction with PAR-1) plays a necessary role in the pathogenesis of I/R injury. Furthermore, based on evidence we have developed with regard to signaling pathways involved in myocardial I/R injury, we have been able to pharmacologically reduce infarct size in our mouse model of I/R injury. Blockade of p38 activity with a proprietary compound significantly attenuates infarct size after ischemia and reperfusion compared to mice treated with vehicle alone. Thus, we have been able to apply what we have determined about the basic science of myocardial I/R injury to potential clinical development.

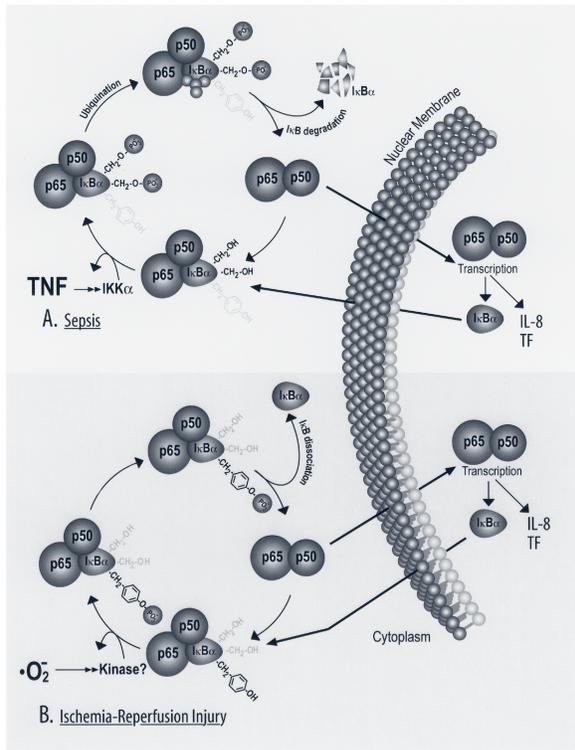


figure 1: **Parallel pathways of NF-κB activation.** In (A), septic stimuli, such as TNF-α (or, IL-1 or LPS), activate transmembrane signaling pathways in responsive cells leading to the phosphorylation of a serine/threonine kinase, IKKα. This kinase in turn phosphorylates IκBα on serine residues 32 and 36. IκBα is an inhibitor of NF-κB, but upon phosphorylation undergoes degradation. Degradation of Ser-phosphorylated IκBα requires the addition of ubiquitin molecules that target proteins for degradation in proteasomes.

After IκBα degradation, NF-κB, consisting of two subunits, p65 and p50, then translocates to the nucleus, where it binds to specific DNA sequences in the 5'-flanking region of several genes that encode proteins mediating inflammatory reactions (for example, IL-8), coagulation (for example, tissue factor [TF]), and immunologic reactions. Of interest, NF-κB regulates transcription of new IκBα, which functions in a negative feedback loop to down-regulate this particular cellular response.

In (B), reactive oxygen intermediates activate signaling pathways yet to be determined that lead to tyrosine phosphorylation of IκBα. The tyrosine kinase (or kinases) responsible for this reaction has not yet been identified. Tyr-phosphorylated IκBα, in contrast to Ser-phosphorylated IκBα, dissociates from NF-κB without degradation. NF-κB subsequently translocates to the nucleus to promote transcription of a similar set of genes as shown in (A), including IκBα. This figure shows the molecular basis for the inflammatory reaction induced by ischemia-reperfusion injury, or any other injury in which reactive oxygen intermediates are formed. The figure also indicates that it may be possible to suppress an inflammatory reaction associated with ischemia-reperfusion injury that may be detrimental to the patient, without blocking the patient's ability to generate an inflammatory reaction when required to contain microbial invasion.

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- IMMUNOMODULATION OF THE ALVEOLAR MACROPHAGE
- THE CYTOKINE PROFILE OF BURN PATIENTS RECEIVING PLASMAPHERESIS
- PRE-HOSPITAL MANAGEMENT OF THE DIFFICULT AIRWAY
- RIB FRACTURE MANAGEMENT
- VARIATIONS IN THE CARE OF HEAD INJURED PATIENTS
- THE USE OF ANABOLIC STEROIDS IN THE CHRONICALLY VENTILATED SURGICAL PATIENT

AWARDS:

American College of Surgeons
Committee on Trauma Competition, Best Basic Science Paper 1995 & 1999, Finalist 1996
Washington State Chapter Henry N. Harkins Resident Paper Competition, 2nd place (1994)
Helen and John Schilling Resident Research Symposium, First Place (2000)
Seattle Surgical Society Award
Shock Society
Young Investigator Award, Finalist (1996)

FUNDING:

American Association for the Surgery of Trauma/Wyeth-Ayerst Research Scholarship
Clinical Nutrition Research Unit, University of Washington
Pilot & Feasibility Award
Medic One Foundation
Northwest Burn Foundation
Washington State Council of Firefighters

Based on a strong interest in trauma and critical care, my research has focused on addressing important clinical questions regarding patient management, and elucidating the cellular biology of the systemic inflammatory response. My laboratory efforts, in collaboration with Dr. Ronald V. Maier, 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. Nicole Gibran seeks to explore the cytokine physiology associated with the response to plasmapheresis in the severely burned patient. On the clinical front, I have been interested in the pre-hospital management of the difficult airway, impact of rib fractures in the elderly, variations in the care of head injured patients, the use of anabolic steroids in the ICU.

Immunomodulation of the Alveolar Macrophage

ARDS is a process of acute inflammatory lung injury which affects a diverse array of surgical and medical patients. The etiology of this process is thought to involve an excessive, overexpression of the inflammatory response leading to the destruction of host tissue. The alveolar macrophage is a key cell in the coordination of this response. Our laboratory has focused on all aspects of this response using endotoxin as a prototypic inflammatory stimulant. In previous studies we have demonstrated that treatment of alveolar macrophages

with certain antioxidants, *in vitro*, results in significant inhibition of the macrophage cytokine response. This work was extended to an *in vivo* model of enteral Vitamin E supplementation in rats with similar results and a recently completed prospective, randomized trial of high dose enteral Vitamin E and C vs. placebo in the surgical ICU.

Recently we have also investigated the use of platelet activating factor acetylhydrolase (PAF AH) *in vitro*. PAF is a pro-inflammatory lipid mediator which has been implicated in several animal models of lung injury. PAF AH is the endogenous enzyme for PAF metabolism. These studies have demonstrated profound inhibition of cytokine production by macrophages treated with PAF AH prior to and following LPS stimulation. We are currently exploring the intracellular signaling pathways interrupted during this process and have extended these studies to animal models of ARDS. PAF AH has recently been studied in a phase II clinical trial for trauma and septic patients at risk for ARDS with encouraging results. The PAF AH treated group was found to have a significant decrease in 28 day mortality, the development of ARDS, and length of ICU stay compared to placebo controls.

The Cytokine Profile of Burn Patients Receiving Plasmapheresis

Burn mortality has dramatically decreased over the past twenty years due to improvements in ICU manage-

ment and better skin coverage. However, patients with large burns still face a high mortality during the first 48 hours of resuscitation. Severe burn injury is associated with a systemic inflammatory response which results in increased capillary permeability. As a result, these patients require a massive fluid resuscitation.

Several formulas have been developed to help estimate the fluid requirements during the first 24 hours, however, some patients, especially those with large, deep burns or inhalation injury, exceed these estimates and thus have evidence of ongoing inflammation which is not self-limiting. These patients have a higher mortality. Anecdotal experience suggests that these patients benefit from a plasma exchange which results in cessation of the capillary leak and decreased fluid requirements after therapy. In collaboration with Dr. Nicole Gibran, we are investigating the cytokine profile and degree of oxidative stress of these patients, both before and after plasma-pheresis, to better define the mechanism responsible for the clinical improvement seen with this therapy.

Pre-hospital Management of the Difficult Airway

The introduction of endotracheal intubation to the pre-hospital arena in the 1970s has resulted in definitive airway control for the majority of critically ill and injured patients, leading to a significant improvement in

the pre-hospital use of paralytic agents to facilitate endotracheal intubation in the combative patient or one with significant muscular spasm.

The Seattle Medic One program has been on the forefront of advanced field care by providing training and access to the techniques of surgical airway access and extensive experience with the pre-hospital use of paralytic agents since 1970. This experience provides the opportunity for a population based study of the indications for pre-hospital intubation and the use of paralytic agents. We have recently completed an analysis of all pre-hospital intubations in Seattle over the past three years with detailed investigation regarding the management of the "difficult airway patients." In addition, we are collecting prospective data for every prehospital intubation event. Our goal is to optimize the field management of these complex 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

Burn mortality has dramatically decreased over the past twenty years due to improvements in ICU management and better skin coverage. However, patients with large burns still face a high mortality during the first 48 hours of resuscitation.

morbidity and mortality. There remain, however, patients who have a "difficult airway" in that they can not be successfully intubated by conventional techniques.

These include patients with prohibitive head and neck habitus or anatomy such as trauma patients with significant facial and neck injuries and medical patients with severe upper airway inflammation or obstruction. These are patients in whom orotracheal or nasotracheal intubation, with or without the use of chemical paralysis, is impossible to accomplish. Successful definitive airway control for these patients requires advanced surgical airway access techniques: cricothyroidotomy, tracheostomy, or retrograde intubation. There also remains considerable controversy in the literature concerning

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 and a 31% rate of pneumonia. For an elderly patient with >6 rib fractures mortality was 33% with a pneumonia rate of 51%.

One of the key strategies in the management of these patients involves the ability to obtain adequate pain control to optimize pulmonary status. To further investigate the issues surrounding pain management for these patients, we are currently enrolling patients into a prospective, randomized trial of thoracic epidural vs. intravenous narcotics. We hope to use this

data to develop an optimal management strategy for these patients.

Variations in the Care of Head Injured Patients

In 1995 the Brain Trauma Foundation compiled a series of evidenced-based guidelines for the care of the head injured patient. That same year, a survey of the clinical management of the head injured patient, nationwide, revealed considerable variation in care.

In this study we sought to determine the current status of variations in care, since these guidelines have been widely distributed, with a particular focus on the controversy surrounding intracranial pressure monitoring. We have analyzed data from 34 academic trauma centers of the University HealthSystem Consortium regarding the management of patients with severe brain injury (GCS < 8). Centers were classified as "aggressive" if they placed intracranial pressure monitors in more than 50% of those patients meeting the Brain Trauma Foundation guidelines for monitoring. We have found that management at "aggressive" centers is associated with a significant reduction in mortality.

The Use of Anabolic Steroids in the Chronically Ventilated Surgical Patient

Multisystem traumatic injury results in a hyper-metabolic state which leads to a stress-induced catabolism and the accelerated breakdown of protein stores. If this process continues unchecked it results in loss of lean body mass which can lead to muscle weakness and depression of the immune response, making the patient more susceptible to infectious complications. Weakness of the respiratory musculature can inhibit ventilator weaning and lack of protein leads to significant impairment in wound healing. These complications are observed with a loss of only 10-15% of lean body mass. A loss of lean body mass greater than 40% is usually fatal due to infectious complications.

Recognition of these concerns has led to an appropriate emphasis on early nutritional support including replacement of protein losses. Despite this approach, however, several studies have shown that aggressive nutritional support alone does not prevent substantial body protein loss during the catabolic state of severe illness. As a result, attention has turned to the development of adjuvant nutritional therapies which when administered, in conjunction with aggressive protein support, will help reverse the catabolic state. These include the use of recombinant human growth hormone and anabolic steroids.

Oxandrolone is an oral anabolic steroid with enhanced anabolic activity and minimal androgenic activity when compared to testosterone. In chronically malnourished patients including renal dialysis patients, COPD patients, and HIV patients, anabolic steroids, in combination with an enhanced protein diet, have been shown to significantly improve lean body mass and muscle strength. In burn patients, oxandrolone use has been improvements in lean body mass and strength training during the rehabilitation phase.

Based on these studies, oxandrolone has achieved FDA approval as an adjunctive therapy to promote weight gain after extensive surgery, chronic infections, and severe trauma. Despite this approval, this agent has not been well studied in the acute trauma population. We believe that post-surgical or trauma patients who require a prolonged period of mechanical ventilation (>7 days) may benefit from oxandrolone therapy. To test this hypothesis we are currently enrolling patients in prospective, randomized, blinded trial of oxandrolone vs. placebo in this patient population.

In addition to the clinical arm of this trial, we are simultaneously investigating the effect of anabolic agents on monocyte function, *in vitro*, and receiving circulating monocyte samples from the patients in this study to evaluate their activation status.

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Nicole Gibran, M.D.

- BURN WOUND REPAIR
- CYTOKINE RESPONSE TO THERMAL INJURY
- NEUROINFLAMMATORY RESPONSE TO WOUND REPAIR
- GENETIC APPROACH TO HYPERTROPHIC SCAR

FUNDING:

National Institutes of Health

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

WA State Association of Fire Fighters Burn Foundation

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

Burn Wound Repair

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

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

We designed our basic science efforts to define cellular and extracellular inflammatory processes in normal burns. Our aim has been to better understand what deviations result in non-healing wounds or in

abnormal scars in order to know when to perturb the healing process with a repair accelerant.

We have studied the temporal and spatial localization of dermal inflammatory cells, basic fibroblast growth factor, macrophage chemoattractant protein-1, and collagenase during repair. Collectively, our data support the theory that the skin itself is a component of the immune system and that non-inflammatory cells may contribute to the initiation and maintenance of the inflammation at the wound site. Furthermore, these studies have 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.

Cytokine Response to Thermal Injury

Our latest therapeutic approach to the acute care management of patients with thermal injury has been to reintroduce plasmapheresis into the care plan of patients with large burns that are failing resuscitation. With advances in wound closure we are able to treat patients effectively if we can help them to survive the initial resuscitative phase — or 48 hours after injury. Over the past year we have had favorable experience using plasmapheresis on selective patients with large burns. Since these patients represent anecdotal evidence that plasmapheresis may have a role in the management of patients with large burns, we are pursuing an in depth clinical and basic science study of the effect of plasmapheresis. We will look at cytokine levels in the plasma of the patients before and after their plasmapheresis has been completed to determine which mediators are elevated during the inflammatory response to injury. We will

correlate these results with the clinical course of patients that undergo plasmapheresis compared with control subjects matched in age and burn size.

Neuroinflammatory Response to Wound Repair

Our lab has been dedicated to defining the neuro-inflammatory response to wound repair. The sensory nerves in skin regulate not only pain transmission, but also a local inflammatory response within the wound bed. We have identified the normal temporal and spatial distribution of pain fibers in human burn wounds.

Following injury, sensory nerves are absent within the injury site. With time there appears to be a transient abnormal increase in neuroinflammatory mediator

These studies are currently focussed on intracellular signaling pathways that mediate substance P mediated changes to the endothelial cell.

Activated endothelial cells stimulate reinnervation of the injury site. We have defined this process to be a neuro-endothelial axis and believe that it may contribute to the pathophysiology of hypertrophic scar formation. Our latest effort has been to determine the mechanism by which substance P upregulates an inflammatory response. We have evidence that change in substance P-induced cell shape with the accompanying reorganization of the cytoskeleton may be an intermediary step. These studies have been funded by the NIH.

Our latest therapeutic approach to the acute care management of patients with thermal injury has been to reintroduce plasmapheresis into the care plan of patients with large burns that are failing resuscitation.

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. These data were presented at the Wound Healing Society annual meeting in 1994 and were published in the *Journal of Burn Care and Rehabilitation* in October 1996.

We have demonstrated that patients with sensory deficits due to both spinal cord injury and diabetes mellitus have a dramatic reduction in cutaneous sensory nerves, especially in the wound beds. We have also recently determined that activity levels of neutral endopeptidase, a membrane bound enzyme that degrades substance P, is elevated in the wounds and skin of patients and mice with diabetes. Therefore, it was not a surprise to us that exogenous substance P shortens time to healing in a model of delayed wound repair in diabetic mice.

Our lab is focused on determining the 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.

Genetic Approach to Hypertrophic Scar

Another approach to understanding hypertrophic scar formation is to assume that it is genetic. We know that some patients are at risk based on their skin tone, and others do not develop hypertrophic scars. We also know that hypertrophic scars form with more frequency on some body parts and less frequently on others.

Investigators have compared differences in gene expression between cells from hypertrophic scars and normal scars, but they have routinely focused on the expression of a specific gene. Our goal is to identify differentially expressed known and unknown genes in hypertrophic scar tissue compared with normal tissue. We are using a technique known as cDNA microarray analysis to screen for differences in gene expression between hypertrophic scar, normal scar and normal skin. As part of this project, we will use this technology to determine whether gene expression from normal uninjured skin varies between otherwise healthy individuals. This study has been funded by the Washington State Council of Firefighters Burn Foundation.

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Gregory J. Jurkovich, M.D.



- NATIONAL STUDY ON COSTS AND EFFECTIVENESS OF TRAUMA CARE
- FUNCTIONAL OUTCOMES FOLLOWING BLUNT HEAD AND EXTREMITY INJURY
- PROSPECTIVE, RANDOMIZED STUDY OF ELDERLY RIB FRACTURE MANAGEMENT
- IMPACT OF TRAUMA SYSTEM ON ACUTE CARE IN RURAL HOSPITALS
- RANDOMIZED COMPARISON OF ULTRASOUND WITH DPL IN THE INITIAL EVALUATION OF BLUNT TRAUMA
- WASHINGTON STATE TRAUMA REGISTRY AND CENTRAL REGION CQI

FUNDING:

American Trauma Society

- Trauma Prevention Research

Centers for Disease Control and Prevention

- National Center for Injury Prevention and Control

National Highway Traffic Safety Administration

Nestle Clinical Nutrition, USA

University of Washington Clinical Research Unit

National Study on Costs and Effectiveness of Trauma Care

The University of Washington and Johns Hopkins University were recently co-awarded 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, entitled “The National Study on Cost and Effectiveness of Trauma Center Care” has as its principle investigator at Johns Hopkins University Dr. Ellen MacKenzie, Professor of Health Policy, Senior Associate Dean for Academic Affairs in the School of Public Health, and Director of the Johns Hopkins Center for Injury Research. The Principle Investigators at the University of Washington are Dr. Gregory J. Jurkovich, Professor of Surgery, Chief of Trauma at Harborview, and Director of the Acute Care Section of the Harborview Injury Prevention and Research Center (HIPRC) and Dr. Fred Rivara, George Atkins Prof. of Pediatrics, and Director of Harborview Injury Prevention and Research Center.

The purpose of the three-year, \$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:

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

This is an ambitious project, which we hope will provide answers to numerous questions regarding the cost-effectiveness of trauma system design and implementation.

Functional Outcomes Following Blunt Head and Extremity Injury

This study is a collaborative study of multiple hospitals participating in the University Health Systems Consortium (UHC) Trauma Benchmark Project. The UHC is a consortium of hospitals which collect, analyze and share data on process and outcome of care. A recent and ongoing UHC project involves benchmarking trauma care at approximately 50 University Level I Trauma centers. As an addendum to the UHC project, we have embarked on a study that aims to examine the functional outcome of patients 12 months after combined head and lower extremity injury.

While much work has been done on the development of functional outcome scales for use with non-trauma patients and the general population, none have

been well-validated in a trauma population. In this study we propose to use a number of these existing outcome scales (such as the SF-36, Sickness Impact Profile, and the Functional Capacity Index), plus some injury-site specific measures, to determine patient-reported functional outcome. We hope to relate these outcomes to characteristics of the patients, their injuries, and the hospitals and care received. Funds for this study are from the CDC-sponsored injury research center at Harborview and Johns Hopkins University, and the National Highway Traffic Safety Administration (NHTSA).

The specific aims of the study are to:

1. Demonstrate the feasibility of tracking trauma patients post-discharge to assess their functional status, and the use of post-acute care services;
2. Examine the validity and responsiveness of currently available measures of health status for evaluating outcome following major trauma. Emphasis will be placed on identifying short, practical instruments that can be administered over the phone.
3. Begin developing benchmarks for functional outcomes following trauma and assessing variations in outcome from care.

Prospective, Randomized Study of Rib Fracture Management

Rib fractures are common in blunt trauma victims, occurring in perhaps 10% of blunt trauma hospital admissions. In the elderly trauma victim, this seemingly minor injury can have a devastating outcome, with reported mortality rates of 12% and pulmonary complications of 33%. A key factor in the care of patients with

younger cohort. Additionally, mortality in the elderly patients with rib fractures was 22%, compared to only 10% in the younger patients. These differences are particularly noteworthy since the pattern of injury, the mean severity of chest injury, and the total injury score was nearly identical between elderly and young patients. Nonetheless, the elderly with rib fractures had more ventilator days, more ICU days, and longer hospital length of stay. As illustrated in the figures, a nearly linear relationship between the number of rib fractures and the development of pneumonia and mortality was observed in the elderly cohort, distinctly different from the morbidity and mortality pattern observed in younger patients.

This retrospective study could not convincingly demonstrate an advantage to one type of pain control, although epidural anesthesia patients had lower mortality, despite a greater number of rib fractures and more severe overall injuries. The next phase of the study, currently underway, is a joint Anesthesia Pain Service - Trauma Service prospective, randomized trial of epidural vs. no-epidural for the management of thoracic pain in patients with rib fractures. Trauma patients at Harborview Medical Center are currently being enrolled in this study, which may become a multi-center effort during the next year.

Impact of Trauma System on Acute Care in Rural Hospitals

Integrated statewide trauma systems are believed to improve the outcome of injured patients, but most supporting evidence for the effectiveness of trauma systems has come from urban populations. The goal of this study is to assess the impact of Oregon's statewide

A key factor in the care of patients with rib fractures is felt to be adequate pain control.

rib fractures is felt to be adequate pain control. Several different strategies of pain relief have been employed, including intravenous narcotic, local rib block, and epidural analgesia. A recent retrospective cohort study was undertaken at Harborview Medical Center to examine the impact of rib fractures and morbidity and mortality, particularly in the trauma patient over the age of 65.

Of the 277 elderly patients with rib fractures, 31% developed pneumonia compared to only 17% of the

trauma system on the outcome of injured patients in the rural setting. The state legislatures of Oregon and Washington have established trauma systems that encompass large geographic regions with diverse needs. The trauma systems in these two states were implemented five years apart. This difference in timing and the natural similarities between these states make comparison of patient outcomes a valuable measure of the impact of statewide trauma systems on clinical practice and system effectiveness.

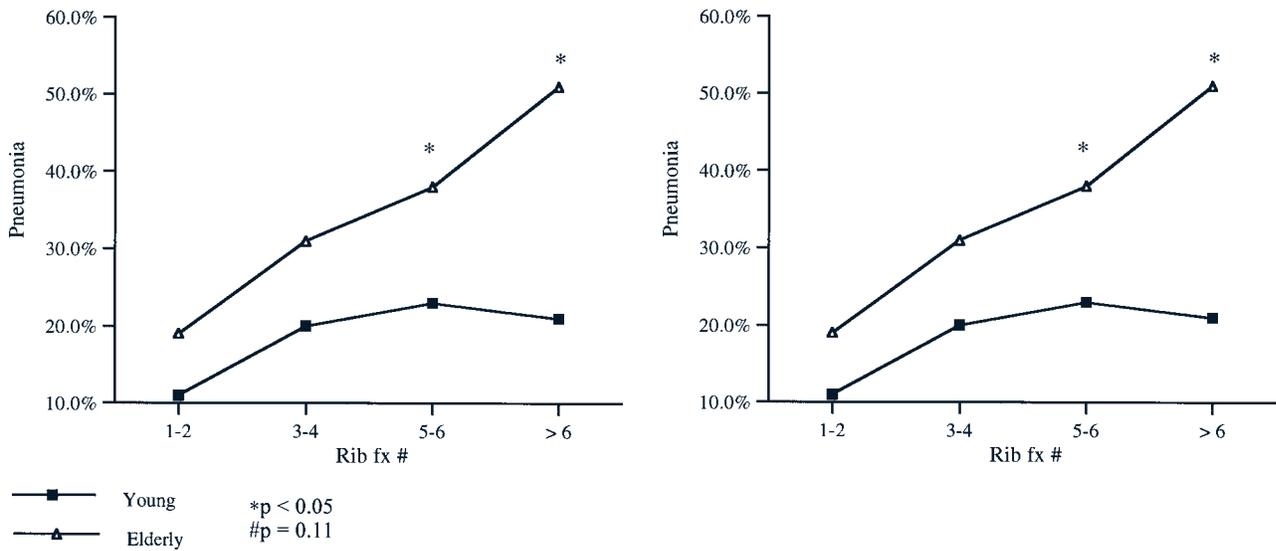


figure 1: Pneumonia and mortality rate increases per occurrence of rib fractures in young and elderly patients.

The specific aims are to:

1. Determine if implementation of statewide trauma systems reduces the risk-adjusted death rates for hospitalized injured patients.
2. Determine if trauma systems were associated with alterations in the statewide hospitalization patterns of severely injured patients.
3. Using data abstracted from rural hospital medical records and transferred into a unique Rural Trauma Registry, determine if trauma systems alter the acute care of head injured patients.

Randomized Comparison of Ultrasound with DPL in the Initial Evaluation of Blunt Trauma

Abdominal trauma remains an important cause of morbidity and mortality in trauma, and unrecognized intraabdominal injury remains the primary cause of preventable trauma deaths. Diagnostic techniques used in abdominal trauma include diagnostic peritoneal lavage (DPL), ultrasound (US), and computed tomography (CT). Each diagnostic technique has advantages and disadvantages. Ultrasound and DPL are most comparable, as both are done at the bedside, provide rapid interpretation with poor specificity but high sensitivity, and both are relatively inexpensive. While DPL has long been the standard diagnostic technique used in urban trauma centers in North America, ultrasound has been the standard in Europe and Japan. Although interest in US as an alternative to DPL has recently been evident in many North American trauma centers, to date no study has prospectively randomized patients to these two

techniques. Furthermore, studies conducted to date have focused on diagnostic accuracy and effect, with no assessment of overall patient outcome or therapeutic effects in protocols comparing US, DPL and CT.

We are pursuing a prospective randomized study of adult blunt trauma patients who are in need of an adjunct to the physical examination of the abdomen. After the need for a diagnostic adjunct is determined by the surgery team, patients will be randomized into one of two arms: DPL and US. The primary endpoints of the study are therapeutic effects (length of stay, time to laparotomy, number of laparotomies, repeat or delayed laparotomy, findings at laparotomy, blood product utilization) and patient-oriented outcomes (morbidity and mortality, missed injuries, wound infections). Ultrasound is performed by ER radiology residents or staff; DPL by surgery residents or staff. Enrollment is currently complete, with approximately 1000 patients in each arm of the study, giving the study the power to detect at least a 5% difference in outcome. Analysis of data is underway.

A significant spin-off of this study is the training of UW Surgery residents in the performance of the Focused Assessment Ultrasound in Trauma (FAST exam). Currently the R3, R4, and R5 residents have been given the opportunity to participate in a FAST training course, which will be offered yearly to the R3 residents.

Washington State Trauma Registry and Central Region CQI

As Washington State continues to evolve and expand its statewide trauma system, hospitals and pre-hospital agencies that are designated patient care providers are required to submit information to a statewide trauma registry. Central Region (conforming geographically to King County) is one of eight designated trauma and emergency medical regions in the state, and has been collecting such information for the past four years.

The Central Region Quality Assurance Committee oversees the collection and analysis of this data, in an effort to analyze and improve trauma care and outcomes in the Central Region. This committee, along with personnel from the Harborview Injury Prevention Center and the State Department of EMS and Trauma Care, is analyzing the data in an effort to address a variety of trauma system issues which remain largely

unanswered in today's trauma systems. These include such questions as "How long is too long in the pre-hospital phase of care?"; "How many patients and of what severity are essential to maintain skills and good outcome?"; and "When should you bypass the closest lowest level trauma center for the highest level trauma center?"

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, and Airlift Northwest landing zone delays by site location. 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.

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Ronald V. Maier, M.D.

- HARBORVIEW INJURY PREVENTION AND RESEARCH CENTER
- CLINICAL TRIALS IN THE SURGICAL INTENSIVE CARE UNIT AT HARBORVIEW MEDICAL CENTER
- MODULATION OF THE EXCESSIVE INFLAMMATORY RESPONSE TO BIOMATERIALS
- MODULATION OF THE TRAUMA-RELATED MACROPHAGE INFLAMMATORY RESPONSE TO PREVENT ARDS, MOFS, AND DEATH

AWARDS:

National Institutes of Health

- NIGMS Institutional NRSA

Shock Society

- Young Investigator of the Year

American Trauma Society

- William S. Stone Award

FUNDING:

Centers for Disease Control

- National Center for Injury Prevention and Control

National Institutes of Health

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

National Science Foundation

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

Harborview Injury Prevention and Research Center

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

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

1. *Evaluation of the Washington Pre-hospital Trauma Triage Instrument in a Population-Based Study:* Triage decisions are made by emergency medical care providers to distinguish patients who require care at a fully equipped trauma center from those whose injuries are less extensive. Excessive triage assignment to a major trauma center overburdens such centers with victims who could be effectively treated at a facility closer to their home. On the other hand, triage which underutilizes the major trauma centers in favor of local hospitals may place patients in facilities with inadequate resources and increased risk of poorer outcomes. Using selected population-based trauma registry data consisting of pre-hospital and hospital records, we will determine the accuracy of the state trauma triage instrument for distinguishing major trauma patients for care in major trauma centers, and modify patient distribution as appropriate.

2. 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 logistic issues. We are

currently investigating two distinct cohorts of trauma patients to evaluate whether there is an institutional volume threshold at which optimal outcomes can be achieved for critically injured patients.

tion use tidal volumes of 10–15 ml/kg of body weight. These volumes are much larger than those in normal subjects at rest, but are frequently necessary to achieve normal values for partial pressure of arterial carbon dioxide and pH. Since atelectasis and edema reduce aerated lung volumes, inspiratory airway pressures are often excessively high to achieve these parameters, suggesting the presence of excessive distension or “stretch” of the remaining aerated lung.

Thus, this traditional approach to mechanical ventilation may exacerbate or perpetuate lung injury, and in contrast, the use of lower tidal volumes during ventilation may reduce or prevent this deleterious process. Previous uncontrolled studies suggest that

Until now, therapeutic approaches have focused on control or inhibition of single components of the overall inflammatory response.

currently investigating two distinct cohorts of trauma patients to evaluate whether there is an institutional volume threshold at which optimal outcomes can be achieved for critically injured patients.

Clinical Trials in the Surgical Intensive Care Unit at Harborview Medical Center

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 the 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:

1. *Low Tidal Volume Ventilation in ARDS:* The mortality rate from acute lung injury and ARDS is approximately 40–50%. Traditional approaches to mechanical ventila-

tion use tidal volumes of 10–15 ml/kg of body weight. These volumes are much larger than those in normal subjects at rest, but are frequently necessary to achieve normal values for partial pressure of arterial carbon dioxide and pH. Since atelectasis and edema reduce aerated lung volumes, inspiratory airway pressures are often excessively high to achieve these parameters, suggesting the presence of excessive distension or “stretch” of the remaining aerated lung.

Thus, this traditional approach to mechanical ventilation may exacerbate or perpetuate lung injury, and in contrast, the use of lower tidal volumes during ventilation may reduce or prevent this deleterious process. Previous uncontrolled studies suggest that

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

An HMC study demonstrated that supplementing 3 gram/day of Vitamin C and 3 gram/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 reference range. The significance of Vitamin C deficiency in these patients is illustrated by a study of 78 patients with 105 fractures of the mandible treated at HMC: those patients who had fracture complications (infection, malunion) had significantly lower serum Vitamin C concentration than those with a good fracture outcome. In addition, patients with ARDS have been shown to have high levels of oxidants and suppressed levels of antioxidants, such as Vitamin C and Vitamin E, in bronchoalveolar lavage (BAL) specimens.

We hypothesize that plasma and tissue Vitamin C and E concentrations are significantly low in patients admitted to the intensive care units at HMC, and that routine supplementation of Vitamin C and E will elevate levels. Elevated levels of these two potent antioxidants may well protect against oxidant-induced injury in these severely injured and stressed patients and avoid the diffuse insult predisposing to ARDS and other organ dysfunction, and also to secondary nosocomial infections such as ventilator associated pneumonia and wound infections.

The study design is a prospective, observational study in which all trauma admissions to HMC ICU will have plasma Vitamin C and E levels determined at time of admission. Patients are randomized to receive standard care or antioxidant supplemented care with 1 gm q 8h orally Vitamin E and 1 gm q 8h IV of Vitamin C. These populations will be followed to determine their incidence of ARDS, duration in the ICU, mortality and infectious complications. In addition, BAL samples will be obtained to determine the effect on oxidant levels, cytokine production and activation state of the alveolar macrophage regarding intracellular signal transduction pathways.

Modulation of the Excessive Inflammatory Response to Biomaterials

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

biomaterials and that cause the subsequent chronic inflammatory response that leads to non-healing and extrusion of biomaterials.

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

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

In additional experiments, we will test the effect of end products of macrophage activation and modulation of macrophage activation. Using a chorioallantoic membrane fractal dimension and grid intersection assay we will monitor angiogenesis as a crucial component of both normal and abnormal wound healing and incorporation, or "healing," of biomaterials. The ultimate goal is to modulate the surface characteristics of biomaterials so that they may be adapted as "compatible" and elicit a normal host response and normal wound healing with incorporation of the biomaterial — "true healing."

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

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

ARDS and MOFS are the major determinants of late death following trauma.

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

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

production of multiple inflammatory cytokines.

The goal is to develop therapeutic interventions based on controlling these intracellular transduction pathways and to modulate the over-aggressive macrophage response and the subsequent auto-destructive immunoinflammatory 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, SAPK, and r38. 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 inhibitors, such as cytochalasin D, to modify the cellular response to inflammatory stimuli. 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.

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Charles Mock, M.D., Ph.D.

- STRENGTHENING TRAUMA SYSTEMS IN DEVELOPING COUNTRIES
- CRASH INJURY RESEARCH AND ENGINEERING NETWORK (CIREN)

FUNDING:

American Trauma Society

Medic One Foundation

National Highway Traffic Safety Administration (NHTSA)

United States Agency for International Development (USAID)

In all societies, the leading cause of death was once infectious diseases; however, in developed countries, this pattern changed over the past two centuries, with decreases in infectious diseases and increases in life expectancy. Unfortunately, some of these gains were offset by increases in other diseases, including chronic diseases and injury. Today, injury is the leading cause of years of life lost in almost every developed country.

Similar trends are underway in today's less developed countries. In middle income countries, as in East Asia and Latin America, injury has become a leading cause of years of life lost. In low income countries, such as in South Asia and Africa, infectious diseases continue to predominate because of their continued high toll in younger children. However, even in these locations, injury is usually one of the leading causes of death among working age adults.

In many developed countries, injury mortality rates have fallen in recent decades, as a result of both improved prevention efforts and improved trauma treatment capabilities. Such well-organized approaches to prevention and treatment have not been carried out in less developed countries. Moreover, basic information about the incidence, mechanisms, and causes of injury in such locations is lacking.

Co-workers from several countries and I have helped to address these concerns by working to improve the spectrum of trauma system activities:

1. Surveillance and research on the basic epidemiology of injury.
2. Injury Prevention.
3. Prehospital Care.
4. Hospital Based Trauma Care.

We have worked on these activities in several developing countries, including Ghana, Mexico, and Vietnam. During the conduct of this work, the UW

Department of Surgery has served as my home base. The advice and expertise of colleagues in the Department and at the Harborview Injury Prevention and Research Center has also represented a valuable resource for my work.

Strengthening Trauma Systems in Developing Countries **Surveillance and research on the basic epidemiology of injuries in developing countries**

In developed countries, the usual sources of data on the incidence and consequences of injury include vital statistic registries, police accident reports and health care records. In many less developed countries, these sources are inadequate. Many or most deaths are not reported to the government. Many injured persons may never receive formal medical care, making health care records an incomplete source of data as well.

To better ascertain the incidence and consequences of injury in Ghana, co-investigators at the University of Science and Technology in Kumasi and I undertook a community-based survey of injuries in this country. Using a defined random sampling strategy, known as two-stage cluster sampling with probability proportional to size, a denominator of 21,105 persons living in 432 separate urban (city of Kumasi) and rural (Brong-Ahafo region) sites were selected.

Through direct household visits and interviews, we sought information on any injury which had resulted in one or more days of lost activity during the prior year (including fatalities). A total of 1,597 injuries were reported and analyzed. Information was obtained on the mechanism, specific body part injured, type of medical care obtained, cost of treatment, and outcome of injury, including length of disability. Information was also obtained on the economic consequences of the injury to the family of the victim.

In the urban area, the major causes of injury included falls, accidental lacerations, and transport related injuries. However, transport related injuries were more severe than the other causes, as indicated by a longer mean period of disability (64 days per injury), compared to all other injuries (37 days per injury). In the urban area, 38% of injured persons received treatment at a hospital (either emergency room visits or inpatient admissions); 30% received treatment at a government or private clinic, and 32% received no formal medical treatment.

In the rural area, the major causes of injury included agricultural injuries (30% of all injuries), falls, and transport related injuries. Compared with the urban area, significantly fewer injured persons received any type of formal medical care. Only 20% of injured persons received hospital based treatment; 31% received treatment at one of the network of non-physician staffed primary health care clinics; and nearly half (49%) of all injured persons in the rural area received no formal medical care.

Detailed information on the characteristics and outcomes of the transport related injuries has been shared with the road safety officials in Ghana. Information on health service utilization patterns has been shared with the Ministry of Health of Ghana. Data from this survey has been the basis for multiple publications on mechanisms and causes of injury, pediatric injuries, economic consequences of injury, trauma treatment, and epidemiologic methodology. Further publications will hopefully include injury related disability and occupational injuries.

The data from this survey has also been useful for efforts to improve the existing information sources for trauma in Ghana. For example, a comparison of the incidence rates of pedestrian injuries in the city of Kumasi as derived from the survey with the incidence rates as derived from police reports, showed that only about 10% of actual injuries were being recorded in police records. These data have prompted efforts to improve the existing information sources.

In addition to the above survey, we have undertaken research regarding trauma mortality patterns in developing countries. The goals of this study were to provide information that would help with decisions regarding trauma system development in developing countries. In developing a trauma system, decisions must be made as to the extent to which limited resources should be allocated to injury prevention programs, prehospital care, emergency room care, or other aspects of hospital

based care. Hence, there is a need to know where in a nation's trauma system the greatest mortality lies.

Assessment of where the greatest improvements are to be made could be assisted by comparing such mortality patterns to those of an industrialized nation with a well-developed trauma system. In our study, we compared the trauma mortality patterns in three cities in countries at different economic levels: Seattle, WA, USA (industrialized country), Monterrey, Mexico (middle income country), and Kumasi, Ghana (low income country).

The main finding of this study was that with decreased socio-economic status, the overall rate of death among seriously injured patients increased from 35% in Seattle to 55% in Monterrey, Mexico to 63% in Kumasi, Ghana. This was not unexpected. What was surprising however was the way in which this happened. The majority of the changes in mortality between the various cities were accounted for by changes in deaths in the field (e.g., the prehospital setting). The proportion of deaths occurring in the field increased with decreasing socio-economic status, from 59% in Seattle, to 72% in Monterrey, to 81% in Kumasi. The study pointed out the importance of injury prevention efforts and pre-hospital care in the setting of developing countries.

Injury prevention

We have carried out research on factors contributing to injuries in Ghana in collaboration with others involved in road safety in that country, including the National Road Safety Committee, the Building and Road Research Institute, the Motor Traffic Unit of the Ghana Police, and the Ghana Police Hospital.

One study involved a random roadside breathalyzer study to assess the prevalence of drunk driving. This used methodology similar to that developed by the National Highway Traffic Safety Administration (NHTSA) in the United States. A total of 722 drivers were randomly tested on the major roads leading into the capital city of Accra, Ghana. A total of 149 (21%) of these were positive for any level of blood alcohol. Furthermore, 53 (7.3%) had blood alcohol concentration of 80 mg/dl or higher, indicating alcohol impaired driving. This prevalence of alcohol impaired driving is higher than that reported by similar methodology in developed countries (0.4 - 3.4%). It is notable that 3.7% of bus drivers and 8.0% of truck drivers had blood alcohol concentrations of 80 mg/dl or higher.

These data indicate that alcohol impaired driving is likely to be a major contributor to motor vehicle crashes

in this country. Data from this study has been used by the National Road Safety Committee in its educational campaigns and has been presented to the Ghanaian Parliament in efforts to stimulate updating of Ghana's drunk driving laws.

In Mexico, injury prevention work has involved a collaborative effort of the Harborview Injury Prevention and Research Center (HIPRC) and several local institutions in the city of Monterrey, Nuevo Leon. These include the Hospital San Jose and the TEC de Monterrey School of Medicine. As part of these efforts, we have developed a program providing injury prevention counseling for parents. This focuses on improving parents' knowledge and practices of childhood safety in the Mexican environment. It has involved adaptation of existing educational materials developed by the American Academy of Pediatrics. Thus far we have carried out pilot work in this and have put on educational seminars that have had the participation of nearly 1200 parents

In Mexico, we have developed a training course in injury prevention work for health care professionals. The course has now been given three times in Monterrey over the past two years. Around 150 persons, including doctors, nurses, public health professionals, teachers, and others have taken the course. We are in the process of undertaking further research and development of this course and hope to eventually export it to other areas of Mexico and other countries in Latin America.

In Vietnam, similar work is underway. The HIPRC has entered into a project in partnership with the Hanoi School of Public Health to design a program to improve injury prevention and control training and capabilities throughout Vietnam.

Prehospital care

My efforts in the development of prehospital care capabilities in developing countries have involved

In developing a trauma system, decisions must be made as to the extent to which limited resources should be allocated to injury prevention programs, prehospital care, emergency room care, or other aspects of hospital based care.

in the Monterrey area. We have received a grant from the American Trauma Society to further this work. In particular the grant is for research and development of injury prevention counseling materials oriented for the different socio-economic levels in Mexico.

Worldwide, injury prevention and control work has often been misconstrued as merely admonitions to be careful. However, it is a scientific field like any other. As such, it has a need for expertise in a variety of fields: for example, epidemiologists who can handle injury data in the development of injury surveillance systems; psychologists, media experts, and public health personnel who can develop social marketing strategies to effectively improve safety related behavior; medical personnel who can undertake outcomes research and effect changes in trauma system design based on such research. Perhaps one of the most important things that workers from developed countries can do in assisting developing countries is to increase and strengthen local expertise. Along with others at HIPRC, I have been undertaking two programs for the development of local expertise in injury prevention and control, in Mexico and Vietnam.

Ghana, Mexico, and, more recently, Vietnam.

In Ghana as in many low income countries, there is no formal emergency medical system (EMS). Ill or injured persons are usually brought to the hospital by relatives, using whatever type of transportation is available. In a review of the mode of transport for injured persons treated at the main hospital in Kumasi (the Komfo Anokye Teaching Hospital), the great majority (70%) were brought in by some form of commercial transportation (taxis or buses); 22% were brought in by a private vehicle; 5% were brought in by the police; and only 3% were brought in by an ambulance. All of the latter were transferred from a smaller rural hospital and a hospital ambulance was used for the transfer.

Currently, efforts to institute a formal prehospital system include plans to place ambulances along the major inter-urban roads and to build up the capability of groups such as the Red Cross and the Fire Service. Plans for EMS development are hampered by the paucity of telephones and other telecommunications in the country. Hence, current efforts also include

building upon the foundation of what prehospital care does exist, namely the commercial drivers, who bring in the majority of injured persons.

I am involved with pilot training programs which are being conducted through the University of Science and Technology and the Ghana Private Road Transport Union, to which most commercial drivers belong. These training programs are evaluating the educational background of commercial drivers and their experience with transporting injured persons, as well as providing them with basic first aid instruction. Approximately 400 drivers have been given first aid instruction as part of this program.

As part of the research and development aspects of this program, we are interviewing drivers 1 year after having taken the course. Thus far, we have obtained follow-up information on 71 drivers. Before the course, few drivers provided any type of first aid to injured persons they transported. After the course, 61% of drivers indicated they had provided first aid during the interval year. Improvements included: airway management (2% before vs. 21% after*), bleeding control (4% vs. 25%*), splint application (1% vs. 10%*), and triage (7% vs. 21%*) (* $p < 0.05$). The course has cost \$4 per driver trained.

In Mexico, as in many other middle income countries, there are usually basic ambulance services, at least in the urban areas. My Mexican colleagues and I have been involved in ongoing efforts to improve the ambulance systems in the Monterrey metropolitan area over the past eight years. Efforts to upgrade this EMS have included introduction of the Prehospital Trauma Life Support course (PHTLS). Introduced in 1994 for paramedics in the Green Cross ambulance service, this course has been conducted annually since that time.

In our evaluation of this program, we documented an improvement in both the process and outcome of prehospital trauma care after the PHTLS course. Airway maneuvers for patients in respiratory distress increased from 18% before the course to 43% after ($p < 0.05$). IV fluids for patients with BP < 100 increased from 44% to 81% ($p < 0.05$). En route mortality declined from 8.2% to 4.7% ($p < 0.05$). Regular PHTLS courses have cost \$2600 per year (0.5% of the EMS budget). Hence, the improvements in both Ghana (a low income country) and Mexico (a middle income country) have been low cost and sustainable within the context of the local economies.

We are currently working on a project funded by the Medic One Foundation in Seattle to further the

EMS development work in Mexico. This project builds upon the foundation that was started with the PHTLS project by specifically addressing increased training for advanced airway maneuvers, including endotracheal intubation.

We have also recently embarked on a program to conduct similar EMS development in Vietnam. In particular, we have received a grant from USAID to establish a link between the world renowned Medic I program in Seattle and the Hanoi Emergency Transport Center. This center contains some highly motivated individuals. However, it is still at a rudimentary level and is estimated to meet only 15% of the need for EMS in the city. The upcoming program, which is due to start in January 2002, is to provide upgraded training for prehospital trauma care for ambulance personnel in Hanoi. This will be done through exchanges of personnel between the two cities. Of special note is the fact that this is one of the first times that USAID has funded an EMS or trauma related project in a developing country.

Hospital based care

Experience with the Advanced Trauma Life Support Course (ATLS) of the American College of Surgeons has shown that using a structured educational approach, with well-planned teaching materials and evaluation of the course's effectiveness, can improve the process and outcome of trauma care in the U.S. and in developing nations with higher economic status, such as Trinidad.

However, in nations at the lower end of the economic spectrum, such as Ghana, facilities needed to implement the ATLS guidelines (including CAT scans and consultations with neuro- and general surgeons) are extremely limited. In rural areas, hospitals are staffed almost exclusively by general practitioners. Opportunities to refer patients are limited by poor roads and financial restrictions. Hence, training in this setting needs to be expanded beyond the early resuscitation and diagnostic work-up of the "Golden Hour" to include definitive treatment which general practitioners might be expected to perform in isolated rural hospitals. The experience of the ATLS program in the U.S. indicates that a similar approach, oriented for the particular circumstances of developing nations, could improve trauma care in these locations.

During the past five years, in collaboration with the Department of Surgery at the University of Science and Technology in Kumasi, I have conducted several postgraduate lecture series on trauma management.

These have formed the basis for the development of a more organized, standardized continuing medical education (CME) course. The material in these lectures has been updated based on surveys conducted in rural hospitals to ascertain general practitioners' needs and desires for CME. Research is currently underway to assess how the material taught in this course has been used by course participants and whether the course has improved the process of trauma care in rural hospitals.

The lessons learned from this one country's experience as well as from similar programs in other countries need to be expanded internationally. In addition, other issues, such as staffing, equipment, supplies, organization, and administration need to be addressed. In so doing we can build upon the experience gained by the WHO and others in international health. Working within the same tight financial constraints, these organizations have made considerable progress in several disease entities by developing the concept of "essential services." These are services that are highly effective, low cost, and which should realistically be available to most members of a given population.

Several programs have developed, refined, and promulgated such essential services, including the Expanded Program on Immunizations, the Essential Drug List, and the Safe Motherhood Initiative. I and others working in trauma feel that it is time for a similar approach to trauma care. In this regard the International Society of Surgery, through its trauma section (International Association for Trauma and Surgical Intensive Care) this year created a "Task Force for Essential Trauma Care," with me as chair, to specifically address this issue.

Crash injury research and engineering network (CIREN)

In addition to my work in less developed countries, I am active in research on injury prevention in the U.S. Harborview Medical Center and its associated HIPRC are part of a network organized by NHTSA which includes six other trauma centers nationwide. At each center, persons injured in motor vehicle crashes are identified, and a team of investigators examines the involved vehicles for crash deformation patterns. The automotive findings are correlated with the patient's injuries, and hypotheses are generated regarding the biomechanical etiology of the injuries. Data from this process is fed back to NHTSA to help with the develop-

ment of safety regulations and to the automobile manufacturers to help with safety engineering design.

In collaboration with the NHTSA, our center (HIPRC) has investigated several issues pertinent to vehicle safety design and related regulations. We have investigated biomechanical thresholds for femur fracture and shown that, on average, femurs tend to fracture at lower energy loading thresholds than previously suspected from cadaver tests. This has implications for the crash test standards that are currently used for frontal impact.

We have also investigated the effects of varying body sizes and found an increased risk of death and serious injury to larger occupants. This has implications for safety design as most crash testing has been done using dummies of 70 kg size. There has been a push lately for more testing using small size dummies, to better account for the crash biomechanics of smaller size women and children. However, our research has shown that more attention may need to be given to larger size occupants as well.

We have investigated the effectiveness of different seatbelt systems and found that minimal protection was afforded by using a shoulder harness alone, without the associated lap belt. This is an issue as many people assume that an automatic shoulder harness is protecting them and do not bother to buckle up their lap belt as well.

We have looked at the veracity of the safety ratings provided by the New Car Assessment Program (NCAP) of NHTSA. NCAP rates vehicles on their safety based on the forces transmitted to dummies in standardized crash tests. These forces are compared with the estimated thresholds for major head and torso injuries, as derived from cadaver tests. Assessment of these thresholds in real world crashes has been infrequent. Utilizing data from the CIREN project, we have determined that the relationship between forces in vehicle crashes and injury thresholds are more complex than initially appreciated.

On preliminary analysis, it appears that the likelihood of head injury has been over-estimated for some vehicles, especially those that appeared most unsafe and had the highest forces to the head during standardized crash tests. However, the likelihood of head injury has been under-estimated for some vehicles, especially those that appeared the most safe on crash tests. Such information is being fed back to NHTSA and its NCAP.

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Avery B. Nathens, M.D., Ph.D., MPH

- EFFECT OF ORGANIZED SYSTEMS OF TRAUMA CARE ON MOTOR-VEHICLE CRASH MORTALITY
- RELATIONSHIP BETWEEN TRAUMA CENTER VOLUME AND OUTCOME
- TRAUMA PATIENT IN AN URBAN COUNTY HOSPITAL: BENEFIT OR BURDEN?
- EFFECT OF PRE-HOSPITAL TRIAGE TO A LEVEL I TRAUMA CENTER VS. A LEVEL III/IV TRAUMA CENTER
- PROSPECTIVE RANDOMIZED CONTROLLED TRIAL OF ANTIOXIDANT THERAPY IN CRITICALLY ILL SURGICAL PATIENTS

AWARDS:

American College of Surgeons, Committee on Trauma Region X

- Best clinical paper, 1999
- Best clinical paper, 2000

American College of Surgeons, Committee on Trauma National

- Runner up paper competition, 1999
- Runner up paper competition, 2000

FUNDING:

Centers for Disease Control and Prevention

Surgical Infection Society

Effect of Organized Systems of Trauma Care on Motor-Vehicle Crash Mortality

During 1976 through 1995, 22 states developed organized systems of trauma care with the intent of reducing injury-related mortality. Despite calls for wider national implementation, the effectiveness of an integrated approach to trauma care at a regional or state level remains unproven.

This study was designed to assess the impact of trauma system implementation on mortality due to motor-vehicle crashes across the United States between 1979 and 1995. The primary endpoint was the rate of death of front-seat occupants of passenger vehicles aged 15 through 74. Crash rates were compared before and after trauma system implementation in states with

crash mortality of 13% (95% CI, 9-16%) while relaxation of state speed limits increased mortality by 6% (95% CI, 3-9%). These data suggest that implementation of an organized system of trauma care reduces deaths due to motor-vehicle crashes. The effect takes several years to manifest, a finding that is consistent with the maturation and development of trauma triage protocols, inter-hospital transfer agreements, organization of trauma centers, and ongoing quality assurance.

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

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.

organized systems of trauma care. After controlling for secular trends in crash mortality and implementation of traffic safety laws (restraint laws, maximum posted speed limits, laws designed to limit drinking and driving), trauma systems had a significant impact on deaths due to traffic crashes. Eight years following initial trauma

system implementation, mortality due to traffic crashes began to decline; about 15 years following trauma system implementation, mortality was reduced by 9% (95% CI, 2-15%) (Figure 1).

By contrast, legislative initiatives geared toward enforcing restraint laws result in an early reduction in

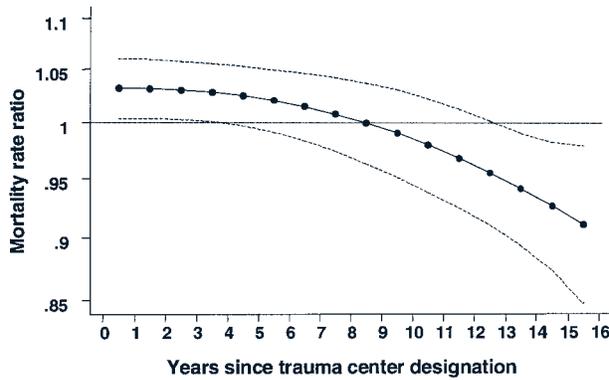


figure 1: Adjusted mortality rate ratio attributable to a trauma system as a function of time from first trauma center designation. The dashed lines represent upper and lower bands of the 95% confidence interval.

experience translates into better outcomes. This study evaluated the effect of trauma center volume in two distinct cohorts of patients admitted to one of 31 academic trauma centers across the country. These cohorts included patients with isolated penetrating abdominal trauma and patients with a combination of lower extremity long bone fractures and closed head injury.

The relationship between trauma center volume and outcome depended on the severity of illness. For example, there was no association between volume and outcome in penetrating abdominal trauma patients without shock or in blunt multisystem trauma patients without coma. However, in patients with shock or coma there was a marked reduction in the risk of death (Figure 2).

Similar advantages were also evident when hospital lengths of stay were assessed. The greatest benefits to these high-risk patients occurred when they were cared for in centers with greater than 650-700 major (ISS \geq 15) trauma admissions per annum.

In summary, these data provide further support emphasizing the importance of regionalization of trauma care, and provide guidelines for estimating the number of trauma centers required per unit population. Trauma system care should ensure triage of the most severely injured patients to relatively few dedicated trauma centers. Consideration should be given to consolidation of urban trauma programs to maximize institutional volume. Further work is needed to identify differences in the process of care, the impact of individual surgeon volume, the role of fellowship training programs, trauma research activities and other factors that may be contributing to the observed outcome benefit at high volume trauma centers.

Trauma Patient in an Urban County Hospital: Benefit or Burden?

The high cost of uncompensated trauma care is the principal obstacle to trauma system development. Designation as a regional Level I trauma center may burden an institution with an unprofitable mix of uninsured patients with severe injuries. This burden may weigh heavily on an inner-city hospital already taxed by the payer mix of non-trauma patients and may undermine the sustainability of large urban trauma centers. To assess the potential burden of the trauma patient on an urban Level I trauma center, we evaluated the payer-mix of trauma patients relative to non-trauma patients at different levels of trauma care in a mature trauma system. Patients admitted to hospital in the state of Washington over a two year period were classified as either trauma (ISS \geq 9) or non-trauma and by insurance status as either commercial insurance (CI) (e.g. managed care) or non-commercial insurance (e.g. Medicaid or self-pay). Medicare patients were excluded from analysis.

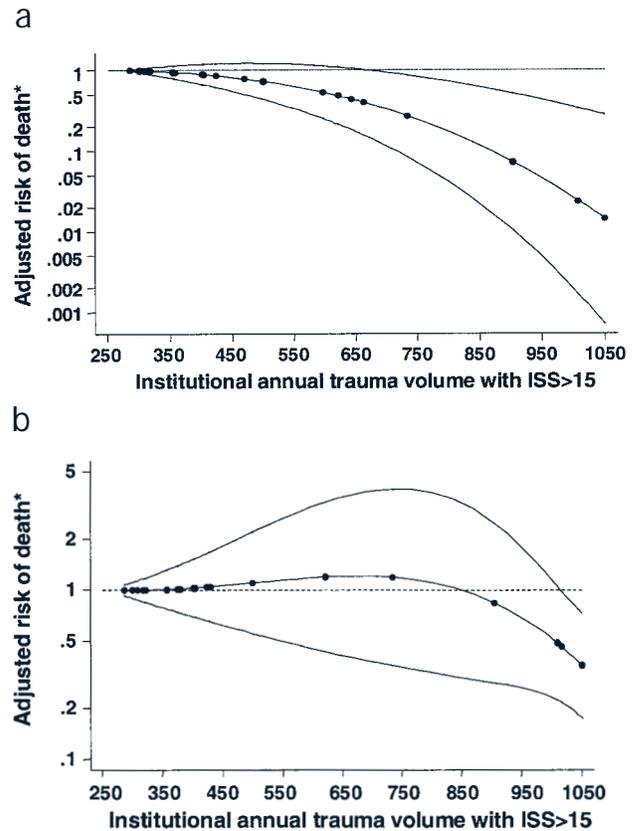


figure 2: Relationship of the risk of death to trauma center volume in patients admitted in shock with penetrating abdominal injury (A) and in patients with coma and multisystem blunt trauma (B). Lines without • represent 95% confidence bands for the estimated odds ratio.

*Adjusted risk of death compared to the lowest volume institution.

There were 10,386 trauma admissions and 474,944 non-trauma admissions to 87 centers. Trauma patients were less likely to have commercial insurance than non-trauma patients (69% vs. 74%, $p < 0.001$). The proportion of trauma patients with commercial insurance treated at the urban Level I trauma center was significantly less than at other centers. However, trauma patients treated at the Level I trauma center were far more likely to have commercial insurance than non-trauma patients treated at this same center (52% vs. 30%, $p < 0.001$). By contrast, there was no relationship between payer and trauma status at other levels of care.

These data suggest that referrals from across the state result in a disproportionate number of trauma patients with commercial insurance relative to non-trauma patients at this urban Level I trauma center. In this environment, designation as a Level I trauma center may actually improve care for inner city non-trauma patients by ensuring the ready availability of acute care services that follows designation as a trauma center and by means of cross subsidization of non-trauma care through trauma care reimbursement.

Effect of Pre-Hospital Triage to a Level I Trauma Center vs. a Level III/IV Trauma Center

Little is known about the effectiveness of regionalized, tiered trauma systems and whether clinical outcomes of trauma patients differ by the initial destination of the trauma patient. Level I trauma centers are designed to handle the most complicated and severe trauma patients, and Level III/IV facilities are designed to admit less severely injured patients and to stabilize severely injured patients before transfer to a Level I center.

The purpose of this population-based retrospective cohort study is to determine if injured patients who receive uniform care by pre-hospital advanced life support in the field, and who are transported directly from the field to a Level I trauma center, have better

outcomes than those who are transported from the field to a Level III or IV center, and then transferred to the Level I center. The cohort will be restricted to all patients injured in King County transported by an Advanced Life Support crew to one of 8 trauma centers in the county (4 Level IV, 3 Level III, 1 Level I) during the years 1995 to 1998. It is anticipated that the results and conclusions derived from this analysis will identify subgroups of patients who are best served by direct transport to a Level I as well as those in whom optimal outcomes are achieved by triage at the Level III/IV prior to transfer.

Prospective Randomized Controlled Trial of Antioxidant Therapy in Critically Ill Surgical Patients

Oxidant-mediated tissue injury induced by activated neutrophils or following ischemia-reperfusion injury is thought to be one of the key mechanisms leading to Acute Respiratory Distress Syndrome (ARDS) and multiple organ failure. This project was designed to evaluate the effectiveness of antioxidant supplementation in critically ill surgical patients admitted to the intensive care unit.

Patients were randomized to receive either standard care or administration of alpha-tocopherol (3000 units daily) and ascorbic acid (3 gms daily) for the duration of their ICU stay. Primary clinical endpoints are the development of ARDS and pneumonia. A subset of these patients underwent bronchoalveolar lavage to assess the impact of antioxidant supplementation on markers of alveolar injury (alveolar fluid protein and neutrophil content, and F_2 isoprostanes, a marker of oxidative tissue injury), the alveolar inflammatory response including alveolar cytokine levels, and markers of alveolar macrophage activation. Enrollment for this study has recently been completed after enrolling over 300 patients. Once available, the results should provide further insight into the role of oxidant-mediated tissue injury in the manifestations of critical illness.

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Robert K. Winn, Ph.D.

- ISCHEMIA-REPERFUSION INJURY:
SEPSIS (OVERWHELMING INFECTION)
ADULT RESPIRATORY DISTRESS SYNDROME

FUNDING:
National Institutes of Health

The focus of our research is to better understand the cellular and molecular events that lead to organ dysfunction and organ failure in severely ill patients. Two causes of organ failure are severe infection or sepsis, and ischemia followed by reperfusion (i.e., following severe traumatic injury, hemorrhagic shock, myocardial infarction, stroke, organ transplantation, etc.). We are interested in understanding cellular and molecular events in those patients who suffer from these severe pathologic events.

It has been known for some time that these types of injuries result in an inflammatory response and that this response might contribute to organ dysfunction. More recently it has been observed that a portion of the cellular injury is the result of programmed cell death or apoptosis and this has led to increased research into this area. Our effort is directed toward understanding both inflammation and apoptosis as well as possible interactions or overlap of these cellular events. We are currently investigating the following areas:

Ischemia-Reperfusion Injury

We and others have shown that ischemia followed by reperfusion can result in a neutrophil (PMN) induced reperfusion injury, and that a portion of the injury can be ameliorated with monoclonal antibodies that recognize leukocyte adhesion molecules. These pre-clinical experiments led to a number of clinical trials using anti-adhesion therapy in an attempt to reduce injury following myocardial infarction, stroke and hemorrhagic shock following traumatic injury. Unfortunately these trials did not demonstrate the expected protective effect of anti-adhesion therapy in spite of the very strong pre-clinical data.

In an attempt to understand why the clinical trials failed, we examined the ischemic times in the experi-

mental setting designed to model myocardial and cerebral ischemia-reperfusion and found them to be generally less than 1.5 hours with the majority of times being between 30 min and 1 hour. Since the time to treatment for myocardial infarction and stroke is considerable longer than 1 to 1.5 hour, we questioned whether the duration of ischemia in the experimental setting was too short. In preliminary experiments, when skeletal muscle was made ischemic for 60 minutes or less, the injury was partially reduced by blocking a major PMN leukocyte adhesion molecule (CD18).

Additionally, preliminary results showed that the injury could no longer be reduced by blocking adhesion molecules if the ischemic time was increased to 90 minutes. The CD18 independent portion of the injury was associated with DNA strand breaks consistent with apoptosis but the earlier CD18 dependent portion of the injury had a reduced component of apoptosis. The extended ischemia that is independent of adhesion blockade was reduced by blocking apoptosis or by blocking the complement system, suggesting a mechanism for therapy. We are continuing investigations of these two potential mechanisms of injury with the hope of defining potential therapeutic agents.

Sepsis (overwhelming infection)

Sepsis or septic shock is a potentially lethal consequence of bacterial infection and is a significant complication in victims of traumatic injury. It is one of the leading causes of death in patients requiring intensive care. There are multiple bacterial products implicated as pathogenic molecules including bacterial lipoproteins, lipopolysaccharide, (LPS), lipoteichoic acid, peptidoglycans, cell wall products, etc. Sepsis was shown to activate the intrinsic cell "suicide" program leading to apoptosis of multiple cell types. Insights into the mole-

cular basis of cellular activation/apoptosis in response to sepsis are under intense investigation in the hope of finding new approaches to therapy.

Signaling by bacterial products occurs through the recently described Toll-like receptors (TLRs) on the surface of cells. Intracellular pathways leading to activation proceed along similar pathways for TLR-2 and TLR-4 (the two receptors shown to respond to bacterial products). However, the apoptotic pathways have received less attention. We are examining sepsis-induced apoptosis and a novel activation pathway in cell culture as well as the effect of gene alterations that are expected to lead to decreased apoptosis in monocytes, lymphocytes and endothelial cells *in vivo*. These gene alteration experiments will help to identify cells that are critical in responding to invading organisms associated with sepsis.

Recent clinical investigations aimed at reducing the death rate in patients suffering from sepsis have been disappointing as a number of potential therapeutics have

Neutrophils are thought to have an inherently limited lifespan in tissue (i.e., they undergo a constitutive programmed cell death), but recent evidence suggests that their survival in tissues can be regulated to some extent by local factors including adhesion, cytokines, and chemokines. Neutrophil persistence in the lung may be an important determinant of acute lung injury since the longer neutrophils are present in the lung tissue, the greater the possibility that they may provoke lung injury by release of proteases and reactive oxygen intermediates. While the resolution of acute lung inflammation ultimately depends upon the clearance of neutrophils, the mechanism(s) of clearance may also affect the duration and severity of lung inflammation.

Necrosis of neutrophils releases toxic products extracellularly, thereby perpetuating the inflammatory response and further damaging tissue. In contrast, apoptosis of neutrophils with their monocyte-derived macrophages may terminate the inflammatory reaction.

Sepsis or septic shock is a potentially lethal consequence of bacterial infection and is a significant complication in victims of traumatic injury.

not shown any benefit in this disease. We hope to better define the process leading to organ dysfunction and organ failure in patients suffering from sepsis or sepsis syndrome. An understanding of the cellular and molecular events of this process is expected to provide information that will allow the development of therapeutics for the treatment of this devastating syndrome.

Adult Respiratory Distress Syndrome

Adult respiratory distress syndrome (ARDS) is a major complication in patients who have suffered severe traumatic injuries and in patients with sepsis or sepsis syndrome. Patients suffering from ARDS have increased pulmonary edema resulting from endothelial and epithelial permeability that is thought to result from hyperactive leukocytes. Considerable progress has been made in inflammation; however, the factors regulating the fate of transmigrated neutrophils *in vivo* are not as well understood.

We are investigating the role of neutrophil apoptosis in determining the severity and duration of acute lung inflammation. We hypothesize that factors promoting neutrophil apoptosis and engulfment by macrophages will lead to more rapid resolution of lung inflammation, while those that prevent apoptosis will prolong the inflammatory response and increase the probability of acute lung injury.

It is hoped that these studies will yield new information on the molecular mechanisms involved in the resolution of acute lung inflammation and perhaps yield new approaches to the therapy of ARDS. In these studies we are particularly interested in understanding the role of neutrophil apoptosis in septic lung injury. The goal of these investigations is to identify potential molecular mechanisms that can provide protection from the development of ARDS.

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PEDIATRIC SURGERY

ROBERT S. SAWIN, M.D.



Robert S. Sawin, Ph.D.

• NEUROBLASTOMA IN THE PEDIATRIC PATIENT

AWARDS:

Robert E. Condon Surgical Resident Competition, 1998
Wisconsin Surgical Society
• Resident Competition (Scientific Division), 1998

FUNDING:

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

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

that is expressed in both adult and pediatric tumors that are derived from neural crest cells. Our work has shown that GRP and its receptor, GRP-R, are both expressed in abundance by neuroblastoma cells in culture and by tumor cells removed from children. Our cell culture studies have also shown that inhibitors of GRP retard neuroblastoma growth.

We are presently working collaboratively with the Clinical Research Institute at Madigan Army Medical

Perhaps the most intriguing feature of neuroblastoma is the well-documented spontaneous maturation of highly malignant tumors to a more differentiated benign variant, called ganglioneuroma.

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

Our laboratory effort has focused on a particular peptide growth factor, gastrin releasing peptide (GRP),

Center to define the quantitative differences of GRP and GRP-R expression in neuroblastoma as compared to ganglioneuroma. Our hypothesis is that these differences account for the virulence of the behavior of a given tumor. If verified, this observation would suggest that GRP antagonists might be useful clinically to stimulate maturation of neuroblastoma cells.

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PLASTIC AND
RECONSTRUCTIVE SURGERY

LOREN H. ENGRAV, M.D.

F. FRANK ISIK, M.D.



Loren H. Engrav, M.D.

- OUTCOMES OF EARLY EXCISION AND GRAFTING OF FACE BURN
- UW BURN INJURY REHABILITATION MODEL SYSTEM
- HYPERTROPHIC SCARRING
- A PIG MODEL OF HYPERTROPHIC SCARRING

FUNDING:

Washington State Council of Firefighters

Department of Education

• National Institute on Disability and Rehabilitation Research

During the past 23 years, Dr. David Heimbach and I and, during the past decade, Dr. Nicole Gibran have initiated and/or participated in significant changes in burn care. Survival is at an all time high and length of stay and postburn impairment are at all time lows. In spite of this enormous progress, there are equally enormous gaps in the current burn knowledge, some of which we are addressing in our current clinical, model system, and laboratory research.

Clinical Topics

Everyone knows the Baxter formula (4cc/kg/\%total body surface area) for calculating burn resuscitation fluids. It has probably been in every inservice examination for the past two decades. But does it reflect how things really are? We anecdotally observed that we frequently exceeded the predictions of the formula and wondered if this was true over time and unique to us.

require more fluids, and/or 4) burn care has changed. Burn surgeons are obligated to explain this discrepancy.

Outcomes of Early Excision and Grafting of Face Burn

Early excision of burns has become commonplace, but early excision of face burns has not. Many or most burn centers still permit face burns to heal over weeks, accept the grotesque result, and refer the patient for reconstruction. A recent review of the English-language literature revealed only eight manuscripts containing actual photographs of only 15 postoperative results.

We began early excision of face burns in 1979 and have now accumulated a 20+ year experience with essentially one method and one surgeon and 100 patients. We are in the process of summarizing the outcome of this experience. What will it demonstrate? The method is not a panacea. The technique is operator dependent and the method requires dedicated surgeons.

Early excision of burns has become commonplace, but early excision of face burns has not.

Many or most burn centers still permit face burns to heal over weeks, accept the grotesque result, and refer the patient for reconstruction.

We studied 50 patients from our burn center and others in the United States. We found that 58% exceeded 4.3 cc/kg/\%TBSA , far more than the 12% reported by Baxter. These findings suggest that in actual practice, fluid volumes administered are often larger than the Baxter formula predicts. And why is this? The possibilities include: 1) the sample is not representative; 2) the formula is used improperly; 3) burns have changed and

Hypertrophic scar and graft contraction are still problems which require reconstruction. But the magnitude of the reconstructions is far less than that following spontaneous healing of face burns, and the time from burn to completion is significantly diminished.

Like peritoneal lavage, which was accepted slowly, we anticipate that early excision of face burns will in time become the standard method.

*Model System Topics***UW Burn Injury Rehabilitation Model System**

There is very little data available on the long term outcomes of burns. In 1993 and again in 1997, the National Institute on Disability and Rehabilitation Research (NIDRR) of the Department of Education funded model systems in burn care to obtain this data. The UW Burn Center successfully competed on both occasions, so now we have a seven-year history with burn model system research matched only by the burn center at UT Southwestern. Funding is \$295,000 per year for five years. The majority of this money funds four personnel to gather and process clinical and research data.

The model system research conducted at the UW Burn Center at Harborview covers burn care from injury to discharge from outpatient care with particular attention to rehabilitation and outcomes. Two projects of particular interest at UW are "efficacy of pressure garments" and "time off work after burns."

For decades burn survivors have worn pressure garments to minimize scarring. These garments are expensive and uncomfortable, and there has never been a controlled study establishing their efficacy. We have nearly completed a study in which pressure is applied to 1/2 of an arm burn for one year. Clearly this is a major effort for the patients and they are paid \$1000 upon completion of the study (this figure is among the highest patient reimbursements approved by the HSRC.) The study is ongoing but we estimate that it will be completed in 1-2 years. Our first pass at analysis revealed no efficacy of the garments. If this observation holds upon completion, it will have a major impact on burn care.

In spite of all the current interest in costs and outcomes and in spite of improved survival, time off work after burns has never been determined with a large sample. The Model System multicenter national database permitted us to analyze this and we found the mean to be 14 weeks in a sample of 363 patients working at the time of injury. This translates into an estimated \$170 million in lost wages per year, a substantial sum. This was presented to the American Burn Association.

*Laboratory Topics***Hypertrophic Scarring**

Hypertrophic scarring, which follows injury to the deep dermis, may be the worst outcome of burns. For decades researchers have tried to find a solution to this problem by studying the fibroblast and collagen and this has led to essentially nothing. Clearly a new approach is necessary.

We have determined that the fat dome of skin is a deep dermal structure which is seldom described in textbooks and is located in the same anatomic areas where hypertrophic scarring occurs. It may suggest a clue to the cause of hypertrophic scarring. We have now clarified the histological anatomy of the fat domes in normal skin, burned skin, mature and hypertrophic scars, fetal skin, rats, rabbits, and pigs, and the material has been presented to the Plastic Surgery Research Council and is currently being prepared for publication. It is our hope that understanding this histology will redirect research in hypertrophic scarring to a more productive path and that we can obtain funding to pursue this matter.

A Pig Model of Hypertrophic Scarring

Perhaps the main reason that hypertrophic scarring is so poorly understood is the lack of an animal model. In 1976, Silverstein, Goodwin, Raulston and Pruitt reported that the female, red Duroc pig develops hypertrophic scar. Nothing ever came of this report. Was it not reproducible? Missed? Not useful?

We decided to clarify this matter and made tangential wounds of varying depths on two of these pigs which were then allowed to heal spontaneously. We observed scars of up to 11mm in thickness. Encouraged by this, we did two more pigs and again found thick scars and reported this to the Plastic Surgery Research Council. One might ask why only two pigs, and only two at a time? These are enormous animals and the cost and effort is quite significant.

We plan to do two more pigs to confirm our results. The next step will be to analyze the scar tissue for molecules that have been implicated in human hypertrophic scar formation including versican, decorin, nitrous oxide, collagenase and TGF- β to establish that it is more than merely thick scar. If the observations hold, an animal model, albeit costly and difficult, will facilitate hypertrophic scar research.

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• WOUND HEALING

FUNDING:

Department of Defense & VA
National Institutes of Health
Plastic Surgery Education Foundation

Much of what we see in surgical practice — especially in plastic surgery — involves and relies on the tissue's response to injury. When the response to injury is normal, wounds heal without complication. However, a multitude of factors such as neoplasms, infection, and radiation injury disrupt normal responses to injury and often necessitate reconstructive surgery to transfer healthy tissue.

Wound healing represents a series of well-orchestrated events, including inflammation, angiogenesis, epithelialization and matrix remodeling. Our laboratory is interested in determining the cell-cell signaling and the changes in gene expression during normal wound healing. Each of the projects represents our efforts to understand the normal healing process in order to better understand and treat aberrant healing processes.

Angiogenesis: Role of Proteases

One of the cellular events of normal wound repair is angiogenesis, the formation of new blood vessels. Blood vessels are assembled by either vasculogenesis, in which a primitive vascular network is established from mesenchymal progenitor cells during development or angiogenesis, in which new blood vessels arise from preexisting vessels. Though vasculogenesis is limited to development, angiogenesis occurs both in the embryo and in adults. For angiogenesis to occur, endothelial cells must detach from adjacent endothelia, proliferate, migrate, and assemble into tubes.

Wound repair angiogenesis requires activation of fibrinolytic enzymes for cellular migration of microvascular endothelial cells through a fibrin matrix. These fibrinolytic enzymes include the plasminogen activator system, which consists of urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA), balanced by plasminogen activator inhibitor-1 (PAI-1) and vitronectin. It has been hypothesized that dysregulation of the plasminogen activator system can result in

abnormal wound healing. We have studied the plasminogen activator system in models of excisional wound healing to clarify the role of the plasminogen activator system during normal healing

Transgenic mice deficient for vitronectin, uPA, or uPAR (urokinase plasminogen activator receptor) genes appear phenotypically normal, but we have found marked disturbances in response to tissue injury. Our data indicate that vitronectin serves to limit fibrinolysis following injury and that elimination of vitronectin from the provisional wound matrix leads to microvascular hemorrhage and results in delayed wound healing.

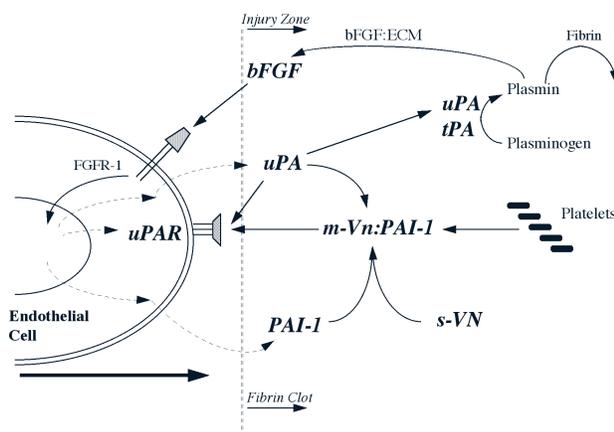


Figure 1: Urokinase plasminogen activator receptor.

In addition, limited function of uPA or uPAR genes disturbs cell migration. This disturbance results in decreased wound angiogenesis and decreased wound epithelialization, leading to significant differences in the histologic appearance of the final wound. Interestingly, in spite of significant effects on wound microvasculature, deletion of uPA, or uPAR genes does not result in delayed healing. The murine wounds with uPA, or uPAR deletions compensate with increased wound contraction.

Angiogenesis: Role of Matrix

Tissue injury is immediately followed by coagulation, changing the extracellular matrix (ECM) environment of endothelial cells from a collagen and laminin rich matrix to a fibrin and vitronectin rich matrix. These newly deposited extracellular matrix molecules bind to integrins, a class of cell surface receptors that anchor the cell cytoskeleton to the surrounding extracellular matrix and provide cellular signals about the matrix environment to the endothelial cell.

Using cDNA microarrays, we have identified the changes in gene expression of endothelial cells exposed to different ECM, including vitronectin. Change in the matrix alone resulted in greater than a 3-fold difference in expression among 430 out of 4,400 genes examined. The quiescent state (characterized by laminin or collagen type IV) increased the expression of signaling molecules such as phospholipase C- α and PI 4,5 kinase, whereas the injury-associated matrices (characterized by vitronectin and fibrin) enhanced the signaling molecules PKC- α and multiple serine-threonine kinases. In addition, vitronectin increased the expression of DNA-damage repair proteins that protect cells from apoptosis. Finally, there were global increases in multiple transcription factors including POU-2, TF3, E2F-5, MEF2 and the transcription regulator, ISGF3.

Interestingly, we also found marked up-regulation of several growth factor receptors after exposure to an injury-associated matrix, such as vitronectin. These data show that changes in the matrix environment alone can result in large-scale gene expression changes that favor endothelial cell proliferation and survival. Together, these studies support evidence that manipulating the wound matrix environment alone may augment wound repair and have obvious implications for tissue bioengineering.

Angiogenesis: Role of Growth Factor Receptors

Angiogenesis is initiated by paracrine signals that stimulate the proliferation and migration of endothelial cells. Among the angiogenic ligands are fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). A growth factor must bind to its specific endothelial cell surface receptor for a cellular response. FGF receptors on endothelium include FGFR-1 and FGFR-2; VEGF receptors include VEGFR-1 and VEGFR-2. Although normal adult dermal microvessels do not constitutively express these receptors, we have shown by our cDNA microarray studies that changes in

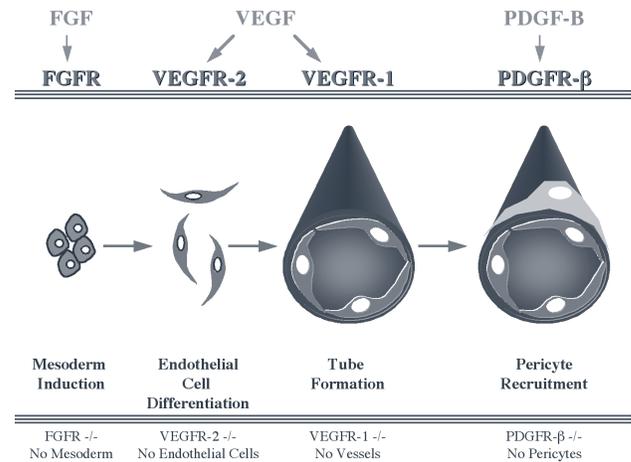


Figure 2: **VEGFR-2 and FGFR-1 systems.**

the matrix environment following tissue injury induce endothelial cells to express these specific growth factor receptors.

Mice carrying homozygous disruptions in FGFR-1, FGFR-2, VEGFR-1, or VEGFR-2 die in utero, highlighting the critical role of these receptors during vasculogenesis and developmental angiogenesis. To better understand the role of these receptors during repair, we are currently delivering truncation mutant growth factor receptors to normal mice wounds using a retroviral system. Current ongoing work suggests that the VEGFR-2 and FGFR-1 system appear to be critical for the proliferation of multiple cell types during wound repair, including microvascular endothelial cells.

Gene Expression Profiling of Normal Human Wound Healing

Response to acute cutaneous injury is dependent on the temporal activation and silencing of thousands of genes. Gene expression profiling using cDNA microarrays allows for simultaneous comparison of thousands of genes. In these studies, our aim is to analyze and catalogue the gene expression profile of human skin during the first few hours following cutaneous wounding.

We have found significant gene up-regulation 30 min. after wounding: expression of 334/4000 genes was increased >3 fold. Within the first 30 minutes, we found up-regulated expression of genes involved in cell signaling inhibition, including suppressor of cytokine signaling (SOCS) and suppressor of ras-1. In addition, there was silencing of cell cycle genes (e.g. Rb), proteases (e.g. uPA), and growth factor-related pathways. At 1 hour post wounding, 471/4000 genes were increased > 3 fold with a predominant reversal of the 30 min.

pattern: we found down-regulation of transcriptional and signaling inhibitors, and up-regulation of multiple transcriptional activators. A searchable web site is being constructed to disseminate this data.

Our data demonstrate the complexity of the gene activation/suppression processes that occur early in the normal human wound healing process. Most of these genes have never been examined in wound healing research. Using this database in gene expression, new targets may emerge that could provide further insight into the study of normal response to injury.

Hematopoietic Stem Cells in Normal Wound Healing

Whereas morphogenesis in tissue repair has often been compared and contrasted to the morphogenesis

cells in wound repair and the signaling pathways that regulate their path of differentiation. From our cDNA microarray studies of gene expression in normal healing human skin wounds we have determined that one of the genes that is activated belongs to the family of signaling molecules that are highly expressed during development but become dormant in adults.

We have found that Wnt genes are re-expressed in response to tissue injury. We are beginning to examine the role and contribution of HSC to murine wound repair and define the contribution that the Wnt signaling plays in the response to cutaneous injury. HSC may be a valuable source of regenerative capacity for a wound, especially when the local resident cells have been damaged by irradiation.

Our laboratory is interested in determining the cell-cell signaling and the changes in gene expression during normal wound healing. Each of the projects represents our efforts to understand the normal healing process in order to better understand and treat aberrant healing processes.

during development, normal wound repair has always been thought to involve proliferation and migration of terminally differentiated cell types. Recent evidence suggests that normal cutaneous repair involves recruitment of non-resident undifferentiated cells from distant sources, such as the bone marrow. Populations of progenitor cells have been identified as valuable sources of uncommitted cells, capable of reconstituting multiple cell types in various tissues, including skin. This pool of cells may represent the opportunity to induce tissue regeneration in sites of injury similar to the morphogenesis seen in development. The ability to manipulate these cells may provide a previously unrecognized means of therapeutic intervention in patients with non-healing wounds

The most-studied progenitor cell type is the hematopoietic stem cell (HSC) from the bone marrow. We have recently found that HSCs migrate to sites of dermal injury, differentiate into several cell phenotypes and incorporate into the cutaneous wound long-term. These recent findings raise important and unanswered questions regarding the actual role of these progenitor

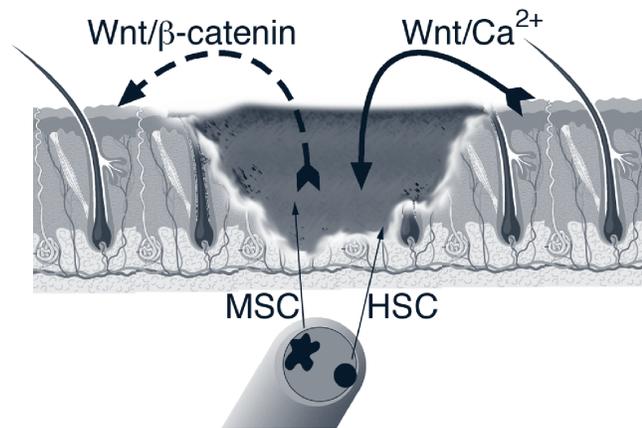


figure 3: **Graphic demonstration of our hypotheses.** We think stem cells in the epidermis and stem cells from bone marrow can provide the missing cell types following loss due to injury. However, we think that the lack of morphogens in the wound may account for the lack of regeneration seen in wound repair. We propose that the epidermal stem cells regulate the deeper dermal stem cell's fate, and that the deeper dermal cells regulate the epidermal stem cell's fate, based on the two Wnt signaling pathways.

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TRANSPLANT SERVICE

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- IDENTIFICATION OF GENES RESPONSIBLE FOR IMMUNOLOGIC TOLERANCE
- IMMUNOLOGIC TOLERANCE IN A LARGE ANIMAL MODEL
- LYMPHOCYTE DEVELOPMENT AND DIFFERENTIATION: THE ROLE OF THE NOTCH GENE

AWARDS:

American Foundation of Urologic Disease Fellowship, 1997
National Institutes of Health
• Career Investigator Development Award, 1999

FUNDING:

National Institutes of Health
• National Institute of Diabetes, Digestive and Kidney Diseases

Transplantation has matured to become the treatment of choice for end-stage renal and hepatic disease. Despite many advances in immunosuppression, the majority of transplant grafts continue to be lost to immunologic causes. Of these, rejection, a lymphocyte-mediated response to foreign tissue, is a leading factor. Our research is directed toward understanding the factors responsible for this

both from human patients who have developed tolerance and in a transgenic mouse model of tolerance. We expect that the patterns of gene expression novel to the tolerant versus the non-tolerant state could provide a tool to determine when the tolerant state is reached. Additionally, individual genes that are differentially regulated between these two states may lead to insights into the mechanisms of tolerance induction.

We expect that the patterns of gene expression novel to the tolerant versus the non-tolerant state could provide a tool to determine when the tolerant state is reached.

immune response and developing techniques to abrogate them. Our laboratory focuses on the control of development and differentiation as it pertains to lymphocytes and hematopoietic stem cells. We are using large-scale cDNA array techniques to ascertain the regulatory genes involved in these processes. In addition, we are interested in developing tolerance strategies in a large animal transplant model using knowledge gained from this work.

Identification of Genes Responsible for Immunologic Tolerance

Tolerance describes a state of 'donor-specific unresponsiveness.' This develops *de novo* in some patients after organ or stem cell transplant. The events responsible for this have not been elucidated and would provide important insights into therapies that would mitigate the effects of chronic non-specific immunosuppression. We are actively seeking answers to these questions by employing cDNA arrays (Fig. 1) of lymphocyte subsets

Immunologic Tolerance in a Large Animal Model

This part of our work involves a large animal transplant model. In collaboration with Drs. Rainer Storb and Beverly Torok-Storb at the Fred Hutchinson Cancer Research Center, we have successfully created dog models that are hematopoietic chimeras through hematopoietic stem cell transplantation. These animals have accepted renal transplants in the absence of immunosuppression from their DLA matched littermate donor, and currently have excellent renal function more than 1 year after renal transplantation (Fig. 2). We are exploiting this animal model to examine both the induction of tolerance and the robustness of hematopoietic chimerism as platform for organ transplantation in the absence of immunosuppression.

Lymphocyte Development and Differentiation: The Role of the Notch Genes

Lymphocyte development proceeds along a pathway characterized by a series of gene rearrangements that

impart antigen specificity. Alterations in these pathways can contribute significantly to the development of autoimmune and immunodeficiency states. Understanding the control of lymphocyte development and maturation will lead to important insights into antigen specificity and immune dysregulation, and could be exploited to alter the immune response.

One phylogenetically conserved family of transmembrane receptors with known importance in cell-fate decisions is Notch. Originally identified in *Drosophila melanogaster*, Notch family members subsequently have been identified in other invertebrates, and four mammalian homologues are now known. Their function involves control of developmental cell-fate decisions through Notch receptor signaling which is thought to delay or block differentiation of uncommitted cells. The mammalian Notch family members are ubiquitously expressed and all are expressed in lymphoid tissue. Notch1 has been shown to influence the development of T lymphocytes, and Notch2 has recently been found to inhibit a transcription factor (E47) that is necessary for B lymphocyte. Our preliminary work shows that while

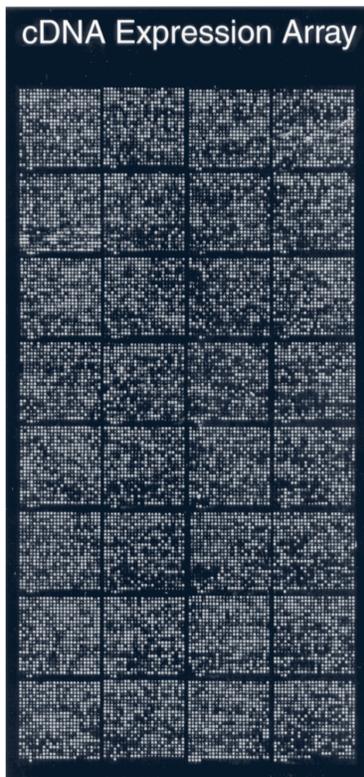


figure 1

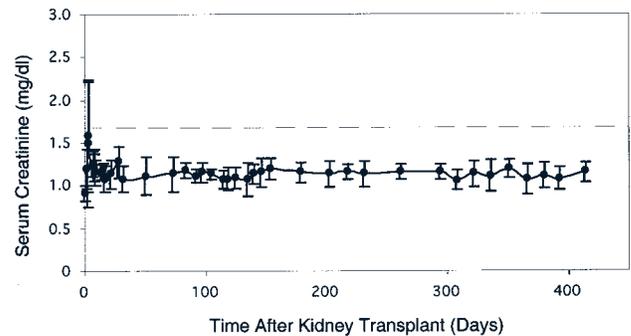


figure 2

the Notch family members are expressed in developing B lymphocytes, Notch2 expression is highest, suggesting unique activity in this cell population.

The focus of our work is to determine the role that Notch family members have in controlling lymphocyte development. To this end we have generated mice which overexpress the constitutively active intracellular portion of the Notch2 and Notch3 genes. We are currently analyzing the animals to characterize the phenotypic changes resulting from increased Notch activity. We plan to make use of cDNA array technology to identify downstream effectors of Notch, which remain to be fully characterized in mammals.

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• IMMUNOLOGIC TOLERANCE

FUNDING:

Boeing Company

Fujisawa USA, Inc.

Immunex

Juvenile Diabetes Foundation International

MedImmune, Inc.

Novartis, Inc.

Roche Pharmaceuticals

Our clinical goal is to induce donor specific immunologic tolerance in solid organ transplant recipients. We use a strategy that combines recipient infusion with tolerizing regulatory leukocytes and 'pro-tolerant' minimal immunosuppression. The tolerant recipient would accept the transplanted organ as "self," thus eliminating the side effects and chronic graft rejection which continue to limit the optimal effectiveness of solid organ transplantation.

Our *initial* step to this goal was the development of a clinical trial combining donor stem cell infusion with organ transplantation, *A Study Evaluating the Safety and Efficacy of Stem Cell Infusion in Solid Organ Transplant Recipients for the Induction of Microchimerism and Tolerance*. This pilot study examined the effect of cadaveric donor stem cells infused after solid organ kidney or kidney/pancreas transplantation.

The key to the success of this project has been the collaborative efforts of LifeCenter Northwest (the regional organ procurement agency), the Northwest Tissue Center (for procuring donor vertebral bodies) and Dr. Shelly Heimfeld's Cryobiology Lab at the Fred Hutchinson Cancer Research Center (for isolating the CD2 depleted and CD34+ stem cells from the bone marrow). The stem cells infused into the recipients were derived from the same donor who gave the kidney or kidney/pancreas. The infusions have been well tolerated by the twelve patients enrolled to date, and all patients have had good clinical outcomes. We are evaluating long-term patient and graft survival and rejection rates, and processing surrogate assays of tolerance.

Our *second* step has been the development of a pro-tolerant immunosuppression trial that will support donor cell therapy, *Steroid Free, Calcineurin Inhibitor Sparing, Mycophenolate and Sirolimus Based Regimen For Renal Transplantation*. Unlike standard immunosuppressive therapy, steroid therapy is avoided. Steroids have many

side effects and recent findings show steroids can prevent immunologic tolerance. Also, standard high dose calcineurin inhibitor therapy (tacrolimus) very effectively prevents organ rejection by blocking the early events in recipient T-cell recognition of donor tissue. However, initial immune recognition is required for tolerance. Thus, tacrolimus immunosuppression is delayed for two weeks, and then delivered at a low dose.

The synergism between sirolimus and tacrolimus allows for lower doses of both drugs, helping to avoid the nephrotoxicity of tacrolimus. Our experience with the 22 enrollees in this trial shows we can safely prevent acute rejection without the use of steroids. Steroid free immunosuppression is now the standard of care at the UW for kidney and pancreas transplantation and will be used for islet transplantation. To further enhance immunosuppressive support for peri-transplant donor cell therapy, we have developed the, *Sirolimus and Mycophenolate Immunosuppression For Low Risk Solid Organ Transplant Recipients* trial.

Elimination of steroids and calcineurin inhibitors may significantly enhance the induction of tolerance during the initial post-transplant period. Also, the considerable inflammation associated with surgery may inhibit the effect of our donor tolerizing cells. Therefore we are planning alternative tolerance induction strategies wherein patients would receive calcineurin inhibitor immunosuppression for several months post-transplant during the diminution phase of transplant inflammation. Thereafter, patients would be converted to a minimal immunosuppression for an infusion of tolerizing regulatory donor leukocytes and/or regulatory T-cells (TR).

The *third* step to our goal is to develop a tolerizing donor/recipient cell preparation to combine with pro-tolerant immunosuppression. To implement this step, we submitted a proposal entitled *Role of Stem Cell Therapy*

in the Generation of Tolerance in Clinical Islet Transplantation, and won funding from the Boeing Company. Boeing recognized the merit in our goal to ensure survival of insulin-producing donor islets in transplant recipients. To accomplish this we are first building and testing specific donor-derived stem and dendritic cell therapies in our research laboratory and then implementing the cell based therapies in our clinical kidney transplants and later islet transplants.

The aforementioned laboratory work will be to develop and test a population of tolerance inducing cells and then develop procedures for ex-vivo isolation of sufficient cells for human clinical trials. By using a directed donor dendritic cell (DC) and pro-TR cytokines, our goal is to efficiently produce TRs (regulatory T-cells) for post-transplant infusion into tissue or organ recipients under the protection of pro-TR immunosuppression therapy.

DC and/or TR cells for infusion into human transplant patients.

We believe a therapeutic approach, cognizant of the mechanisms of natural tolerance, which combines donor cell infusion and targeted 'pro-tolerant' short course or single agent, low dose immunosuppression, will achieve donor specific immunologic tolerance and free patients from life-long immunosuppression.

We envision a day when cells collected from cadaveric or living organ donors and combined with recipient cells will produce graft protective cells. The recipients would receive the cells as part of a 'tolerance regimen.' Later the level of tolerance would be assayed to determine if immunosuppression can safely be withdrawn. The treatments could be extended to living related patients pre-transplant to prepare them for an organ or tissue and to all post-transplant patients if suitable donor cells or antigen can be obtained.

Immunologic tolerance to transplanted tissue and organs may well depend on donor cell therapy. A specific class of lymphocytes has the capacity to 'introduce' environmental antigen, such as food or inhaled protein, to host regulatory T-cells as a benign entity.

We are analyzing the ability of a promising donor lymphoid antigen-presenting DC fraction to induce donor specific alloprotective regulatory T-cells (TR) in transplant recipients. We are testing the donor DC and TR preparations in our established animal models of heart and pancreatic islet transplantation. Ex-vivo TR production for infusion into tissue and organ recipients has the potential to jumpstart natural tolerance in all transplant and post-transplant patients. In addition, ex-vivo TR production allows easier characterization of the gene expression markers characteristic of recipient cell recruitment into the TR phenotype. This marker set will allow determination of sufficient TR development in culture and in transplant patients.

For the clinical effort, we are developing DC and TR purification methods. We are involved in the clinical development of a large-volume, laser-based cell identification and isolation system termed the 'rapid cell sorter' currently under construction in the Clinical Research Center in the UW Medical Center. We will adapt the technology and experience gained with the high-speed cell sorter to isolate and purify tolerogenic

The significance of this research is that tolerance induction leading to the early prevention of acute rejection will enhance long-term graft survival, now the leading cause of transplant recipient mortality. Tolerance induction will also reduce complications related to immunosuppression, which is the leading cause of patient avoidance of transplantation. These efforts should improve patients' quality of life and reduce the morbidity and mortality of solid organ transplantation. The significance and interest of this work has given rise to numerous clinical research projects and collaborations, including the transplantation of human islets by the Human Islet Transplantation in Seattle (HITS) consortium.

The success of the islet transplantation effort by the HITS consortium will depend to a large degree on the pro-tolerant islet friendly immunosuppressive strategies developed at the University of Washington. The consortium consists of researchers and clinicians from the Pacific Northwest Research Institute, the University of Washington, Virginia Mason Medical Center, Puget Sound Blood Center, Fred Hutchinson

Cancer Research Center and Swedish Medical Center. The consortium is led by Dr. R. Paul Robertson of the Pacific Northwest Research Institute. The islet isolation effort is being led by Dr. Joanna Reems, scientific director of the Northwest Tissue Center Islet Isolation Core, with Core scientist Dr. Shinichi Matsumoto. (Dr. Matsumoto also has an appointment in the UW Dept of Surgery). Dr. Tom Hefty at Virginia Mason and Dr. Bill Marks at Swedish are leading the clinical efforts at their institutions.

Dr. Christopher Marsh is leading the effort at the UW, with Dr. John Borsa, in the Department of

Interventional Radiology, and Dr. Connie Davis, in the Division of Nephrology and Transplantation. Dr. Marsh is heading a trial wherein patients will receive both an islet cell and kidney transplant. The patients are diabetics who have suffered kidney damage as a result of their disease. These transplants will be done at the UW, Swedish Medical Center, and Virginia Mason starting as early as December of 2001. The Juvenile Diabetes Foundation and the National Institutes of Health are providing funding for the trials and the FDA has approved of these projects.

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James D. Perkins, M.D.

- THE ROLE OF THE LIVER, DENDRITIC CELLS, AND CYTOKINE REGULATION ON ORGAN TRANSPLANT TOLERANCE INDUCTION AND MAINTENANCE

The Goal in Transplant Immunology Research

The great challenge of transplant surgery has always been the surmounting of an immunological barrier, rather than the perfecting of surgical technique. The body's immune system resists a transplanted organ in the same manner as it resists infection. Unfortunately, current immunosuppressive therapy overrides the body's immune system equally for an allograft and an infection, resulting in many adverse side effects, including opportunistic infections, an increased rate of malignancy, and end-organ toxicities.

Our overall goal of transplant immunological research is attempting to identify methods to induce donor-specific transplant tolerance with preservation of recipient immunocompetence. Knowing how to induce

immature lymphocytes encounter antigens and are deleted (the process of *negative selection*, also called *clonal deletion*, *programmed cell death*, or *apoptosis*).

Peripheral tolerance occurs in peripheral lymph organs, such as the lymph nodes and spleen, where mature lymphocytes encounter antigens under particular conditions. Three principle mechanisms contributing to peripheral tolerance are: 1) *clonal deletion*, 2) *clonal anergy* (functional inactivation of lymphocytes without cell death), and 3) *immune regulation* (*suppression* of lymphocyte activity by regulatory T cells). These three mechanisms are not mutually exclusive.

Dendritic Cells and Cytokines

As with all the biological sciences, research has shown that immunological functions occur with tremen-

Knowing how to induce a state of tolerance in transplant recipients would eliminate the need for immunosuppressive agents with their associated complications.

a state of tolerance in transplant recipients would eliminate the need for immunosuppressive agents with their associated complications. A greater understanding of tolerance induction would allow development of methods for decreasing the sensitization of patients while awaiting transplant. More broadly, the ability to achieve tolerance could be used in the treatment of some autoimmune diseases.

Mechanisms of Tolerance

The vital balance of the immune system between specific reactivity and specific unresponsiveness relies on a multitude of mechanisms (Fig.1). Immunological tolerance is of two types: central and peripheral. *Central tolerance* is induced in generative lymphoid tissue, namely, the thymus. Central tolerance occurs when

dous complexity at the cellular and molecular level. *Dendritic cells (DCs)*, acting as professional antigen presenting cells, located throughout the body, have been found to be highly efficient initiators and regulators of immune responses. They play diverse roles in the regulation of the immune response, depending on their maturation status, signals from antigens, and the cytokine microenvironment. Immature DCs express low levels of MHC class II and co-stimulatory molecules, downregulating immune responses and potentially inducing immune tolerance.

DCs can quickly become mature in response to appropriate inflammatory stimuli and significantly enhance their stimulatory properties for naïve CD4⁺ and CD8⁺ T cells, promoting rejection. There is increasing evidence that certain subtypes of DCs, such as liver-

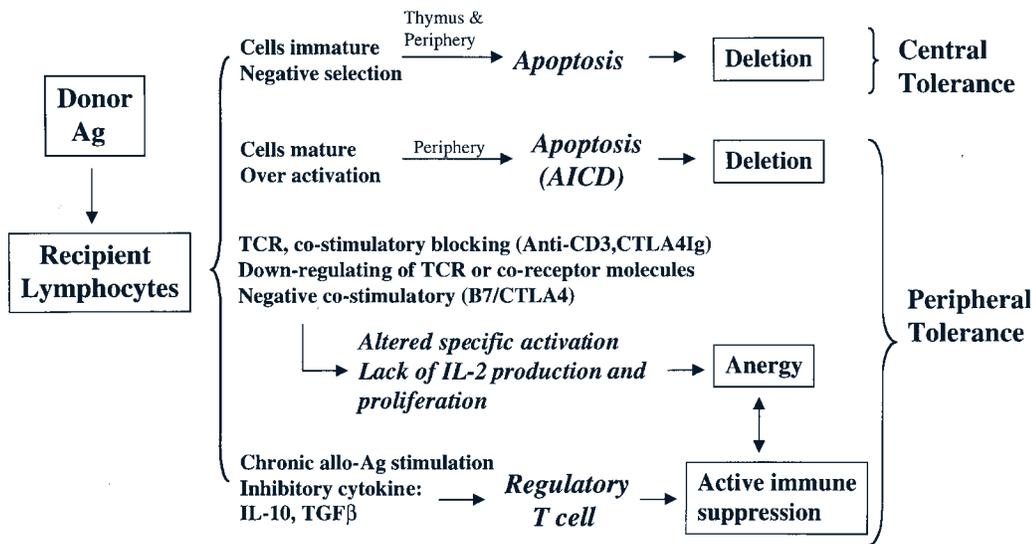


figure 1: Mechanisms of tolerance.

derived DCs, IL-10 modulated DCs or CD8a⁺ lymphoid-derived DCs, are able to downregulate the immune response. These may play an essential role in inducing and modulating regulatory T cells both *in vivo* and *in vitro* (Fig. 2).

Cytokines are known to modify immune responses. Recent studies have shown that regulatory T cells play a key role in maintaining tolerance; they do this via tolerogenic cytokines such as IL-10 and TGFβ, as well as by DC polarization. This suggests a possible new strategy for the induction of either antigen-specific or non-specific tolerance. Therefore, an understanding of how to manipulate DCs and cytokine balance *in vivo* towards active suppression of allo-reactive T cells will be our next goal in transplant immunology research.

The Role of the Liver in Peripheral Tolerance

Liver tolerogenicity has been known for a long time. Successful liver transplantation induces donor specific tolerance across major histocompatibility complex (MHC) barriers with no immunosuppression in virtually all mouse and several rat strain combinations, as well as in a significant proportion of outbred pigs. Even across species barriers, liver grafts can survive longer than other types of organ transplants. In spite of this, the tolerance induced by the liver can specifically protect the subsequent donor heart or skin graft from acute — even chronic — rejection. In humans, liver transplant patients experience fewer episodes of acute rejection and are more easily rescued than in other types of organ transplantation. Patients receiving a combined

kidney and liver transplant experience significantly less rejection of the transplanted kidney than patients receiving a kidney alone. Certain vascularized allografts have improved survival with the venous drainage via the portal vein into the liver.

The mechanism of the liver’s role in peripheral tolerance remains unclear. It has been suggested that it may be due to the organ’s combination of hematopoietic activity, production of growth-promoting and immunoregulatory cytokines (GM-CSF, IL-10, TGFβ), and comparatively large numbers of potentially tolerogenic antigen-presenting cells, particularly dendritic cells (DCs). The liver has also been considered as a site of T cell death. Evidence has been found that continuing cytotoxic T lymphocyte apoptosis occurs in the liver allografts after transplantation in mice.

We will develop a mouse model, based on mouse orthotopic liver transplantation and oral antigen, to investigate the liver’s role, particularly the liver and gut DCs, in the different mechanisms of inducing and maintaining peripheral tolerance. Aims of our research include: to elucidate the mechanisms of liver tolerogenicity and explore an optimal method for Ag delivery on organ transplant tolerance induction; to determine the critical condition of tolerance transferred by the liver; and to examine whether liver tolerance can be induced in a sensitized mouse.

The liver’s involvement in any of the mechanisms of peripheral tolerance in this oral tolerance model is unknown. Through the ability to remove and insert various liver combinations and stages of tolerance, we

can begin to seek the answer to the liver's role in peripheral tolerance. As these are clearly defined, our research could potentially lead to human trials.

Summary

Our project seeks to learn which of the three mechanisms of tolerance (deletion, anergy, or suppression)

predominates in the liver, and, at the microscopic level, to assess the role of hepatic dendritic cells and the production of cytokines on the induction and maintenance of tolerance. It will be exciting to learn if a particular mechanism predominates in the liver, because ways can then be found to exploit that mechanism for clinical applications.

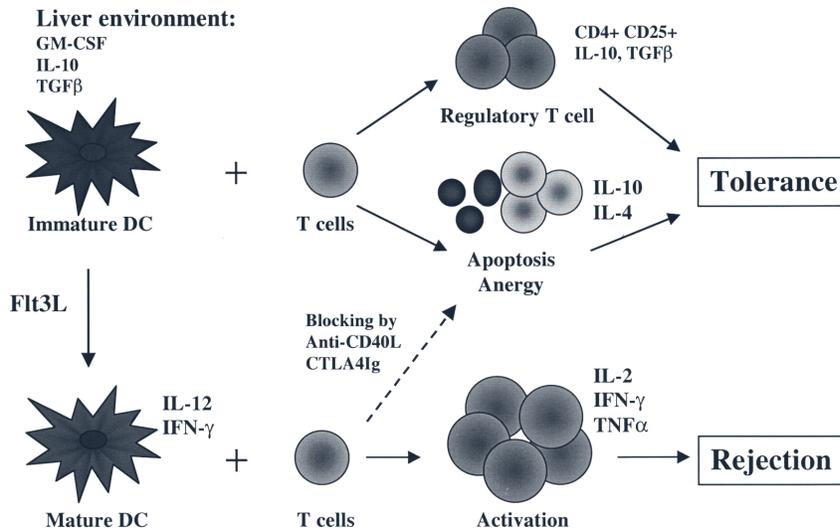


figure 2: Mechanism of DC on T cell proliferation and tolerance induction.

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• ADVANCING THE CLINICAL SCIENCE OF SURGERY USING OUTCOMES RESEARCH TOOLS

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Over the last decade “outcomes” research became a catch phrase for healthcare administrators, providers and researchers. Outcomes research means different things to different people, however. For some it is viewed as a way to provide more services for fewer dollars; for others it means finding ways to regulate physician practice 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.

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.

1. 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

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.

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,

what should be considered the community standard, who are the outliers (both good and bad) and what techniques work out 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.

Research I’ve been involved with during the last year 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.

To study outcomes from antireflux procedures we studied over 86,000 records and found that while the rates of splenectomy have decreased significantly with time the rate of in-hospital mortality and esophageal injury have not. Furthermore, while the rate of adverse outcome identified was 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. This population-based research is both the first step in assessing what needs to be fixed in the system and the last step in determining whether changes have had the expected effect.

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.

2. What are the Avoidable Factors Associated with These Adverse Outcomes?

The next step is figuring out what avoidable factors contribute to the adverse outcome. 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 are important next steps in the quality improvement process.

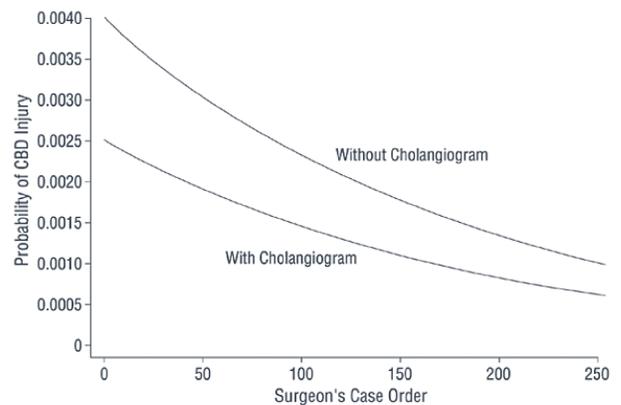


Figure 1: The predicted probability of CBD injury after laparoscopic cholecystectomy based on surgical experience and use of an intraoperative cholangiogram. As the level of surgical experience (case order) increases the predicted probability of CBD injury decreases. Failure to use an intraoperative cholangiogram is negatively associated with CBD injury at all levels of surgical experience but this effect is best demonstrated in the surgeon's first 20 procedures.

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 (the surgeons' 1st through 19th cholecystectomy) 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 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 1.) This may be a more effective way of "informing" the informed consent process.

We recently completed a similar analysis looking at early experience and its association with adverse out-

comes after anti-reflux surgery. We found the same relationship of surgeon experience to almost all adverse outcomes. Another study using statewide data from New York, Florida, New Jersey and South Carolina will address whether states that have effectively concentrated antireflux procedures in the hands of the most experienced surgeons have better overall outcomes than states with more even distribution of procedures/surgeon.

In looking at the association of CT scan use on the adverse outcome of misdiagnosis in appendicitis we are currently studying over 5000 patients undergoing appendectomy at Group Health Cooperative over the last two decades to determine the relationship between CT scan use and misdiagnosis. This should help address the question, why has misdiagnosis remained stable all these years if we have such a reportedly good diagnostic test?

For incisional hernia surgery we have tried to link better outcomes (shortened length of hospitalization and lower reoperative rates) to recent developments in laparoscopic technology. We would like to determine whether or not laparoscopy should replace open hernia as the procedure of choice. Using available data we have been unable to identify differences in outcome when comparing the pre and post-laparoscopic eras. This information is helpful because it demonstrates the need for a randomized trial in assessing the importance of this new technology.

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 medicolegal) 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 general surgical division, however, we are hoping to develop and get funding for randomized trials in the management of appendicitis (routine versus selective CT scan use) and for incisional hernia (laparoscopic versus open).

4. 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 have reviewed the ~200,000 patients over age 65 undergoing cholecystectomy per year (n=1.6 million patients) to determine if our findings regarding cholangiogram and CBD injury are identified in this population. If so, these administrators would like 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.

Involving the financial stakeholders may be the most effective way in improving 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, for 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.

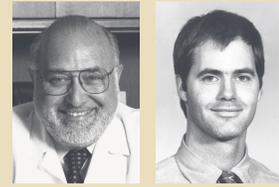
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- GERD AND AIRWAY DISEASE
- INEFFECTIVE PERISTALSIS

AWARD:

Society for Surgery of the Alimentary Tract

- Ross Resident/Fellow Research Award, 2001

The Department of Surgery, along with the University of Washington Medical Center, established the Swallowing Center in 1995. Under the direction of Dr. Carlos Pellegrini the center has grown to see over 1,000 patients a year, making it one of the largest esophageal labs in the country. It was established as a clinical lab, but generates a large amount of information for research purposes. Investigators in the departments of Gastroenterology, Pulmonology, and Otolaryngology have participated with us in many research endeavors.

A major interest of ours is the relationship of gastroesophageal reflux and airway injury. We have the world's largest experience in the measurement of acid exposure in the pharynx. This has proven useful to us and our colleagues in other departments in diagnosing the etiology of cough, hoarseness, asthma, and many other respiratory symptoms. We perform esophageal operations on many patients who come through our Swallowing Center. Thus we can evaluate and report on the effect of these operations in patients with motility disorders of the esophagus. In these and other areas, our comprehensive functional study of the upper gastrointestinal tract answers questions across medical disciplines.

GERD and Airway Disease

Pharyngeal pH monitoring and laryngoscopy are used to diagnose gastroesophageal-pharyngeal reflux as a cause of respiratory symptoms. Though their use seems intuitive, their ultimate role remains to be defined.

We performed pharyngeal pH monitoring and laryngoscopy on a group of 76 patients with suspected reflux induced injury and on 11 normal subjects. The most common symptoms reported by patients were hoarseness (87%), cough (53%), and heartburn (50%). The normal subjects had a significantly lower reflux

finding score (RFS) — (2.1 vs. 9.6, $p < .01$) — and fewer episodes of pharyngeal reflux (PR) — (0.2 vs. 3.4, $p < .01$) — than did the patients. No subject had more than one episode of PR. Fifty patients (66%) were RFS+ and 26 (34%) were RFS-. Thirty-two patients (42%) were PR+ and 44 (58%) were PR-. Fifteen patients had a normal RFS and no pharyngeal reflux (Group I=RFS-, PR-). Forty patients had discordance between the laryngoscopy findings and the pH monitoring (Group II=RFS-, PR+ or RFS+, PR-). Twenty-one patients had both an abnormal RFS and pharyngeal reflux (Group III=RFS+, PR+).

The heartburn score in Group I was 1.3; Group II, 0.9; and Group III, 1.8 ($p < .05$ for Group II vs. Group III). The mean distal esophageal acid exposure (% time $\text{pH} < 4$) was 3% in Group I, 3.3% in Group II, and 5.9% in Group III ($p < .05$ for Group II vs. Group III).

An abnormal PR or RFS differentiates normal subjects from patients with laryngeal symptoms. Agreement between PR and RFS helps establish or refute the diagnosis of gastroesophageal reflux as a cause of laryngeal symptoms. Patients who are RFS+ and PR- may have laryngeal injury from another source; whereas patients who are RFS- and PR+ may not have acid enter the larynx, despite pharyngeal reflux. Patients who are RFS+ and PR+ have more severe gastroesophageal reflux disease. Laryngoscopy and pharyngeal pH monitoring should be considered complementary studies in establishing the diagnosis of laryngeal injury induced by gastroesophageal reflux.

We then operated on a small group of patients with suspected reflux induced airway injury, and performed pharyngeal pH monitoring pre and post-operatively.

All patients had GERD and respiratory symptoms; 60% had evidence of pharyngeal reflux pre-operatively. Antireflux procedures resulted in a significant decrease

in pharyngeal reflux (7.9 to 1.6 episodes/24hr; $p < 0.05$) and esophageal acid exposure (7.5% to 2.1%. $p < 0.05$). In patients with pharyngeal reflux and complete postoperative testing, three had improvement of respiratory symptoms and resolution of pharyngeal reflux. In two patients with recurrent respiratory symptoms after surgery, persistent pharyngeal reflux was detected.

Our study showed that operative treatment of GERD is effective in controlling extraesophageal reflux, measured both subjectively and objectively. Evidence of pharyngeal reflux on pH testing helps to identify which patients with respiratory symptoms will benefit from an antireflux procedure.

groups (preop 2.8, postop 0.65; $p < 0.05$). Dysphagia was 1.1 preoperatively and 0.62 postoperatively ($p = \text{NS}$) in the Toupet group and 1.2 preoperatively and 0.3 postoperatively ($p < 0.05$) in the Nissen group. Furthermore, none of the patients in the Nissen group developed new dysphagia and none required dilatation.

Distal esophageal acid exposure normalized in both groups after operative treatment (DeMeester score median 72.3 vs. 11.3, $p < 0.05$ Toupet; 57.1 vs 6.3, $p < 0.05$ Nissen). Distal esophageal amplitudes averaged 27.8 mm Hg preoperatively and 35.6 mmHg ($p = \text{NS}$) in the Toupet group. In the Nissen group the average was 28.2 mm Hg preoperatively vs 49.0 mm Hg postopera-

Evidence of pharyngeal reflux on pH testing helps to identify which patients with respiratory symptoms will benefit from an antireflux procedure.

Ineffective Peristalsis

Partial fundoplication has traditionally been indicated for patients with gastroesophageal reflux disease (GERD) who have defective peristalsis (DP). Because some studies had found that partial fundoplication was less effective than total fundoplication to control acid reflux, in 1997 we stopped performing partial fundoplications for patients with DP and switched to a floppy total fundoplication. This study analyzes the results of our new strategy and compares it with our older one.

We performed total (Nissen) fundoplication in 57 patients with DP (distal amplitude < 30 mmHg or failed propagation in more than 40% of swallows) and compared clinical and physiologic outcomes to 39 patients previously treated with partial (Toupet) fundoplication. Heartburn scores improved in both

tively ($p < 0.005$). Two patients with a previous Toupet required a conversion to Nissen fundoplication. No postoperative dilation was required in either group.

Our study shows that both partial and total fundoplication are effective in controlling the symptoms of GERD in patients with defective peristalsis. Dysphagia is significantly improved after a total fundoplication, but not after partial fundoplication. Although both operations brought acid reflux to within normal limits, the effect was more pronounced with Nissen fundoplication. Nissen, but not Toupet fundoplication, produced a significant increase in amplitude of esophageal peristalsis, which may explain the subjective improvement during deglutition. Total fundoplication should be the treatment of choice in patients with GERD and defective peristalsis.

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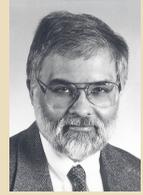
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• CENTER FOR VIDEOENDOSCOPIC SURGERY

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Minimally invasive surgery (MIS) involves a multi-dimensional series of tasks requiring a synthesis between visual information and the movement (kinematics) and forces (dynamics) of the surgical tools. Analysis of these sources of information is a key step in mastering MIS surgery but may also be used to define objective criteria for training and characterizing surgical performance.

The **BlueDRAGON** is a new system based on a simple pantograph design. We have designed and built a two-handed model for acquiring the kinematics and the dynamics of two endoscopic tools along with the visual view of the instruments performing within the minimally invasive surgical scene. It includes two four-bar mechanisms equipped with position and force torque sensors for measuring the positions and the orientations (P/O) of two endoscopic tools along with the forces and torques applied by the surgeon's hands.

Together with the pantograph, we have developed a methodology for deconstructing surgical tasks based on a

fully connected, finite-state model (28 distinct states, 14 for each hand) where each state corresponds to a fundamental tool/tissue interaction. State interactions are defined operationally by the tool kinematics and associated unique force/torque (F/T) signatures. Transitions from state to state are analyzed using a statistical methodology called Markov modeling, commonly used for complex pattern recognition tasks such as voice recognition.

Our current experimental protocol includes seven MIS tasks performed on an animal model (pig) by 30 surgeons at different levels of their residency training. Preliminary analysis of these data show that major differences between residents at different skill levels include: (i) the types of tool/tissue interactions being used, (ii) the transitions between tool/tissue interactions being applied by each hand, (iii) time spent while performing each tool/tissue interaction, (iv) the overall completion time, and (v) the variable F/T magnitudes being applied by the subjects through the endoscopic

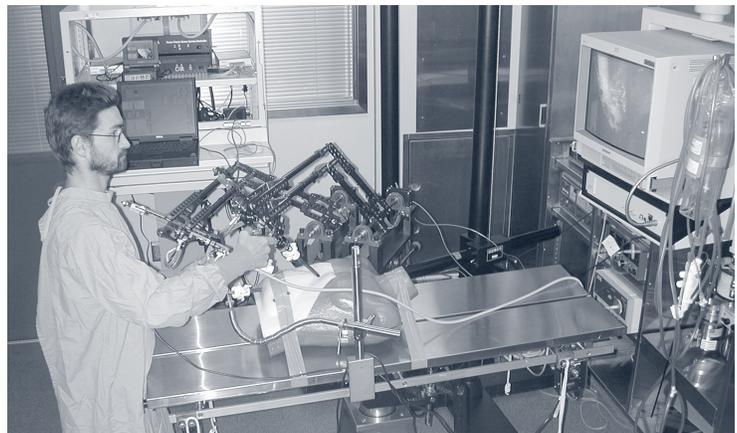
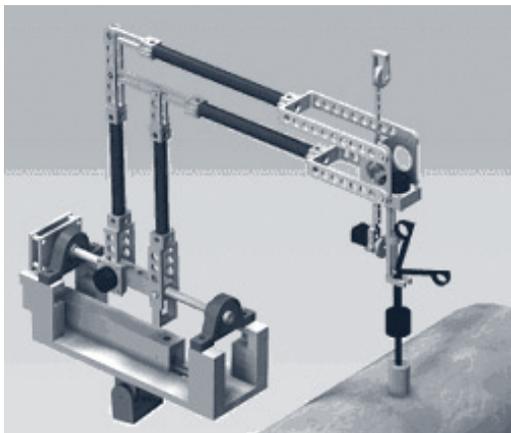


Figure 1: Schematic pantograph (left) and a two-handed model BlueDRAGON (right).

We have designed and built a two-handed model for acquiring the kinematics and the dynamics of two endoscopic tools along with the visual view of the instruments performing within the minimally invasive surgical scene.

tools. Further data collection is ongoing to validate and extend these initial impressions, then to integrate the objective data on performance derived from such a system into next generation surgical tools and simulation training.

Systems like surgical robots or virtual reality simulators that inherently measure the kinematics and the dynamics of the surgical tool may benefit from including the proposed methodology for analysis of efficacy and objective evaluation of surgical skills during training.

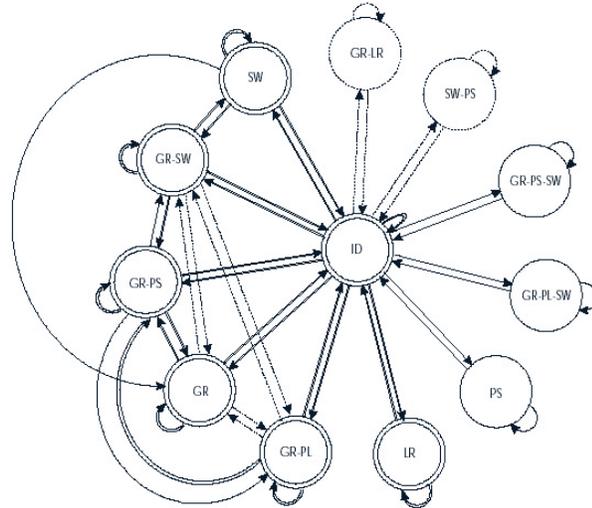


Figure 2: Schematic of state to state transitions around the central, idle state.

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VAPSHCS/GENERAL SURGERY

KEVIN G. BILLINGSLEY, M.D.

MATTHIAS STELZNER, M.D.



Kevin G. Billingsley, M.D.

- SURGICAL ONCOLOGY AT VAPSHCS: OVERVIEW
- NORTHERN ALLIANCE CANCER CENTER AT VA PUGET SOUND HEALTH CARE SYSTEM
- STUDY OF COMBINED MODALITY TREATMENT OF ESOPHAGEAL CANCER IN THE VA SYSTEM
- HEPATIC MALIGNANCIES
- CLINICAL STUDIES IN COLORECTAL CANCER
- COLORECTAL CANCER CARE AMONG VULNERABLE PATIENT GROUPS

FUNDING:

- Department of Veterans Affairs New Clinical Initiatives Program
- Pharmacia Upjohn
- National Institutes of Health
 - National Cancer Institute

Surgical Oncology at VAPSHCS: Overview

The surgical oncology program at the VA Puget Sound Health Care System is focused on the delivery of optimal multidisciplinary cancer care to veterans residing throughout the Pacific Northwest. The primary research goal of the program is to develop the use of telemedicine and other emerging technologies to facilitate patient access to multidisciplinary cancer care. Additional research projects are designed to study outcomes and quality measures in the multimodality treatment of gastrointestinal cancer, as well as racial and ethnic disparities in cancer care.

Northern Alliance Cancer Center at VA Puget Sound Health Care System

The Northern Alliance Cancer Center at VA Puget Sound Health Care System is funded by the VA New Clinical Initiatives Program. This funding has allowed us to develop a regional telemedical cancer center that provides clinical oncology services to the veterans of the Pacific Northwest using telemedical technology. The center is located at VA Puget Sound Health Care System, Seattle Division. Contact is maintained with all satellite facilities via a telemedicine network (Figure 1).

The telemedical outreach service is designed to allow patients with cancer to undergo initial evaluation at their closest VA facility. Local providers will present the relevant clinical information to a multidisciplinary tumor board at the Seattle VA. A comprehensive evaluation and treatment plan is then developed, and patients either come to Seattle for further treatment, or they are treated at their home facility if feasible. We have

designed this system to optimize patient care by providing consistent multidisciplinary consultation, and eliminate unnecessary appointment scheduling and patient travel.

The telemedicine network also serves to link the facilities for educational purposes. A monthly multidisciplinary oncology conference is presented to all participating facilities using the telemedicine system. Participating providers currently receive CME credit for their participation.

VISN 20 TELEMEDICINE NETWORK



figure 1: VISN Telemedicine Network

Since the inception of the cancer center, 85 patients have received telemedical oncology consultation. Sixty-two percent of the patients were treated at their closest facility. Thirty-eight percent were referred to the cancer center at the Seattle VA. Patients presented to the telemedicine tumor board had diagnoses that included the entire clinical spectrum of malignant

table 1: Disease sites treated at the cancer center

DISEASE SITE	PATIENTS	PERCENTAGE
Leukemia/Lymphoma/Myeloma	21	25%
Lung	17	20%
Head and Neck	7	8%
Colon	6	7%
Skin	5	6%
Rectum	4	5%
Breast	3	4%
Benign Disease	2	2%
Bile Duct	2	2%
Bladder	2	2%
Brain	2	2%
Esophagus	2	2%
Soft tissue sarcoma	2	2%
Stomach	2	2%
Testicle	2	2%
Anemia	1	1%
Liver	1	1%
Liver and Kidney	1	1%
Pancreas	1	1%
Thyroid	1	1%
Unknown Primary	1	1%
Total	85	100%

disease. (Table 1) Our preliminary results indicate that a telemedical cancer center is feasible within the VA health care system, and it appears to improve patient access to multidisciplinary cancer care.

Study of Combined Modality Treatment of Esophageal Cancer in the VA System

The optimal therapy for patients with cancer of the esophagus remains controversial. Utilizing data from the VA Patient Treatment File (PTF), Outpatient Clinic File (OPC) and Beneficiary Identification Record Locator System (BIRLS), we are studying the outcome of surgical and multimodality treatment of esophageal cancer.

We have previously reported our study of trimodality therapy in esophageal cancer (Billingsley et al.) We have now shifted our focus to include study of the spectrum of therapy of esophageal cancer within the VA system. We are currently studying the role of definitive chemoradiation in these patients. Our results demonstrate that definitive chemoradiation is the most common non surgical treatment for esophageal cancer in the VA system (Table 2).

Although our data is retrospective, logistic regression analysis suggests that the addition of surgery may provide a survival advantage over treatment with chemo-

therapy and radiation alone. We hope that these findings will motivate additional clinical trials for this difficult disease.

Hepatic Malignancies

The Department of Surgery at the University of Washington has been selected to be the coordinating center for a five site clinical trial entitled, *A Phase II study of Adjuvant Irinotecan following resection with or without radiofrequency ablation of hepatic metastases from colorectal carcinoma*. This trial is sponsored by Pharmacia Upjohn. Currently, there is no proven role for chemotherapy after resection of colorectal liver metastases.

This trial aims to define the disease-free and overall survival in this group of patients when they receive postoperative chemotherapy with the drug irinotecan. Molecular markers, including p53, topoI, p27, BAX, and thymidylate synthetase will be correlated with clinical outcome. Additional participating institutions include the University of Toronto, Case Western Reserve University and the University of Texas at San Antonio.

table 2: Esophageal Carcinoma Treatment for Veterans Affairs patients (1992-1997)

TREATMENT	NUMBER	PERCENTAGE
No treatment	1459	34.6%
Concurrent chemo + radiation	689	16.4%
Surgery alone	534	12.7%
Radiation alone	519	12.3%
Chemo alone	329	7.8%
Chemo + radiation (sequential)	242	5.8%
Trimodality therapy*	161	3.8%
Surgery + chemo	123	2.9%
Surgery + radiation	80	1.9%
Surgery + radiation +chemo†	75	1.8%
Total	4211	100%

* Concurrent chemotherapy and radiation followed by surgery

† Radiation and chemotherapy delivered in sequential fashion following surgery

Clinical Studies in Colorectal Cancer

We have established a prospective colorectal database in the department of surgery at the VA Puget Sound Health Care System. This database is designed to serve as a resource to facilitate clinical as well as translational studies. Early studies using this database have demonstrated that up to 30% of veterans treated within the system do not receive stage appropriate adjuvant chemotherapy (Cummins et. al.) The reasons underlying this observation are complex. They include medical

The primary research question will focus on the documentation of usage of stage appropriate adjuvant therapy for colorectal cancer among ethnically and geographically vulnerable patient groups.

comorbidities and patient misperceptions regarding the toxicity of chemotherapy.

A study of the utility of preoperative CT scanning in patients undergoing surgery for colorectal cancer demonstrates that the clinical management of this group of patients was affected in 38% of the cases by the preoperative scan. Management was altered to include management of metastatic disease, treatment of coincident pathology, and, in some cases, *en bloc* resection of adjacent organs.

Colorectal Cancer Care Among Vulnerable Patient Groups

Our research group, which includes members from surgery, family medicine, medicine, radiation oncology, and health services research, has received funding to study disparities in colorectal cancer treatment among vulnerable patient populations. The analytic database for this study will be constructed by merging data from the SEER (Surveillance, Epidemiology, End Results)

registry with Medicare claims data. The primary research question will focus on the documentation of usage of stage appropriate adjuvant therapy for colorectal cancer among ethnically and geographically vulnerable patient groups. If we document differential usage of adjuvant therapy among these groups of patients, the study will examine processes of care which may contribute to this disparity.

A supplemental study has also been funded which will focus specifically on the quality of surgical care for colorectal cancer among different patient groups. This study aims to use the SEER/Medicare database to identify differences in perioperative morbidity and mortality following colon and rectal resection. The goal of this investigation is to identify either provider or system features which may compromise the quality of surgical care that is delivered to rural or minority patient groups.

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Matthias Stelzner, M.D.



• STEM CELL TRANSPLANTATION AS TREATMENT OF BILE ACID MALABSORPTION

FUNDING:

American College of Surgeons

• G.H.A. Clowes Research Career Development Award VA Merit Review Grant

Bile acid malabsorption is a major complication in patients undergoing ileal resection for Crohn's disease and in patients with ileal bladder reconstructions following bladder resections for cancer. Bile acid malabsorption leads to severe diarrhea and – in the longterm – to the formation of gallstones and kidney stones.

Our laboratory has developed a novel method to transplant ileal stem cells, which could be used to prevent bile acid malabsorption after ileal resection. In our experiments, ileal stem cells are harvested from donor intestines and then transplanted in the proximal gut, i.e. the jejunum. Here they give rise to a „neo-ileal“ mucosa. This neo-mucosa expresses ileal bile acid transport protein and is capable of active bile acid transport, a feature unique to ileal mucosal cells. Our current work focusses on the optimization of engraftment rates and on tests of the neo-ileal mucosa in clinical settings.

and secreted into the duodenum with the bile stream to aid fat absorption in the lumen of the small intestine. Biosynthesis of these detergents in the liver requires considerable energy and most animals have developed a recycling mechanism for bile acids in the terminal small intestine.

After bile acids have fulfilled their duties as emulsifiers in the gut lumen and after fat absorption is complete, over 90% of the bile acid molecules are reabsorbed in the terminal ileum by an active membrane transport process. They are then shuttled back to the liver via the portal vein and again excreted in the bile. This is called enterohepatic circulation of bile acids. The transport protein responsible for the ileal uptake process is called IBAT (i.e., ileal bile acid transporter). IBAT is expressed solely in the mucosa cells of the terminal ileum but not in the more proximal sections of the intestine. This has great clinical importance.

Bile acids are the most important detergent substances in the body.

This scientific work has importance beyond its clinical application for bile acid absorption. Successful stem cell transplantation provides a potential methodology to apply gene therapy using stem cells in the future. For example, an intact cystic fibrosis gene could be introduced into stem cells to prevent the intestinal symptoms of cystic fibrosis. In addition, intestinal stem cell transplantation will enable us to study interactions between the epithelial cells and the connective tissue cells of the mucosa. While there is increasing evidence that such interactions exist, this field remains to be explored.

Bile acids are the most important detergent substances in the body. They are synthesized in the liver

Patients who have undergone resections of the terminal ileum or who have suffered disease-related changes of the ileal mucosa, develop severe bile acid malabsorption (BAM). The bile acid malabsorption syndrome is characterized by diarrhea with voluminous bowel movements. In addition, it leads to the development of gall stones and kidney stones in the long term.

The most common cause for bile acid malabsorption is ileal resection. Operative removal of the ileum from the alimentary tract may be required for severe inflammation of the ileum, e.g., with Crohn's disease, or when the ileum is used for bladder reconstructions after the bladder was removed for bladder cancer. Other diseases may also cause bile acid malabsorption. These

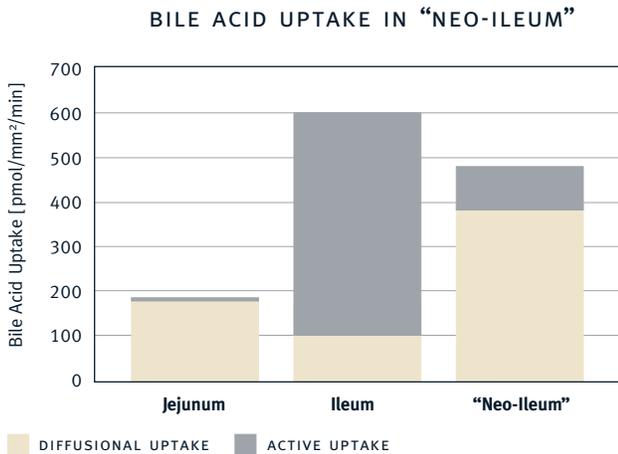


figure 1: **Neo-ileal segments are able to absorb bile acids actively, unlike native jejunum. The neo-ileum thus has features characteristic of native ileum. This suggests successful transplantation of ileal cells into the jejunum.**

include AIDS-related enteropathy, cystic fibrosis, post-cholecystectomy diarrhea, radiation enteritis and hyperthyroidism.

Our laboratory has studied intestinal bile acid transport for many years. Our research group has mapped the distribution of bile acid absorption in detail in different animal species as well as in humans for the first time, and studied the regulation of intestinal bile acid uptake. During the course of this work, we developed novel methods for bile acid uptake measurements in small mucosal specimens and generated specific antibodies to detect IBAT protein from different species. This expertise put us in a unique position to develop and evaluate techniques for ileal stem cell transplantation.

In recent years, it has become possible to harvest stem cells from donor intestines, maintain these cells in culture, and successfully transplant them into the subcutaneous tissue or onto the colon musculature of suitable donors. Based on this work, we developed techniques to transplant ileal mucosa cells into the proximal jejunum. We tested different methods to strip

the resident mucosa out of the recipient jejunal segment. Mechanical stripping did not yield uniform, reproducible results. Chemical stripping by perfusing with divalent ion chelators, however, was much more successful and resulted in good engraftment rates. We prepared ileal stem cell clusters from neonatal donor intestines using gentle enzymatic digestions and gravity sedimentations.

Four weeks after engraftment, a neo-ileal mucosa developed that resembled native ileal mucosa morphologically. Using reverse-transcription polymerase chain reactions and semiquantitative immunohistochemistry with our anti-IBAT antibodies, we were able to demonstrate transcription of IBAT mRNA and the expression on IBAT protein in this new epithelium. We were also able to detect active bile acid transport in this neo-mucosa, a feature that is unique to the terminal ileum (Figure 1.)

At present, we are concentrating on a further improvement of engraftment rates by optimizing the debridement of the recipient sites and by testing the effects of different epithelial growth factors.

Our work has been made possible only through the tremendous expertise, generosity and scientific support of our friends and collaborators in the fields of gastroenterology and pediatrics listed at the margin of these pages. Our laboratory and our research efforts are connected to them not only in our work on stem cells but also through our collaborations on the topics such as insulin gene therapy, effects of erythropoietin on intestinal mucosa cells, long-term culture of non-transformed epithelial cells and transdifferentiation of biliary epithelial cells. Thus on numerous occasions we have obtained invaluable practical and logistical help, in the form of helpful comments, critical discussions and generous words of encouragement. Our gratitude is greater than we could possibly convey.

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VASCULAR SURGERY

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- TISSUE PULSATILITY IMAGING FOR THE DETECTION OF SOLID TUMORS
- ULTRASOUND REFRACTION IN BREAST IMAGING
- ULTRASOUND TRANSDUCER FILTER FOR HARMONIC IMAGING IN HIGH INTENSITY FOCUSED ULTRASOUND
- ULTRASOUND ATTENUATION IMAGING
- ULTRASOUND LATERAL COHERENCE IMAGING
- THE EFFECT OF DOPPLER ANGLE ON DOPPLER VELOCITY WAVEFORMS AT THE FEMORAL BIFURCATION
- ARTERIAL VELOCITY AND BRUIT FREQUENCY

FUNDING:

DARPA/Office of Naval Research
National Cancer Institute

Investigators at the University of Washington have developed noninvasive ultrasound examination technologies and protocols which have been accepted throughout the world. One result is that two major medical systems companies have selected the Seattle area for their international headquarters.

Under the leadership of D. Eugene Strandness, Jr, M.D., the noninvasive vascular laboratory in the Department of Surgery has developed the primary ultrasonic Doppler diagnostic methods used throughout the world. The Department of Surgery in collaboration with the new UW Center on Industrial and Medical Ultrasound and the Department of Bioengineering, is continuing to develop innovative ultrasound examination technologies for vascular diseases and abnormalities.

Tissue Pulsatility Imaging for the Detection of Solid Tumors

Forty years ago, Judah Folkman postulated that solid tumors could not grow larger than 1 mm in diameter without generating a new blood supply. That process is called angiogenesis. Now it is accepted that angiogenesis is a marker for growing tumors. The new blood vessels are abnormal: large, poorly innervated, and arranged in irregular patterns. We believe that they also pulsate with the cardiac cycle with a greater amplitude than surrounding normal tissues. Our preliminary data suggests that the pulse amplitude of breast tumors is three times as great as normal surrounding breast tissues. Normal pulsations in tissue are about 0.1% by volume.

Using ultrasound methods, we can measure volume pulsations of 0.005%. The National Cancer Institute Unconventional Innovations Program is supporting the construction and testing of an ultrasound instrument to form 3-dimensional pulsation images which are expected to display breast tumors as regions of increased pulse amplitude. Patients having breast biopsy are asked to volunteer to have 3-dimensional ultrasound "Tissue Pulse Images" prior to the biopsy under a Human Subjects approved protocol.

Figure 1 shows the tissue motion. The pulse expansion will be computed from this for each position

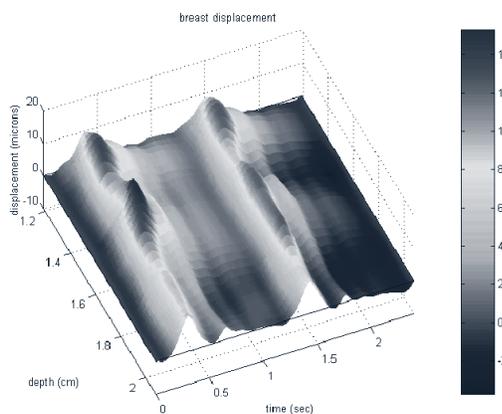


figure 1: Pulsations in Breast of a Patient with Mammographic Calcifications. Over a range of depths from 1.2 to 2 cm (left scale), two pulsations can be seen over a period of 2.5 seconds (bottom right scale). The displacement amplitude (shown by gray scale and by the vertical axis) is 16 micrometers (microns).

in the breast. Positions with abnormally large pulse amplitude will be marked in a 3-dimensional rendering of the breast.

Ultrasound Refraction in Breast Imaging

Our goal is to create a 3-dimensional ultrasound examination system which can image the natural pulsations in breast, which will allow us to identify different tissue types. Conventional ultrasound images of the breast show structures in considerable detail (Figure 2).

As a first step in the design process, images were made of a small reflector through breast to see the effect of breast tissue on the image of a small reflector (Figure 3).

The figure demonstrates lateral smearing of the reflector image (which should appear as a small spot). This amount of lateral smearing is typical of all ultrasound images of the breast, and is a limit of ultrasound imaging. No technology is currently available to prevent or compensate for this lateral smearing.

High Intensity Focused Ultrasound is being developed at the University of Washington, Center for Industrial and Medical Ultrasound for the treatment of bleeding.

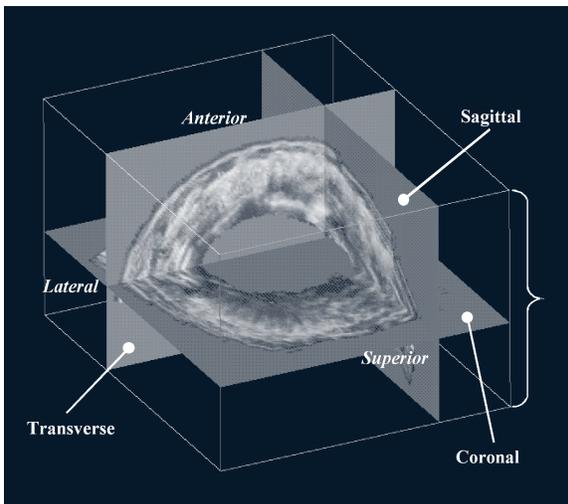


figure 2: **Three Dimensional Image of Breast.**

Such images appear to have good resolution. However, ultrasound methods usually have much better resolution in the depth direction (depth resolution = 0.1 mm) than in the directions parallel to the skin (lateral resolution = 2.0 mm).

Conventional "real-time" 2-dimensional ultrasound instruments cannot easily be converted to image pulsations in three dimensions because pulse imaging requires that the ultrasound transducers remain stationary for several seconds at a time; conventional "real-time" ultrasound scanheads are constantly in motion. It is therefore necessary to construct an ultrasound system designed for breast pulsatility imaging. The most difficult design consideration is making the best (smallest) possible lateral resolution.

Measurement of breast pulsation is still possible even though this lateral smearing (due to the refraction of ultrasound in the adipose tissues under the skin) of the image is present. Thus the poor lateral resolution will not detract from the diagnostic ability of the instrument.

Ultrasound Transducer Filter for Harmonic Imaging in High Intensity Focussed Ultrasound

High Intensity Focused Ultrasound (HIFU) is being developed at the University of Washington, Center for Industrial and Medical Ultrasound (CIMU) for the treatment of bleeding. HIFU uses ultrasound intensities of 1000 Watts/cm². For comparison, sunlight has an

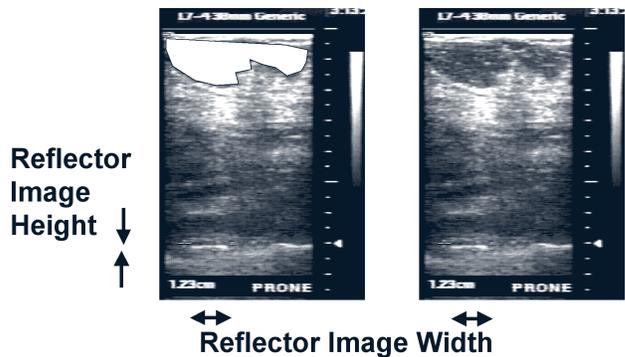


figure 3: **Image of a 1 mm Reflector Through Breast.**

The same image of breast is duplicated here with the skin on the top. On the left, a white area was drawn to show the area and shape of fat under the skin which is seen as a dark region on the right. The bright reflector, indicated by the height and width markers on the image margins, should be 1 mm high and 1 mm wide. The image measures 1 mm high and 12.3 mm wide. The lateral spread (lateral smearing) of the image is due to refraction of the ultrasound by the undulating fat layer under the skin.

intensity of 0.5 Watts/cm² and diagnostic ultrasound has an intensity of 0.1 Watts/cm². HIFU can cauterize tissue at depths of 5 cm in a treatment volume 10 mm long and 1 mm in diameter. This treatment has been tested successfully in liver, pre-cauterizing a plane of resection. In those tests, only vessels larger than 3 mm continue to bleed as the resection is performed.

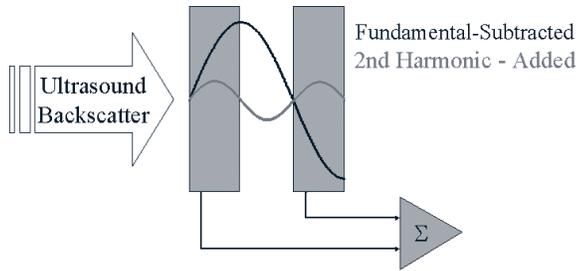


figure 4: **Transducer Designed to Reject the HIFU Frequency and Accept the Second Harmonic of the HIFU Frequency.** Piezoelectric ultrasound transducers are shown as gray rectangles. The amplifier that adds the voltages from the transducers is shown as a triangle. The backscattered ultrasound echo pressure waves are shown as sine waves passing through the transducers.

The HIFU treatment of an organ like the liver can be done through the skin. If properly aimed, the cautery of the treatment volume raises the treated tissue temperature by 50°C and is complete in two seconds. Aiming of the treatment volume is critical and monitoring the treatment volume for motion during treatment is also essential. Unfortunately, during the HIFU application, conventional ultrasound imaging cannot be done due to interference from the HIFU.

To monitor the treatment volume position during HIFU, we have developed a transducer that detects harmonic echoes generated in the treatment volume that are at twice the HIFU ultrasound frequency. To assure that the weak harmonic echoes from the treatment volume can be detected when the strong HIFU ultrasound frequency is present, we have designed an ultrasound transducer that rejects the HIFU frequency, accepts the harmonic at double the HIFU frequency and rejects other frequencies up to the 6th harmonic. The design is a two layer transducer (Figure 4). By adding the signal from the first layer to the signal from the second layer, the HIFU “fundamental” frequency is subtracted away and the second harmonic from the treatment volume is added to enhance the desired signal.

A graphical representation of the conditions of sensitivity of the transducer (Figure 5) shows the frequencies that the transducer can detect and those that it will not detect. The lower portion of the graph shows the

effect of changing the transducer thickness while keeping the spacing interval equal to half of the fundamental HIFU ultrasound wavelength. Figure 5 shows that a conventional single layer transducer that is sensitive to the desired second harmonic is also sensitive to the fundamental. However, the layered transducer can completely reject the fundamental (HIFU) frequency allowing the electronics connected to the transducer to process the harmonic echoes only.

Ultrasound Attenuation Imaging

Conventional ultrasound imaging shows the strength of the ultrasound echo as brightness on the ultrasound image. The rate of ultrasound attenuation in tissue is another property that can help identify tissue type. For instance, both fat and cystic fluid provide weak ultrasound echoes and therefore appear dark on ultrasound images, but fat attenuates ultrasound more effectively than cystic fluid. High ultrasound frequencies are attenuated by fat more than low frequencies. Therefore measuring the frequencies of the ultrasound echoes

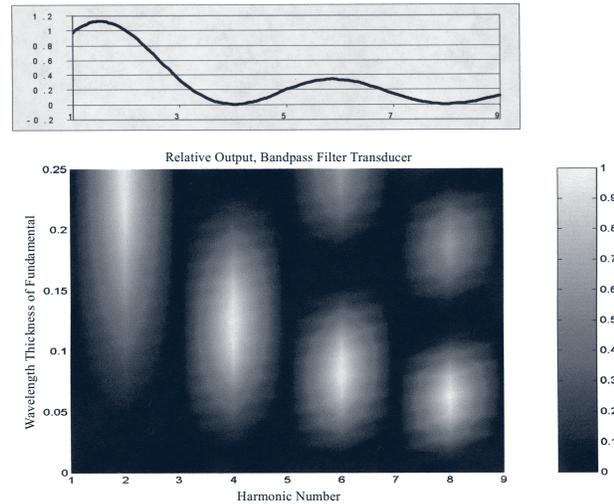


figure 5: **Sensitivity of the Ultrasound Transducer to Different Frequencies.** The height of the UPPER TRACE shows the sensitivity of a conventional single layer transducer to different frequencies of ultrasound if the transducer thickness is 0.25 times the wavelength of the HIFU ultrasound. The sensitivity to the HIFU frequency (1) and the second harmonic (2) are both near 1.0, and the sensitivity to the third harmonic (3), the fifth harmonic (5) and the seventh harmonic (7) is near 0.2. The transducer is not sensitive to the fourth harmonic (4) and eighth harmonic (8). The brightness of the LOWER PANEL shows the sensitivity of the layered transducer to different frequencies of ultrasound depending on element thickness. No matter what the element thickness, the transducer is not sensitive to the HIFU frequency because of the spacing between the elements. The transducer is most sensitive to the desired second harmonic (2) when the element thickness is 0.25 times the HIFU wavelength. That element thickness is represented by a horizontal line along the upper edge of the lower panel.

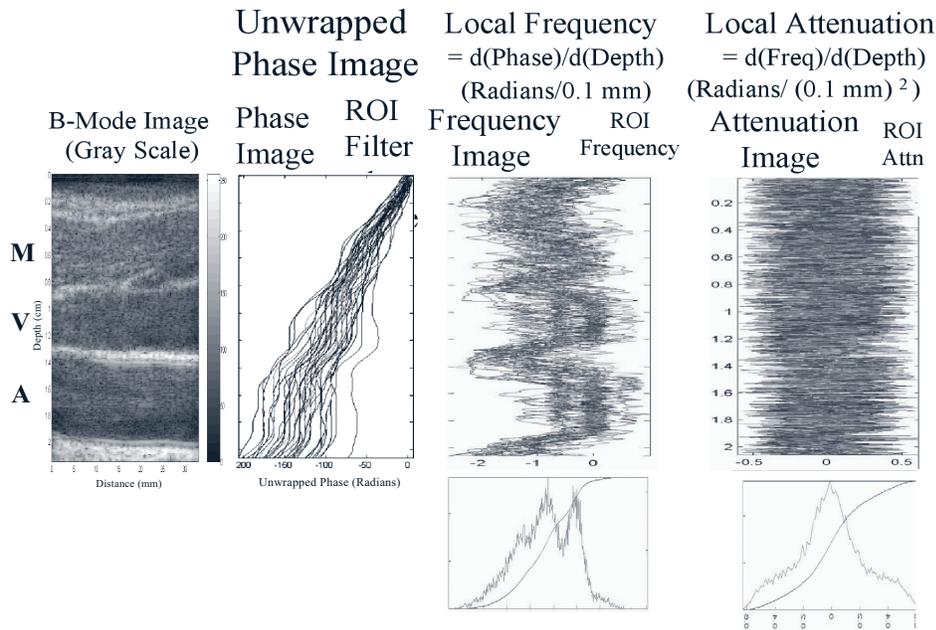


figure 6: **Ultrasound Echo Frequency Compared to Tissue Type**

FAR LEFT: Conventional 2-dimensional B-mode ultrasound showing muscle (M), vein (V) and artery (A).

CENTER LEFT: Cumulative phase of the ultrasound echo for the 30 ultrasound beams near the center of the image. Note that although the total phase change for each line is very different, in the muscle and in the wall between the vein and artery lumens, the negative shift in phase is very steep but there is little phase change in either the venous or the arterial blood. Because of the small size of isolated blood cells compared to the wavelength of ultrasound, blood reflects high ultrasound frequencies much more strongly than low frequencies.

CENTER RIGHT: Estimated frequencies along the 30 ultrasound beams showing high (toward the right) echo frequencies from the depths of the arterial and venous blood and the lowest (toward the left) from the depth of the wall between the artery and vein.

FAR RIGHT: Derived attenuation plot from the 30 lines. No pattern of “calculated attenuation” is obvious in this plot. A colored image (not shown here) showing “calculated attenuation” does show some of the anatomy corresponding to the image at the left.

that return from deep tissues may provide information to differentiate fat from cystic fluid. Echoes that come from deep in fat are expected to have lower frequencies than those that come from deep in cystic fluid.

To measure the frequency, the phase, (timing of the peaks and valleys of the wave) is measured at each depth. If that phase decreases with depth, the echo frequency is low, if it increases with depth, the echo frequency is high. The rate of change of frequency equals, in theory, attenuation. Figure 6 is a test of that theory in tissue with an artery and vein.

Many other investigators have attempted similar analyses without success. Using methods that are more sensitive to small frequency changes, we believe that we have a chance of some success with this approach.

Ultrasound Lateral Coherence Imaging

While exploring the Radio Frequency (RF) echoes from arterial walls, we found that the ultrasound echoes that return from arterial wall have aligned phase at adjacent locations in the image (Figure 7). This phase alignment is likely due to the laminar structure of the

arterial wall. The random phase of echoes from deeper muscle and superficial blood suggests that these structures do not have a laminar structure. The phase alignment of adjacent ultrasound echo lines is called lateral coherence.

By measuring the lateral coherence of tissues in ultrasound images, we expect to be able to detect and measure total arterial wall thickness and to differentiate “normal” (laminar structure) arterial wall from atherosclerotic arterial wall. The coherence can be detected by using the “phase angle” of the echo which shows the alignment of the peaks and valleys in the RF. The phase is measured in degrees from -180 to +180 or in radians from -p to +p. The phase of echoes from adjacent lines in muscle will each show an angle between -p and +p as a random distribution. Echoes from the arterial wall at a depth of 12 millimeters (Figure 8) have a nearly constant phase near 0.2 radians for echoes from most of the width of the image demonstrating coherence.

It is surprising that coherent ultrasound echo phase can remain nearly constant over such great widths of the ultrasound image.

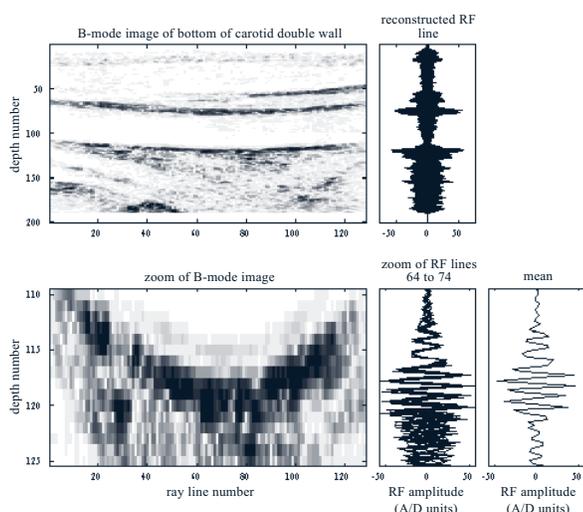


figure 7: **Coherence of Ultrasound Echoes from the Deep Arterial Wall**
UPPER LEFT: 2 dimensional B-mode ultrasound image of an artery. The artery is the light colored region running horizontally across the center of the image. The dark streaks at the upper and lower boundaries of the artery are the artery walls. Muscle is superficial and deep to the artery.
UPPER RIGHT: “Radio Frequency (RF) echo from one line in the center of the 2 dimensional (2D) B-mode image. Strong signals (wide parts of the pattern) are shown as dark on the 2D ultrasound image, weak signals (narrow parts) are shown as light on the 2D image.
LOWER LEFT: Magnification in the vertical direction of the deep wall of the artery from the 2D image.
LOWER CENTER: Details of the 11 RF lines (lines 64 to 74) around the deep wall of the artery. Notice that echoes from the wall are all aligned, echoes from the blood (portion above) and muscle (portion below) are not aligned.
LOWER RIGHT: Horizontal average of the 11 RF lines.

The Effect of Doppler Angle on Doppler Velocity Waveforms at the Femoral Bifurcation

Ultrasonic Doppler blood velocity waveforms can be obtained noninvasively from almost all arteries in the body. Many of these arteries are parallel to the skin so the Doppler ultrasound beam intersects the artery at an acute angle. For uniformity, we usually select to use an angle of 60° between the ultrasound beam pattern and the vessel axis for consistency.

Bifurcations are interesting locations in the arterial system because these are preferred locations for the formation of atherosclerotic plaque. Hemodynamics at bifurcations is often complex. Using our multiview “Vector Doppler” system, we explored the femoral bifurcation to see some details of the bifurcation hemodynamics (Figures 9 and 10).

If the blood velocity vectors are directed down the artery parallel to the artery axis, then the “Doppler equation” which computes the velocity from one angle on the basis of the velocity at another angle provides a

correct result as in the superficial femoral artery (Figure 9). In contrast, at the origin of the profunda femoris artery, the blood velocity vector is angled differently at different times during the cardiac cycle. This leads to focal regions of high and low shear on the artery walls. Such regions of shear anomaly may contribute to focal atherosclerosis.

The hemodynamics at the profunda femoris origin are, in part, due to the early reversal of flow in the profunda resulting from the short distance to the capillary bed which causes reflection of the blood. The reversal in the superficial femoral artery is expected to be 100 milliseconds later because of the 50 cm greater length of the superficial femoral artery and its branches.

Arterial Velocity and Bruit Frequency

Peripheral artery bruits sometimes have a “seagull” sound, a “pure” changing frequency during systole. These bruits are associated with a significant arterial stenosis. The bruit originates from the distal (post-stenotic) side of the stenosis. The cross-stream velocity waveform in the post-stenotic region in these cases oscillates from positive to negative at the frequency of the bruit in the region near the arterial wall (Figure 11, left). This bruit frequency is highest at peak systole. The blood velocity varies during systole.

By dividing the bruit frequency by the center stream arterial velocity at each time in the cardiac cycle, a rescaled value is obtained. That rescaled value, frequency/velocity, is constant throughout the cardiac cycle (area inside circle in right panel). This result suggests that as the systolic arterial velocities increase and decrease during systole, the size of the eddies in the flow remains constant, and the change in bruit frequency is due simply to the velocity at which the eddies pass along the artery. This generalization will help to compute the relationship between bruit frequency and the severity of arterial stenosis in the lower extremity.

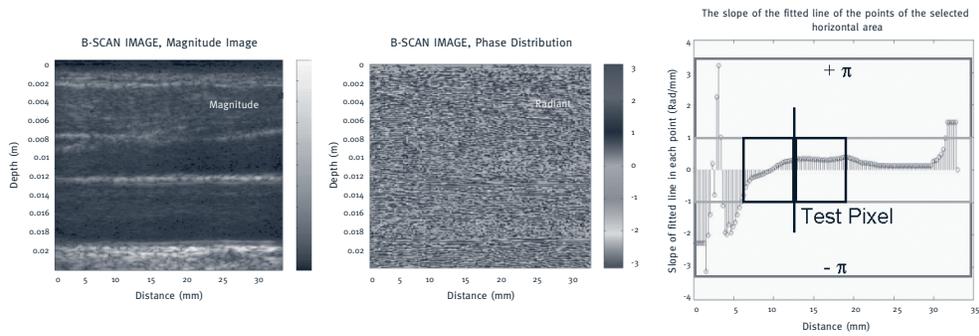


figure 8: 2-Dimensional B-Mode Image (left), 2-D Phase Image (middle) and Phase Value (right).

LEFT: Echo strength is displayed for all depths (vertical direction) and lateral positions (width direction) as varying shades of brightness with white showing the strongest echoes.

MIDDLE: Echo phase is displayed for all depths (vertical direction) and lateral positions (width direction) as varying shades of brightness. The scale relating angle to brightness is not monotonic (varying continuously from one value to another) because this image was converted from color to monochrome.

RIGHT: The plot shows the phase of the echo from each of 128 ultrasound beams at a depth of 12 mm in each. On the left edge of the image, the phase begins at -2.2 radians and is constant for the leftmost 2 mm, then the phase takes on a range of values between $-\pi$ and $+\pi$ between 2 mm and 4 mm. From 4 mm to 9 mm, the phase makes a continuous change to near ZERO radians. Between 30 and 35 mm, the phase angle increases to 1.3 radians. The box with a vertical line in the right panel shows a region to test whether the phase is coherent (stays within the box) or is incoherent (wanders outside the box).

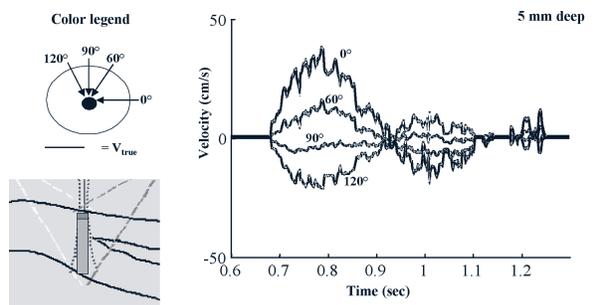


figure 9: Superficial Femoral Artery Doppler Velocity Waveforms Viewed from Different Angles

As expected, the Doppler waveforms taken from different angles have different amplitudes but similar shapes. The waveform taken at a Doppler angle of 90° (perpendicular to flow) is flat as expected, showing that the blood is neither coming toward nor away from that transducer.

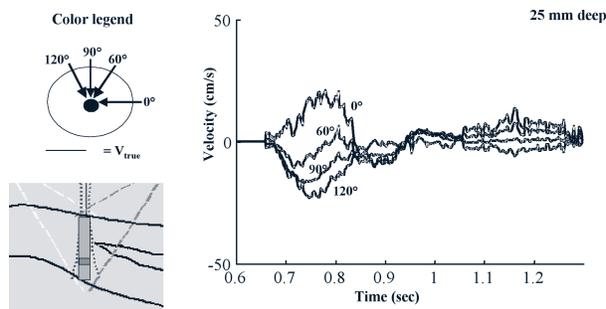


figure 10: Profunda femoris Artery Doppler Velocity Waveforms Viewed from Different Angles

Doppler waveforms taken at this depth from different angles have different wave shapes. The time of peak systole varies with the Doppler examination angle between the ultrasound beam and the vessel lumen.

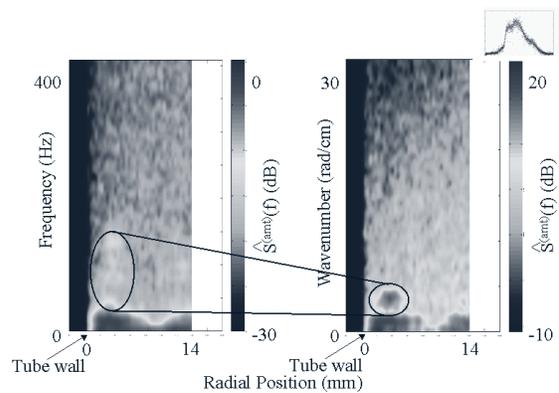


figure 11: Intensity of Post Stenotic Frequencies with Lumen Radius in a Phantom of an Arterial Stenosis in Pulsatile Flow.

LEFT: Blood velocity oscillation frequencies during the cardiac cycle: frequency along vertical axis, artery radius along horizontal axis, oscillation amplitude shown as gray scale.

RIGHT: Blood velocity oscillation frequencies divided by central blood velocity to yield wave number shown along the vertical axis, artery radius along the horizontal axis.

Note the concentration of acoustic power between 2 and 6 mm radius from the artery wall centered around a wave number of 3 (inside circle).

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Alexander W. Clowes, M.D.



• REGULATION OF VASCULAR SMOOTH MUSCLE CELL GROWTH

AWARDS:

National Heart, Blood and Lung Institute MERIT Award

National Institutes of Health

- Vascular Surgery/Cardiology Training Grant

FUNDING:

National Institutes of Health

Vascular surgical procedures are designed to rebuild diseased blood vessels and improve blood flow. While these procedures restore the circulation, they also cause injury. This injury induces a wound healing response that in some instances is associated with accumulation of scar tissue (intimal hyperplasia) and significant luminal narrowing (e.g. 20-40% of coronary arteries treated by angioplasty). Smooth muscle cells living in the arterial wall proliferate in response to injury and are largely responsible for the intimal hyperplasia (see Figure 1). Further surgery is required since no pharmacology is available to inhibit this process. 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.

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 receptor and the expression and the activity of proteases needed for the degradation of extracellular matrix.

Recent studies in the laboratory have defined a novel pathway of smooth muscle cell activation which depends on the EGF receptor. Thrombin can induce cell growth by interacting with its G-protein coupled

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.

receptor. 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.

Smooth Muscle Cells and Gene Therapy: As an approach to gene therapy, we are seeding modified smooth muscle cells into injured arteries and vascular grafts in several different species (rat, dog, baboon).

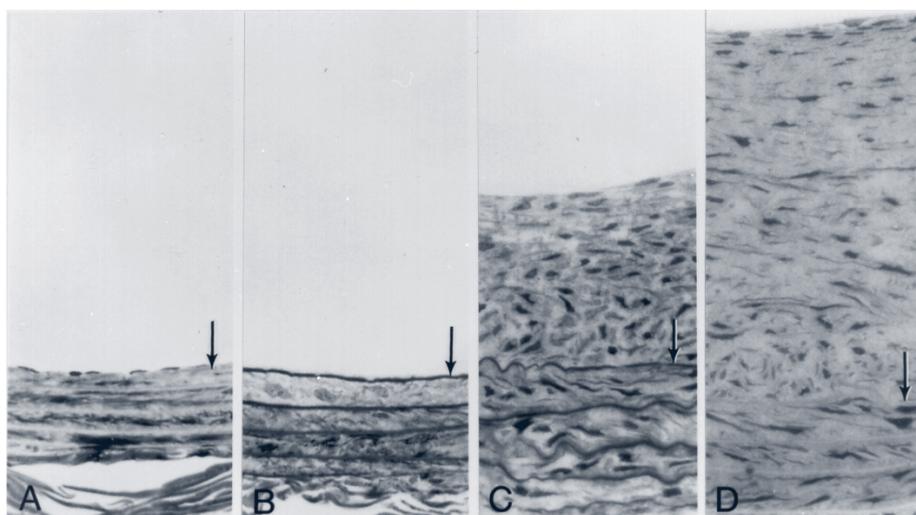


figure 1: This series of photographs shows how a normal rat carotid artery (panel A-histologic cross-section) responds to injury. Angioplasty of the artery removes the surface endothelium (panel B). By two weeks (panel C), smooth muscle cells have migrated from the media into the intima (region above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

These cells have been modified to express proteins of therapeutic or pathological importance by prior infection with replication-defective retroviruses encoding the corresponding genes. These experiments will allow us to evaluate the utility of seeded smooth muscle cells as a means to correct defined metabolic deficiencies.

We are using the seeding technique to introduce pharmacological growth inhibitors into the wall (e.g., over-expression of endothelial nitric oxide synthase) and to model certain pathological states (e.g., over-expression of tissue factor to mimic atherosclerotic plaque thrombosis).

Regulation of smooth muscle growth in grafts by blood flow: We have found that smooth muscle cell proliferation and neointimal hyperplasia in primate PTFE grafts are exquisitely regulated by changes in blood flow. In the grafts, smooth muscle cells proliferate when

endothelial cells are present, whereas in injured arteries they proliferate only when 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 (e.g., PDGF, nitric oxide). Recent experiments using a mouse monoclonal antibody that recognizes and blocks the beta form of the PDGF receptor (PDGFR- β) have demonstrated conclusively that intimal hyperplasia in grafts as well as in injured arteries depends on PDGF. In collaboration with Celltech, Ltd., and ZymoGenetics, Inc., this antibody has been genetically engineered to resemble a human immunoglobulin; this "humanized" antibody is currently being tested in human trials for the prevention of restenosis after coronary stent angioplasty.

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• MODULATION OF GROWTH FACTOR SIGNALING IN VASCULAR SMCs BY OXIDATIVE STRESS

FUNDING:
National Institutes of Health

In the normal vessel wall, arterial smooth muscle cells (SMCs) are quiescent because of the lack of activating factors and the presence of growth inhibitory stimuli produced by the endothelium (e.g. nitric oxide). In atherosclerosis, SMCs are exposed to a different environment that activates cells and induces migration, proliferation, or programmed cell death (apoptosis). All of those contribute to pathological aspects of the disease.

In the early stages of atherogenesis, for example, SMC proliferation contributes to plaque formation, whereas at later stages, SMC death may increase the risk of plaque rupture and the formation of occlusive thrombi. A major determinant of SMC function in atherogenesis appears to be oxidative stress. Low levels of oxidative stress are pro-proliferative and may also be part of growth factor signaling. In contrast, oxidative stress over time results in lipid peroxidation products which kill SMCs. Our laboratory is interested in identifying molecular mechanisms induced by oxidative stress that regulate SMC function.

The Small G-protein Ras is a Key Element in Regulating SMC Proliferation and Survival

The membrane associated small G-protein Ras is an essential component of all growth factor signaling pathways. Ras exists in an inactive, GDP-bound, and an active, GTP-bound state. Most growth factors that signal through receptor tyrosine kinases (RTKs) or heterotrimeric G-protein-coupled receptors, stimulate Ras by recruiting the guanine-nucleotide exchange factor Sos to the membrane. Sos exists in a complex with the adapter protein Grb2. Upon receptor activation, the Grb2/Sos complex is translocated to the membrane by binding of Grb2 to tyrosyl-phosphorylated residues in RTKs or additional adapter proteins. GTP-loaded Ras binds to Raf thereby initiating the activation of a protein kinase cascade consisting of Raf, MEK (ERK kinase), and ERK, (extracellular signal-regulated kinase)..

The importance of the MEK/ERK signaling module for cell growth has been demonstrated by different approaches: the expression of ERK antisense RNA, or a dominant negative ERK mutant, inhibits proliferation in fibroblasts; similarly, when the activation of MEK/ERK is prevented by the specific inhibitor PD098059, cell growth is inhibited. On the other hand, the expression of a constitutively active MEK in fibroblasts is sufficient for cell transformation.

Ras dependent survival (or anti-apoptotic) signaling has been observed in many cell systems. The Ras effector involved is phosphatidylinositol-3-kinase (PI3K). Upon translocation to the membrane by binding to tyrosyl phosphorylated RTKs or to active Ras, PI3K phosphorylates the inositol moiety of phosphatidylinositol-4,5-bisphosphate (PI(4,5)P₂) on position 3 to generate PI(3,4,5)P₃. This lipid second messenger has two functions: it binds to PKB and activates the lipid dependent protein kinase PDK1. It has been suggested that PI(3,4,5)P₃ binding to PKB is required for its phosphorylation and activation by PDK1. PKB appears to mediate survival by blocking Bad, an inhibitor of the anti-apoptotic proteins Bcl-2 and Bcl-x_L.

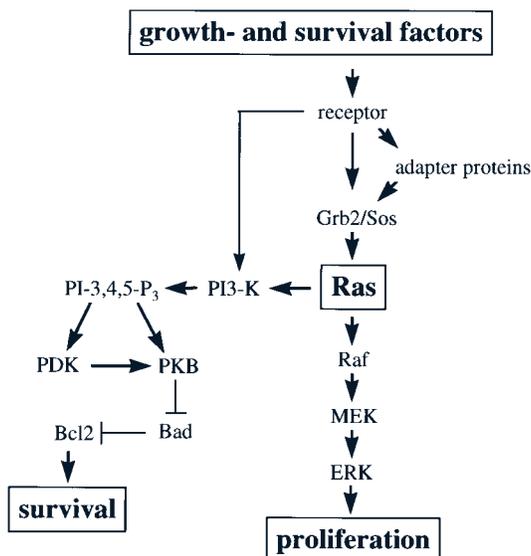


figure 1: Growth and survival factors

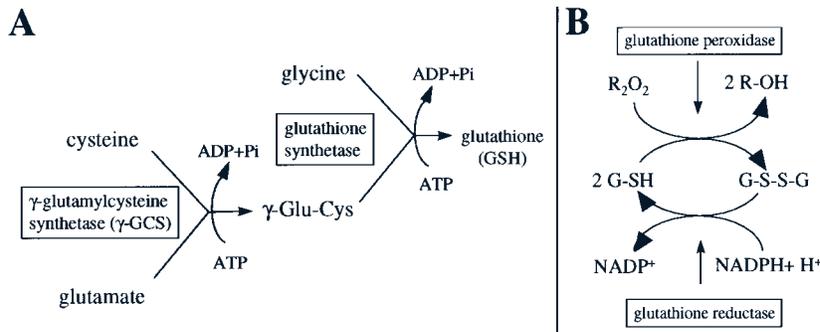


figure 2: Glutathione synthesis (A) and role in peroxide detoxification (B)

Oxidative Stress and Cellular Defense

Oxidative stress is a general term to describe reactions that lead to an increase of the oxidation potential in cells. Typically, oxidative stress is generated by free radicals, such as superoxide, that are deleterious to cells as they oxidize and damage biological molecules including DNA, proteins, and lipids. Critical for protein function is a reducing environment that prevents inter- and intramolecular formation of disulfide bonds by oxidation of cysteine residues. Compounds preferentially oxidizing free thiol groups include per(oxo)-vanadate (a mixture of vanadate and hydrogen peroxide) and arsenite.

used experimental strategy to investigate effects of oxidative stress. GSH levels increase when cells are given N-acetyl cysteine (NAC) which serves as a cysteine donor in the γ -GCS reaction. They decrease when GSH synthesis is blocked by buthionine sulphoximine (BSO), an inhibitor of γ -GCS.

Oxidative Stress Inhibits Ras Activation in SMCs

Considering the pivotal role of Ras in proliferation and survival, we are exploring the influence of oxidative stress on the Ras signaling pathway. Using pervanadate and arsenite as model systems for thiol oxidants, we found that both prevent the activation of Ras by growth

In the early stages of atherogenesis, for example, SMC proliferation contributes to plaque formation, whereas at later stages, SMC death may increase the risk of plaque rupture and the formation of occlusive thrombi.

Cellular defense mechanisms against oxidative stress include the expression of specific enzymes to neutralize free radicals (e.g. superoxide dismutase that converts superoxide to peroxide). Most importantly, cells maintain high levels (~10 mM) of the tripeptide glutathione (γ -glutamylcysteinylglycine, GSH). GSH is synthesized in two steps; first, γ -glutamylcysteinyl synthetase (γ -GCS) forms an unusual γ -amide bond between glutamate and cysteine. In the following reaction, glutathione is synthesized by glutathione synthetase that adds a glycine to the dipeptide (Figure 2A).

GSH reduces disulfides and is used by the glutathione peroxidase to detoxify peroxides. GSH is regenerated by glutathione reductase that reduces GSSG utilizing NADPH as the reducing equivalent (Figure 2B). The manipulation of GSH concentrations is a widely

factors (shown for arsenite in Figure 3). As expected, the signaling elements downstream of Ras, MEK and ERK, are also inhibited by the oxidants. Activation of PKB is partially blocked by arsenite consistent with the concept that the PKB activator, PI3K, can be activated by binding to both RTK and Ras (Figure 1).

To confirm that oxidative stress is involved in the arsenite-induced inhibition of Ras, we have treated SMCs with the anti-oxidant NAC before challenging with arsenite. With increasing concentrations of NAC, the inhibitory effect of arsenite is decreased. Notably, lowering glutathione levels by blocking glutathione synthesis with BSO kills SMCs suggesting that these cells are highly susceptible to oxidative stress.

From these observations, we conclude that oxidative stress inhibits Ras signaling which causes cell cycle

arrest and possibly promotes apoptosis by decreasing survival signaling through PKB. Current experiments are being performed to identify how arsenite prevents Ras signaling. We are investigating whether arsenite or pervanadate form an inhibitory adduct with Ras or its activator Sos. Another possibility is that these compounds initiate a signaling pathway that involves the stimulation of stress-activated protein kinases (SAPKs). SAPKs have been known in various systems to mediate apoptotic cell death and it is an intriguing hypothesis that stress signaling interferes with growth factor signaling by blocking Ras activation. Overall, we hope

to elucidate and eventually manipulate the molecular mechanisms by which oxidative stress in the vessel wall promotes SMC death.

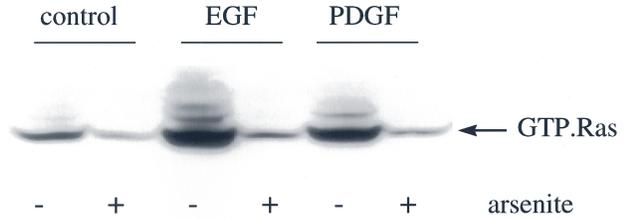


figure 3: Arsenite inhibits growth factor-induced GTP loading of Ras.

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• MAGNETIC RESONANCE IMAGING OF THE HIGH-RISK ATHEROSCLEROTIC PLAQUE

FUNDING:

Astra-Zeneca Pharmaceutical
National Institutes of Health
VA Merit Review Grant

Our research work involves a collaborative effort between members of the Departments of Medicine, Pathology, Radiology, and the Division of Vascular Surgery. Our goals are two-fold: 1) to define the role of atherosclerotic carotid plaque rupture in the pathogenesis of stroke, and 2) to develop and test non-invasive diagnostic imaging techniques that will identify structurally unstable carotid lesions.

Background: According to the National Center for Health Statistics, cardiovascular disease is the leading cause of death in the United States, and over 70% of these deaths are atherosclerosis related. Atherosclerotic cerebrovascular disease is the third leading cause of death and the leading cause of major disability amongst adults. Improved methods of diagnosis, treatment, and prevention of atherosclerotic coronary and cerebrovascular disease would result in major savings in health care costs and significant improvement in quality of life.

Traditionally, the severity of arterial stenosis has been used to identify the high-risk atherosclerotic plaque. However, in 1988, Ambrose and Little demonstrated in angiographic studies that mild-to-moderate coronary artery stenoses may lead to acute myocardial infarction, and suggested that lumen narrowing was not the sole predictor for thrombotic events. Based on histopathologic studies, Davies, Falk, Fuster, and others suggested that plaque erosion or disruption was the critical feature in these moderately stenotic, high-risk lesions.

table 1: Test performance of multispectral MRI for identifying regions of lipid-rich necrotic core and acute intraplaque hemorrhage. Accuracy (95% C.I.) = 87% (80-94%), sensitivity = 85% (78-92%), specificity = 92% (86-98%), and Kappa = 0.69 (0.53-0.85).

	histology +	histology -
MRI +	56	2
MRI -	10	22

HISTOLOGIC STATE OF THE FIBROUS CAP

mr appearance of the fibrous cap	thin or ruptured	thick	column totals
Thin or Ruptured	25	6	31
Thick	6	54	60
Row Totals	31	60	91

table 2: 2 x 2 performance table for identifying an unstable fibrous cap: comparing the MR findings with the histologic standard. Of the 91 exam locations, the MR findings suggested the presence of 33 unstable fibrous caps, twenty seven of which were confirmed histologically. The data result in a sensitivity of 0.81± 0.06, specificity of 0.90± 0.02, and a calculated Kappa statistic of 0.71 (95% CI = 0.56 to 0.86).

Falk noted that more than 75% of major coronary thrombotic events were precipitated by rupture of the fibrous cap that overlies the lipid-rich plaque core, resulting in exposure of thrombogenic subendothelial plaque constituents. In a study involving 44 carotid endarterectomy specimens (25 asymptomatic, 19 symptomatic), Carr noted fibrous cap rupture in 74% of symptomatic plaques, compared to 32% of asymptomatic lesions. Fibrous cap thinning was noted in 95% of symptomatic lesions and 48% of asymptomatic plaques.

In order to better understand the mechanisms leading to fibrous cap thinning and to establish the risk of thromboembolic complications associated with cap rupture, serial examination of the plaque is needed. To accomplish this goal *in vivo*, accurate, reproducible imaging methods for characterizing the fibrous cap are required. MRI is ideally suited for serial examination of the lesions of atherosclerosis, as it is non-invasive, has high resolution and has superior capability for distinguishing tissue types within the plaque. Using custom-made phased array coils, we have developed a high-resolution magnetic resonance imaging (MRI) technique that is capable of quantifying plaque volume (interscan error = 3.8%, able to detect a 11% change in

Atherosclerotic cerebrovascular disease is the third leading cause of death and the leading cause of major disability amongst adults.

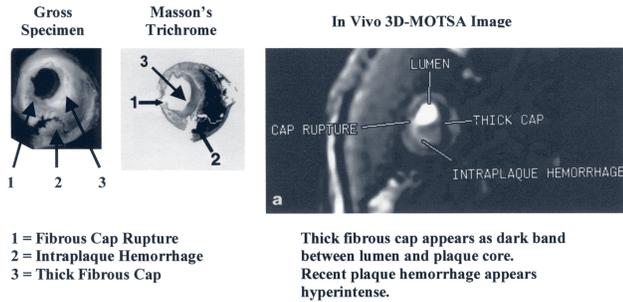


figure 1: Appearance of a thick fibrous cap and adjacent region of fibrous cap rupture (at the 8:00 position) on MRI, with corresponding gross and histological cross sections.

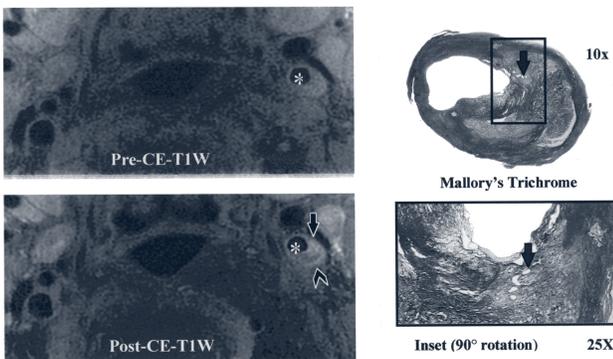


figure 2: Enhancement in the common carotid artery (asterisk in lumen) at the 2:00 position seen after administration of gadolinium contrast. The enhanced region corresponds to an area rich in neovasculture seen on the histological section.

plaque volume with 95% confidence), identifying the lipid-rich necrotic core (Table 1), distinguishing thick, thin and ruptured fibrous caps (Figure 1, Table 2), and identifying the presence of neovasculture within the plaque (Figures 2 and 3).

Conclusions: Our findings indicate that high-resolution MRI can quantify plaque volume with high precision, identify fibrous cap features and the lipid rich necrotic core with good sensitivity and high specificity, and identify regions of neovasculture within the plaque. Serial examination with MRI will further our understanding of the pathogenesis of the vulnerable plaque. Furthermore, by identifying the high-risk lesion, MRI will permit better selection of patients for intervention,

which will reduce overall health care costs. Lastly, high-resolution MRI provides a non-invasive tool to directly assess the effect of pharmacological interventions, such as aggressive lipid lowering therapy, on the morphologic and compositional features of the lesions of atherosclerosis.

We currently have two studies underway using MRI to monitor changes in carotid atherosclerosis. In the first study (PRIMARI) we are performing serial carotid artery MRI on individuals with moderate carotid stenosis with the goal of identifying changes in the plaque over time, that may predict a higher risk for stroke. In the second study, we are enrolling individuals with mild to moderate carotid stenosis and mild hypercholesterolemia to determine whether aggressive LDL-cholesterol lowering results in carotid atherosclerosis regression, as detected by MRI.

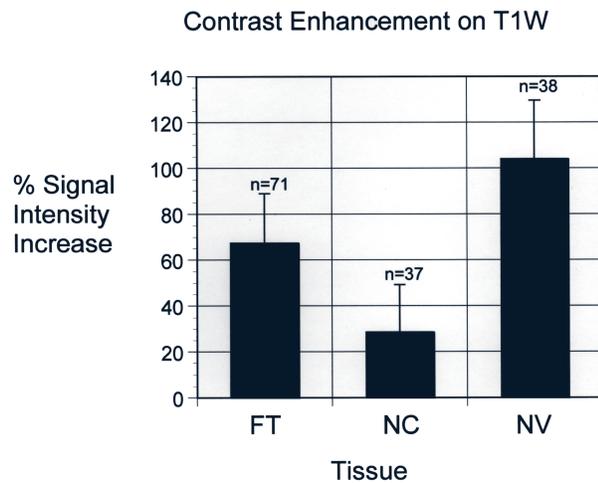


figure 3: Percent signal intensity increase in post-contrast enhanced T1W MRI compared to pre-contrast, for fibrous tissue (FT), necrotic core (NC) and regions with neovasculture (NV). The differences between tissue types was statistically significant.

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Ted R. Kohler, M.D.

- ENDOVASCULAR THERAPY
- EFFECT OF BLOOD FLOW ON INTIMAL HYPERPLASIA AND ACCESS GRAFT FAILURE
- DIALYSIS ACCESS GRAFTS

FUNDING:

National Institutes of Health
Northwest Kidney Foundation

Endovascular therapy

Endovascular therapy is an exciting new approach to aneurysm repair that uses a catheter-based delivery system rather than conventional open techniques. Patient morbidity and hospital stay are dramatically decreased. Endovascular grafts are held open and in proper position by attached metallic stents and are placed by a simple arterial cutdown or, in some cases, percutaneously. These devices have been very successful in early clinical trials and are soon to be approved for market release by the FDA. It remains to be seen, however, if these devices will perform as well over the long term (decades) as conventional grafts. The primary concern is whether or not the devices will remain well attached to the native artery at either end despite the native vessel's tendency to dilate over time.

(range 4.0-12 cm). Mean case length was 3.3 hours (range 1.5-6), and length of stay was 4.2 days (range 1-13). The mortality rate was 9.7%, and morbidity rate 29%. There were no intraoperative deaths and three deaths within 30 days (one respiratory failure in a pulmonary cripple with a ruptured iliac aneurysm, one arrhythmia, and one renal failure). There were 10 early complications (3 renal segmental infarcts, and one arrhythmia, readmission for vomiting, groin infection, biliary stasis, iliac artery rupture, and two iliac limb thromboses). There was an early endoleak rate of 19%, and a late endoleak rate of 6%. In all, there were 8 endoleaks, all Type II. Six were noted at the initial post-procedure computerized tomography scan (CT), one was noted one month after stent placement, and one was not apparent until a follow-up CT one year following stent placement. Two spontaneously closed. Seven

We have found that standard grafts fail within two to three months due to narrowing, which is much more pronounced at the venous end. Active thrombosis along the graft surface, particularly at the venous end, appears to be a major contributing factor.

Dr. Kohler and Dr. David Glickerman, from interventional radiology, began the endovascular therapy program at the University, using the new endovascular suite at the VA hospital. We are one of several centers in the country participating in an FDA-sponsored trial of the AneuRx endovascular graft. Our experience has been similar to that of other institutions. We have placed thirty-two stent grafts. All patients were male with a mean age of 74.4 years. Follow-up has ranged from 1 to 22 months (mean 8.6). Mean aneurysm size was 6.1 cm

embolizations were attempted in 4 patients, one of which was successful in treating the endoleak. One patient required open ligation of a middle sacral artery following multiple embolizations. One patient required placement of a proximal cuff at six months to treat separation of an aortic cuff from the main stent-graft body. There were no aneurysm ruptures during the study period, but 26 have been reported worldwide. In summary, our initial results in this high-risk group compare favorably with those of other centers. The

primary benefit is shortened length of stay. The high rate of endoleak and difficulty treating this complication add significantly to the cost and morbidity of this procedure. Close follow-up is essential and costly. For more information on our endovascular program, visit our web site at <http://faculty.washington.edu/kohler/>.

Effect of Blood Flow on Intimal Hyperplasia and Access Graft Failure

Vascular surgery has made tremendous advances in the last few decades. Bypass grafts, angioplasty, and stents are now standard treatment for arterial insufficiency and aneurysm disease in peripheral arteries. However, long-term success of these procedures is limited by a process of wound healing called intimal hyperplasia, in which wall thickening from smooth muscle cell proliferation narrows the lumen.

Intimal hyperplasia causes failure of almost one-third of all vascular reconstructions. Much research has been devoted to understanding the cellular pathology of this process and to developing ways to combat it with drugs, new devices, and genetic modification of the cells involved. Our laboratory is studying the effects of altered blood flow on intimal hyperplasia, and is evaluating new vascular devices to reduce restenosis.

Dialysis Access Grafts

Effective renal dialysis requires several hundred cc's per minute of blood flow. To accomplish this, a fistula is created between an artery and vein, typically in the arm. This provides a high-flow conduit just under the skin surface where it can be accessed by needle puncture. Unfortunately, these fistulae have a high failure rate, even higher than other vascular grafts. Re-operation for

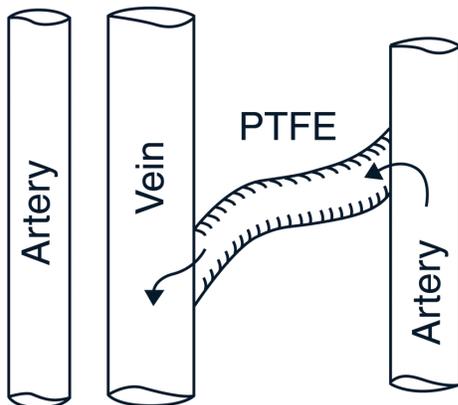
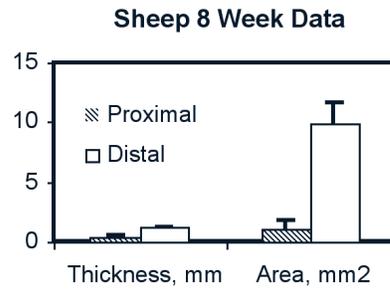


figure 1: **Fistula created between artery and vein to provide high-flow conduit.**



graph 1: **Sheep Eight Week Data**

failed access is a major cause of morbidity, prolonged hospital stay, and increased cost in the treatment of renal failure. Most access failures are caused by intimal hyperplasia at the venous end of the graft. This is very surprising since in animal models we have found that increased blood flow reduces wall thickening after placement of prosthetic arterial grafts.

We are studying this problem in an animal model. Polytetrafluoroethylene (PTFE) grafts like those used in humans are placed in the neck of sheep, and measure-

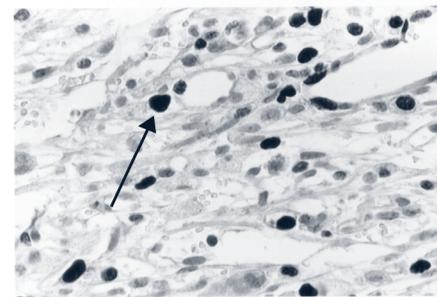


figure 2: **PCNA-positive nucleus.**

ments are made of the narrowing at the junction of the graft and native vessels. We have found that standard grafts fail within two to three months due to narrowing, which is much more pronounced at the venous end (see graph). Active thrombosis along the graft surface, particularly at the venous end, appears to be a major contributing factor. Thickening is greatly reduced if the grafts are sewn into an artery instead of a vein, even if blood flow is increased by creation of an artery-to-vein fistula beyond the graft. We have also found that special coating of the graft surface with phospholipids can stop this thickening process.

The three principle components of graft healing and lumen narrowing are endothelial ingrowth, smooth muscle cell proliferation, and thrombosis. These are evaluated using scanning electron microscopy, mor-

phometry, and immunohistochemistry. We can also use simulated dialysis to assess the potential role in graft failure of the various components of the dialysis procedure.

Like the clinical specimens, the sheep lesions have focal regions of prominent cellular proliferation, often adjacent to thrombus and in granulation tissue surrounding the graft. This can be seen in Figure 2, showing a proliferating-cell-nuclear-protein (PCNA)-positive nucleus marked by an arrow.

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

We have determined that tissue factor (a stimulant of thrombosis) is increased along the length of the access graft, at both early and late times, possibly in response to this injury (Table 1). Elevated levels of this clotting factor may explain the thrombosis we have observed. Studies are underway to determine the cellular source of this enzyme and whether local drug infusion can block its production and therefore the development of intimal hyperplasia.

We will compare standard PTFE grafts with grafts that are more porous. Increased porosity allows in-growth of capillaries across the graft to the lumen, where

they spread and form an endothelial lining that may protect against thrombosis and intimal hyperplasia. We are also using this model to study the use of arterial grafts to deliver gene therapy.

PTFE grafts are seeded with smooth muscle cells that have been transduced with the erythropoietin (epo) gene (Dr. William Osborne, PI). Erythropoietin, normally made by the kidney, stimulates production of red blood cells. Patients with renal failure do not make enough of this hormone and as a result are anemic. We will use a uremic sheep model to find out if epo made by cells placed in dialysis access grafts can reverse the anemia of chronic renal failure.

location	tissue factor activity (+/-sd)	tissue factor protein	fibrin
Normal Artery	22.0 +/- 18.0	-	-
Graft near Artery	113.5 +/- 10.9 *	++	+
Graft near Vein	194.5 +/- 15.2 *	+++	+
Normal Vein	32.0 +/- 1.5	-	-

(N=4. *=p< .05, one-tailed Mann-Whitney comparison)

table 1: **Tissue Factor Levels in Sheep Access Grafts**

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Daniel F. Leotta, M.D.

- VEIN GRAFT SURVEILLANCE USING 3D ULTRASOUND IMAGING
- MEASUREMENT OF ABDOMINAL AORTIC ANEURYSM WITH 3D ULTRASOUND
- TUMOR MEASUREMENT AND LOCALIZATION WITH 3D IMAGING

FUNDING:

National Institutes of Health
National Cancer Institute

Quantitative evaluation of anatomy from medical images has applications in clinical diagnosis, monitoring, drug development and research. Ultrasound is a safe, non-invasive and relatively inexpensive imaging modality that produces a tomographic image of a thin tissue slice within a region of interest. Standard real-time ultrasound systems, however, do not maintain a record of the spatial relationship between sequential 2D images. Therefore, measurements of size and shape are often based on geometric assumptions and may be operator dependent.

We use a custom ultrasound imaging system that preserves the relationship of the 2D image planes in space, thereby allowing reconstruction of structures in a 3D coordinate system. Accurate 3D reconstructions provide better quantification of geometric parameters, enhancing comparisons of data both over time and between imaging modalities. In addition, realistic and intuitive displays can assist in the transfer of information between the multiple groups often involved in patient care.

Vein Graft Surveillance using 3D Ultrasound Imaging

Vein grafts are placed to bypass diseased arteries in the lower limb when symptoms such as pain during walking, rest pain, and tissue necrosis occur. While vein grafts provide effective relief of lower extremity ischemia for the majority of patients, approximately 30–40% of these grafts fail due to focal stenoses caused by myointimal hyperplasia. Because these lesions can be effectively corrected, their early detection is crucial.

A component of our laboratory's research in vein graft failure is the development of 3D ultrasound techniques for vein graft monitoring. Arteriograms and conventional ultrasound imaging produce only 2D views of vessels. Lesions at sites of complex geometry are difficult to monitor with 2D methods, and spatial

relationships over time are not preserved. Three-dimensional imaging, however, can produce a full representation of the vessel geometry, allowing assessment of changes over time at specific sites. We are continuing to focus our work on the extraction of quantitative measurements from 3D data sets, including cross-sectional area and wall thickness, to measure progressive changes in vein graft geometry.

Our 3D ultrasound imaging system is based on a standard ultrasound imager modified with a magnetic tracking system to register 2D ultrasound images in a 3D coordinate system. The tracking system records the location and orientation of the ultrasound scanhead during imaging, from which a 3D computer reconstruction of the vessel can be derived. Cross-sectional area measurements in planes normal to the center axis of the vessel are calculated from the 3D surface reconstructions.

Data sets from serial studies are registered in a common coordinate system using anatomical reference points, and cross-sectional area measurements are then compared. The sequential area measurements, com-

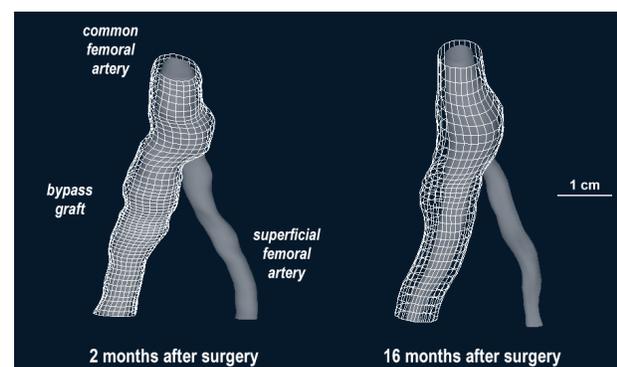


figure 1: 3D surface reconstructions of the proximal anastomosis of a femoral to popliteal saphenous vein bypass graft imaged 2 months (left) and 16 months (right) after surgery. The outer wall of the graft is displayed as a mesh; the lumen is displayed as a surface.

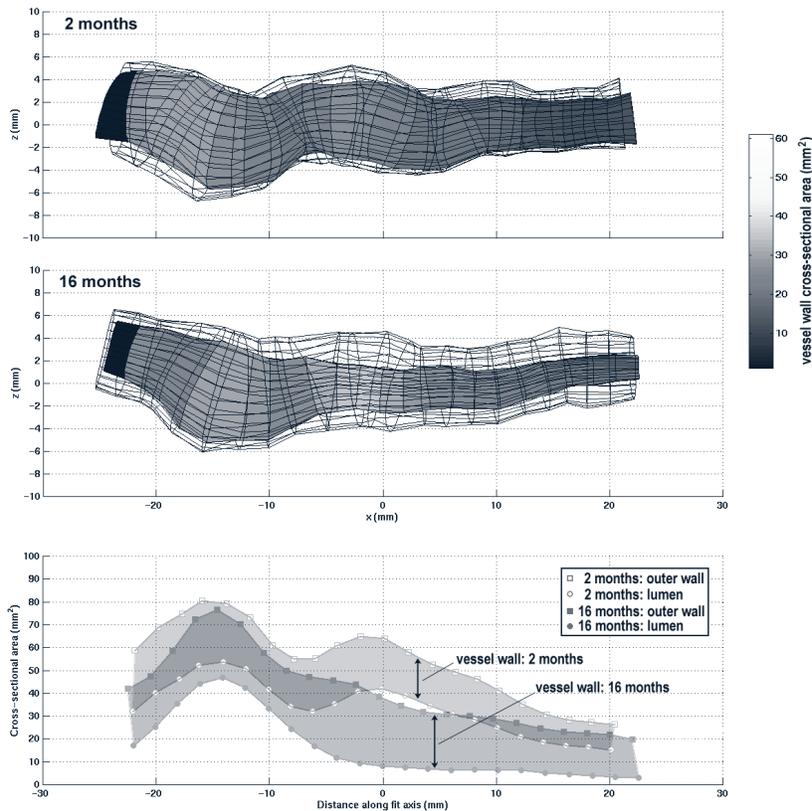


figure 2:

TOP: Surface reconstructions of the graft segment shown in Figure 1 at 2 months (upper) and 16 months (lower) after surgery. The outer wall is displayed as a mesh; the lumen is displayed as a surface, and the lumen surface is shaded according to the cross-sectional area of the vessel wall at each point along the axis.

BOTTOM: Cross-sectional area measurements of the total vessel (outer wall) and the lumen for the two studies shown above, plotted as a function of distance along the graft center axis.

bined with 3D surface displays, provide a record of remodeling patterns and rates at specific sites within the grafts.

Figure 1 shows 3D reconstructions of the proximal anastomosis of a femoral to popliteal saphenous vein bypass graft imaged at 2 months and 16 months after surgery. Surface reconstructions were generated for both the outer wall and the lumen of the vessel. Figure 2 shows the corresponding cross-sectional area measurements registered in a common coordinate system. Cross-sectional area of the total vessel and lumen are presented as a graph registered with the vessel surface reconstruction; the cross-sectional area of the vessel wall is mapped as a gray level to the lumen reconstruction. Changes are quantified as the mean percent change in cross-sectional area relative to the first post-operative study. The total vessel area decreased by 22%, the lumen area decreased by 45%, and the vessel wall area increased by 21%.

Measurement of Abdominal Aortic Aneurysm with 3D Ultrasound

Abdominal aortic aneurysms (AAAs) are dilations of the aorta occurring between the renal and the iliac arteries. Reliable quantitative evaluation of AAAs is

required both for diagnosis and in the follow-up studies needed to avoid life-threatening rupture. Small aneurysms enlarge at an average rate of 0.5 cm in diameter per year, and they require close tracking by serial measurements to assure suitable treatment before risk of rupture is significant. A recent development in AAA treatment is endovascular repair, which is a minimally-invasive procedure to exclude the aneurysm from the circulation.

In contrast to the traditional open surgery, an endovascular graft is deployed using a catheter system passed into the aorta through the femoral arteries. This procedure is associated with significantly reduced morbidity and recovery time. However, extended post-treatment monitoring is generally required to ensure that the endograft is stable and that there are no leaks. While decrease in aneurysm size indicates its successful exclusion from the circulation, post-implant expansion indicates the presence of a leak and a risk of aneurysm rupture.

Ultrasound is an attractive imaging modality for screening and monitoring AAA patients. Since it does not involve radiation or contrast agents, ultrasound offers a safe, non-invasive and relatively inexpensive method of measuring AAAs and following changes over

time. However, dimensional measurements made with conventional 2D ultrasound are sensitive to image plane orientation. In addition, the orientation and placement of the imaging planes change from visit to visit, which contributes to measurement variability in studies over time. Therefore, we are using the 3D ultrasound imaging system described above to generate computer reconstructions of the aorta from which quantitative measurements can be extracted. Three-dimensional reconstruction minimizes the variability in measurement due to ultrasound image plane orientation, and the 3D data sets also provide documentation of the aneurysm size along its entire length.

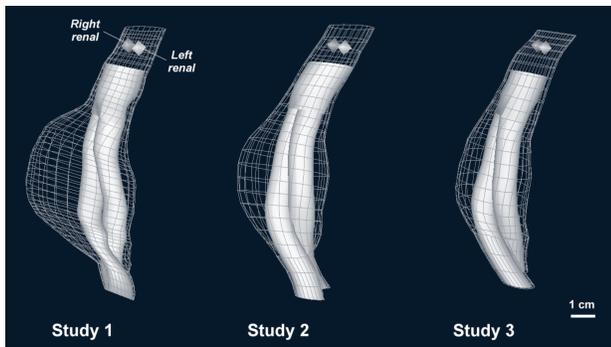


figure 3: Serial study of an AAA repaired by an endovascular graft. The 3D reconstructions show both the aneurysm (outer mesh) and the graft (inner surface). The studies were performed 1) 2 weeks, 2) 6-months, and 3) 1 year after graft placement. Diamond markers show the origins of the renal arteries.

Computer reconstructions of an AAA are presented in Figure 3 for a series of 3D ultrasound studies after endovascular repair, showing both the aneurysm sac and the graft. Aneurysm size is quantified as the cross-sectional area and maximum diameter as a function of distance along the length of the aorta, and the volume of the aneurysm sac. Figure 4 displays the aneurysm cross-sectional area measurements, and the table below summarizes the diameter and volume measurements. The overall maximum diameter decreased by 23% in 1 year, and the volume decreased by 33%.

	time after repair	maximum diameter	change in maximum diameter	volume	change in volume
Study 1	2 weeks	5.7 cm	—	132.4 ml	—
Study 2	6 months	5.0 cm	-12%	106.0 ml	-20%
Study 3	1 year	4.4 cm	-23%	88.6 ml	-33%

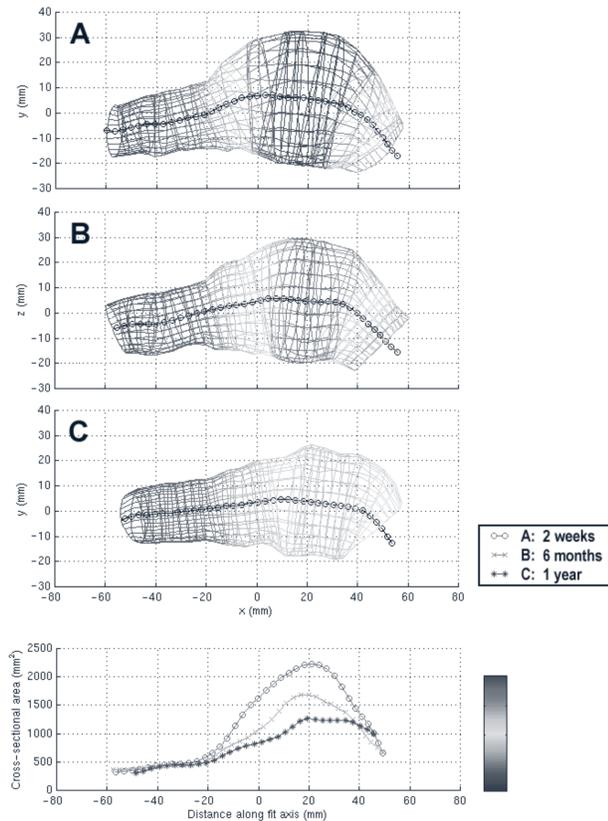


figure 4: Cross-sectional area measurement of an AAA after endovascular repair. At the top are mesh representations of the aneurysm imaged at three time points, with shading corresponding to the cross-sectional area. Below are plots of the cross-sectional area measured at each sample point along the vessel center axis for each of the studies. The cross-sectional area measurements are mapped to the surfaces according to the gray scale at the right. A cross-sectional area of 2000 mm² is approximately equivalent to a diameter of 5 cm.

Tumor Measurement and Localization with 3D Imaging

A National Cancer Institute project under Dr. Kirk Beach in the Division of Vascular Surgery is investigating the use of ultrasound to detect small tumors by their pulsatility relative to surrounding tissues. As part of this effort, we are developing 3D displays of tumor location, and methods of quantitatively monitoring tumor size and blood flow. Preliminary studies of 3D ultrasound scanning of tumors were performed in collaboration with Shahram Vaezy in the

Three-dimensional imaging can produce a full representation of the vessel geometry, allowing assessment of changes over time at specific sites.

UW Center for Industrial and Medical Ultrasound, who is working on tumor treatment methods using high-intensity focused ultrasound (HIFU).

Figure 5 shows a surface reconstruction of a uterine fibroid derived from manual traces of a series of gray-scale images. The volume of this tumor, calculated from the surface reconstruction, is 1.1ml. A study of a second uterine fibroid was conducted in the power Doppler mode to assess tumor blood flow. The color (blood flow) and gray scale (anatomy) data were reconstructed separately and displayed in a common 3D coordinate system (Figure 6). The structure of the vascular network associated with the tumor is well visualized in the 3D reconstruction. Our goal for future work is to track both the growth of tumors and their response to HIFU treatment using measurements of tumor volume, vascularity, and pulsatility.

We have also adapted our software for 3D reconstruction of freehand ultrasound scans to produce 3D reconstructions of clinical MRI breast scans. Images were analyzed from a contrast-enhanced MRI breast study, including scans before and after the administra-

tion of gadolinium contrast agent, and a derived image set representing the difference between the pre- and post-contrast scans. Manual outlines on successive sagittal images were made of the skin surface of the breast and the regions in which contrast enhancement was evident. A large anterior region and a smaller posterior region of enhancement were observed in the subtraction data. Figure 7 shows the resulting 3D surfaces, with the skin surface represented by a mesh and the regions of enhancement visualized within the context of the breast anatomy. Volumes of the enhanced regions were calculated from the surface reconstructions. The depths of the enhanced regions relative to the skin surface of the breast were also extracted from the 3D data. These depths were then mapped to the skin surface as a test of potential treatment guidance displays.

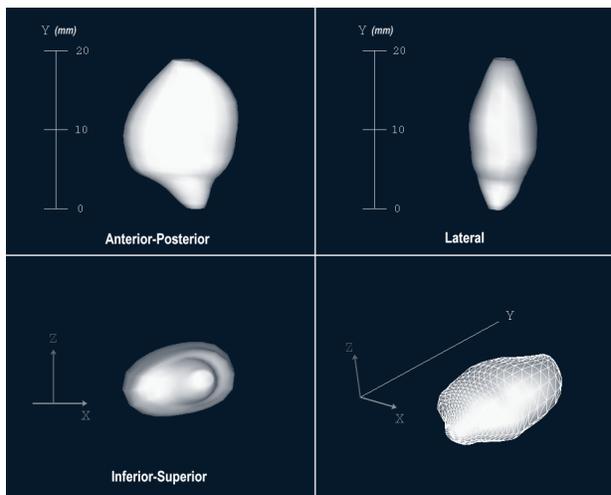


figure 5: Orthogonal views of a 3D surface reconstruction of a uterine fibroid, and an oblique view (lower right) explicitly showing the triangular patches that define the surface. The tumor volume of 1.1 ml is calculated from the 3D reconstruction.

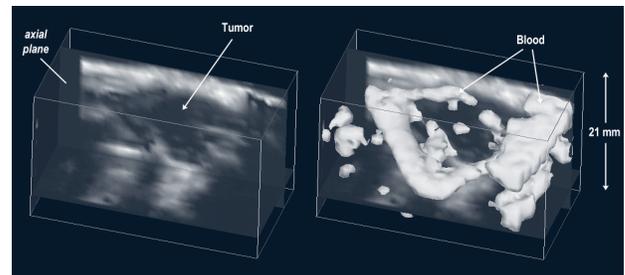


figure 6: 3D volume reconstruction of a uterine fibroid imaged with power Doppler. Orthogonal planes through the gray scale data are shown on the left; the blood flow detected by power Doppler imaging is superimposed on the right. Voxel size = 0.6 mm. Volume size = 36 x 59 x 32 voxels = 21 x 35 x 19 mm.

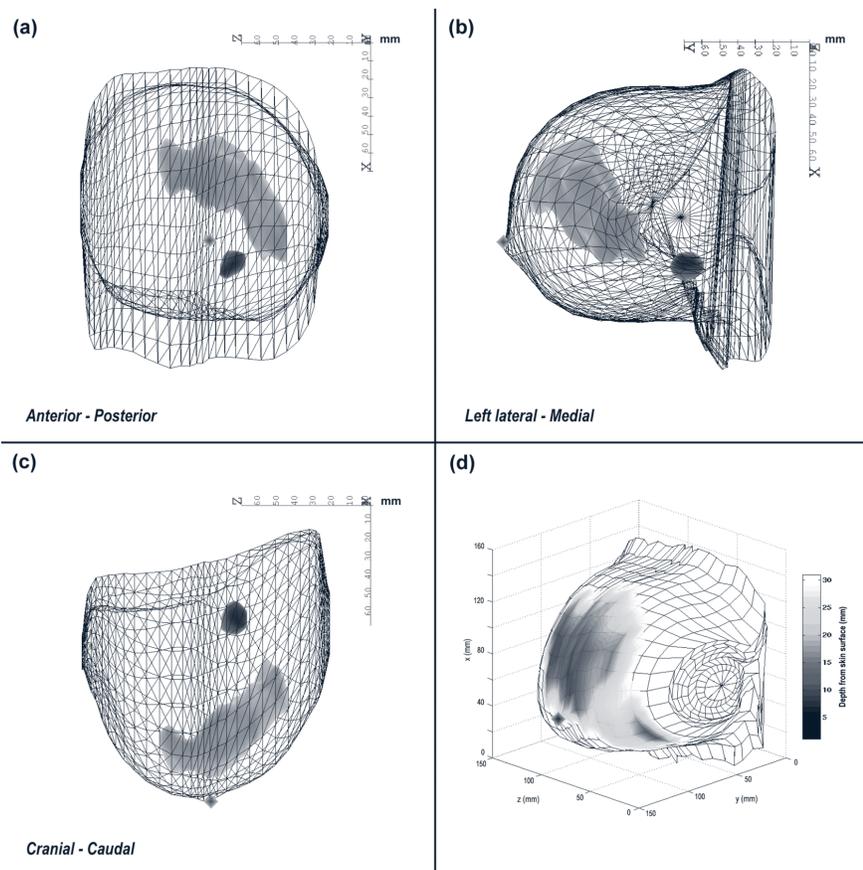


figure 7: (a–c) Orthogonal views of 3D reconstructions of the skin surface and two regions of contrast enhancement derived from manual outlines of a stack of sagittal MR breast images. The skin surface is displayed as a mesh to show the surface-rendered regions of enhancement. The volume of the larger region is 47.1 ml; the volume of the smaller region is 2.3 ml. (d) The depth within the breast of the larger mass is mapped to the skin surface according to the gray scale to the right. Diamond marker: nipple.

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