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Lower image: A prostate biopsy core showing prostate cancer.

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Cleveland Clinic Ranked No. 1 in Urology and Nephrology

U.S. News & World Report’s “America’s Best Hospitals”

Glickman Urological & Kidney Institute now has the distinction of holding two No. 1 positions in U.S. News & World Report’s hospital rankings, with No. 1 rankings in both urology and nephrology.

The 2012 “America’s Best Hospitals” survey recognized Cleveland Clinic as one of the nation’s best overall, ranking the hospital as No. 4 in the country. Cleveland Clinic ranked in all 16 of the specialties surveyed by the magazine.

For details, visit clevelandclinic.org.
Dear Colleagues,

Welcome to another edition of the Glickman Urological & Kidney Institute’s Urology & Kidney Disease News. I hope that you find this issue informative and inspiring.

We are very pleased to report that we received some very good news, times two, this past summer – that you, our peers and colleagues, rated us as No. 1 in both urology and nephrology in U.S. News & World Report’s Best Hospitals annual survey. It’s especially gratifying that you hold our staff and programs in such high regard.

The Glickman Urological & Kidney Institute’s activities encompass a unique combination of high-volume and challenging clinical cases, extensive basic and translational scientific activities, and credible laboratory research within an environment that nurtures the future leaders of our specialties.

Our 93 physicians and scientists offer in-depth expertise in every subspecialty area. In 2011 alone, the faculty was responsible for providing almost 75,000 outpatient visits, more than 14,000 dialysis treatments and performed more than 20,000 surgical procedures while publishing 433 peer-reviewed manuscripts, 62 book chapters and five textbooks and receiving $10.5 million in research funding.

In addition to leading-edge research, we offer a full range of urological and kidney care for adults and children. Most of the institute’s physicians have subspecialty training in one or more of the following areas: bladder, prostate, kidney and testicular cancer; voiding dysfunction; stone disease; chronic urinary tract infections and obstructions; dialysis; hypertension; acute and chronic kidney disease; kidney and pancreas transplantation; male fertility; pediatric urology and nephrology; prostate disease; sexual dysfunction/impotence; and genitourinary reconstruction. This year also marked the beginning of a new program, Translational Urology and Nephrology, to help patients born with genitourinary malformations transition into adulthood. This program is another example of how our institute structure allows us to leverage the expertise of two subspecialties to benefit patients.

Within the pages of this issue, we tell you some of our stories in these areas. Among many exciting advances, robotic surgery has made great strides in many applications; we provide a variety of studies related to this evolving area of surgical practice.

Treating cancer is a complicated process requiring careful diagnosis, staging procedures and a thorough evaluation of the best approach to management. This requires input from multiple physician specialists to define the optimal treatment plan for an individual patient to arrive at the best outcome. And, as technology has improved, patients now have more options for managing their disease, and for the medical community to provide fast and accurate diagnoses and treatments. In this vein, we follow up on last year’s cover story about fluorescent cytoscopy and how it reveals cancers of the bladder not recognized before. We also touch on more about the PSA controversy and how we approach that test in our practice.

We also touch on a trial of one of the most exciting advances in the treatment of blood pressure disorders – renal denervation – and report on the progress of the SYMPLICITY trials.

I hope that you find the information in this publication to be useful in your practice. Please do not hesitate to contact me with any questions, concerns or suggestions at 216.444.5591 or kleine@ccf.org.

Eric A. Klein, MD
Chairman
Glickman Urological & Kidney Institute
Novick Center

The Novick Center for Clinical and Translational Research supports the research efforts of all members of the Glickman Urological & Kidney Institute. Headed by Daniel Shoskes, MD, and Sankar Navaneethan, MD, the center has 30 full-time employees, including a research manager, research nurses, study coordinators, database managers and IT support personnel. The center manages a number of clinical trials and 15 disease-specific databases that serve as a source for clinical projects and outcomes reporting. Biostatistical support is provided through the Department of Quantitative Health Sciences, led by Michael Kattan, PhD, and center personnel frequently collaborate with scientists in Cleveland Clinic’s Lerner Research Institute.

J. Stephen Jones, MD, Appointed Chief of Surgical Operations at Fairview Hospital

J. Stephen Jones, MD, was recently appointed Chief of Surgical Operations at Fairview Hospital, a Cleveland Clinic hospital located on Cleveland’s West Side. The appointment took effect Oct. 1, 2012. He stepped down as Chairman of Regional Urology and relocated his urology practice to Fairview Hospital. Dr. Jones joined Cleveland Clinic in 2000. He has served in a number of leadership positions at Cleveland Clinic, including an appointment to the Board of Governors. In 2011, he was named the holder of the new Leonard Horvitz and Samuel Miller Distinguished Chair in Urologic Oncology Research for the Glickman Urological & Kidney Institute.

Robert Heyka, MD, Becomes Interim Chair of Nephrology

Robert Heyka, MD, was recently appointed as interim chairman of the Department of Nephrology and Hypertension at the Glickman Urological & Kidney Institute. Dr. Heyka replaces Martin J. Schreiber Jr., MD, who has returned to the full-time medical staff. A national search is being conducted for a new chairman; an appointment is expected in early 2013.

Upcoming Events – Save These Dates

March 22, 2013
Ambulatory Urology Course
Led by J. Stephen Jones, MD, and Edmund Sabanegh Jr., MD

March 23, 2013
Ninth Annual Glickman Urological & Kidney Institute Nursing Conference
Led by the Nursing Conference Committee

Sept. 6, 2013
Kidney Stones: Medical, Surgical and Dietary Approaches
Course Director: Manoj Monga, MD

Sept. 13-14, 2013
Fifth Annual International Symposium on Robotic Kidney and Adrenal Surgery
Course Director: Jihad H. Kaouk, MD

Please visit ccfcme.org for more details on these events.
Now Available: Cord Denervation for Testicular Pain

Men suffering with chronic orchialgia (long-term testicular pain) now have a new treatment option: microscopic cord denervation (MCD).

“Chronic orchialgia is a common and frustrating condition with limited treatment options,” says Cleveland Clinic urologist Daniel Shoskes, MD. “Studies show the condition accounts for 1 percent of visits to Canadian urologists and 2.5 percent of visits to Swiss urologists, with an incidence of 4 in 1,000 men.”

If ongoing testicular pathology is ruled out, the pain may be neuropathic, coming from branches of the genitofemoral nerve that run in the spermatic cord, explains Dr. Shoskes. If so, a spermatic cord block, which injects local anesthetic into the cord, can give complete although temporary pain relief. Patients who get relief from a spermatic cord block are potential candidates for MCD.

Dr. Shoskes now offers MCD for Cleveland Clinic patients. MCD is an outpatient procedure requiring general anesthesia. During MCD, the cord is delivered through a small subinguinal incision and dissected with the aid of an operating microscope. Arterial, venous and lymphatic branches are preserved, as well as the vas deferens if the patient desires ongoing fertility. The remaining tissue, which contains the genital nerve branches, is then transected.

“We have had excellent success with the procedure to date, with most patients pain-free and all significantly improved,” says Dr. Shoskes.

For more information, contact Dr. Shoskes at 216.445.4757 or shosked@ccf.org.

Resident Awards


Karen C. Baker, MD, won the Crile Research Fellowship Award for highly meritorious basic science research proposals for “Nanoparticle Mediated Drug Delivery to the Testicular Tissue,” March 2012.

Christina B. Ching, MD, was awarded the George and Grace Crile Traveling Fellowship, June 2012.

Brandon K. Isariyawongse, MD, PhD, was one of the winners of the Resident Abstract Competition at the Ohio Urological Society meeting, April 2012.

Michael C. Lee, MD, won the 2012 Outstanding Laparoendoscopic Resident Award. He was nominated for demonstrating great promise in laparoscopic, endoscopic and minimally invasive surgery.

Devon Snow-Lisy, MD, won the Society of Male Reproduction Traveling Scholars Award for the research project “Novel Technique for Nanoparticle Delivery to the Testis in the Rat Model,” May 2012; the Society for the Study of Male Reproduction/Sexual Medicine Society Traveling Fellowship, May 2012 and was appointed treasurer of the House Staff Association and member of the Graduate Medical Education Committee, 2011-2012.

Mary Katherine Samplaski, MD, won the Bruce Hubbard Stewart Award for Humanistic Medicine, June 2012.

Yuka Yamaguchi, MD, was awarded the 2011 Society of Women in Urology Traveling Award, January 2012.
New Staff  The Glickman Urological & Kidney Institute welcomes the following new staff members:

Joseph Africa, MD, received his medical degree from University of Santo Tomas Faculty of Medicine & Surgery in Manila, Philippines. He completed internships at both Capitol Medical Center in the Philippines and Jacobi Medical Center, Albert Einstein College of Medicine. He also completed a residency in general surgery at University of Santo Tomas Hospital and a fellowship in kidney and pancreas transplant surgery at Washington Hospital Center. Dr. Africa serves patients at the Charleston Urology Office.

Juan Calle, MD, received his medical degree from Instituto de Ciencias de la Salud CES in Medellin, Colombia. He completed a residency in internal medicine at Mount Sinai Medical Center in Miami Beach, Fla., and a fellowship in nephrology and hypertension at Mayo Clinic. Dr. Calle serves in the Department of Nephrology and Hypertension at main campus.

Diana Deitzer, DO, received her medical degree from the New York College of Osteopathic Medicine. She completed a residency in internal medicine and a fellowship in nephrology and hypertension at Cleveland Clinic. Dr. Deitzer sees patients at main campus.

Kripa Kavasseri, MD, received her medical degree from Wayne State University School of Medicine. She completed a residency in urology at Wayne State University/Detroit Medical Center. She joins the Department of Urology at Hillcrest Hospital, Twinsburg Family Health and Surgery Center, and the Mentor Medical Office Building.

Michael Lee, MD, received his medical degree from Northwestern University Feinberg School of Medicine. He completed both an internship and residency in urology at Cleveland Clinic. Dr. Lee serves patients at Lorain Family Health Center, Wooster Specialty Center and the Medina Hospital South Medical Office Building.

Michael Lioudis, MD, received his medical degree from State University of New York at Buffalo School of Medicine. He completed an internship in internal medicine at Michigan State University Kalamazoo Center for Medical Studies and a residency in internal medicine/pediatrics at Penn State Geisinger-Milton S. Hershey Medical Center. He also completed a fellowship in nephrology at Hospital of the University of Pennsylvania. Dr. Lioudis has offices at both main campus and the Twinsburg Family Health and Surgery Center.

Audrey Rhee, MD, received her medical degree from the Albert Einstein College of Medicine. She completed an internship in general surgery at Temple University Hospital and a residency in general surgery/urology at the Medical College of Georgia Hospital (MCG Health). She also completed a fellowship in pediatric urology at Riley Hospital for Children. Dr. Rhee sees pediatric patients at main campus, the Richard E. Jacobs Health Center in Avon and the Independence Family Health Center.

Mark Stovsky, MD, received his medical degree from Northwestern University Medical School. He completed an internship in general surgery and a residency in urology at University Hospitals of Cleveland. Dr. Stovsky has offices at Euclid Hospital, Willoughby Hills Family Health Center and the Mentor Medical Office Building. He also works one day a week in Innovations, Cleveland Clinic’s corporate venturing organization.
Study Shows Prostate Cancer Screening Cuts Risk of Developing Advanced Cancer

A Cleveland Clinic study suggests prostate cancer screening decreases a patient’s risk of developing metastatic disease. Patients treated before routine prostate-specific antigen (PSA) screening was advocated had a 10-year metastasis-free survival rate of 74 percent while those treated after routine PSA screening was advocated had an 82 percent 15-year metastasis-free survival rate, according to the study published in August 2012 in the journal *Urology*.

“The study shows that routine PSA screening reduces the risk of developing advanced cancer, which is associated with additional treatment-related costs and morbidity,” says Jay P. Ciezki, MD, the study’s author and a radiation oncologist in Taussig Cancer Institute at Cleveland Clinic.

The impact of screening for prostate cancer has been widely debated since 1992 when the use of PSA testing was first advocated in the United States. Opponents of routine screening argue that it has not resulted in a meaningful improvement in survival.

In this particular study, researchers from Cleveland Clinic’s Taussig Cancer Institute and Glickman Urological & Kidney Institute determined that a more meaningful way to evaluate the effectiveness of screening was to examine its ability to decrease the development of metastatic disease after treatment.

The study was based on data from more than 1,700 prostate cancer patients who were treated at Cleveland Clinic with either radiation therapy or surgery to remove the prostate gland and surrounding tissues. To assess the impact of screening, the patients were divided into two groups according to when they were treated: a prescreening era (1986-1992) and a post-screening era (1993-1996). Patients were classified as having high-, intermediate-, or low-risk disease to determine which groups may have benefited from prostate cancer screening. The 10- and 15-year rates were used to account for changes in the population caused by screening. The benefit of screening was seen across all risk groups.
In the Editor’s Words

It is not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is the most adaptable to change.

—Charles Darwin

In the quarter century since I started medical school, the future of medicine has never appeared more gloomy. This designation is not without competition from other difficult times, but also not without reassurance to be gleaned from the seeming cataclysms that came — and quickly faded — before. Things seem gloomy, but are they actually so?

The arrival of Medicare in the 1960s was decried by most physicians as a governmental assault on the very core of modern medicine. Instead of poverty and an end to the profession, Medicare actually ushered in the era where payment was finally ensured for most services. When diagnosis-related groups (DRGs) were created in an attempt to rein in the resulting explosion of medical spending, this presumed precursor of socialized medicine made many of my fellow medical students question whether we were following the wrong career path. Although DRGs made the job market tighter for those of us who began to practice in the early ‘90s, we learned to benefit from efficiencies and business practices that allowed us to provide better care for more patients while still making an even better living. The Clinton health plan (with its flawed and ultimately unsustainable twin, capitation) soon made us young practicing physicians fear a medical nuclear winter. However, we worked hard and improved care, and our profession became the most important and sustainable economic engine in America. As other industries wilted during the Great Recession, urologists continued to make a good living while doing honorable and deeply satisfying work.

Nevertheless, have no doubt that the risks of an uncertain healthcare reimbursement environment are real. Healthcare spending now approaches one-fifth of the U.S. gross domestic product. A resolve to rein in those costs is growing in government, industry and among the public. Therefore, it probably will not matter whether challenges come in the form of the Affordable Care Act or accountable care organizations, or simply from market forces such as insurance reductions and transfers of cost. The form of the threats may be unpredictable — their inevitability is not.

Furthermore, our ability to control our medical world continues to diminish. In this so-called era of radical transparency, consumers and regulators now know more about the workings of almost every field. This is even truer of the medical field — the most heavily regulated industry. Patients can increasingly compare individual physician quality and patient satisfaction measures online. Regulations will continue to increase costs to us as providers while reimbursements drop.

However, the successful navigation by physicians and hospitals through the challenges of the past offers valuable lessons to address this daunting future. The key point is that smart, hardworking people thrive as long as they adapt. As an example, a resident in my urology externship told me that the standard TURP would put my children through college. He was wrong; I haven’t performed one since my children were in elementary school, and even if I had, the operations would have generated hardly enough revenue to cover the overhead. However, in the interim, urologists have made our specialty sustainable through innovations in the care of almost every urological condition. We have embraced minimally invasive treatments and have fought through initial disappointments to create new standards of care. Our practices have developed successful business strategies, and we continue to drive a critical American industry. We have done well by doing good for the patients who depend on us.

The lesson is that our specialty is full of — dare I say dominated by — smart, hardworking, innovative people. Those who adapt to the evolving world around us will thrive regardless of the challenges presented by a continually evolving though deeply rewarding environment. This is not the time for fear. Rather, it is the time for us to forge the urological future that we, our patients and our co-workers deserve.

J. Stephen Jones, MD
Chief, Surgical Operations,
Fairview Hospital
From the Interim Chairman, Nephrology and Hypertension

The field of medicine, and within it nephrology, is, as are most things, ruled by change rather than permanency. The most relevant change building on the horizon involves the dynamic reimbursement environment: the current bundling of payments for end-stage renal disease (ESRD) services. This new challenge is similar to past changes in the diagnosis-related group (DRG) hospital environment. The impact of this new environment looms large and represents a new seascape that we need to learn to navigate.

In January 2011, under the authority of MIPPA (Medicare Improvements for Patients and Providers Act), the Centers for Medicare & Medicaid Services (CMS) introduced a prospective payment system (PPS) that bundles dialysis sessions with antibiotics; laboratory work ordered by the managed care physician; previously billable items such as erythropoietin (EPO) stimulating agent, vitamin D and intravenous iron; and oral medications. Adjustments were made for certain acute and chronic case-mix adjusters (CMAs) and time elapsed since the start of ESRD treatments. CMS also introduced quality parameters under the Quality Improvement Project (QIP) that can affect payments up to 2 percent yearly if not met.

Most dialysis organizations around the country decided to fully opt into the system rather than phase in over a four-year period.

Several PPS issues loom large. These include CMA adjustments, addition of cinacalcet and phosphate binders to the bundle in 2014, and changing quality indicators for the QIP. These indicators include clinical and reporting parameters, such as ICH-CAHPS (In-Center Hemodialysis-Consumer Assessment of Healthcare Providers and Systems), release and use of the CROWN web-central database, lab coverage, and pharmacy issues.

On the positive side, the PPS should increase the number of home patients, promote activities to reduce catheters, and increase efforts to reduce hospitalizations and keep patients in their dialysis facilities.

PPS caused several immediate changes as facilities transitioned to intravenous medications that were both effective and economical. PPS reduced the use of local labs and challenged doctors to capture comorbid conditions that could affect reimbursement. Most units implemented anemia management protocols and increased iron administration based on mandated lower targets to maximize the response for a given dose of EPO.

Although the future is still cloudy and we have no crystal ball to give us answers, several things seem certain. Dialysis organizations and physicians will need to continually evaluate the implementation of these changes. The CMS-mandated targets are likely to be a work in progress and to change over time. We need to anticipate that they will be adjusted and ensure that the dialysis community has adequate input into the changes. We must ensure that the time frames selected by CMS allow adequate evaluation of interventions. We need to study the effects of PPS implementation in the non-Medicare population. We will see continued movement to the electronic record and more computerized physician order entry and electronic prescribing. Unit-based quality initiatives will focus more on CMS-mandated goals, and we must be certain that they are workable and the best indicators for quality. Currently selected comorbid conditions are likely to undergo adjustment as the true cost of comorbid conditions is better evaluated.

Eventually, access to pharmacy services in each unit may be commonplace in an attempt to master quality and cost. It is possible that pharmacists will become directly involved in the care of dialysis patients to improve medication reconciliation, look for drug-drug interactions, attempt to improve compliance and assist patients with overall costs.

Dialysis organizations are likely to become more interested in joint venture partnerships to increase the involvement of renal physicians in quality processes. The monthly capped payment may be included in the bundle soon. Pay-for-performance opportunities will increase, with a wider focus especially in the areas of catheter use and arteriovenous fistula placement. Unit and physician scoring will be widely available.

Unfortunately, there is an increased risk of some dialysis clinics closing, especially smaller facilities that are unable to average out their costs and remain solvent. A potentially large risk is lack of cooperation between dialysis organizations and physicians as the boat we are all floating in becomes smaller. Another pernicious effect may be attempts to cherry-pick existing patients and accept or reject new patients based on their compliance and comorbidities that affect the bottom line.

Add in the uncertainties of accountable care organizations and the impact of that structure on the ESRD population, and we are in for quite a ride.

The nature of the PPS and QIP will require a more active nephrology community than in the past to protect our patients and colleagues. I hope the PPS will encourage continuous evaluation of results and improvement of patient outcomes. Either way, sales of headache medications to nephrologists are sure to increase.
A Prostate Biopsy Antibiotic Prophylaxis Protocol

Howard B. Goldman, MD

Infectious complications from prostate biopsy requiring hospitalization have historically ranged from 1 to 2 percent. In recent years, rates as high as 4 percent have been reported in the literature.

There appears to be growing resistance to fluoroquinolones, with some studies reporting rates of resistant E. coli in the fecal flora in as many as 22 percent of men. Because a fluoroquinolone is one of the recommended (and easiest to administer) antibiotics for prostate biopsy prophylaxis – suggested by both the American Urology Association (AUA) Best Practices Report and the Surgical Care Improvement Project – this trend of resistance is worrisome.

A number of factors appear to predispose men to antibiotic resistance. A history of fluoroquinolone use in the prior six months, recent international travel or work in a healthcare environment has been associated with quinolone resistance. In response to these concerns, the Glickman Urological & Kidney Institute, in consultation with Cleveland Clinic infectious disease specialists and cognizant of the various healthcare requirements, has developed a new prostate biopsy antibiotic prophylaxis protocol. Both oral levofloxacin (24-hour coverage) and intramuscular gentamicin (weight-adjusted dosages) are administered prior to biopsy. In patients with risk factors for quinolone resistance, ceftriaxone is substituted in place of levofloxacin. Based on specific patient requirements and clinical judgment, other options are also available.

Furthermore, there is often confusion about antibiotic use in patients with orthopedic hardware or cardiac conditions. Our protocol addresses these concerns and reinforces the use of appropriate antibiotics.

Other strategies have been suggested to deal with this problem - for example, stool cultures with sensitivity-guided antibiotic use. While this appears to be a promising approach, the literature is still scant and we await further data.

Our protocol is provided below.

---

Prostate Biopsy Antibiotic Protocol

Howard B. Goldman, MD

Levaquin® 750 mg PO x 1 dose and gentamicin IM as dosed below:

<table>
<thead>
<tr>
<th>Actual body weight</th>
<th>1.5 mg/kg dose of gentamicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 70 kg</td>
<td>80 mg</td>
</tr>
<tr>
<td>70–90 kg</td>
<td>100 mg</td>
</tr>
<tr>
<td>&gt; 90 kg</td>
<td>120 mg</td>
</tr>
</tbody>
</table>

In rare instances for very obese patients, the dose may need to be higher. Note: The antibiotic that meets the SCIP measure is the Levaquin. Gentamicin is our addition.

If allergic to Levaquin: ceftriaxone 1g IM plus gentamicin as above.

See table below for other options if the above are not feasible due to allergies, etc.

Table. Official AUA antibiotic recommendations for prostate biopsy prophylaxis

<table>
<thead>
<tr>
<th>Anti-microbial(s) of choice</th>
<th>Alternative anti-microbial(s)</th>
<th>Duration of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoroquinolone* or 1st-/2nd-/3rd- generation cephalosporin</td>
<td>Aminoglycoside (or aztreonam) in patient with renal insufficiency (2 g IV) + metronidazole (500 mg IV) or clindamycin (600 mg IV)</td>
<td>≤ 24 hours</td>
</tr>
</tbody>
</table>

*Note: only fluoroquinolones are acceptable orally.
A Working Definition of Meaningful Use

The federal Health Information Technology for Economic and Clinical Health (HITECH) Act is the component of healthcare reform that provides federal incentive payments to doctors and hospitals when they adopt electronic medical records (EMRs) and demonstrate their use in ways that can improve quality, safety and effectiveness of care.

Incentive payments began in 2011 and will continue at diminishing levels for up to six years, ending once the incentive pool is depleted. After that, penalties will be assessed for nonqualification, resulting in reduced provider payments for services.

Defining Meaningful Use

The term “meaningful use” implies that clinicians, beyond merely using the EMR, must ensure that the EMR plays a meaningful role in care delivery. The Centers for Medicare & Medicaid Services has developed metrics designed to signify that the EMR is used to improve patient care.

For year 1 (2011), qualifying clinicians had to use e-prescribing to 1) directly deliver at least 40 percent of prescriptions from the EMR to the patient’s chosen pharmacy, 2) print an after-visit summary (AVS) documenting the diagnosis and care plan for more than 80 percent of patients, and 3) maintain an active problem list that must be reviewed or updated for 80 percent of ambulatory visits.

Despite external pressure, clinicians may delay adoption of EMRs if they perceive the meaningful use processes as contributing to decreased efficiency for most encounters.

The Glickman Urological & Kidney Institute adopted an EMR system almost a decade ago both for documentation and e-billing as part of an enterprise-wide effort. As a result of this early adoption, we were prepared to combine energies toward achieving the meaningful use qualification for institute physicians.

New Work Flow: Smart Sets

The first step of the EMR implementation was to identify the meaningful use measures and build them into the work flow of the Epic® EMR system on an institute basis. A clinical support analyst was hired to educate and assist the urologists and nephrologists through a combined program to ensure that every staff member was qualified within the first year.

Smart sets were developed to facilitate documentation, diagnosis entry and computerized physician order entry (CPOE) in one or two motions. This allowed standardization where appropriate and decreased duplication of data entry. Finally, pharmacy data were entered and confirmed for each patient prior to all visits.

The next step was to educate both nephrologists and urologists on building the three meaningful use measures (e-prescribing, AVS and problem list maintenance) into standard work flows. Early experience was challenging, as many physicians had difficulty following all three, but starting months before the measures became official enabled us to have most physicians qualified by the time the program began in the fall of 2011.

Monthly reports were supplied to all physicians letting them know how close they were to achieving meaningful use status. Stragglers received one-on-one assistance with the COA until all were qualified.

Across all three departments (Nephrology, Urology and Regional Urology), 98 percent of physicians qualified by the first quarter of 2012. One major lesson learned was that physicians were slowest to adopt e-prescribing, one of the common features made available during the decade of EMR adoption.

But once they used it for just short spans of time, few considered returning to paper prescriptions based on the efficiency, accuracy and legibility of e-prescribing. Notably, most physicians expected that patients would resist letting go of paper prescriptions, but fewer than 5 percent of patients requested written versions.

Paid Incentives and Outcomes

Even more remarkable was the positive impact on the organization. Providers were awarded on average $16,498, and the institute expects a slightly higher amount in 2012. This success was mirrored across Cleveland Clinic.

Notably, Cleveland Clinic employs only 0.35 percent of all eligible providers in the U.S., but 7.7 percent of all qualifying funds in the United States went to Cleveland Clinic, demonstrating dedication to qualification across the organization. Twelve percent of all qualifying urologists in the United States were from the Glickman Urological & Kidney Institute.

The major lesson was that infrastructure was critical to success. This took time and labor investment upfront, but at this point all the steps in each encounter are quick and our prescribing process is incomparably improved, including the accuracy of medication reconciliation throughout the enterprise.

As more measures are introduced, we will build on this infrastructure to further harness the power of the evolving EMR, with the hope that it will improve both individual and population outcomes.

A version of this article originally appeared in the June 2012 issue of Renal & Urology News.
Kidney Transplant Milestone Reached – Pancreas Transplants on the Way

Alvin Wee, MD

Cleveland Clinic’s Indiana partner – St. Vincent Transplant Center in Indianapolis, has reached another milestone with our 150th kidney transplant completed in June 2012. This milestone was reached in less than three and one-half years. Through the St. Vincent program, Cleveland Clinic provides comprehensive, state-of-the-art renal transplant services in Indianapolis. The program has a dedicated 11-bed inpatient unit at St. Vincent Indianapolis Hospital as well as an outpatient clinic.

In February 2012, we started to evaluate patients for pancreas transplantation; a waiting list for this procedure at our center is rapidly growing. St. Vincent Transplant Center is now one of only two centers that provide pancreas transplantation in Indiana.

In the area of kidney transplantation, we continue to far exceed national averages for waiting times. Since our program began in 2009, 41 percent of patients on a transplant waiting list have received a kidney within six months at St. Vincent – compared with only 13 percent nationwide. Our center has one of the shortest waiting times for transplant in the nation.

Patients who list at their local center and at our center increase their chance of getting a donor organ in a practice known as multi-listing. By doing so, it increases the chance of patients getting exposed to more donors and therefore increases the chance of transplantation in a shorter time. The goal is to list them six weeks from evaluation so they can start accumulating time on the waiting list.

Important to the success of our program is our patient evaluation process. We use a patient-centered evaluation system - instead of patients being scheduled for multiple appointments to see different members of the healthcare team, the patients are scheduled on a day when he or she will be seen by all the members of the team. With strong collaboration with other subspecialties such as cardiology, gastroenterology, radiology, and even dentistry, we are able to make it convenient for patients to be evaluated, since most of the procedures that they will need can be scheduled for the same day.

This success has in part resulted in this transplant center being recognized and honored by the U.S. Department of Health & Human Services for better than expected outcomes in all transplant categories.

The St. Vincent program has transplanted kidneys in patients from numerous states. Patients come from as far away as Texas and California to be transplanted in our facility.

For published results, visit clevelandclinic.org/SRTReport.
Jihad H. Kaouk, MD

The incidence of renal cell carcinoma (RCC) has been rising globally during the past several decades. Primarily a disease of the seventh and eighth decades of life, RCC has the highest reported incidence—approximately 56 per 100,000—in people 75 to 84 years old. In addition, elderly patients tend to have more aggressive renal tumors compared with their younger counterparts. These realities highlight the need to obtain quality data on treatment type and outcomes in elderly patients with RCC.

Current guidelines issued by the American Urological Association for the treatment of stage 1 renal tumors identify nephron-sparing surgery (NSS) as the standard of care for the treatment of T1a and most T1b tumors. A recent meta-analysis on the management of small renal masses suggests that active surveillance is more likely to be adopted for elderly patients with a T1 renal mass compared with younger patients. Interestingly, a recent examination of active surveillance conducted in 172 patients with renal tumors showed a delayed intervention rate of 39 percent. Further evaluation of surgical treatments for T1 masses in the elderly is therefore required, because a large number of patients on active surveillance might require active treatment later. A few studies suggest that NSS can be performed with a low rate of perioperative morbidity and that it should remain the standard of care for elderly patients, with acceptable risk. The benefits of minimally invasive NSS, including faster convalescence and decreased postoperative narcotic use, make it an attractive option in patients with advanced age and numerous comorbidities.

**Table 1. Perioperative outcomes**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Age &lt; 70 y (n=38)</th>
<th>Age &gt; 70 y (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operative time (min)</td>
<td>180 (110-540)</td>
<td>197.5 (120-330)</td>
</tr>
<tr>
<td>WIT (min)</td>
<td>20 (7.5-35)</td>
<td>18 (0-36)</td>
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<tr>
<td>EBL (mL)</td>
<td>200 (50-1400)</td>
<td>200 (30-900)</td>
</tr>
<tr>
<td>Blood transfusions, n (%)</td>
<td>5 (13.5)</td>
<td>6 (15.8)</td>
</tr>
<tr>
<td>Conversions to open</td>
<td>2 (11.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Length of stay (days)</td>
<td>3 (3.5)</td>
<td>4 (3.5)</td>
</tr>
<tr>
<td>Positive surgical margin, n (%)</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Intraoperative complications, n (%)</td>
<td>3 (7.9)</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Postoperative complications, n (%)</td>
<td>8 (21.1)</td>
<td>12 (31.6)</td>
</tr>
<tr>
<td>Change in creatinine (mg/dL)</td>
<td>9.5 (-17.9 to 42.3)</td>
<td>18.6 (-34.9 to 84.7)</td>
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<td>1 mo</td>
<td>20.8 (-36.9 to -12.7)</td>
<td>12.7 (-28.4 to 28.3)</td>
</tr>
<tr>
<td>Overall causes of deaths</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Follow up (mo)</td>
<td>7.1 (4.6 to 9.9)</td>
<td>9.5 (1.9 to 14)</td>
</tr>
</tbody>
</table>

Values expressed as medians ± interquartile range unless otherwise specified.

Key Point:

Perioperative and postoperative outcomes appear similar between patients 70 years and older and those younger than 70 years who undergo robotic partial nephrectomy for small solitary renal masses. Older age therefore should not be viewed as a contraindication to robotic nephron-sparing surgery.

There is a paucity of outcomes data, however, with NSS in elderly patients, which prompted our analysis of perioperative outcomes of robotic partial nephrectomy (RPN) in a contemporary cohort of elderly patients at our institution. A retrospective review was performed of 250 consecutive patients who underwent RPN for a solitary renal tumor from June 2006 to May 2010. Outcomes were compared between a group of patients ≥70 years and a control group of patients <70 years matched for American Society of Anesthesiology (ASA) score, Charlson comorbidity index (CCI), preoperative creatinine level, glomerular filtration rate (GFR), and nephrometry score. All patients underwent preoperative cross-sectional imaging to evaluate tumor size, location, depth of invasion, and vascular anatomy. Cross-sectional imaging was also used to determine nephrometry score (RENAL score 4-6, 7-9, and 10-12) and clinical TNM staging. All cases were performed via transperitoneal approach using the da Vinci® Surgical System (Intuitive Surgical, Sunnyvale, Ca.) according to a previously described technique.
Table 2. Postoperative complications in this study population

<table>
<thead>
<tr>
<th>Group</th>
<th>Complication (n)</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt; 70 (n=38)</td>
<td>Urine leak (1)</td>
<td>Foley catheterization and drainage</td>
</tr>
<tr>
<td></td>
<td>Lymphatic leak (1)</td>
<td>Prolonged drainage</td>
</tr>
<tr>
<td></td>
<td>Ileus (1)</td>
<td>NPO and IV fluids</td>
</tr>
<tr>
<td></td>
<td>Acute renal failure (1)</td>
<td>Hydration</td>
</tr>
<tr>
<td></td>
<td>Bleeding (5)</td>
<td>Transfusion</td>
</tr>
<tr>
<td></td>
<td>Dyspnea (1)</td>
<td>Home oxygen therapy</td>
</tr>
<tr>
<td></td>
<td>Atrial fibrillation (1)</td>
<td>Pharmacologic control</td>
</tr>
<tr>
<td></td>
<td>Pulmonary embolism (1)</td>
<td>Anti-coagulation therapy + IVC filter*</td>
</tr>
<tr>
<td>Age &lt; 70 (n=38)</td>
<td>Atelectasis (1)</td>
<td>Incentive spirometry</td>
</tr>
<tr>
<td></td>
<td>Urinary retention (1)</td>
<td>Foley catheterization</td>
</tr>
<tr>
<td></td>
<td>Bleeding (4)</td>
<td>Transfusion</td>
</tr>
<tr>
<td></td>
<td>DVT (1)</td>
<td>IVC filter</td>
</tr>
<tr>
<td></td>
<td>Bleeding (1)</td>
<td>Angioembolization</td>
</tr>
</tbody>
</table>

NPO = nothing by mouth; IV = intravenous; IVC = inferior vena cava; DVT = deep vein thrombosis
*Patient developed bleeding secondary to anticoagulation therapy requiring angioembolization and IVC filter placement.

Median follow-up was 7.1 months (range 4.6-9.9 months) and 9.5 months (range 1.9-14 months) in the control and elderly groups, respectively (p=1.0).

Table 1 summarizes the perioperative outcomes. Median operative time was similar between groups (180 vs. 197.5 minutes, p = 0.80). No significant difference existed in median warm ischemia time (20 vs. 18.0 minutes, p = 0.76), estimated blood loss (200 vs. 200 ml, p = 0.20), and conversions to open nephrectomy (2 vs. 0, p = 0.12). Table 2 depicts postoperative complications. There was no difference in the rates of intraoperative complications between the groups. The length of hospital stay was similar between the two groups (p = 0.37). There was one positive surgical margin in one patient in the control group, whereas no positive surgical margin was found in the group of elderly patients. Each group had one patient with a recurrence of renal cancer.

Five deaths occurred overall, with a median time to death of 22 months. Three deaths occurred in the group of elderly patients: two from unknown causes and one secondary to cardiovascular complications. Two deaths occurred in the control group: one secondary to cancer and one caused by cardiovascular accident.

RPN offers a safe treatment option for patients 70 years and older with small renal masses, with no increase in perioperative and postoperative morbidity and mortality. Age alone cannot be regarded as a contraindication for robotic NSS. Further investigation is warranted to elucidate the actual role of RPN in the management of small renal masses in the elderly.

For references, please email the editor.
Is Robotic Partial Nephrectomy Safe for Morbidly Obese Patients?

Jihad H. Kaouk, MD

Obesity represents a major health problem in industrialized countries, where its prevalence has dramatically increased during the past two decades. Obese patients have a higher risk of developing renal cell carcinoma (RCC) and higher mortality from RCC than nonobese patients.

Renal surgery is steadily evolving toward nephron-sparing minimally invasive approaches. Despite a suggestion that the degree of obesity could be associated with increased difficulty during laparoscopic kidney surgery, similar or even better perioperative outcomes have been obtained with laparoscopic partial nephrectomy than with open partial nephrectomy in obese patients. Robotic partial nephrectomy (RPN) has recently been shown to be safe and feasible in obese patients, who trend toward greater blood loss and a longer duration of surgery and warm ischemia time.

The aim of the present study was to assess the impact of body mass index (BMI) on the surgical outcomes of patients undergoing RPN in a tertiary care institution.

Our ongoing institutional review board-approved, prospectively maintained database was used to identify the study population. The medical records of 250 consecutive patients treated with RPN from 2006 to 2010 at Cleveland Clinic were reviewed retrospectively. Patients were categorized into four groups according to their BMI, as follows: group 1, normal weight (BMI < 25 kg/m²); group 2, overweight (BMI 25–29.9 kg/m²); group 3, obese (BMI 30–39.9 kg/m²); and group 4, morbidly obese (BMI > 40 kg/m²).

The RPN followed our standard technique, which has been previously described elsewhere. In obese groups, extra padding is used for pressure points. The patient is placed in flank position at about 80° (vs. 45°–60° in patients with normal BMI). In obese patients, the trocars are placed more laterally, the double arm board is placed more superiorly to avoid clashing with robotic arms, and the use of longer trocars is adopted as needed (Figure).

Key Point:
With slight modification in trocar placement and patient positioning, the surgical outcomes of robotic partial nephrectomy are not compromised in morbidly obese patients.

Of the 250 patients, 43 (17.2 percent) were nonobese, 104 (41.6 percent) were overweight, 75 (30 percent) were obese, and 28 (11.2 percent) were morbidly obese. Morbidly obese patients had a higher American Society of Anesthesiologists (ASA) score than other groups (p = 0.002), and larger tumors, with a median of 3.6 cm (interquartile range [IQR]: 2.5–4.5), than those in the normal BMI (p = 0.003), obese (p = 0.01) and overweight groups (p = 0.01).
<table>
<thead>
<tr>
<th>Outcome</th>
<th>Normal (BMI &lt; 25 kg/m²) (n = 43)</th>
<th>Overweight (BMI ≥ 25 to &lt; 30 kg/m²) (n = 104)</th>
<th>Obese (BMI ≥ 30 to &lt; 40 kg/m²) (n = 75)</th>
<th>Morbidly obese (BMI ≥ 40 kg/m²) (n = 28)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (IQR) EBL, mL</td>
<td>150 (100–200)</td>
<td>200 (100–300)</td>
<td>200 (100–350)</td>
<td>250 (150–375)</td>
<td>0.1</td>
</tr>
<tr>
<td>Median (IQR) operation duration, (min)</td>
<td>180 (140–200)</td>
<td>180 (150–210)</td>
<td>180 (150–210)</td>
<td>200 (180–240)</td>
<td>0.1</td>
</tr>
<tr>
<td>Median (IQR) WIT, (min)</td>
<td>17 (14–24)</td>
<td>17.5 (13.3–23.8)</td>
<td>19 (12–26)</td>
<td>20 (16–26)</td>
<td>0.5</td>
</tr>
<tr>
<td>Any postoperative complications (n [%])</td>
<td>9 (21)</td>
<td>27 (26)</td>
<td>27 (36)</td>
<td>11 (39)</td>
<td>0.2</td>
</tr>
<tr>
<td>Postoperative transfusion n (%)</td>
<td>5 (12)</td>
<td>9 (9)</td>
<td>8 (11)</td>
<td>5 (18)</td>
<td>0.6</td>
</tr>
<tr>
<td>Mean (SD) change in GFR (latest – preoperative)</td>
<td>−9.8 (19.8)</td>
<td>−7.7 (15.6)</td>
<td>−6.7 (16.6)</td>
<td>−11.5 (15.8)</td>
<td>0.6</td>
</tr>
<tr>
<td>Median (IQR) hospital stay, days</td>
<td>3 (2.8–4.0)</td>
<td>3 (3–4)</td>
<td>3 (3–4)</td>
<td>4 (3–4.3)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

There was no significant difference in log (operation duration) between BMI categories, after adjustment for age, ASA score, Charlson comorbidity index, tumor size and nephrometry score. Estimated blood loss was higher in morbidly obese patients than in all other groups (mean: 250 ml vs. 150 ml, 200 ml, and 200 ml for normal weight, overweight and obese patients, respectively, p = 0.04). Tumor size also contributed to increased blood loss (p = 0.002). Warm ischemia time was not significantly higher with increased BMI (p = 0.8). Mean hospital stay was longer in the morbidly obese group, at four (IQR: 3–4.3) days, than in all other groups (mean = 3 days), although this association did not reach statistical significance (p = 0.4). Increase in BMI was not significantly associated with the occurrence of postoperative complications (p = 0.3), while increased tumor size (p = 0.004) and longer operation duration (p = 0.001) were correlated with the occurrence of complications on multivariate analysis. There was no significant association between changes in renal function among groups (p = 0.3).

In a tertiary referral center, surgical outcomes of RPN in obese and morbidly obese individuals are similar to those in normal-weight individuals. Thus, for patients with BMI up to 60 kg/m², RPN represents a viable treatment option for renal masses amenable to nephron-sparing surgery.

For references, please email the editor or Dr. Kaouk at kaoukj@ccf.org.
Transvaginal Hybrid Natural Orifice Transluminal Surgery (NOTES) Robotic Donor Nephrectomy: First Clinical Application

Jihad H. Kaouk, MD, Ali Khalifeh, MD, Humberto Laydner, MD, Riccardo Autorino, MD, Kamol Panumatrassamee, MD, Charles Modlin, MD, and Howard Goldman, MD

Laparoscopic living donor nephrectomy (LDN) with vaginal extraction has the potential to reduce postoperative pain. The transvaginal route also allows insertion of instrumentation and significant steps of the procedure to be performed within the realm of natural orifice transluminal surgery (NOTES)-assisted laparoscopic techniques.

Our group first reported the successful completion of a completely transvaginal NOTES nephrectomy in 2010. By integrating previous experience gained in laparoscopic single-site surgery (LESS), NOTES and robotics, we conceptualized a transvaginal hybrid NOTES robotic approach for LDN and performed the first clinical case with this novel, minimally invasive approach.

An overall healthy 61-year-old female volunteered to donate her kidney to her brother. Her baseline estimated glomerular filtration rate (eGFR) was 94 mL/min/1.73 m², and she had equal bilateral kidney function according to the renal scan. Specific institutional review board approval was obtained prior to the procedure. Donor and recipient were both thoroughly informed about the technique and its presumed risks, and consented to the surgery.

Commercially available instruments and the da Vinci Si™ (Intuitive Surgical, Sunnyvale, Calif.) robotic platform were used. The patient was given one 500-mg dose of metronidazole and 1.5 g of cefuroxime intravenously after induction of general anesthesia. She was placed in a modified right decubitus position, with legs abducted, and the left hip minimally externally rotated, resting both legs on stirrups to maximally expose the genital area and to minimize anticipated clashing (Figure 1).

Through a 4-cm intra-umbilical incision, the abdominal cavity was entered using the Hasson technique. An 8-mm robotic trocar was tunneled through a fascial incision in the superior aspect of the abdominal wound. To this port, the left robotic arm was docked. A SILSTM (Covidien, Mansfield, Mass.) port was also inserted through the fascia, accommodating one 12-mm trocar through which the robotic scope was inserted as well as two 5-mm trocars for assistance. Pneumoperitoneum was achieved at 15 mm Hg.

Using a 30 degree down scope placed through the 12-mm umbilical trocar, the pelvic pathway was inspected. A sponge on a stick was positioned in the posterior vaginal fornix. With the aid of a cystoscope to observe the base of the bladder, the vaginal apex was infiltrated with a local anesthetic and a 2.5 cm transverse incision was made. Through this incision, the rectum was dissected bluntly off the posterior vaginal wall, simultaneous with a rectal examination to avoid any injury. The peritoneum was then grasped and penetrated with a confirmatory air leak. Two stay sutures were placed at both corners of the vaginal incision that was extended to 3 cm, and a GelPOINT™ platform (Applied Medical Resources Corp., Rancho Santa Margarita, Calif.) was placed properly with a 12-mm bariatric port within it. An 8-mm robotic port was placed within the bariatric vaginal port, and the right robot arm was docked.

Monopolar robotic curved scissors were introduced through the vaginal port, while the ProGrasp™ forceps (Intuitive Surgical, Sunnyvale, Calif.) was inserted through the umbilical port. A laparoscopic suction was used through the SILS port by the bedside assistant.

Once the colon was reflected, the spleen was dissected off the upper pole of the left kidney and the pancreatic tail mobilized medially. Then, the left gonadal vein and ureter were identified at the level of the left common iliac artery. With the assistant gently retracting both the ureter and gonadal vein laterally, the plane up toward the renal hilum was developed. At the level of the hilum, the main renal artery and vein were identified and skeletonized. In addition to two lumbar veins, a large left gonadal vein and left adrenal vein were double clipped and divided, allowing better exposure of the main renal artery that was circumferentially dissected.

At this point, the right and left robotic instruments were switched to permit a better angle to peel the adrenal gland off the left kidney upper pole, followed by releasing the kidney from its lateral and posterior attachments.

By alternating the scissors with the robotic Hem-o-Lok® clip applier (Teleflex Medical, Research Triangle Park, N.C.) through the vaginal port, the ureter and the gonadal vein were clipped and transected at the iliac crossing level.

As the kidney was completely mobilized except for its renal artery and vein attachments, the robot was undocked, and a 15-mm Endo Catch™ bag (Covidien, Mansfield, Mass.) was brought in through the vaginal GelPOINT. The kidney was placed into the Endo Catch bag from lateral to medial and
the bag partially closed over the kidney except for the renal hilum. Mannitol was administered intravenously. The umbilical robotic port was replaced with a 12-mm laparoscopic port, which was used to introduce the vascular Endo GIA™ stapler (Coviden, Mansfield, Mass.). Without modifying the patient’s position, the renal artery was stapled first, followed by the renal vein (Figure 2). The kidney was then delivered within the Endo Catch bag through the vagina under laparoscopic vision. Upon delivery, the kidney was collected by the transplant team. The main renal artery and vein lengths were 3.5 cm and 3.0 cm, respectively.

Following removal of the GelPOINT, the colpotomy was closed transvaginally. The abdomen was checked for hemostasis after removal of ports and trocars, and the incisions were closed.

The procedure was successfully completed without intraoperative complications. Overall operative time was 240 minutes. Blood loss was minimal. The umbilical incision measured 4 cm, mostly included in the umbilical groove. On the first postoperative day, the patient resumed oral intake and ambulation. She was deemed suitable for discharge after an uneventful 48-hour hospital stay with no postoperative complications and an eGFR of 61 mL/min/1.73 m².

The graft had delayed function due to biopsy-proven acute graft rejection, which was managed by administration of pulse steroids. The recipient was discharged 14 days postoperatively, without signs of infection.

The transvaginal hybrid NOTES robotic LDN was successfully performed without complications. Clashing of equipment and difficult working angles experienced during the standard LESS approach were overcome by placing the second robotic working arm through the vaginal port. The robotic platform allowed simultaneous control of two surgical instruments that were inserted a significant distance apart.

Preoperative, intraoperative and postoperative steps were managed to minimize any potential risks. The vaginal extraction of the graft did not cause infection to the donor or the recipient. The donor recovered rapidly postoperatively, and resumption of sexual activity was permitted at four weeks without dyspareunia. At three-month follow-up, both recipient and donor had a normal eGFR.

In conclusion, transvaginal hybrid NOTES robotic LDN is feasible and can be safely completed. Advances in purpose-built instrumentation may further develop this approach.

For references, please email the editor.
Robotic Partial Nephrectomy for Multiple Tumors in the Same Kidney

Humberto Laydner, MD, and Jihad H. Kaouk, MD

Multifocality of renal cell carcinoma can be defined by the presence of two or more tumoral foci in the same kidney, separated by a strip of normal tissue. The incidence of multifocal renal tumors ranges from 4.3 to 25 percent. Since its development, nephron-sparing surgery has increasingly gained acceptance, showing oncological outcomes similar to those obtained with radical nephrectomy. Currently, nephron-sparing surgery is considered in many major guidelines as the primary treatment modality in the management of small renal tumors.

Since the first robot-assisted partial nephrectomy (RPN) was described in 2004, there has been an exponential growth in the use of RPN, as reported in the literature. The advantages of the EndoWrist® (Intuitive Surgical, Sunnyvale, Calif.) coupled with increasing surgeon experience have led to the use of RPN for complex cases. Herein, we describe our experience with RPN for patients with two or more ipsilateral tumors, by reporting surgical technique and outcomes.

We reviewed our institutional review board-approved database and the charts of eight consecutive patients with ipsilateral multifocal kidney tumors who underwent RPN at our institution between April 2007 and July 2010. We reviewed data from imaging, operative, anesthesiology and pathology reports, as well as from the discharge and laboratory records. Estimated glomerular filtration rate (eGFR) was calculated using the abbreviated Modification of Diet in Renal Disease study equation. Complications were evaluated using the Clavien classification system.

Our surgical technique for RPN has been described previously. Usually, the artery and vein were both clamped individually with bulldog clamps (Figure). If the hilum had a complex anatomy with multiple branches, however, or if for any reason the hilar dissection was exceedingly difficult, a Satinsky clamp was used and the hilum was then clamped en bloc. Tumors that were mostly exophytic with minimal cortical involvement were resected without hilar clamping, using Hem-o-lok clips to control bleeding vessels from the parenchyma.

Eight patients were subjected to nine procedures (one patient underwent bilateral RPN) and had a total of 19 tumors removed. Seventy-five percent of the patients were men, and the mean patient age was 67 ± 11 years (median 69 years; range 44–83 years). Twelve masses were on the right side in six patients, and seven masses were on the left side in three patients. Mean operative time was 199 ± 47 min (median 200 min; range 150–300 min). All patients had at least two or more tumors removed. Mean size of the dominant lesion was 3.0 ± 1.1 cm (median 2.7 cm; range 1.6–4.8 cm), and overall mean tumor size was 2.2 ± 1.2 cm (median 1.9 cm; range 0.4–4.8 cm). Six of 19 tumors did not require hilar clamping.

Excluding the off-clamp procedures, mean warm ischemia time was 21 ± 9.2 min (median 21 min; range 10–35 min). Mean estimated blood loss was 250 ± 154 mL (median 200 mL; range 100–500 mL), and no patient required blood transfusion. There were no intraoperative complications and no conversions to pure laparoscopic or open surgery. In the postoperative period, one case of atrial fibrillation was successfully managed with anti-arrhythmic drugs. The patient did not require transfer to the intensive care unit and was discharged home on the fifth postoperative day.

Mean length of stay was 4.2 ± 1.0 days (median 5 days; range 3–5 days). Table 1 lists intraoperative and postoperative outcomes. Mean follow-up was 14 months. Of the 19 resected tumors, 16 were renal cell carcinomas on final histopathological reports. All tumors had negative margins. Table 2 details renal function outcomes. The eGFR was decreased by an average of 4 percent, which translates into an optimal preservation of the baseline renal function and favorably compares to reported laparoscopic series. In this regard, it can be speculated that the easier dissecting, cutting and suturing maneuvers allowed by the robotic platform could possibly have a role in these findings in this particularly challenging subgroup of patients.

For references, please email the editor or Dr. Kaouk at kaoukj@ccf.org.
Table 1. Intraoperative and postoperative outcomes

<table>
<thead>
<tr>
<th>Intraoperative</th>
<th>Postoperative</th>
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<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>Patient</td>
<td>No. of tumors</td>
</tr>
<tr>
<td>1</td>
<td>R</td>
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<tr>
<td>L</td>
<td>3</td>
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<td>7</td>
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</table>

*Grade II according to Clavien classification. WIT = warm ischaemia time; EBL = estimated blood loss; PRBC = packed red blood cells; LOS = length of stay

Table 2. Renal function outcomes

<table>
<thead>
<tr>
<th>Patient</th>
<th>Preoperative creatinine (mg/dL)</th>
<th>6-month postoperative creatinine (mg/dL)</th>
<th>Preoperative eGFR (mL/min/1.73m²)</th>
<th>6-month postoperative eGFR (mL/min/1.73m²)</th>
<th>Variation of eGFR (%)</th>
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<tbody>
<tr>
<td>1</td>
<td></td>
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<td></td>
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<tr>
<td>R</td>
<td>0.96</td>
<td>1.09</td>
<td>82.8</td>
<td>71.5</td>
<td>-11.3</td>
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<tr>
<td>L</td>
<td>1.27</td>
<td>1.31</td>
<td>59.8</td>
<td>57.7</td>
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</tr>
<tr>
<td>2</td>
<td>0.74</td>
<td>1.04</td>
<td>90.6</td>
<td>99.9</td>
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</tr>
<tr>
<td>3</td>
<td>0.79</td>
<td>0.88</td>
<td>91.5</td>
<td>80.8</td>
<td>-10.7</td>
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</tbody>
</table>

eGFR = estimated glomerular filtration rate

Jihad H. Kaouk, MD, Riccardo Autorino, MD, and Humberto Laydner, MD

More than 1,000 laparoendoscopic single-site surgery (LESS) procedures have been reported in urology. However, limitations such as restricted triangulation, clashing of instruments and ergonomic discomfort may prevent wider adoption of this technique. The application of the da Vinci Surgical System to LESS helps to overcome some of these drawbacks, but the current robotic system and instruments were not originally designed for this specific application. Our group previously reported the results of experimental procedures in the porcine model testing the first generation of single-site robotic instruments, developed specifically for robotic LESS (R-LESS). The single-site instruments and accessories are composed of the port, curved cannulae and 5-mm semirigid instruments. They were designed to reduce external collisions and instrument crowding. Here we describe our early experience with the second generation of single-site instruments for R-LESS for kidney procedures in a cadaver model.

The multichannel port (redesigned to correct defects of the first generation) contains one insufflator channel, one straight channel for the 30-degree down 8.5-mm scope, a 12-mm channel for the assistant port, and two curved tunnels to specifically fit the crossing, curved cannulae for the robotic instruments (Figure 1A-C). These cannulae are

Key Point:
A variety of extirpative and reconstructive kidney procedures are feasible in the cadaver model using a purpose-built set of instruments for single-site robotic surgery. The main features of this novel instrumentation are the restored intra-abdominal triangulation and the avoidance of external clashing, while the lack of wrist articulation represents the main limitation currently.

Figure 1. (A-C) Multichannel port with trocars and instruments; (D) first- (longer) and second- (shorter) generation single-site trocars; (E) single-site instruments; (F) multichannel port inserted in the umbilicus

Figure 2. The assembled device
shorter than ones used previously (35 vs. 55 mm), which makes them more suitable for urologic single-site surgery (Figure 1D). The 5-mm instruments include Resano and Cadiere forceps, curved scissors, clip applier, needle driver, Maryland bipolar forceps, cautery hook, and suction irrigator (Figure 1E).

**Technique**

A 2.5-cm transumbilical incision was made to allow the insertion of the port. A lower abdominal standard port was also inserted, for recording purposes only (Figure 1F). With the cadaver in the flank position, left side up, the robot was docked with the camera oriented approximately in line with the target organ. Although the robotic instruments were crossed at the entrance site, the instruments were automatically reassigned by the system software, so that the surgeon’s right hand would control the right-hand instrument and the same would apply for the left side (Figure 2).

**Pyeloplasty:** The left ureter was transected at the ureteropelvic junction and then spatulated. A running suture previously placed through the assistant port was used for the ureteropelvic anastomosis (Figure 3A).

**Partial Nephrectomy:** The hilum was prepared for clamping, simulating resection under warm ischemia. The left kidney was defatted, and the resection margin was scored with electrocautery. Hilar occlusion was obtained by using a vessel loop tourniquet secured with a Hem-o-lok clip. Lower pole partial nephrectomy was performed (Figure 3B-D). Renorrhaphy was accomplished using a sliding clip technique.

**Nephrectomy:** The renal artery and vein were controlled using the robotic clip applier and divided with robotic scissors. The kidney was dissected away from its attachments and the specimen was extracted in an Endo Catch bag.

At the completion of the procedures, the port was removed and the robot was undocked. A laparotomy was performed to examine for any visceral injuries that may have gone unnoticed.

**Surgical Outcomes**

Time for setup, including positioning, multichannel port insertion, robot docking and insertion of instruments, was 40 minutes. Three left-side procedures were completed successfully without the addition of extra ports. Time to complete the ureteropelvic anastomosis during pyeloplasty was 39 minutes. For partial nephrectomy, simulated warm ischemia time was 21 minutes. For nephrectomy, time to complete the procedure was 13 minutes. No tearing of the multichannel port and no significant gas leakage were noticed during the entire time of the procedures. No injuries to intra-abdominal organs or vessels occurred.

We demonstrated the feasibility of robotic kidney procedures using second-generation robotic instruments designed for R-LESS in a cadaver model. Although the favorable ergonomics provided by these instruments may safely enable R-LESS for extirpative procedures by more surgeons, the lack of wrist articulation is an obstacle for complex reconstructive procedures.

For references, please email the editor or Dr. Kaouk at kaoukj@ccf.org.
Urologic Laparoendoscopic Single-Site (LESS) Surgery: Recent Advances

Riccardo Autorino, MD, and Jihad H. Kaouk, MD

The concept of laparoendoscopic single-site (LESS) surgery was introduced into urology five years ago. Soon after, we reported a large series showing the feasibility of LESS across almost the entire spectrum of urological procedures. Although the application of robotics to LESS (R-LESS) was expected to overcome the limitations of the technique, the benefits of R-LESS have yet to be demonstrated. Recent advances in LESS in urologic surgery, however, appear to have improved complication rates associated with LESS and expanded its indications.

Novel Instrumentation

Intuitive Surgical Inc. has developed a novel set of instruments and accessories specifically dedicated to LESS (Figure). The set includes a multichannel access port with room for four cannulae and an insufflation valve. Two curved cannulae have been developed for robotically controlled instruments. The other two cannulae are straight; one is 8.5 mm and accommodates the high-definition 3-D endoscope, and the other is a 5-mm bedside-assistant surgeon port. Triangulation is achieved by crossing the curved cannulae midway through the access port. Same-sided hand-eye control of the instruments is maintained through assignment of the da Vinci Si system software.

The second part of the platform is a set of semirigid, non-wristed instruments based with standard da Vinci instrument tips. The semirigid flexible shaft allows for insertion down the curved cannula and triangulation of the anatomy. Robotic arm collisions are minimized externally because the curved cannulae angle the robotic arms away from each other. The single-site instruments and accessories do not have a wrist at the distal end of the instrument. Our group described the first laboratory experience with Intuitive Surgical’s ViSPA robotic instruments for urologic applications in a porcine model.

More recently, we reported the use of a second generation of the da Vinci single-site instruments for R-LESS to perform different kidney procedures in the cadaver model. Three types of left-side kidney procedures were performed (one pyeloplasty, one partial nephrectomy and one nephrectomy). The procedures were completed successfully without the addition of more ports.

Key Point:

Outcomes with laparoendoscopic single-site (LESS) surgery demonstrate that a broad range of procedures can be performed effectively and safely, given the physician’s solid laparoscopic surgical background and stringent patient selection criteria. The recent introduction of purpose-built instrumentation is likely to further foster the application of robotics to LESS. Further improvements are needed before this technique can be widely adopted.

Outcomes of Urologic LESS

Worldwide experience

A recent collaborative analysis led by Cleveland Clinic’s Center for Laparoscopic and Robotic Surgery aimed to provide an overview of indications, techniques and outcomes of urologic LESS in various hospital settings worldwide. Overall, 1,076 consecutive procedures done between 2007 and 2010 at 18 participating institutions were included in this analysis. The most common procedures were extirpative or ablative operations in the upper urinary tract. The da Vinci robot was used to operate on 143 patients (13 percent). This study provides a global view of the evolution of LESS in the field of urologic surgery by showing that a broad range of procedures has been performed effectively, primarily in the academic setting, and that when LESS is performed by experienced laparoscopic surgeons, the risk of complications remains low when stringent patient selection criteria are applied.

Complications

From the already mentioned worldwide multi-institutional project, a detailed analysis of the incidence of and risk factors for complications and conversion of urologic LESS was reported. Included in the analysis were 1,163 cases. Intraoperative complications occurred in 3.3 percent of cases. The overall conversion rate was 19.6 percent, with 14.6 percent, 4 percent and 1.1 percent of procedures converted to reduced port laparoscopy, conventional laparoscopic/robotic surgery and open surgery, respectively. On multivariable analysis, the factors significantly associated with the risk of conversion were oncologic surgical indication (p = 0.02), pelvic surgery (p < 0.001), robotic approach (p < 0.001), high difficulty score (p = 0.004), extended operative time (p = 0.03) and an intraoperative complication (p < 0.001). A total of 120 postoperative complications occurred in 109 patients (9.4 percent), with major complications in only 2.4 percent of the entire cohort. Reconstructive procedure (p = 0.03), high difficulty score (p = 0.002) and extended operative time (p = 0.02) predicted high-grade complications. The authors concluded that urologic LESS can be performed with a low complication rate, resembling that in laparoscopic series.
Advanced Indications for LESS
Increasing experience and the proven safety and feasibility of LESS have allowed for the expansion of indications to include complex reconstructive procedures. Recently, our group reported intermediate-term outcomes for patients undergoing reconstructive LESS procedures. Median follow-up was 24.4 months for pyeloplasty, 35 months for ileal interposition, 29.4 months for ureteroneocystostomy and 20 months for retrocaval ureter repair. At last follow-up, 24 of 25 patients treated with pyeloplasty, two of three with ileal ureter, three of three with ureteroneocystostomy and one of one with retrocaval ureter reported being asymptomatic or improved after the procedure.

Robotic LESS
The amount of clinical outcomes data with R-LESS has grown considerably. Our group detailed the technique of R-LESS radical nephrectomy and reported the comparative outcomes vs. the current gold standard laparoscopic procedure. There was no difference between R-LESS and conventional laparoscopy in terms of operative time, estimated blood loss, visual analogue scale score, or complication rate. The R-LESS group had a lower median narcotic requirement during hospital admission (25.3 vs. 37.5 morphine equivalents; p = 0.049) and a shorter length of stay (2.5 vs. 3 days; p = 0.03). Study limitations included the small sample size, short follow-up period, and the retrospective study design.

When first reporting an initial feasibility study of LESS radical prostatectomy in humans, we acknowledged the limitation of embarking on this procedure due to challenges related to ergonomics and intracorporeal suturing. More recently, we detailed the surgical technique and reported the outcomes of 20 R-LESS radical prostatectomies. Steps of the procedures resembled those of standard robotic procedures. A positive margin was found in four cases, two of them being in the first three cases, which supports a learning curve. The limited follow-up did not allow a reliable oncologic assessment, but early postoperative continence rates were encouraging.

We also reported our R-LESS cumulative experience. Overall, 50 patients were scheduled to undergo urologic R-LESS during the study period, representing 36 percent of the total patients undergoing LESS. Specifically, 24 patients underwent renal surgery and 26 underwent pelvic surgery.

Conclusions
Significant advances have been achieved in the field of urologic LESS since the first reported clinical series in 2007. Despite unresolved challenges, LESS can be regarded as an emerging trend in minimally invasive urologic surgery, and it has evolved significantly, becoming a widely applicable technique in a relatively short time.

For references, please email the editor.
Robot-Assisted Laparoscopic Partial Nephrectomy: Step-by-Step Contemporary Technique

Jihad H. Kaouk, MD, and Riccardo Autorino, MD

A robot-assisted laparoscopic partial nephrectomy (RALPN) program was started in 2006 at our institution, and it has been consistently implemented since then. Herein we describe our contemporary surgical technique.

Surgical Technique

The da Vinci Surgical System is generally used in a three-arm configuration. The patient is positioned in a modified flank position at approximately 60 degrees. Pressure points are carefully padded with pillows and foam pads, and the patient is secured to the table with tape. The surgical table is mildly flexed and positioned in a slight Trendelenburg position.

A transperitoneal approach is used. A similar port configuration is used for both right and left sides. The abdomen is insufflated to 15 mm Hg with a Veress needle at the lateral border of the rectus muscle across from the 12th rib. This serves later as the site for a 12-mm port through which the robot scope is inserted. An 8-mm robot port is placed at the lateral border of the ipsilateral rectus muscle, about 3 cm below the costal margin. A second 8-mm robot port is placed about 5-7 cm cephalad to the anterior superior iliac spine. An assistant 12-mm port is placed along the lateral border of the rectus muscle in the lower abdominal quadrant. On the right side, an additional 5-mm port is placed in the subxiphoid area to retract the liver.

For upper pole tumors, all the ports can be shifted 1-2 cm cephalad. Moreover, an extra 5-mm assistant port can be placed between the camera and the right robotic port to allow the assistant better access to the operative field.

The robot is positioned over the patient’s shoulder in order to have the camera oriented in line with the kidney. On the right side, liver retraction is obtained by introducing a locking Allis clamp through the 5-mm subxiphoid port. With monopolar curved Endo Wrist® (Hot Shears™, Intuitive Surgical, Sunnyvale, Calif.) scissors in the right hand and a ProGrasp forceps in the left hand, the surgeon sharply cuts the peritoneum along the white line of Toldt. The bowel is mobilized medially, developing a plane anterior to Gerota’s fascia and posterior to the mesocolon by using both sharp and blunt dissection. Attachments to the spleen or liver are released as necessary.

The gonadal vein is an important anatomical landmark when proceeding toward the renal hilum. On the right side, the gonadal vein is kept medially towards the vena cava, whereas on the left side the gonadal vein is lifted along with the left ureter to expose the lower margin of the left renal hilum.

Dissection proceeds along the psoas muscle, with anterior elevation of the ureter and/or gonadal vein to allow identification of the renal hilum (Figure 1). The renal vein can be identified by tracing the gonadal vein proximally to its insertion in the renal vein on the left side or to its insertion in the inferior vena cava just caudal to the hilum on the right side. A flexible robotic Doppler probe can be used to identify hilar vessels before clamping, especially in cases involving multiple renal arteries or early branching. The main hilar vessels are circumferentially dissected to allow adequate clamping.

Key Point:

The standardization of each surgical step of our contemporary technique of robot-assisted laparoscopic partial nephrectomy has allowed optimization of the procedure. The robotic approach offers the option of minimally invasive nephron-sparing surgery, which is likely to duplicate the safety and effectiveness of the current open reference technique.
Gerota’s fascia is opened in an area far from the tumor to find the capsule, and dissection is performed along the renal capsule until the mass is exposed. The fat is then cleared circumferentially around the mass, allowing for visualization of 1-2 cm of normal parenchyma for future renal reconstruction. A laparoscopic ultrasound probe, which is introduced, held and maneuvered manually by the surgical bedside assistant, is used to plan the excision margins by allowing accurate identification of the location, depth and borders of the tumor. A recently introduced drop-in flexible ultrasound probe was specifically developed for robotic surgery, and it can be directly controlled by the console surgeon by grasping a notch on its ventral aspect. Live intraoperative images are displayed as a picture-on-picture display on the console screen using the TilePro™ (Intuitive Surgical, Sunnyvale, Calif.) functionality. Margins of resection of the renal capsule are scored with cautery to delineate the resection boundaries.

Before hilar occlusion, 12.5 g of mannitol is given intravenously to aid in renal protection by facilitating osmotic diuresis. Hilar clamping can be performed using either laparoscopic bulldog clamps or a Satinsky clamp. If bulldog clamps are used, the renal artery is clamped first, followed by the vein. In selected cases, resection may be performed by clamping the renal artery only. Recently, robotic bulldog clamps, applied by the console surgeon using the robotic ProGrasp forceps, have become available. The hilum is occluded and the tumor resected along the previously scored margin using cold scissors (Figure 2).

Renorrhaphy is performed in two layers using robotic needle drivers. A 20-cm 2-0 Vicryl™ (Ethicon, Somerville, N.J.) suture on an SH-1 needle with a knot and Hem-o-lok clip to the free end is used as a running suture of the tumor excision bed to oversew larger vessels as well as entries into the collecting system. The suture is brought through the renal capsule with the final throw and secured with two sliding Hem-o-lok clips. The renal capsule is reapproximated using a continuous, horizontal mattress 0 Vicryl suture on a CT-1 needle with a sliding Hem-o-lok clip placed after each suture passed through the capsule (Figure 3).

After the completion of renorrhaphy, the hilum is unclamped, and the renal excision bed is inspected for hemostasis with pneumoperitoneum pressure lowered. Whenever possible, the hilum is unclamped before capsular suturing in an early unclamping technique to minimize warm ischemia time.

The defect can be covered with oxidized cellulose and a fibrin sealant. Gerota’s fascia is closed by using Hem-o-lok clips. A laparoscopic entrapment sac is introduced by the assistant; the specimen is placed in it and removed from an extended lower quadrant port site. All 12-mm incisions are closed with 0 Vicryl suture by using the Carter-Thomason device. A Jackson-Pratt drain is placed through a lower lateral port.

Intravenous fluids, analgesics, antibiotics and prophylaxis for deep vein thrombosis are given as per institutional protocol. Hemoglobin levels and hematocrit are monitored in the postoperative period. The Foley catheter and drain are usually removed on the morning after surgery, ambulation is encouraged and the diet is advanced.

(For outcomes obtained with RALPN at Cleveland Clinic, see part two of this article on page 28.)

For references, please email the editor.
We describe Cleveland Clinic’s cumulative surgical outcomes associated with robot-assisted laparoscopic partial nephrectomy (RALPN) at our high-volume tertiary center. Medical charts of consecutive patients who underwent RALPN at Cleveland Clinic between June 2006 and November 2011 were reviewed from a prospectively maintained, institutional review board–approved database. (A description of the technique appears in part one of this article on page 26.)

A total of 400 patients were included in this analysis (Table 1). Mean age was 58.5 (± 11.9) years, body mass index was 30.7 (± 7.2) kg/m2, and the mean American Society of Anesthesiologists score was 2.5 (± 0.5). Based on preoperative imaging, mean tumor size was 3.17 (± 1.64) cm, and the mean RENAL score was 7.2 (± 2). In six cases (1.5 percent), the patient presented with a solitary kidney, and in 247 cases (62.2 percent) there was a history of previous abdominal or pelvic surgery.

Perioperative outcomes are summarized in Table 2. Mean warm ischemia time was 19.2 (± 10.72) minutes. In 38 cases an unclamped technique was used (9.5 percent of cases). After a mean follow-up of 12.4 months (± 12.2), there was a decline of 9.2 (± 26.56) mL/min/1.73 m2 in the estimated glomerular filtration rate. The tumor pathology is detailed in Table 3. Most (74.5 percent) of the masses were malignant. Renal cell carcinoma with a clear cell histology represented the most frequent malignant diagnosis (64.4 percent of cases). In most of the cases the tumor stage was T1a or T1b. A positive margin was observed in nine cases (2.25 percent), one of them being in a case of angiomyolipoma.

A total of 11 intraoperative complications (2.7 percent) occurred. Overall, in eight cases the procedure was not completed as planned, due to conversion to open or laparoscopic partial nephrectomy (six cases; 1.5 percent) or to robotic radical nephrectomy (one case; 0.25 percent).

### Table 1. Demographics of the study cohort

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<th>Patients, n</th>
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<tr>
<td>Mean (SD)</td>
<td>58.5 (11.9)</td>
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<td>Median (IQR)</td>
<td>59 (51-67)</td>
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<tr>
<td>Gender, n (%)</td>
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</tr>
<tr>
<td>Male</td>
<td>233 (58)</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
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<tr>
<td>Mean (SD)</td>
<td>30.7 (7.2)</td>
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<td>29.2 (25.8-34)</td>
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<td>Mean (SD)</td>
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<td>Solitary kidney, n (%)</td>
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<td>6 (1.5)</td>
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<td>Previous abdominal surgery, n (%)</td>
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<td>248 (62)</td>
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<td>Preoperative creatinine, mg/dL</td>
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<tr>
<td>Mean (SD)</td>
<td>0.96 (0.3)</td>
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<tr>
<td>Median (IQR)</td>
<td>0.9 (0.7-1.1)</td>
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<tr>
<td>Preoperative eGFR, mL/min/1.73m2</td>
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<td>Mean (SD)</td>
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<td>Median (IQR)</td>
<td>85.16 (69.8-97.5)</td>
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<td>Tumor size, cm</td>
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<td>Mean (SD)</td>
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<tr>
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<td>Tumor side, n (%)</td>
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<td>Mean (SD)</td>
<td>7.2 (2.0)</td>
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<tr>
<td>Median (IQR)</td>
<td>7 (5-9)</td>
</tr>
</tbody>
</table>

BMI = body mass index; ASA = American Society of Anesthesiologists; CCI = Charlson comorbidity index; eGFR = estimated glomerular filtration rate

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**Key Point:**

The standardization of each surgical step of our contemporary technique of robot-assisted laparoscopic partial nephrectomy has resulted in an improvement in perioperative, intraoperative and postoperative outcomes at Cleveland Clinic.
A postoperative complication occurred in 61 cases (15.3 percent). The majority of these complications were of low grade, with major (grade 3 and 4) ones occurring only in 3.2 percent of the entire cohort. There were no cases of multiple-organ dysfunction (grade 4b) or death (grade 5).

Notably, this represents the largest single-center RALPN series to date. The standardization of each surgical step has allowed optimization of the procedure and, ultimately, an improvement of its outcomes. The robotic approach offers the option of minimally invasive nephron-sparing surgery, which is likely to duplicate the safety and effectiveness of the current open reference technique.

For references, please email the editor.

### Table 2. Perioperative outcomes

<table>
<thead>
<tr>
<th>Intraoperative variables</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
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<tbody>
<tr>
<td>Operative time, min</td>
<td>190.3 (57)</td>
<td>180 (150-210)</td>
</tr>
<tr>
<td>Unclamped, n (%)</td>
<td>38 (9.5)</td>
<td></td>
</tr>
<tr>
<td>Early unclamping, n (%)</td>
<td>80 (20)</td>
<td></td>
</tr>
<tr>
<td>Warm ischemia time, min</td>
<td>19.2 (10.72)</td>
<td>19 (14-25)</td>
</tr>
<tr>
<td>Estimated blood loss, mL</td>
<td>260.2 (277.2)</td>
<td>200 (100-300)</td>
</tr>
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</table>

### Table 3. Pathology

<table>
<thead>
<tr>
<th>All patients</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant, n (%)</td>
<td>298 (74.5)</td>
</tr>
<tr>
<td>Benign, n (%)</td>
<td>102 (25.5)</td>
</tr>
<tr>
<td>Pathologic tumor size, cm Mean (SD)</td>
<td>3.05 (1.66)</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>2.6 (1.9-3.9)</td>
</tr>
<tr>
<td>Positive surgical margins, n (%)</td>
<td>9 (2.25)</td>
</tr>
<tr>
<td>Malignant</td>
<td>n (%)</td>
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<tr>
<td>RCC, clear cell</td>
<td>192 (64.4)</td>
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<tr>
<td>RCC, papillary</td>
<td>67 (22.5)</td>
</tr>
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<td>RCC, chromophobe</td>
<td>19 (6.4)</td>
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<tr>
<td>RCC, mixed</td>
<td>8 (2.7)</td>
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<tr>
<td>RCC, unclassified</td>
<td>9 (3)</td>
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<tr>
<td>Other malignant</td>
<td>3 (1)</td>
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<td>RCC pathologic stage</td>
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<tr>
<td>pT1a</td>
<td>215 (72.9)</td>
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<tr>
<td>pT1b</td>
<td>58 (19.7)</td>
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<tr>
<td>pT2</td>
<td>4 (1.4)</td>
</tr>
<tr>
<td>pT3a</td>
<td>18 (6.1)</td>
</tr>
</tbody>
</table>

| Benign patients                | n (%)      |
| Oncocytoma                     | 43 (42.1)  |
| Benign cyst                    | 23 (22.5)  |
| Angiomyolipoma                 | 22 (21.5)  |
| Adenoma                        | 5 (4.9)    |
| Others                         | 9 (8.8)    |

| Pathologic stage               | n (%)      |
| pT1a                           | 215 (72.9) |
| pT1b                           | 58 (19.7)  |
| pT2                            | 4 (1.4)    |
| pT3a                           | 18 (6.1)   |
| Benign patients                | n (%)      |
| Oncocytoma                     | 43 (42.1)  |
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| Others                         | 9 (8.8)    |

| Pathologic stage               | n (%)      |
| pT1a                           | 215 (72.9) |
| pT1b                           | 58 (19.7)  |
| pT2                            | 4 (1.4)    |
| pT3a                           | 18 (6.1)   |
| Benign patients                | n (%)      |
| Oncocytoma                     | 43 (42.1)  |
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| Angiomyolipoma                 | 22 (21.5)  |
| Adenoma                        | 5 (4.9)    |
| Others                         | 9 (8.8)    |

| Pathologic stage               | n (%)      |
| pT1a                           | 215 (72.9) |
| pT1b                           | 58 (19.7)  |
| pT2                            | 4 (1.4)    |
| pT3a                           | 18 (6.1)   |
| Benign patients                | n (%)      |
| Oncocytoma                     | 43 (42.1)  |
| Benign cyst                    | 23 (22.5)  |
| Angiomyolipoma                 | 22 (21.5)  |
| Adenoma                        | 5 (4.9)    |
| Others                         | 9 (8.8)    |
A Simplified Approach to Non-Ischemic Robotic Partial Nephrectomy for Small Renal Masses

Anthony N. Avallone, MD, Kathleen Mechir, MD, and Jihad H. Kaouk, MD

Partial nephrectomy, whether performed through open surgery or minimally invasive techniques, has replaced radical nephrectomy for the treatment of most small renal masses. As our ability to treat small renal tumors with nephron-sparing surgery has matured, with oncologic results equivalent to those of radical nephrectomy, further emphasis is being placed on minimizing the impact of treatment on renal function.

Furthermore, minimally invasive robotic partial nephrectomy (RPN) has continued to grow in popularity as the 3-D vision and articulated instruments simplify the suturing required for renal reconstruction. In the absence of effective methods to cool the kidney, however, RPN requires a period of warm ischemia time (WIT) to accomplish tumor excision in a bloodless field. If prolonged, WIT increases the risk of subsequent nephron loss.

We have found that in selected patients with small exophytic renal tumors, preplacing renorrhaphy sutures allows partial nephrectomy to be accomplished without hilar clamping, thereby eliminating renal ischemia. Others have attempted to isolate and control segmental renal vessels to avoid global renal ischemia, but the technical difficulty involved, even in experienced hands, limits the procedure’s widespread adoption insofar as the technique is not easily transferred to other surgeons. Our procedure, in contrast, is easily transferable because it utilizes techniques familiar to most surgeons performing robotic renal procedures.

Additionally, patients presenting with multiple small renal tumors are usually treated with open partial nephrectomy because, in this setting, cold ischemia can be used, lengthening the time available to operate without subsequent renal damage. Hilar clamping during RPN to remove multiple tumors can result in an excessive WIT, causing nephron loss. In many cases, however, multiple renal tumors are small, exophytic and amenable to the technique outlined below utilizing preplacement of renorrhaphy sutures without hilar clamping.

The technique we now use in selected patients with solitary or multiple small exophytic renal tumors is begun by mobilizing the colon and identifying and isolating the renal hilum. The tumor location is then identified, Gerota’s fascia opened and dissection carried out to the base of the tumor. Using care to avoid violating the renal capsule, the surrounding fat is freed for several centimeters from the base of the tumor. At this point, intraoperative ultrasound can be employed to determine the depth of the tumor and
ascertain that it is not too deep for suture preplacement. We have used the Ethicon CT needle on a 0 Vicryl-coated suture as a renorrhaphy suture, as it is larger than the CT-1 needle, allowing larger masses to be excised. The depth of this needle is about 12 mm, so exophytic tumors slightly larger than 2 cm can be removed.

The suture needs to be placed deep into the tumor and must include a small margin of normal renal parenchyma, which is facilitated by using the larger CT needle. Application of the renorrhaphy sutures can be done with or without bolsters, depending on surgeon preference. In addition, the renorrhaphy sutures and bolsters can be applied completely before tumor excision, or gradually. In the latter case, the first renorrhaphy sutures are positioned, tumor excision is begun, another renorrhaphy suture placed, more of the tumor is excised and so on until the entire tumor is removed.

After the renorrhaphy sutures have been placed and the tumor completely excised, we have found it necessary to utilize one or several running sutures of 2-0 Vicryl on an SH needle or 3-0 Vicryl on an RB-1 needle to secure any vessels that could be present at the base of the defect. It is necessary to place these before final tightening of the renorrhaphy sutures to allow enough room for their placement. We then apply hemostatic agents such as a mesh bolster to ensure hemostasis. The pressure of the pneumoperitoneum is then reduced to assess for bleeding.

Although renorrhaphy sutures and bolsters are widely employed for renal reconstruction after tumor removal during RPN, our approach of placing them prior to tumor excision without hilar clamping avoids subjecting the kidney to any renal ischemia. This lack of WIT minimizes the potential for renal damage. Furthermore, this approach to nephron-sparing surgery utilizes familiar techniques and is easily mastered by surgeons currently performing robotic renal surgery.
An Update on Blue Light Fluorescence Cystoscopy

Ryan K. Berglund, MD, and J. Stephen Jones, MD

Bladder cancer is prevalent in the U.S., and is the ninth most common cancer worldwide. It is also one of the most expensive cancers to manage. Nonmuscle-invasive bladder cancer (NMIBC) has a low mortality rate (< 5 percent at five years) if diagnosed early and managed aggressively, but it also has a high recurrence rate, with up to 80 percent of cases recurring within five years. With recurrence, up to 50 percent of cases eventually progress to muscle-invasive disease, and early recurrence within three months of initial diagnosis is associated with worse overall outcomes.

Endoscopic resection remains the mainstay of early management of NMIBC. Early recurrence after resection may be related to tumors not seen on routine white light cystoscopy, or incomplete resection of a visualized tumor at the initial resection. This leads to repeated resection, which results in increased surgical risk and expense to the patient. There is a need to improve detection and intervention to reduce the risk of future recurrence and progression.

Cysview® (Photocure ASA, Oslo, Norway) is an optical imaging agent indicated for use in the cystoscopic detection of nonmuscle-invasive papillary cancer of the bladder among patients suspected or known to have a lesion(s) on the basis of a prior cystoscopy. Hexvix® is used with the Karl Storz Photodynamic Diagnostic D-Light C System to perform cystoscopy with the blue light setting (mode 2) as an adjunct to the white light setting (mode 1).

The agent is instilled into the bladder one hour prior to initial cystoscopic resection. It is not indicated for repeat use. The most common adverse reaction reported in patients who received Cysview was bladder spasm, occurring in < 3 percent of patients, followed by dysuria, hematuria, bladder pain, procedural pain, urinary retention and headache, all occurring in ≤ 2 percent of patients.

In a randomized North American and European trial (study 305), Cysview found at least one additional tumor in 16 percent of patients, while increasing by 32 percent the discovery of patients who had concurrent carcinoma in situ (CIS). Of the additional tumors identified, 43 percent were either high-grade or invasive (stage T1 or greater). Interestingly, the false-positive rate was similar in both the white and blue light setting (around 10 percent). Other studies have demonstrated increased cancer yields in the Ta/T1 setting of 20–29 percent and in the CIS setting of 16–27 percent.

For references, please email the editor.
Locally advanced renal cell carcinoma (RCC) has been classically managed with aggressive surgery, and this is still the standard treatment in this situation. Despite remarkable advances with targeted molecular treatments of patients with advanced RCC, these treatments are not curative, and RCC remains primarily a surgical disease. Complete surgical resection of patients with locally advanced RCC can be curative, and cytoreductive nephrectomy in patients with systemic metastases can extend survival. However, some patients with locally advanced RCC may be unresectable due to various combinations of factors including bulky lymphadenopathy, high-level inferior vena cava thrombi, proximity or invasion of vital structures, and extremes of tumor size. Innovative approaches are greatly needed for this challenging patient population.

We recently reported our experience using sunitinib, a tyrosine kinase inhibitor (TKI), in an effort to downsize the tumor and facilitate subsequent surgery in patients with unresectable RCC. Our series included 30 patients treated in a phase 2 clinical trial, and that experience proved to be highly informative. Median downsizing in the primary tumor was 22 percent, corresponding to a median absolute size reduction of 1.2 cm. While this does not sound impressive, many patients had more robust responses, and 13 patients (45 percent) were able to move forward with surgical resection.

**Effectiveness of Treatment in the Trial**

Patients with clear cell RCC (n = 22) were much more likely to respond to the treatment, emphasizing the importance of tumor mass biopsy to define histology prior to treatment. In the subgroup of patients with clear cell histology, the median downsizing of the primary tumor was 28 percent, and 59 percent were able to proceed with surgical resection. No substantial responses were observed in the patients with non-clear cell histology.

Renal mass biopsy for locally advanced retroperitoneal tumors can be subject to misdiagnosis due to the broad differential diagnosis (not only RCC, but also urothelial carcinoma, adrenal carcinoma and lymphoma) and the presence of tumor heterogeneity and necrosis. Our protocol was revised to obtain multiple cores, including some specifically targeted toward the peripheral aspects of the tumor.

Despite theoretical concerns about tissue healing and thromboembolic phenomena (sunitinib blocks VEGF, which is involved in wound healing and in the natural regeneration of the vasculature), surgical safety was confirmed. We observed no major complications and, specifically, none in these main categories of concern.

**Dosing Experience**

The usual dosing regimen for sunitinib (four weeks on followed by two weeks off of therapy) had to be modified to a continuous dosing regimen due to rapid tumor regrowth that was observed in some patients while off of therapy. Most patients need only two six-week cycles of therapy. If the patient has not responded by that point, further therapy will not be effective. Those who have responded typically will not achieve substantial additional downsizing if the therapy is extended.

A subset of patients with hilar tumors in a solitary kidney or bilateral hilar tumors tended to respond most impressively, in most cases enabling subsequent partial nephrectomy (See Figure).

In reality, this study provided us with a wealth of information that has impacted patient management and helped us design additional clinical trials. Our current trial of pazopanib, also an orally administered TKI, is focused specifically on patients with hilar tumors who need tumor downsizing to enable partial nephrectomy. Based on our previous experience, this most recent trial is restricted to patients with biopsy confirmed clear cell histology.

While enthusiasm for the neoadjuvant approach is growing, our perspective is that primary surgery should still be prioritized whenever possible, and that neoadjuvant strategies remain investigational. Our experience with this prospective sunitinib trial highlights the value of such an approach, allowing us to optimize patient selection, as well as the efficacy and safety of the treatment protocol.

**Key Point:**

Patients in the study with clear cell RCC were much more likely to respond to treatment with sunitinib, emphasizing the importance of tumor mass biopsy to define histology prior to treatment.
Figure Legend. A 60-year-old man presented with a solitary kidney with a hilar tumor and chronic kidney disease (eGFR of 50 mL/min/1.73 m²). Coronal images at the anterior aspect of the kidney demonstrated extension to the proximal aspect of the main renal vein. After TKI therapy, the tumor has downsized substantially, becoming partially necrotic and pulling away from the hilum, enabling partial nephrectomy. Final pathology showed grade 3 clear cell RCC with negative margins. Nadir GFR was 32 mL/min/1.73 m² on postoperative Day 2, and the ultimate GFR six months later was 38 mL/min/1.73 m².
Sickle Cell Trait and Medullary Renal Carcinoma

Matthew Simmons, MD, and Surafel Gebreselassie, MD

Medullary renal carcinoma (MRC) is a rare and fatal cancer that occurs primarily in patients with sickle cell trait (SCT) and sickle cell disease (SCD). The typical patient with MRC is a young male (mean age of 22 years) of African or Mediterranean descent. Most patients with this disease present initially with gross hematuria, weight loss or abdominal pain. In symptomatic patients, 80 percent will have retroperitoneal lymphadenopathy and visceral metastasis. The disease is uniformly fatal, with a life expectancy from the time of initial diagnosis of only 15 weeks. There are no known effective radiation or chemotherapy treatments for the disease.

Investigation has focused on why MRC behaves so aggressively. Data support that chronic renal medullary hypoxia may play a role in tumorigenesis. Conditions in the renal medulla, including low oxygen tension, high acidity and high osmolarity, promote sickling of red cells in patients with SCT. Sickled cells conglomerate and occlude capillaries. This leads to tubular epithelial hypertrophy and hyperproliferation. The nature and extent of mutations in MRC are variable, but the net result is activation of the hypoxia-inducible factor 1 pathway.

Since the discovery of Hemoglobin S (HbS) by Linus Pauling and colleagues in 1949 and identification of the abnormality in the amino acid sequence by Vernon Ingram in 1956 (replacement of the hydrophilic glutamic acid at position 6 in the globin chain by the hydrophobic valine residue), it has been known that the abnormal polymerization of deoxy-HbS is the main cause for vaso-occlusive crisis involving many organs, including the kidneys, in SCD.

Advances in understanding of RBC dehydration, the roles of nitric oxide, adenosine, and changes in RBC and plasma membrane proteins during sickling have advanced our current understanding of SCD.

The hypertonic, acidic and hypoxic environment in the inner medulla favors RBC sickling, leading to occlusion and necrosis of the vasa recta. The resulting ischemia leads to release of vasodilators such as prostaglandin. This, in turn, increases the glomerular filtration rate (GFR), leading to microalbuminuria, proteinuria and eventually low GFR, particularly among patients in the fourth decade of life. Sickle cell patients can have supranormal proximal tubular function resulting in increased creatinine secretion and hyperphosphatemia. Such patients can also have impaired distal hydrogen ion potassium secretion.

Diminished urinary concentrating ability is one of the early renal involvements in SCD, and this is often irreversible after age 10. Less severe impaired urinary concentrating ability is also seen in patients with SCT, which is a benign carrier condition in which one sickle cell beta globulin gene is inherited along with a normal beta globulin gene. The prevalence of SCT is as high as between 8 and 10 percent in African-Americans. Hematuria is common in patients with both SCD and SCT and could be from papillary necrosis, renal infarct or medullary renal carcinoma, which is seen more often in those with the trait.

Clinical strategies are limited currently to prevention and early detection. Medullary hypoxia in these patients may be lessened with daily bicarbonate supplementation and increased fluid hydration. General screening for MRC would be difficult to justify given the high cost of imaging and the low incidence of disease. Patient selection for screening plays a clear role. Most young males with SCT have gross hematuria with associated papillary necrosis or hypertrophy. Cross-sectional imaging with and without contrast is mandatory in these patients as part of their formal hematuria evaluation.

It could be argued that these patients should be placed on a surveillance protocol. There is no current data to support that early detection impacts survival of those with MRC. Indeed, there are no data that describe outcomes of curative-intent surgery for low-stage localized MRC. Nonetheless, in young patients with SCT and gross hematuria, surveillance seems a prudent course of action.

Key Point:

Medullary renal carcinoma is a rare and fatal cancer that occurs primarily in patients with sickle cell trait and sickle cell disease. Clinical strategies are limited currently to prevention and early detection.
Key Markers of Circulating Prostate Cancer Stem Cells and Their Predictive Value

Tatiana V. Byzova, PhD, and Bethany Kerr, PhD

Considerable controversy surrounds the appropriate treatment choice for patients with newly diagnosed, localized prostate cancer. This is due in part to deficiencies in the diagnostic techniques, which are incapable of accurately predicting which patients may suffer from future disease recurrence. Recent findings raise important questions of overtreatment and overdiagnosis of clinically insignificant prostate cancer and present a need for the development of new markers that can specifically identify those patients at risk of dying from prostate cancer. Our group focuses on the development of novel biological markers to distinguish aggressive prostate cancer from localized low-grade disease.

Over the past decade, the concept of a cancer stem cell has emerged as a possible explanation for the initiation, maintenance and relapse of tumors. Research in this area has shown that these cells constitute a very small fraction of tumor cells, and that they possess unlimited self-renewal properties along with an ability to differentiate and induce a phenotypic copy of the original tumor. These cells may remain dormant for many years and are resistant to commonly used cytotoxic therapies.

Multiple markers for cancer stem cells were proposed, based primarily on animal models. We are interested in tracking the circulating cancer stem cells in prostate cancer patients and using this information to identify patients with advanced prostate cancer, which is likely to spread to other tissues. Identifying such patients early would be beneficial, especially since this would enable them to start “early” systemic therapy. Moreover, identification and detailed characterization of circulating cancer stem cells will be key to preventing their spread to metastatic sites.

Key Point:

We analyzed the presence of circulating stem cells in the blood of patients before and after primary tumor removal, using several previously described markers. We have found that CD117, but not other proposed markers, indicates the presence of tumor-associated cells in circulation.

Our hypothesis was as follows: In patients with advanced prostate cancer, circulating cancer stem cells will be present at high levels but would be expected to decrease when the primary tumor is surgically removed. In patients with metastasis, these cells should stay at high levels despite the removal of the main tumor. The key issue was to find a marker for such cells.

We analyzed the presence of circulating stem cells in the blood of patients before and after primary tumor removal, using several previously described markers, such as CD133, CXCR4, CD34 and CD117. We have found that CD117 (or c-kit, a receptor for stem cell factor), but not other proposed markers, indicates the presence of tumor-associated cells in circulation. Amounts of circulating CD117-positive cells were higher in patients with high-grade cancer compared with low-grade, whereas no difference in CD133-positive cells was found (Figure 1A).

Most important, these numbers decreased after radical prostatectomy and remained low for at least three to six months in patients with low-grade tumors without recurrence (Figure 1B). In patients with high-grade primary tumors experiencing recurrence, the presence of these cells remained unchanged after radical prostatectomy (Figure 1B).

Figure 1A.

Figure 1B.
Thus, the presence of CD117-positive cells in the blood of prostate cancer patients could predict recurrence of cancer in high-risk patients.

We are now focusing on the functional role of CD117 in prostate cancer dissemination. This receptor can be activated by stem cell factor and appears to promote the growth and metastatic potential of prostate cancer cells in experimental models. Tumors derived from the CD117-positive population are substantially larger than those derived from the CD117-negative population of prostate cancer cells. Quantification of vasculature demonstrated that there were 1.9-fold more vessels in CD117-positive tumors compared with CD117-negative tumors. In addition, cells of CD117-positive tumors were proliferating at a higher rate compared with those of CD117-negative tumors.

These data demonstrate that CD117 expression in human prostate cancer cell lines results in increased tumor growth and angiogenesis. Thus, CD117 expression represents a possible marker for prostate cancer severity.

Our group continues to study the role of this receptor in prostate cancer progression and dissemination.

The team of investigators also includes Ranko Miocinovic, MD; Armine K. Smith, MD; Joseph C. Klink, MD; Maria C. Mir, MD; Donna E. Hansel, MD; Eric A. Klein, MD; Andrew Stephenson, MD, and Warren D. Heston, PhD.

Prostate Cancer Screening: The Controversy Continues

Andrew J. Stephenson, MD

Few areas in medicine generate as much controversy among health professionals as prostate cancer screening. Since the introduction of the prostate-specific antigen (PSA) blood test in the late 1980s, more than 50 percent of men older than 50 years have reportedly undergone PSA screening in their lifetime, including a substantial proportion of men older than 80 years. Since the early 1990s, a 35 percent reduction has been observed in age-adjusted prostate cancer-specific mortality; 45 to 70 percent of this reduction may be attributable to PSA screening. However, the age-adjusted incidence rate has more than doubled. Thus, a man’s lifetime risk of dying from prostate cancer has been reduced from 3 percent to 2.6 percent, but his lifetime risk of being diagnosed with prostate cancer has increased from 7.3 percent to 18 percent.

The United States Preventive Services Task Force (USPSTF), an independent panel of nonfederal experts in prevention composed of internists, pediatricians, family physicians, nurses and health behavior specialists, released its updated guidelines and has advised against PSA screening, citing evidence that it is associated with a small or no reduction in prostate cancer-specific mortality and is associated with (often) unnecessary harms related to subsequent evaluation and treatments.

The European Randomized Study of Screening for Prostate Cancer (ERSPC) and the National Cancer Institute’s American Prostate, Lung, Colorectal and Ovarian Cancer (PLCO) Screening Trial reported conflicting results with respect to the benefits screening. PLCO reported a nonsignificant 10 percent increase in prostate cancer-specific mortality at 10 years with screening, in contrast to ERSPC, which reported a 20 percent reduction at 9 years, translating into an absolute risk reduction of 0.07 percent. The Göteborg, Sweden, trial was originally designed as a stand-alone trial but later joined ERSPC. The Swedish investigators recently published the results of their trial separately from ERSPC and reported a 44 percent reduction in prostate cancer-specific mortality at 14 years, translating into a 0.34 percent absolute reduction.

A major limitation of PSA screening is the risk of overdiagnosis (defined as the detection of cancers that would otherwise remain undiagnosed over a man’s lifetime in the absence of screening), which is estimated to be as high as 50 percent. Overdiagnosis is a problem if it leads to overtreatment, as prostate cancer treatments are associated with risks of urinary, bowel and sexual dysfunction; a small risk of mortality; and substantial cost. Overdiagnosis may be minimized if cancer specialists embrace active surveillance as a viable option for low-risk patients and/or those with limited life expectancy. However, the available evidence suggests the vast majority of these patients receive radical therapy, though recent evidence suggests attitudes about active surveillance among patients and cancer specialists may be changing.

Given that the decision to undergo PSA screening is associated with important trade-offs between short- and long-term benefits and harms that are likely sensitive to a man’s risk factors, life expectancy and preferences, a rational approach is to actively engage the patient about the risks, benefits and uncertainties of screening before ordering a PSA test. This shared decision-making approach is currently recommended by the American Cancer Society and the American Urological Association, and it represents a fair and balanced approach that recognizes the controversies surrounding PSA screening. In my opinion, the USPSTF panel has emphasized the risks of screening over the benefits in its recommendations and does not recognize individual risk and preferences. For men with multiple first-degree relatives diagnosed before age 65 and African-Americans, this discussion should occur between ages 40 and 45. For those without these risk factors, this discussion should occur at age 50.

Many online decision support tools are available to which physicians can refer their patients who are uncertain about whether to undergo PSA screening. One such tool is the video “Is a PSA Test Right for You?” The video is sponsored by the Foundation for Informed Medical Decision Making and is available at bit.ly/OJQKxt.

For references, please email the editor.
Magnetic Resonance Imaging of Prostate Cancer

David A. Levy, MD

Magnetic resonance imaging (MRI) of the prostate is being utilized with increasing frequency as an adjunct for therapeutic decision-making in select prostate cancer patients at Cleveland Clinic.

Over the past several years, more than 200 patients have undergone MRI and MRI spectroscopy following the diagnosis of prostate cancer based on site-specific prostate biopsy, in an effort to better direct therapeutic decision-making. Patients fall into several different categories for which MRI of the prostate is employed. Both 3 Tesla endorectal coil technique and body surface coil techniques with 1.5 and 3 Tesla technology are being utilized in specific prostate cancer imaging protocols to study these patients. Correlative assessment of site-specific biopsy results, MRI study interpretation and surgical pathology are ongoing to further delineate the role of MRI in our prostate cancer patient population.

Primary Nerve-Sparing Surgical Therapy

Based on the site-specific prostate biopsy results, which reflect disease burden as indicated by number of cores positive, percent of cores positive and total gland volume, patients who are candidates for nerve-sparing surgical techniques may be offered MRI of the prostate no sooner than eight weeks following the prostate biopsy. The goal of the MRI study in these select patients is to better assess possible disease extension into the region of the neurovascular bundles. If the MRI results suggest such extension, the patient is counseled accordingly and the surgical approach may be altered in an effort to better eradicate the patient’s disease.

High-Volume Aggressive Disease

For those individuals with high-volume aggressive disease detected on the site-specific prostate biopsy, the identification of extracapsular disease extension or seminal vesicle extension is paramount in directing therapeutic decision-making. Imaging results are utilized in multimodality treatment planning with our radiation oncology colleagues for select patients in our high-risk population. MRI and MRI spectroscopy are showing promise in identifying extracapsular disease extension as well as seminal vesicle involvement, and based on the interpretation of the imaging study, patients are being counseled accordingly in treatment decision-making.

Salvage Therapy

For individuals who are being considered for salvage therapy following biopsy confirmation of radiation failure, MRI and MRI spectroscopy are being employed as part of the metastatic evaluation, specifically to assess for extracapsular extension as well as seminal vesicle involvement prior to implementation of salvage therapy. Many of these patients will undergo salvage cryoablation, during which efforts can be directed at treatment of the seminal vesicles to better influence the patient’s progression-free survival.
A Genomic Approach to Active Surveillance: A Step Toward Precision Medicine

Eric A. Klein, MD

In the past 25 years, PSA screening has resulted in a large gap between the likelihoods of being diagnosed with and of dying of prostate cancer, leading to the clinical problems of overdiagnosis and overtreatment. Although there is agreement in urologic circles that early detection and aggressive treatment of higher-grade cancers reduces prostate cancer-specific mortality, the widespread overtreatment of low-grade, nonaggressive disease led the U.S. Preventive Services Task Force to recommend against routine screening. There is accumulating data from several institutions on an alternative management strategy called active surveillance. Active surveillance is defined as expectant management with curative intervention delayed until signs of tumor progression. The main advantage of this approach is that it avoids overtreatment of indolent disease, thereby restricting the cost and morbidity of curative-intent treatments only to those who have potentially life-threatening cancers. Despite the favorable outcomes reported for surveillance, its clinical use is limited, with >90 percent of men in the U.S. diagnosed with potentially indolent disease undergoing immediate treatment with radiation or surgery.

There are several reasons that surveillance has not been more widely adopted — legal (fear of being sued if the window of curability is missed), economic (doctors get paid to intervene, not watch) and emotional (patient and family anxiety over not treating a known cancer). However, the major limitation to wider use of surveillance is the lack of a tool that can distinguish indolent from aggressive prostate cancer at the time of diagnosis, and one that can be used on subsequent biopsies to determine if someone on surveillance has true biologic progression.

Working with Genomic Health, Inc., we have designed a novel strategy built on the approach used to successfully develop molecular profiling of breast and colon cancers, designed to address the challenge of tumor heterogeneity inherent in prostate cancer. Results from the first two studies in this strategy were presented at the 2012 American Society of Clinical Oncology (ASCO) annual meeting (Klein EA, Maddala T, Millward C, et al., abstract #4560). We first conducted a gene discovery study in fixed, paraffin-embedded (FPE) tumor tissue from patients treated by radical prostatectomy (RP), where the relationship between gene expression and clinical tumor recurrence in two separate tumor foci selected to represent the primary and highest Gleason patterns were examined. We found a group of 288 genes in six biological pathways that predict for clinical recurrence, expressed in common by both the primary and highest Gleason patterns. We then conducted a second study to demonstrate that the majority of the most highly predictive genes identified in RP specimens in the first study, when assayed in tumor from prostate needle biopsies, could also predict adverse pathology at the time of prostatectomy.

Figure 1. Gene identification study in RP specimens: novel design to assess gene expression in the context of Tumor Heterogeneity

Key Point:
Active surveillance is defined as expectant management with curative intervention delayed until signs of tumor progression. The main advantage of this approach is that it avoids overtreatment of indolent disease, thereby restricting the cost and morbidity of curative-intent treatments only to those who have potentially life-threatening cancers.

Working with Genomic Health, Inc., we have designed a novel strategy built on the approach used to successfully develop molecular profiling of breast and colon cancers, designed to address the challenge of tumor heterogeneity inherent in prostate cancer.
**Methods**

For the gene identification study, we sampled 441 tumor specimens from a large pool of approximately 2,600 men treated by RP between 1987 and 2004 at the Glickman Urological & Kidney Institute. For the biopsy study, FPE prostate needle biopsy specimens were selected from an additional 167 patients (92 low-risk and 75 intermediate-risk) who had both a prostate biopsy and RP at our institution. All specimens were rereviewed and assigned a Gleason pattern and score using the 2005 International Society of Urological Pathology Consensus guidelines. In the RP study, we sampled two spatially distinct tumor specimens that represented the primary Gleason and highest or secondary Gleason patterns. For the biopsy study, representative tissue blocks were selected for each patient.

For the gene discovery study, 727 candidate genes selected from a meta-analysis of publicly available DNA microarray data sets were analyzed. Candidate genes were assayed for expression by quantitative RT-PCR assays. Eighty-one candidate genes identified in the gene discovery study were assayed using the same methods used in the needle biopsy study.

**Statistical Methods**

For the gene discovery study, the primary objective was to identify genes associated with time to clinical recurrence (local recurrence or distant metastases); and for the needle biopsy study, the presence of adverse pathology (high-grade or non-organ-confined disease) in the RP specimen. Cox proportional hazards regression and logistic regression models were used to evaluate associations between genes and outcome variables. The false discovery rate (FDR) was controlled at 10 percent.

**Results**

In data presented at the 2012 ASCO annual meeting, we identified 288 genes that were similarly predictive of clinical recurrence (as assessed by standardized hazard ratios) in both the primary and highest Gleason patterns. This result demonstrated that certain genes could predict tumor aggressiveness regardless of the Gleason pattern tumor in which they were assessed (Figure 1). These genes span multiple pathways that are differentially associated with aggressiveness — for example, higher expression of the stromal response and proliferation genes is associated with higher risk of clinical recurrence, while for other groups (cellular organization, basal epithelial, androgen and stress), higher expression is associated with lower recurrence risk. After adjustment for AUA risk group (based on pretreatment PSA, T stage and PSA), 198 genes, including representative genes from the six groups identified in univariable analyses, remained strongly associated with clinical recurrence in tumors taken from either the primary or highest Gleason pattern. Importantly, in the second study, expression patterns in these groups were also predictive of adverse pathology on RP in tumor samples taken from needle biopsy specimens (Figure 2). Overall, 58 of 81 (72 percent) tested genes predicted high-grade and/or non-organ-confined disease (FDR < 10 percent).

**Discussion**

In 2011, the Institute of Medicine issued a call for the development of a new system of disease classification that would link molecular data to health outcomes in order to allow more precise clinical decision-making that is tailored to individual patients, a concept termed “precision medicine.” The overdiagnosis of nonlethal prostate cancer by PSA screening, coupled with recent advances in genomic profiling of prostate and other human cancers, represents a significant opportunity to apply the concept of precision medicine to the management of prostate cancer. The major question most newly diagnosed men today face is no longer “What is the best treatment for my cancer?” but rather “Does my cancer need to be treated at all?” Current clinical predictors, including nomograms, lack the discriminative ability to answer this question for most newly diagnosed tumors. We have attempted to address this limitation by studying the biology of prostate cancer as revealed by gene expression profiling from both RP specimens and prostate biopsies. The capacity to predict clinically meaningful outcomes from biopsies is essential for those considering active surveillance, since it is the only material available on which to make a judgment.
Our studies, as presented at the 2012 ASCO annual meeting, have revealed that sampling the expression of genes contained in multiple biological families can enable us to predict outcomes in ways that can be used in inform clinical decision-making. The discovery study identified 288 genes that can predict for the development of metastasis or prostate cancer death, whether they are assayed in the primary or highest Gleason pattern present in prostatectomy specimens; a subset of these genes assayed on biopsy samples also predicted for adverse pathology at RP. Together, these observations suggest that meaningful information on outcomes is contained in the small amounts of tissue obtained at biopsy. The fact that this information can be obtained from either the primary or highest Gleason pattern tumor suggests that the sampling error inherent with needle biopsy consequent to tumor multifocality and heterogeneity may be overcome with this approach (although this conclusion requires a great deal more study). A multigene assay developed from this work is now undergoing validation using biopsy specimens from an independent cohort of patients from another center. If our initial findings are confirmed, gene expression profiling of biopsy samples at the time of diagnosis and subsequently in those initially managed by surveillance could have major clinical impact, bringing precision medicine to the prostate cancer clinic in the near future (Figure 3).

For references, please email the editor.

Acknowledgments
Genomic Health, Inc. provided research funding to Cleveland Clinic for performance of this study. A patent application for the development of a genomic assay based on the genes identified in this study has been submitted by Genomic Health, Inc.

Robert H. Silverman, PhD

Oncolytic viruses are therapeutically used microbes, either natural or genetically engineered, that infect and replicate in cancer cells and lead to the elimination of tumors. Virotherapy, the therapeutic use of oncolytic viruses, was initially conceived based on the observation of transient remission of cancer patients during viral infections. Genetic changes in the cancer cell enhance susceptibility to viral infections, and oncolytic viruses are generally considered safe for humans. Importantly, drug-resistant cancer cells and cancer stem cells retain their susceptibility to oncolytic viruses.

Although treatments utilizing oncolytic viruses for late-stage tumors are very promising, novel strategies are required to help the virus invade and spread within tumors and overcome the cancer cells’ anti-viral defense mechanisms.

Current oncolytic virus research being conducted at Cleveland Clinic’s Lerner Research Institute focuses on sunitinib, an FDA-approved drug that is available in clinical practice for the treatment of renal cell carcinoma. Sunitinib is used to suppress the ability of tumor cells to grow their own blood supply (angiogenesis) and to starve the tumor so that it cannot grow. A side effect of sunitinib that our team discovered is that the drug allows viruses to grow better in tumors.

When used in combination with oncolytic viruses, sunitinib temporarily disables the immune system to the point where it allows the virus to kill every cancer cell. Preliminary studies have shown promising results on a wide spectrum of different cancer cells, specifically brain, breast, kidney and prostate cancers, when grown in plastic dishes or in mice. These results are noteworthy because while sunitinib is used to treat renal cell carcinoma and two rare forms of cancer (gastrointestinal stromal tumors and neuroendocrine pancreatic tumors), other types of tumors are resistant.

Our team’s current research aims to demonstrate in mouse models of cancer that a combination treatment with sunitinib and an oncolytic virus cures highly aggressive prostate tumors and renal cell carcinomas without adverse side effects. Next, our team will investigate the molecular mechanism for the anti-tumor response and extend these efforts to other types of tumors in mice. Our team believes that research focusing on sunitinib and oncolytic viruses could ultimately uncover an effective treatment for a range of late-stage, difficult-to-treat forms of cancer.

Treating Cancer with a Virus and a Drug
The Importance of “Extreme Apical Biopsy” for Prostate Cancer Diagnosis

J. Stephen Jones, MD, and Ahmed El-Shafei, MD

The standard initial prostate biopsy scheme has evolved from its original sextant arrangement to extended biopsies of eight to 14 cores. Although the most common templates currently obtain 12 cores, the optimal number and location remain under investigation as we optimize prostate cancer diagnosis.

We previously reported prospective evidence using site-specific labeling in 181 patients that addition of an extreme apical biopsy core on each side increased prostate cancer detection by 3.3 percent, compared with detection results using our 12-core template. More important, we showed that the extreme apex was the most common site (p = 0.01) of unique cancer detection (i.e., when only a single core demonstrated prostate cancer).

To validate these findings, we assessed cancer detection rates and pathologic outcomes according to biopsy scheme in 1,095 patients who underwent initial prostate biopsy. Patients were divided into two groups based on the number of cores retrieved. The first group underwent 12-core biopsy (n = 896). The second group underwent 14-core biopsy (n = 199 patients). Pathologic outcomes were compared between the two groups, controlling for the type of ultrasound probe used, based on our experience that our prostate cancer detection rates are higher for patients who undergo biopsy using end-fire probes compared with side-fire probes.

Our analysis determined that the total cancer detection rate was 38.7 percent for 12 cores vs. 46.2 percent for 14 cores (p = 0.05). Further analysis showed that the disease burden identified with 14-core biopsy is greater, as evidenced by the number of positive cores (p = 0.0005) and the maximum percentage of cancer in a core (p = 0.00017). We also found that the presence of high-risk benign pathology in the form of high-grade prostatic intraepithelial neoplasia (p = <0.0001) and atypical small acinar proliferation (p = 0.027) was significantly increased by the addition of one core from the extreme apex on each side.

These findings have obvious implications for the potential of unrecognized prostate cancer as well as the risk of being identified with prostate cancer during follow-up. Our results in actual clinical practice in more than 1,000 patients reaffirm our prospective findings that the extreme apex is the most common site for unique cancer detection during initial prostate biopsy, if it is adequately sampled. This site should be intentionally targeted during prostate biopsy, and if there is suspicion of persistent cancer following a negative biopsy, the apex is the most likely place in which cancer has been overlooked.

For references, please email the editor.
Role of Autophagy in the Therapeutic Response of Prostate Cancer Cells

Alex Almasan, PhD

Our laboratory has been interested in the response of prostate cancer (PCa) cells to DNA-damaging agents such as ionizing radiation, a commonly used therapeutic modality. Understanding the cellular response to apoptosis-inducing therapeutics, such as Apo2L/TRAIL administered alone or in combination, for example, with inhibitors of the Bcl-2 family, has been another interest. It has recently been discovered that damage occurs to the cellular content, not just the DNA; therefore, understanding the role of autophagy is important. Autophagy also can be critical to the response to apoptosis-inducing agents by counteracting apoptosis regulators and, over time, in the ensuing resistance that develops following chronic treatment that mimics the course of patient therapy.

Autophagy Defined

Autophagy is a physiological process that can be defined as “self-eating,” wherein the cellular organelles and contents are degraded in a programmed manner to recycle amino acids and help maintain energy balance within the cell. Autophagy is very important for the removal of unwanted cells and cellular material during organisms’ development, without causing any inflammation and damage to the whole organism. Autophagy can be understood simply as a natural process for getting rid of unwanted cellular content in the absence of any severe exogenous stress; rather, it occurs in response to endogenous cues facilitating growth and development. In many cell types, such as various glands and neurons, basal autophagy is always functional in adults. Elevated or deregulated autophagy signaling can lead to various disease conditions, such as myopathy, neurodegeneration, microbial infections and aging disorders.

Autophagy signaling can be classified into three steps: 1) initiation, 2) autophagosome formation, and 3) autophagosome fusion with lysosomes and degradation of the autophagolysosomes. Several proteins are involved in mediating these complex signaling events. The induction of autophagy is sensed mainly by a kinase, Atg1/ULK1, which can be activated through phosphorylation under conditions of nutrient starvation through another kinase, AMPK.ULK1 and AMPK activation leads to inhibition of mTOR kinase, which is required for growth and protein translation signaling. Once the autophagy is activated, protein translation is inhibited. Indeed, the anabolic process is halted when cells undergo a catabolic process such as autophagy, but it is still not clear for most cancers whether the cells can support both protein synthesis and high levels of autophagy to survive longer and sustain the metabolic demands necessary for cellular proliferation.

Key Point:

Once autophagy is initiated, a small double membrane eventually develops in the autophagosome, with the help of E3 ubiquitin ligase-like proteins - Atg3, Atg4 and Atg7 - that modify LC3-I (cytoplasmic form) into LC3-II (lipidated and membrane-bound form) and ubiquitinate the Atg5-12 conjugate. Atg16 provides a larger protein scaffolding through multimerization of the Atg5-12-16 complex and thus facilitates the growth of the double membrane to form a complete autophagosome. Once the autophagosomes are formed, they are trafficked to fuse with lysosomes, at which point their contents are degraded.

Autophagy has a dual role in the process of tumorigenesis. During the early stages of cancer development, autophagy has a tumor suppressor role. However, during the later stages, cancer cells use autophagy signaling to keep up with their higher energy requirements and growth rates. Under these conditions, autophagy facilitates cancer cell survival, and thus autophagy inhibitors can be used to sensitize tumors to various therapeutic regimens.

Autophagy in Response to DNA Damage and Apoptosis

In cancer, autophagy usually antagonizes apoptosis and thus promotes cell survival. Autophagy signaling also can be associated with apoptosis and leads to cell death. Our recent studies have uncovered the mechanism of autophagy induction in epithelial cells following DNA damage - by chronic overexpression of a p18-cyclin E fragment (p18-CycE), an apoptosis-inducing protein. Autophagy was activated through cytoplasmic ATM, leading to sequential activation of AMPK and Atg1/ULK1; autophagy was prevented upon inhibiting ATM and AMPK phosphorylation. Thus, chronic p18-CycE expression induces autophagy, leading to clearance of p18-CycE following DNA damage and induction of senescence (Figure 1). These findings define how chronic apoptotic stress and DNA damage initiate autophagy and regulate cell survival through senescence and/or apoptosis.
By examining the crosstalk between autophagic signaling and apoptosis using a tumor-specific apoptosis inducer, Apo2L/TRAIL, we identified new molecular proteins that can be targeted by autophagy in PCa. In Apo2L/TRAIL-resistant PCa cells, increased autophagic flux led to more efficient clearance of p62-bound polyubiquitinated proteins. We identified GAPDH as a p62-interacting protein that formed cytoplasmic aggregates associated with mitochondria that were degraded by mitophagy. Impaired autophagic clearance of these p62-GAPDH-mitochondrial aggregates led to loss of mitochondrial function and cell death in Apo2L/TRAIL-sensitive cells. In contrast, their efficient autophagic degradation led to cell survival in Apo2L/TRAIL-resistant cells. Inhibiting autophagy signaling increased the accumulation of p62-GAPDH-mitochondrial aggregates and induced cell death (Figure 2). Therefore, autophagic degradation of p62-GAPDH-mitochondrial aggregates can determine the cellular response to Apo2L/TRAIL treatment in PCa.

Our recent studies have further established the role of autophagy in the DNA-damage response. Radiation-induced DNA damage led to autophagy induction in PCa cells, resulting in cell survival. Pharmacological (3-MA, chloroquine) or genetic (Atg7, LAMP2 knockdown) approaches for inhibition of autophagy sensitized cells to radiation-induced cell death mediated through delayed DNA damage repair and accumulation of genomic instability, leading to decreased cellular survival. Since autophagy is known to be elevated in many solid tumors and contributes to cell survival, the potential of autophagy inhibitors in increasing the effectiveness of existing therapies can be exploited.

Autophagy signaling is being examined by determining the expression of key autophagy regulatory proteins, such as p62, LC3, p-AMPK and p-ULK1, in human PCa samples. Our preliminary results suggest that the expression pattern of p62 is cytoplasmic in tumors and nuclear in normal prostate tissue (Figure 3). Establishing the extent of autophagy in different grades of PCa will facilitate the design of better therapeutic modalities, where autophagy inhibitors that are currently in clinical trials might be combined with irradiation and other conventional therapies.

For references, please email the editor.
Epigenetic Perturbation in Prostate Cancer

Angela H. Ting, PhD

The functional genome is made up of both genetic information and nonsequence-altering chemical modifications superimposed on the nascent DNA molecules. These chemical modifications modulate how the DNA is recognized by cellular machineries and therefore dictate the phenotype of a specific cell. The study of such nonsequence-altering chemical modifications and the molecular machineries that regulate them is called epigenetics.

Epigenetic regulation is responsible for generating diverse cellular phenotypes from the same genotype within a person (Figure 1). For example, genes required for epithelial morphology and functioning will have permissive epigenetic modifications to allow expression of these genes, whereas the same genes might harbor repressive epigenetic marks in endothelial or neuronal cell types to facilitate and/or reinforce silencing. DNA cytosine methylation and histone protein post-translational modifications are the primary categories of epigenetic modifications with well-delineated roles in maintaining tissue- and timing-specific expression profiles for normal functioning and development. These same modifications can be altered in cancer, thus providing the molecular basis for the abnormal phenotypes.

Our research is focused on characterizing epigenetic changes associated with prostate cancer, and we have initiated efforts to map genomewide DNA methylation patterns in primary prostate specimens. Our comprehensive analysis of the prostate cancer DNA methylome will help advance our understanding and, ultimately, management of the disease through identification of biomarkers and therapeutic targets.

DNA methylation refers to the covalent addition of a methyl group at position 5 of cytosine. In somatic cells of adult humans, this modification almost exclusively occurs in cytosine followed by guanine, also known as a CpG dinucleotide. DNA methylation has a number of essential roles in normal cells, including gene imprinting, X chromosome inactivation, and condensation of centromeric and subtelomeric DNA. In cancer, the DNA methylation pattern is profoundly altered. A global loss of methylation (i.e., hypomethylation) in cancer cells is associated with a loosening of chromatin and is thought to contribute to genomic instability. Conversely,

Key Point:
Epigenetic regulation is responsible for generating diverse cellular phenotypes from the same genotype within a person.

Dysregulation of DNA methylation in prostate cancer can directly alter many cellular functions through changes in both gene expression and genomic stability and therefore provide key insights into the biology of prostate cancer.

Figure 1. Epigenetic regulation dictates cellular phenotypes. Epigenetic patterns for each cell are unique, and these epigenetic modifications on top of the DNA are essential for packaging the nascent DNA molecule into organized functional structures within the nucleus. Such structures, termed “chromatin,” help determine the phenotype of a cell through regulation of gene expression. In this illustration, the same stretch of DNA sequence is shown for the epithelial, endothelial and neuronal cells. The epigenetic modifications are represented by red circles on the DNA (DNA methylation) and by hexagons on the histone (histone post-translational modifications). Depending on the specific epigenetic pattern comprising DNA methylation and different histone modifications within each cell, differential expression patterns can be observed from the same loci within the genome in different cell and tissue types.
a gain of methylation (i.e., hypermethylation) of promoter in CpG-dense regions leads to transcriptional silencing of tumor-suppressor loci, thus contributing to tumor initiation and promotion. Dysregulation of DNA methylation in prostate cancer can directly alter many cellular functions through changes in both gene expression and genome stability and therefore provide key insights into the biology of prostate cancer.

Our laboratory developed methyl CpG binding domain (MBD)-isolated genome sequencing (Figure 2), a robust, unbiased, high-throughput method to characterize genomewide DNA methylation patterns. MBD is a highly conserved protein domain that specifically binds methylated cytosines in the CpG dinucleotide context. Capitalizing on this unique biochemical property, we selectively capture DNA fragments containing methylation by incubating genomic DNA from prostate tissues with purified recombinant MBD proteins. These methylated DNA fragments are then subjected to the latest sequencing technology, which has the capacity to sequence an entire human genome in just a few days, to allow construction of an individual DNA methylome profile for each study specimen.

Our genomewide approach to characterizing the prostate cancer DNA methylome is vitally important to our comprehensive understanding of how epigenetic changes associated with prostate cancer contribute to tumorigenesis, metastasis and progression. While many hypermethylated genes have been discovered in prostate cancer using a candidate gene approach, DNA methylation changes at noncoding sequences could also affect cellular functions and prostate cancer behaviors. Genomewide association studies revealed several SNPs that are highly associated with prostate cancer risk in a 600-kb noncoding region of 8q24.

Given that genetic variations in noncoding sequences can influence prostate cancer susceptibility, DNA methylation changes at such sequences could also affect prostate cancer biology. Hence, a genomewide analysis of DNA methylation patterns is essential for determining whether altered methylation plays critical roles in prostate cancer development and can dictate prostate cancer aggressiveness. The DNA methylation signatures of tumors could be developed into noninvasive biomarkers for cancer surveillance since DNA methylation from prostate cells can be detected in urine and prostate secretions. Additionally, the biological pathways found to be modulated by differential DNA methylation can be targeted for therapy.

Our team at Cleveland Clinic has developed and applied high-throughput sequencing-based technologies and genomewide analysis approaches to characterize the DNA methylomes of human cancers. Using these state-of-the-art tools, we are mapping the DNA methylation differences between normal and clinically relevant subtypes of prostate cancers. Ultimately, such efforts should lead to a functional understanding of aberrant methylation changes in cancer as well as a mechanistic delineation of the origin of abnormal methylation patterns.

This molecular information will improve our understanding of prostate cancer biology, identify novel therapeutic targets for prevention and treatment, and provide new biomarkers for cancer surveillance and prognostication.

For references, please email the editor.
Corin Shown to Impact Pregnancy Through Trophoblast Invasion and Uterine Spiral Artery Remodeling

Qingyu Wu, MD, PhD

Pregnancy poses a serious challenge for maintaining normal maternal blood pressure. Pregnancy-induced hypertension is a major cause of maternal and fetal deaths, which occur in about 10 percent of pregnancies. During pregnancy, the uterus undergoes profound morphological changes, including trophoblast invasion and spiral artery remodeling. In preeclampsia, impaired spiral artery remodeling is common, but the underlying mechanisms are unclear.

Corin is a cardiac protease that activates atrial natriuretic peptide (ANP), a cardiac hormone important in regulating blood pressure. In mice, lack of corin prevents ANP generation and causes hypertension. In humans, corin variants are associated with hypertension. Unexpectedly, corin expression was detected in the pregnant uterus. As a transmembrane protein, corin is predicted to act at the expression sites, suggesting a possible function of corin in the pregnant uterus.

In a recent study published in Nature, we identified a novel function of corin and ANP in promoting trophoblast invasion and spiral artery remodeling. In this study, we created a mouse model in which a corin transgene was expressed under a cardiac promoter. The transgenic (Tg) and corin knockout (KO) mice were crossed to generate KO/Tg mice expressing corin only in the heart. In these mice, transgenic corin expression restored pro-ANP processing in the heart and normalized blood pressure, indicating that cardiac corin was sufficient to maintain normal blood pressure in nonpregnant mice. In pregnant corin KO/Tg mice that did not have pre-existing high blood pressure, blood pressure increased at approximately 17 days post-coitus and rose further before returning to the normal level after delivery. This phenotype resembled late gestational hypertension in preeclamptic women. The data indicate that cardiac corin expression did not prevent pregnancy-induced hypertension.

Proteinuria is a hallmark of preeclampsia. Wild-type corin KO and KO/Tg mice had similar urinary protein levels before pregnancy and at midgestation. The levels increased, however, in corin KO and KO/Tg mice at late gestation, consistent with reported proteinuria in mouse models of preeclampsia. Ischemic glomeruli, indicated by fewer red

Key Point:

In pregnancy, trophoblast invasion and uterine spiral artery remodeling are important for lowering maternal vascular resistance and increasing uteroplacental blood flow. Here we identify a novel function of corin and ANP in promoting trophoblast invasion and spiral artery remodeling. These results indicate that corin and ANP are essential for physiological changes at the maternal-fetal interface, suggesting that defects in corin and ANP function may contribute to preeclampsia.
blood cells, were found in pregnant corin KO and KO/Tg mice but not in nonpregnant mice. PAS staining revealed increased extracellular matrices and collapsed glomerular capillaries in pregnant corin KO and KO/Tg mice. Electron microscopy showed narrow glomerular capillary lumens and thick basement membranes, suggesting endotheliosis and increased extracellular matrices. Additional pathological features such as necrotic cells and calcium deposits in the placental labyrinth also existed in these mice, indicating insufficient uteroplacental perfusion. Consistently, corin KO and KO/Tg mice had smaller litters. We also showed that trophoblast invasion and spiral artery remodeling were impaired in corin KO and KO/Tg mice and that this defect occurred before blood pressure increased in these mice.

Pro-ANP is expressed in the uterus. If corin acts on pro-ANP to promote trophoblast invasion and spiral artery remodeling, thereby preventing hypertension in pregnancy, ANP and corin KO mice should have similar phenotypes. Indeed, we found similarly increased blood pressure in pregnant ANP KO mice. The mice also had late gestational proteinuria and smaller litters. Thus, ANP and corin KO mice had very similar phenotypes, indicating that the role of corin in pregnancy is likely mediated by ANP.

ANP is known to relax vascular smooth muscles. Recently, ANP and its downstream cGMP-dependent protein kinase were shown to be important in angiogenic processes by promoting endothelial regeneration. Thus, ANP may function locally to remodel uterine arteries and promote trophoblast invasion. In a cell-based experiment, we found that ANP markedly stimulated human trophoblasts to invade Matrigel™ matrices. In these cells, ANP receptor mRNA expression was confirmed and ANP-stimulated intracellular cGMP production was detected.

Our findings underscore the importance of ANP produced locally by corin, which acts on trophoblasts and vascular cells in the uterus. Because heart-derived ANP circulates inside the vessel, our model may explain why cardiac corin failed to promote trophoblast invasion and uterine artery remodeling, as shown in corin KO/Tg mice. To verify this hypothesis, we quantified corin mRNA and protein in human uteruses by RT-PCR and ELISA. The levels were low in nonpregnant women but increased in pregnant women. In preeclamptic women, the levels were significantly lower than in normal pregnancies. These results support a local corin function in the pregnant uterus.

We then sequenced the corin gene in preeclamptic patients and identified a mutation that altered Lys to Glu at position 317 in LDL receptor repeat 2 in one woman and another mutation that altered Ser to Gly at position 472 in frizzled 2 domain in two women from the same family who had preeclampsia. The data were consistent with previous findings that LDL receptor repeats and frizzled domains are critical for corin activity, suggesting that the mutations may impair corin function in the patients, thereby contributing to preeclampsia. Interestingly, corin variants in frizzled 2 domain that impaired corin function have been reported in blacks, a high-risk population for preeclampsia.

In summary, we have identified a novel local function of corin and ANP in promoting trophoblast invasion and spiral artery remodeling to prevent hypertension in pregnancy. Our data suggest that impaired corin expression or function in the pregnant uterus may represent an important mechanism underlying preeclampsia.

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Unusual Clinical and Electrolyte Manifestation of an Aldosterone-Producing Adenoma

Surafel Gebreselassie, MD, Emmanuel Bravo, MD, and Jihad H. Kaouk, MD

Primary aldosteronism was first described by Jerome Conn in 1955 in a patient with aldosterone-producing adrenal adenoma. It’s now a widely recognized cause of secondary hypertension. The following case illustrates the challenges of making a clinical diagnosis of aldosterone-producing adrenal adenoma.

A middle-aged woman with medullary sponge kidney and nephrocalcinosis was first diagnosed with an adrenal mass on a CT done for kidney stone evaluation about 10 years ago. She had normal serum potassium and serum bicarbonate. Subsequently she was found to have elevated blood pressure and was referred to us in the Department of Nephrology and Hypertension for further evaluation. Her serum aldosterone level was 475.5 ng/dL. Following an oral salt-loading test, despite achieving 290 mmol/day of urinary sodium, she had no kaliuresis. Her urinary aldosterone level was 231 ug/day. After further discussion with our colleagues in the Department of Urology, we recommended that the patient undergo a robotic-assisted laparoscopic adrenalectomy. The surgery was successful. The patient became normotensive and required no further anti-hypertensive medication.

Aldosterone is a potent mineralocorticoid secreted by the zona glomerulosa of the adrenal cortex. Unlike cortisol, which is inactivated to cortisone by 11beta-hydroxysteroid dehydrogenase, aldosterone avidly binds to the mineralocorticoid receptor in the distal nephron, leading to sodium retention and resultant anti-natriuresis, volume expansion, hypertension, metabolic alkalosis and renal potassium wasting. Our patient had a very high level of circulating serum aldosterone (475.5 ng/dL) but neither presented with hypokalemia or metabolic alkalosis nor had resistant hypertension, which delayed the diagnosis of aldosterone-producing adenoma. We thought that the renal tubular disease as a result of nephrocalcinosis caused the distal tube resistant to the action of aldosterone even in the presence of high distal sodium delivery and high aldosterone levels.

This case illustrates the challenges of making a clinical diagnosis of primary aldosteronism in the presence of renal tubular disease.
Hypertension is a leading diagnosis of ambulatory care visits to physicians in the United States and is associated with increased cardiovascular morbidity and mortality. Although the rate of hypertension control has increased over the past two decades, population-based studies indicate that it remains suboptimal in spite of the availability of a variety of anti-hypertensive medications. Nonpharmacologic approaches to the treatment of resistant hypertension have emerged as potential adjuncts to existing pharmacologic therapy.

Hyperactivation of the sympathetic nervous system has a major role in the initiation, development and maintenance of hypertension. Renal sympathetic nerves run in a mesh-like pattern around the renal arteries. Using a novel catheter-based percutaneous radiofrequency (RF) ablation device, the renal sympathetic nerves are accessed through the femoral artery and a proprietary algorithm of RF energy is applied to the luminal surface of the renal artery, thereby providing thermal injury selectively to the renal sympathetic nerves without affecting abdominal, pelvic or other lower extremity nerves. Patients undergoing selective renal denervation have been shown to have decreased norepinephrine spillover and decreased muscle sympathetic nerve activity, providing evidence that the procedure reduces sympathetic tone.

The device is available only for investigational use in the United States. Renal denervation is already approved for use in Europe and Australia.

The major clinical trials (SYMPLICITY HTN-1 and SYMPLICITY HTN-2) have indicated significant blood pressure reduction with renal denervation that is sustained over at least a two-year period. In those trials, the primary effectiveness endpoint was change in seated office-based blood pressure from baseline to six months.

In SYMPLICITY HTN-2, which was a randomized controlled trial, office-based blood pressure at six months changed by a mean of -32/-12 mm Hg (SD 23/11 mm Hg) in the denervation group, compared with a mean of 1/0 mm Hg (SD 21/10 mm Hg) in the control group. Forty-one patients who had denervation (84 percent) had a significant decrease in SBP of ≥ 10 mm Hg at six months compared with baseline values. No significant difference was reported in the mean change in renal function at six months.

Recent studies have also reported the efficacy of this procedure in hypertensive patients with chronic kidney disease. Sleep apnea, metabolic syndrome, left ventricular hypertrophy and diabetes may also respond favorably to this procedure.

While there is certainly cause for guarded optimism, further understanding of this therapy with long-term follow-up is needed. Enrollment in the SYMPLICITY HTN-3 study has been under way since January 2012, for what will be the largest trial to date. The study has a targeted randomization of approximately 530 patients using strict enrollment criteria, including the use of maximally tolerated doses of anti-hypertensive medications, including diuretics, and will focus more on the use of ambulatory blood pressure monitoring and subject blinding. This study will help further analysis of this technology in a more diverse population.

SYMPLICITY HTN-3 eligibility criteria include a systolic blood pressure of greater than 160 mm Hg and taking at least three anti-hypertensive medications, one of which must be a diuretic, in the denervation group.

To be eligible for SYMPLICITY HTN-3, patients must:

• Have a systolic blood pressure greater than 160
• Currently be on at least three anti-hypertensive medications, one of which must be a diuretic
• Ideally, patients will be on maximum doses of those medications

The departments of Nephrology and Hypertension and Cardiology at Cleveland Clinic are currently enrolling patients in the SYMPLICITY HTN-3 trial. More than 90 sites across the country are involved in the trial.
Catheter-based renal denervation

The renal denervation procedure
Home Monitoring Technology to Optimize Hypertension Control: Challenges and Potential Solutions

**Martin Schreiber, MD, and George Thomas, MD**

As the public’s awareness of hypertension and its potential complications has increased over the past two decades, the medical profession is moving from encouraging blood pressure (BP) self-monitoring outside the healthcare provider’s office to establishing a BP monitoring platform in the patient’s home.

At Cleveland Clinic, this monitoring system would be linked to a patient’s personal electronic medical record (EMR) within Cleveland Clinic’s health system and could serve to facilitate clinical action, based on data obtained in the patient’s home, to optimize BP control and improve outcomes.

**The Scope of the Problem – by the Numbers**

By 2020, the percentage of the U.S. population age 65 and older is expected to expand to 17 percent of the total population, or 50 million Americans. Within this population, 84 percent of Medicare beneficiaries have at least one chronic condition that can significantly escalate healthcare spending, which is expected to reach 19.3 percent of the nation’s gross domestic product, or $4.5 trillion, by 2019.

The cost-effective management of chronic disease in the home has the potential to reduce healthcare delivery costs through home monitoring initiatives. These new models of care would serve to extend the healthcare provider’s office into the patient’s home with technology that could automate follow-up without the need to travel to the provider’s office, track treatment results and apply treatment modification based on evidence-based treatment algorithms.

This active management process could potentially decrease both admissions and rehospitalization, lower chronic disease complications, decrease lost workdays for patients, and facilitate disease management with less practitioner variability.

The potential success of home monitoring technology (HMT) depends on technology/device costs and ease of use, documentation of scientific proof that this technology can lower costs and improve outcomes, and broad acceptance by patients and insurance companies.

Over the past several years, the Department of Nephrology and Hypertension at Cleveland Clinic has explored different approaches to extending the office into the home to optimize BP control. During that time, a number of challenges have been discovered. Some of these difficulties had been anticipated, while others have been surprising both from the technology standpoint and from the patients’ perspective.

**The Feasibility of Home Monitoring Technology**

In 2008, the departments of Nephrology and Hypertension, Endocrinology, and Internal Medicine in conjunction with the Information Technology Department at Cleveland Clinic evaluated the feasibility of transmitting patient data obtained in the home setting and depositing it in the individual’s healthcare record at Cleveland Clinic. The tools used in this evaluation included Cleveland Clinic’s existing EMR, which uses Epic, the Microsoft Healthcare Vault software and a Microsoft digital BP recorder.

More than 200 patients participated in this initial feasibility trial. Early on in the study, our progress was slowed because our patients experienced difficulty in installing the necessary software on their home computer systems, a process made more difficult due to the wide variety and ages of computers used by our patients. A remote transmitter in the home was then used so that BP data could be successfully transmitted to Cleveland Clinic and then entered into the EMR.

By early 2009, several studies demonstrated that coordinated monitoring of home BP readings by healthcare professionals (e.g., nurse practitioners, pharmacists and physicians) coupled with an active management response strategy could decrease variation in treatment, achieve more optimal control of BP and decrease office visits. In partnership with Vital Stream Health, we designed a study that would compare a group of 37 patients with resistant hypertension, closely monitored via a Virtual Hypertension Clinic (VHC) practice management system vs. a group of 37 resistant hypertension patients receiving standard care who would not be monitored via the VHC.

The overall study objectives were to evaluate whether the intervention group had better outcomes in terms of target BP and to determine whether the cost and time to manage the data streaming into the EMR were used to manage the intervention group to better outcomes was less than those associated with the standard method.

The VHC group utilized a personal wireless BP monitor that transmitted data via a cellphone or in-house modem to a central server in Germany. This data was managed by IEM GmbH and IEM’s subcontractors Avetana GmbH and Hy-line GmbH. At the server, the data was qualified and integrated with the patient’s existing data file where, in accordance with the JNC 7 based clinical rule set, it was displayed in a web-based report card. A nurse practitioner and the study investigator reviewed the BP performance of the entire group in aggregate and the individual patient’s detail. The readings were assessed, and where the results deviated from JNC-7, modified medication and designed behavior change were indicated.
What We Learned

Two major hurdles were uncovered in the study. First, the technology needed more development to fully accomplish the study goals. Second, the biggest hurdle by far related to the patient: a significant percentage of patients with the diagnosis of resistant hypertension declined participation while other patients did not want to take BP readings at the intervals desired. Through the study methodology, we also discovered a surprising amount of medication noncompliance and a range of reasons for this noncompliance, including cost to the patient of medication dosing adjustments, side effects and simple unwillingness to make a change.

With this experience, we subsequently shifted our future focus from resistant hypertension to a more engaged, health-conscious, proactive cohort, and we began exploring futuristic technology that could measure BP without active participation of the patient or disruption in the daily routine.

A New Approach

More recent technology developed and tested internationally by EarlySense leverages an automatic, continuous, contact-free patient monitoring system that documents a patient’s vital signs (heart rate, respiratory rate and movements) without the patient realizing that a measurement is being taken. The contact-free sensor slips under the mattress and never touches the patient. It provides measurements without the need for direct patient contact and provides immediate data transfer to a healthcare provider’s workstation.

Without the need for the patient to actively participate in monitoring their vital signs, and technological advances through software packages, this new option could provide BP monitoring 24 hours a day. Night monitoring is especially important based on what we have learned about the importance of achieving a nocturnal dip in BP to decrease morbidity risk in patients with hypertension who are at risk for heart or kidney end organ disease. We are closely monitoring the advancement of this technology and will review the results of feasibility studies that are planned.

While the potential exists for HMT to decrease healthcare costs and improve cardiovascular disease outcomes, the field needs more monitoring technology development, lower device and system costs, and the design and completion of studies that generate scientific data to support the belief that HMT can improve patient outcomes. This is critical to achieving broader acceptance by patients, healthcare systems, employers and insurers. At Cleveland Clinic we continue to accumulate experience and understanding of the challenges that lie ahead for HMT systems and the solutions that can help this advancement in patient care to achieve the greatest acceptance in the future.
Volatile Organic Compounds Measured in Breath Analysis of Patients with End-Stage Renal Disease, Chronic Kidney Disease and Controls

Sevag Demirjian, MD, Kelly Paschke, Jaime Newman, Raed Dweik, MD, and Robert Heyka, MD

Many organic compounds are retained in renal failure, and those associated with adverse biologic effects are referred to as uremic toxins. No solute has emerged as the sole, primary toxin responsible for the uremic syndrome. In dialysis patients, urea kinetic modeling does not reflect the behavior of many other solutes retained in renal failure, and the removal of urea does not ensure adequate removal of other solutes that differ in their volume of distribution, size and charge.

Several of the solutes that accumulate in renal failure are volatile and could be captured via breath analysis. We are performing a series of pilot studies of exhaled breath analysis of volatile organic compounds (VOCs) in patients on hemodialysis with chronic kidney disease (CKD) or with acute kidney injury (AKI) and a control group. Our goals are to gather information about the composition, concentrations and physical characteristics of VOCs, and to look for associations with other markers of renal function and dialysis adequacy.

The advantages of breath analysis include its noninvasive nature, ease of repeat measurements, and its ability to be used in adult and child populations with severe airflow obstruction when other techniques would be difficult or impossible to perform. It is used to monitor asthma, detect Helicobacter pylori infection, measure blood alcohol concentration, detect transplant organ rejection, and monitor breath gases during mechanical ventilation and anesthesia. With recent advances in sensor technology and mass spectrometry, hundreds of common breath volatiles can be measured. The introduction of selected ion flow tube mass spectroscopy (SIFT-MS) has made it possible to detect VOCs in the magnitude of parts per trillion. SIFT-MS has successfully been applied in studies of patients with CKD and end-stage renal disease (ESRD) by members of our group and others.

Evidence suggests that patients with ESRD have a unique breath profile, possibly reflecting solute retention, especially of nitrogen-containing compounds. Several VOCs have been studied in patients with renal failure. Levels of trimethylamine, ammonia and dimethylamine are elevated in patients with renal failure and removed during dialysis. Methylamines, deemed responsible for uremic breath odor, have a lower reduction ratio compared with urea due to sequestration within red blood cells (similar to creatinine). Water-soluble guanidino compounds, which are similar in size to urea, have a significantly different distribution of volumes compared with urea and do not follow urea kinetics. Isoprene shares a common precursor with cholesterol and steroids – namely mevalonic acid. No breath test is clinically standardized for assessing the ESRD breath profile and the adequacy of hemodialysis.

Our recent studies, in cooperation with Cleveland Clinic’s Respiratory Institute, compared the breath profiles of hemodialysis patients treated at an outpatient unit with those of healthy volunteers. We compared pre- and post-dialysis breath analysis, clinical measures of dialysis adequacy and the profiles of VOCs in stable patients with varying degrees of CKD with the VOC profiles of controls.

Patients and controls underwent a mouth rinse with water prior to exhaled breath collection to reduce external contamination and oral production of VOCs. SIFT-MS was used to analyze exhaled breath samples. The instrument used in this study was a VOICE200® SIFT-MS instrument (Syft Technologies Ltd., Christchurch, New Zealand). Fourteen preselected compounds underwent quantitative selective ion methodology: 2-propanol, acetaldehyde, acetone, acrylonitrile, ammonia, benzene, carbon disulfide, dimethyl sulfide, ethanol, hydrogen sulfide, isoprene, pentane, triethylamine and trimethylamine.

Key Point:
We are performing a series of pilot studies of exhaled breath analysis of volatile organic compounds (VOCs) in patients on hemodialysis with chronic kidney disease or with acute kidney injury and a control group.

Our goals are to gather information about the composition, concentrations and physical characteristics of VOCs, and to look for associations with other markers of renal function and dialysis adequacy.
Results

Preliminary findings show that within a large array of VOCs, a majority of levels are elevated in dialysis subjects compared with healthy controls, and several levels of VOCs are reduced during dialysis, of which a handful reach normal levels. The levels of few VOCs are higher in controls compared with predialysis concentrations. In CKD patients, the concentrations of several VOCs are associated with a decline in estimated glomerular filtration rate.

Significance

The results of these studies have the potential to influence the way we monitor patients with CKD, AKI and ESRD. In addition, these studies will provide insight into the use of fractional exhaled nitric oxide (FeNO) as a biomarker of kidney disease progression using improved FeNO measurement technology and procedures. In the future, VOCs measured through breath analysis may serve as a prognostic marker and may be used to fine-tune therapy in an effort to improve clinical outcomes in patients with renal failure.

Obesity and Intentional Weight Loss Modalities in Chronic Kidney Disease

Sankar Navaneethan, MD, MPH

The prevalence of obesity has increased in the United States, and the number of patients with chronic kidney disease (CKD) has increased concomitantly. Experimental and population-based studies have established the mechanistic links between obesity and CKD. After the development of kidney disease, the presence of obesity is associated with increased risk for both progression of kidney disease and all-cause mortality. Sedentary lifestyle and higher energy intake have been linked to the increase in obesity rates in the general population.

It is unclear whether CKD patients have a similar macronutrient dietary intake and physical activity levels compared to individuals without CKD. Also, whether patients with chronic medical conditions such as CKD pursue weight loss programs at rates similar to those of the non-CKD population has not been examined. Commercial weight loss programs are widely available, and several billions of dollars are spent on weight loss-related products and services every year in the United States. Recommendations for weight loss include both reduced caloric intake and increased physical activity. These recommendations may not be applicable to the CKD population, and the types of weight loss modalities followed by CKD patients in the community is unknown. We therefore studied 1) the lifestyle (dietary composition and physical activity levels) and behavioral factors (desire to weigh less and pursue weight loss) among overweight and obese CKD and non-CKD participants, 2) factors related to pursuing weight loss, specifically the presence of CKD and the various weight loss modalities followed by overweight and obese CKD and non-CKD subjects among a nationally representative sample of U.S. adults who participated in the National Health and Nutrition Examination Survey.

The total daily energy intake of the CKD population was lower than that of the non-CKD group (1,987 kcal/day vs. 2,063 kcal/day), even after adjusting for relevant covariates. However, the percentage of energy derived from protein was similar between the groups. Sixty-six percent of the CKD population did not meet the minimum recommended leisure time physical activity goals compared with 57 percent of non-CKD subjects. CKD participants had lower awareness about dietary guidelines (46.8 percent vs. 52.9 percent, p < 0.01) and the food pyramid (71.2 percent vs. 80.1 percent, p < 0.001) compared with non-CKD participants. When adjusted for relevant confounding variables, CKD participants had significantly lower odds of awareness of dietary guidelines (p < 0.03) but not for awareness of the food pyramid (p < 0.26).

Of the 10,971 overweight and obese participants, 5,453 reported pursuing weight loss. Among CKD participants, 50 percent reported pursuing weight loss compared with 54 percent of non-CKD participants (p < 0.01). When adjusted for demographics, comorbidities and household income, presence of CKD was not associated with pursuit of weight loss (odds ratio 0.97; 95 percent confidence interval: 0.83-1.14). African-Americans, lower-income groups and older adults had lower odds of pursuing weight loss. Presence of diabetes and obesity and female gender were associated with increased odds of pursuing a weight loss program. Among participants pursuing weight loss, modalities utilized by CKD and non-CKD participants, including dietary interventions, were similar. Eight percent of CKD participants used medications to promote weight loss.

In conclusion, among the overweight and obese population, lifestyle and behavioral factors related to obesity and weight loss are similar between CKD and non-CKD participants. Insufficient data exist on the beneficial effects of intentional weight loss in CKD, but our data suggest that a significant proportion of the CKD population follows diets that may have high protein content and uses potentially harmful medications to promote weight loss. These results suggest that clinical trials to evaluate the efficacy of various modalities to treat obesity in the CKD population are warranted.
Sankar Navaneethan, MD, MPH

Chronic kidney disease (CKD) is common. A significant proportion of nondialysis-dependent CKD patients die of cardiovascular disease before they reach end-stage renal disease. Dyslipidemia is a well-established risk factor for cardiovascular disease in the general population and is prevalent among nondialysis-dependent CKD patients. The pattern of lipid abnormalities differs between the CKD and non-CKD populations. Patients with CKD have a higher prevalence of hypertriglyceridemia and small, dense low-density lipoprotein (LDL) particles. The American Heart Association has issued new guidelines for the management of elevated serum triglyceride levels in the general population, acknowledging that serum triglyceride level is an important risk factor for cardiovascular disease and death. Therefore, we examined the association between serum triglyceride level and all-cause mortality and whether this association differs based on age and the presence or absence of comorbid conditions in a large cohort of stage 3-4 CKD patients followed in our healthcare system.

Of 25,828 CKD patients who had lipid levels measured, 38.2 percent (n = 9,867) had serum triglyceride levels >150 mg/dL. African-American descent, increasing age and increasing estimated glomerular filtration rate were associated with lower odds of having higher serum triglyceride levels. Higher body mass index, the presence of diabetes and hypertension, and smoking history were associated with higher odds of having higher serum triglyceride levels.

In the Cox proportional hazards model that included all patients, after adjusting for demographics, comorbid conditions, renal function, lipid parameters and use of anti-hyperlipidemic drugs, a serum triglyceride level of 150–199 mg/dL was not associated with death while a serum triglyceride level > 200 mg/dL was associated with an 11 percent increased hazard for death. When examined as a log-transformed continuous variable, serum triglyceride was not associated with mortality for the overall group (hazard ratio [HR] 1.06 per each log increase in triglyceride, 95 percent confidence interval [CI]: 0.98 –1.14). Age modified the association between triglyceride levels and mortality, with patients < 65 years having a 26 percent higher risk for death (95 percent CI: 1.05 - 1.51) and those > 65 years having no increased risk for death.

Similar results were found when serum triglycerides were examined as a continuous variable (log-transformed values), with patients < 65 years having a higher risk for death (HR 1.26; 95 percent CI: 1.10–1.45) but not for patients > 65 years (HR 0.96; 95 percent CI: 0.89 –1.04) (Figure). Serum triglyceride levels > 200 mg/dL are associated with all-cause mortality among stage 3-4 CKD patients. This effect was prominent among CKD patients < 65 years and not evident among patients ≥ 65 years. Our results suggest that an elevated serum triglyceride level may be considered a risk factor for death in the CKD population. If future studies confirm these findings, clinical trials examining whether lowering serum triglyceride levels can reduce cardiovascular disease burden and mortality among CKD patients < 65 years may be considered.

For references, please email the editor.

Associations between all-cause mortality log hazard and baseline serum triglyceride levels, by age
Key Point:

Polycystic kidney disease (PKD) is among the most abundant single-gene disorders in humans. Its most frequent subtype, autosomal dominant PKD, affects about 1 in 800 people in the U.S. Most current approaches to develop new drugs to treat PKD focus on preventing the expansion of kidney cysts by inhibiting either cell growth or fluid accumulation in the cysts. We believe that concentrating on the first steps leading to cyst formation will result in the discovery of new angles for treating PKD in the future.

Polycystic kidney disease (PKD) is among the most abundant single-gene disorders in humans. Its most frequent subtype, autosomal dominant PKD, affects about 1 in 800 people in the U.S. It is characterized by the formation of fluid-filled cysts that accumulate and expand over time, thus causing kidney malfunction and eventually leading to kidney failure. Contrary to its name, PKD not only affects the kidneys but is actually a multiorgan disease that also leads to the development of liver cysts, cardiovascular complications and intracranial aneurysms, among other comorbidities.

Importantly, even though the genes mutated in human patients, polycystin-1 and polycystin-2, have been cloned for several years, the underlying molecular mechanisms of cyst development are still poorly understood. As a consequence, dialysis and kidney transplantation are still the only approved therapeutic options.

Most current approaches to develop new drugs to treat PKD focus on preventing the expansion of kidney cysts by inhibiting either cell growth or fluid accumulation in the cysts. While such approaches are clinically relevant and can postpone or even alleviate the necessity to clinically interfere with the disease, they only treat the symptoms and will never cure the entire spectrum of PKD symptoms.

My laboratory is tackling this problem by focusing on the initial events that trigger the transformation of a normal renal epithelial cell into a precystic one. We believe that concentrating on the first steps leading to cyst formation will result in the discovery of new angles for treating PKD in the future.

Figure. Bicaudal-C and polycystic kidney disease. (A,B) Schematic diagram of a normal nephron and a nephron in autosomal dominant polycystic kidney disease (ADPKD) with cyst formation along the entire nephron. (C) Flow diagram outlining the proposed mechanism of BicC1 regulating epithelial homeostasis by modulating the expression of polycystin-2. (D,E) Kidney histology of a BicC1 mutant mouse and its wild-type littermate at P0. (F-I) PKD phenotype in Xenopus is characterized by the formation of edema (indicated by arrowheads in G) and the enlargement of the pronephric kidney tubules (pn in I). Xenopus embryos lacking BicC1 (xBic-C-MO1+2) or left untreated and analyzed at stage 4 by morphology (F,G) and histological analysis (H,I).
will result in the discovery of new angles for treating PKD in the future. The hope is that this focus will provide novel therapeutic approaches.

To this end, my laboratory is using a two-model system approach, combining a mouse model with the African clawed frog, Xenopus laevis. While the metanephric kidney in the mouse has been the model system of choice to study PKD, my group was the first to establish the amphibian pronephros as an alternative. With its fast development (a functional kidney forms within two days), its ease of molecular manipulations and the large number of eggs laid in one session, the Xenopus is an ideal companion system to the study of PKD in mice, where genetic studies can easily take a year before definitive answers are obtained. Using the distinct advantages of both organisms greatly facilitated the formulation and experimental testing of a new hypothesis about the role of Bicaudal-C in the kidney and its contribution to the formation of PKD.

Bicaudal-C as a Post-transcriptional Regulator

Among the several projects in my laboratory that study these early steps of cystogenesis in PKD, the one studying Bicaudal-C (BicC1) best illustrates the potential of the approach and the possibilities for novel therapeutic areas. BicC1 encodes an RNA-binding molecule, first identified in Drosophila as a protein-regulating anterior-posterior development. We identified the Xenopus and mouse homologues of BicC1. Interestingly, mouse mutants of BicC1 and Xenopus embryos lacking BicC1 develop kidney abnormalities reminiscent of PKD. However, it was unknown why BicC1 is required to prevent renal cyst formation and how it relates to other molecules that are involved in PKD.

To address the molecular mechanism of BicC1 activity, my laboratory performed an in-depth analysis in BicC1 mutant mice. Knockout of BicC1 in mice resulted in the formation of cysts along the entire length of the nephron, a mouse phenotype remarkably similar to the one of polycystin-1 or polycystin-2 mouse mutants. These epithelial malformations were not caused by increased proliferation.

Instead, using the pronephric kidney of Xenopus and the metanephric kidney of mouse, we could show that BicC1 regulated the expression of polycystin-2, one of the proteins causing PKD in humans. Molecular analyses demonstrated that this effect of BicC1 was caused in a completely unexpected way. BicC1 protein was localized to cytoplasmic foci that were positive for P-body markers such as GW182. These foci have previously been shown to be the sites of post-transcriptional regulation by a novel class of molecules - small noncoding RNAs termed “microRNAs” (miRNAs). These miRNAs bind to the 3’ UTR of a given gene and regulate mRNA stability and/or translation.

Indeed, studies in my laboratory have shown that polycystin-2 mRNA is regulated by the miR-17 miRNA family and that this repressive activity is antagonized by BicC1. Most important, the in vivo relevance of this mode of action was confirmed, again using the Xenopus PKD model. The pronephric kidney defects caused by loss of BicC1 were rescued by reducing miR-17 activity. Based on these data, I proposed that the PKD phenotype in BicC1 mutant mice is caused by dysregulation of a miRNA-based translational control mechanism. This work demonstrated for the first time that post-transcriptional regulation is important in maintaining epithelial structures and that the disruption thereof can result in PKD formation.

One intriguing aspect of these findings is that miRNAs have emerged as a novel class of cellular regulators that frequently function as rheostats, adjusting cells to different environmental conditions. As such, their expression is often altered under disease conditions. This has resulted in a growing field that uses miRNA as biomarkers for disease progression. Furthermore, correcting those expression levels has emerged as a novel therapeutic option, particularly in cancer therapy.

In the case of PKD, similar approaches are still in their infancy. My laboratory is currently gearing up to test the feasibility of the approach using the BicC1 mutant mice for a proof-of-principle experiment.

_for references, please email the editor._
Simultaneous Nephrectomy and Transplantation for Autosomal Dominant Polycystic Kidney Disease

David Goldfarb, MD

In the past, when a patient with autosomal dominant polycystic kidney disease (ADPKD) awaiting a transplant required nephrectomy due to either large kidney size or symptoms, the nephrectomy was traditionally performed as a separate procedure before the transplant. The reason for separating the procedures was the belief that the magnitude of both the nephrectomy and transplant would not be tolerated with the transplant procedure. The next advance was to perform the nephrectomy using minimally invasive techniques, also separately from the transplant.

We are now performing simultaneous nephrectomy and transplantation for selected patients with end-stage renal disease (ESRD) and ADPKD. This practice comes with significant advantages to the patient. First, the patient undergoes only one procedure with a single anesthetic. When the procedures are performed separately, there is always the potential for transfusion. Transfusion in the absence of transplant and the administration of immunosuppression carry the risk of allosensitization. There is a real chance that some candidates could lose potential living donors in this way or have more difficulty in matching with a deceased donor. In the past five years, we have increasingly used simultaneous nephrectomy and transplant in ADPKD, an advance well received by patients.

Kathy, now living out of state, returned to Cleveland Clinic when she learned that she would need a kidney transplant. She had known of her disease for many years, during which time her kidney function was deteriorating, and it was only a matter of time before she would need some form of renal replacement. Fortunately for her, she had many potential donors who had volunteered to give her a kidney. Her daughter turned out to be a match, and plans were made for a transplant.

Kathy's kidneys had become very large over the years. Just before she presented to us, they were causing abdominal distention and made her feel full quickly when she ate a meal. She experienced a constant sense of discomfort that was directly attributable to her large kidney size. She had had several episodes of painful cyst rupture over the past several years, causing hematuria. These episodes had involved both kidneys.

On examination, her kidneys were palpable into the pelvis. Her CT scan confirmed large kidneys that extended into the pelvis and a large polycystic liver. It was clear that we would need to make space in the pelvis to have a successful kidney transplant. She was reluctant to undergo separate surgeries, one to remove her kidneys and one for a transplant of the donated ones. We decided she would be a candidate to undergo simultaneous bilateral nephrectomy and transplantation.

Using a midline incision and special retractors, we were able to remove both kidneys uneventfully. This surgery lasted three hours, during which time her daughter underwent donor nephrectomy. The donor kidney was extracted and perfused by the transplanting team. The kidney was then transplanted into the right iliac vessels of the recipient without any concerns about room to fit the kidney or about the large ADPKD kidney compressing the newly transplanted organ. The transplanted kidney worked immediately.

Kathy was motivated to participate fully in her recovery and was walking the hallways on the first day after the operation. Her total hospital stay was five days, perhaps only one day longer than would be typical with the standard extraperitoneal incision.

We believe that there are advantages to avoiding two separate procedures by simultaneously performing nephrectomy and transplantation in patients with ADPKD. Patients can avoid two anesthetics with recoveries, and avoid the risk of transfusion separate from immunosuppressive therapy. Unilateral nephrectomy can be done when there is a need to make space for a transplant and the opposite kidney is asymptomatic. When kidneys are truly massive or both are symptomatic, it is better to remove both kidneys and then transplant. We have considerably improved the morbidity of this approach over time. While this approach will be appropriate for many patients, not all patients will benefit. Furthermore, not all patients with ADPKD require kidney removal. These decisions are based on the specific circumstances of each case as discussed between the transplant team and the patient.
Regulation of Hyaluronan Synthesis by Heparin and Its Oligosaccharides in Diabetic Nephropathy

Aimin Wang, PhD

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease. In general, DN can be divided into two stages: 1) the genesis stage, with early cellular, structural and functional changes; and 2) the progression stage, with advanced structural and functional changes leading to irreversible renal failure. Mesangial expansion is the principal glomerular lesion in DN that reduces the area for filtration and leads eventually to sclerosis and renal failure.

However, the mesangial extracellular matrix expansion and sclerosis are preceded by an early phenotypic activation and proliferation of the glomerular mesangial cells, followed by a prominent glomerular infiltration of monocytes and macrophages associated with glomerular hyaluronan matrix formation. Glomerular monocytes and macrophages have been prominently identified in DN in both animal models and humans, and appear to have a key role in the induction of mesangial matrix expansion by elevating glomerular TGF-beta, hypercellularity and the onset of proteinuria, which are characterized by inflammatory processes that are not yet clearly understood. Our studies focus on the hyaluronan-based matrix that is synthesized by dividing cells stressed by hyperglycemia, and on its dialogue with associated macrophages, which are derived from circulating monocytes that are recruited into the inflamed tissues. We believe that this monocyte-adhesive hyaluronan matrix and its interactions with the macrophages have a central role in defining the resulting inflammatory pathologies, and that understanding the mechanisms involved will identify ways to ameliorate or prevent the pathological consequences.

Further, under hyperglycemia, elevated glucose metabolites (UDP sugars) are major contributors to pathological responses. Our studies will also reveal significant new insights regarding the potential therapeutic roles of the heparin trisaccharide or oligosaccharides by decreasing intracellular UDP sugars based on the formation of the hyaluronan matrix and the glomerular macrophage infiltration in DN.

Hyaluronan, a high-molecular weight (MW) polysaccharide, is normally synthesized at the plasma membrane by hyaluronan synthases that use cytosolic UDP-sugar substrates and extrude the growing polymer into the extracellular space (Figure 1). Thus, it is important for the synthases to be embedded in the plasma membrane before they are activated.

Our studies showed that rat mesangial cells that divide in hyperglycemic (25.6 mM) glucose activate hyaluronan synthases inside the cell before reaching the plasma membrane. This induces synthesis in intracellular compartments - endoplasmic reticulum (ER), transport vesicles and golgi (Figure 2). The deposition of the high-MW, polyanionic hyaluronan polymer in the ER initiates a novel ER stress autophagy that upregulates cyclin D3 at the end of cell division, and that cyclin D3 activates an autophagosome process that extrudes hyaluronan to form a monocyte-adhesive extracellular matrix.

This pathological hyaluronan response of dividing cells to hyperglycemic medium has been demonstrated in aortic smooth muscle cells and in 3T3-L1 cells, which have been used extensively as a model for normal adipogenesis. It is likely to be centrally involved in many diabetic pathologies, where adipogenesis and pathological matrices are common.

Studies by Gambaro et al. showed that a daily IP injection of a small amount of a low-MW heparin preparation in the
Diabetic rat model prevented the nephropathy and protein-urea over an eight-week period. Our new data show that the presence of 0.2 microM heparin in hyperglycemic medium prevented the intracellular hyaluronan synthesis and the autophagic response in dividing mesangial cells. However, at the end of cell division, the cells produced approximately threefold more monocyte-adhesive extracellular hyaluronan matrix than did cells dividing in hyperglycemic medium alone. This process occurs in diabetic rats that were treated daily with the heparin (Figure 3). The hyaluronan content of the glomeruli from the treated rats increased greatly during Weeks 1-2 and then decreased to near normal levels by Week 6. The animals did not have the nephropathy or proteinurea. The glomeruli in sections from the Week 6 kidney showed no autophagy and very little hyaluronan but contained a large number of macrophages.

Our most recent data show that a heparin oligosaccharide initiates the same reaction as heparin in mesangial cells simulated to divide in hyperglycemic medium. This oligosaccharide, unlike heparin, does not have anti-coagulant activity and will be a valuable reagent in which to study the mechanisms involved in these processes in both in vitro and in vivo models.

**Future Perspective**

Our hypothesis is that dividing cells have a cell surface receptor that interacts with this heparin oligosaccharide whereas nondividing cells do not. This is supported by our previous studies that showed that heparan sulfate oligosaccharides generated by heparanase cleavage during catabolism of heparan sulfate proteoglycans bound to mesangial cells showed arrested growth at the G0/G1 cell division stage, but did not do so in confluent mesangial cells. Heparin also bound similarly and showed a Kd of 1.6 x 10^-8 M. The mammalian heparanase is an endoglucuronidase that exposed nonreducing terminal sulfated glucosamines, consistent with our results with the heparin oligosaccharide.

Our future studies will focus on 1) identifying this receptor, 2) determining the signaling pathway that activates the hyaluronan synthases inside the dividing cell that is blocked in the presence of the heparin oligosaccharide, and 3) determining the signaling pathway that greatly activates hyaluronan synthase activity in the plasma membrane in the presence of the heparin oligosaccharide, which forms the extensive pathological monocyte-adhesive hyaluronan extracellular matrix.

*For references, please email the editor.*
Successful Transvaginal Removal of Intravesical Mesh Perforation

Sandip Vasavada, MD, Howard Goldman, MD, Courtenay Moore, MD, and Raymond Rackley, MD

Patients who have mesh-related complications from vaginal prolapse surgery represent a small but problematic group to manage. Earlier in 2012, the U.S. Food and Drug Administration recommended that additional clinical trials (known as 522 extension studies) be performed on patients willing to undergo the use of vaginal mesh for correction of pelvic organ prolapse. These studies (if done) will likely lead to randomized trials on standard suture-based repair vs. the use of transvaginal mesh in women with prolapse. These trials may take several years to complete; in the meantime, present-day practice entails management of some of the complications of mesh use in the vagina.

Although complications can occur in many ways, one of the more challenging problems to manage is the intravesical mesh perforation (a term preferred over “erosion” as agreed upon by a joint International Urogynecological Association/International Continence Society committee). Traditional approaches may entail open cystotomy and removal of mesh whereas our approach utilizes a transvaginal scheme in the majority of cases. We have previously published on our series of patients who have been managed with a purely transvaginal approach for intravesical mesh complications.

In most cases, we begin with cystoscopy and placement of ureteral catheters on both sides. Further identification of intravesical mesh and its proximity to the ureters is noted. The catheters further aid in intraoperative identification of the ureters. An inverted U incisionfacilitates exposure to the anterior vaginal wall and mesh and allows later flap closure over the mesh perforation and repair. We then dissect down to the mesh and track the mesh toward the bladder entry. If a fair amount of the mesh is in the bladder, one may have to track laterally to locate the full extent of the mesh (even outside the bladder itself). If not easily localized, the incisions may need to be extended laterally toward the obturator to guide the mesh toward the bladder perforation.

A right-angle clamp is placed behind the mesh, and then the mesh is divided in two. The mesh is then dissected away from the midline on both sides toward the lateral portions for a few centimeters. This lateral dissection must be done with care as this portion (intravesical portion) is often intimately associated with the ureter. The mesh is then excised with a border away from the bladder incision. An important caveat is that all mesh in the area of the perforation must be removed to ensure a successful bladder closure.

A multilayer closure is then performed, and the ureteral catheters are allowed to remain in situ or are exchanged for JJ stents depending on proximity or issues with the ureters. A Foley catheter is left in place for 2-3 weeks prior to a cystogram to assure healing.

Successful removal of intravesical mesh can be undertaken in the majority of cases in this fashion. The advantages of this approach are as follows: 1) it allows better access to the mesh placement itself (transvaginal) to facilitate its removal; 2) morbidity is minimal, easing patient healing, compared with the open transvesical approach; 3) it permits identification of the ureters intraoperatively; and 4) management of any simultaneous urethral pathology is possible in the same setting.

For references, please email the editor.
Endoscopic-Guided Access for Percutaneous Nephrolithotomy Decreases Intraoperative Fluoroscopy Time and Improves Accuracy of the Puncture

Manoj Monga, MD

Gaining renal access is the most critical step for effective and efficient percutaneous nephrolithotomy (PCNL). A precise puncture improves the likelihood of a successful outcome and minimizes the risk of intraoperative bleeding.

We retrospectively evaluated outcomes of procedures conducted at Cleveland Clinic with endoscopic-guided percutaneous renal access (EGA) and compared them with the outcomes of a contemporary cohort of patients undergoing fluoroscopic-guided access (FGA).

The surgical steps for the endoscopic-guided procedure are depicted in the Figure. The patient is placed in a prone split leg position. Flexible cystoscopy for males and rigid cystoscopy for females is utilized to cannulate the ipsilateral ureteral orifice with a PTFE-Nitinol™ guidewire with a hydrophilic tip. Using a 10Fr double lumen ureteral dilator, an Amplatz Super Stiff™ guidewire (35 cm length for women, 45 cm length for men) is then placed. A 12/14Fr ureteral access sheath is then placed over the Amplatz Super Stiff wire, unless a ureteral stent had been delivered previously, in which case a 14/16Fr ureteral access sheath is used.

The flexible ureteroscope is advanced into the renal pelvis and manipulated around intrarenal calculi until an appropriate calyx for puncture is identified. A posterior calyx is confirmed by the presence of an air bubble (a in Figure). The tip of the ureteroscope is advanced directly onto the papilla. The C-arm is rotated until the tip of the ureteroscope is seen “head on.” An 18G Chiba puncture needle is visualized, forming a bull’s eye image and introduced to target the tip of the ureteroscope. Once the needle is advanced, the C-arm is rotated back to midline to monitor the depth of the needle advancement toward the tip of the ureteroscope, and the needle is visualized endoscopically entering the collecting system (b, c in Figure).

A floppy-tip Teflon-coated Bentson guidewire is introduced through the needle and grasped with a tipless Nitinol basket (d, e in Figure). The ureteroscope and guidewire are then withdrawn through the ureteral access sheath, gaining secure through-and-through access. The wire is exchanged for a super-stiff guidewire using a 5Fr open-ended catheter. The flexible ureteroscope is reinserted into the site of puncture to monitor balloon dilation of the nephrostomy tract (f, g in Figure) with the X-Force™ balloon dilator (Bard Medical, Covington, Ga.) and advancement of the Amplatz sheath over the balloon (h, i in Figure). Endoscopic monitoring of tract dilation and sheath advancement is performed to prevent underdilation into renal parenchyma or over dilation leading to collecting system perforation.

In our retrospective analysis, 159 patients underwent PCNL between August 2010 and January 2012, 40 percent using EGA and 60 percent using FGA. The groups were comparable in regard to age, American Society of Anesthesiologists score, number of stones, cumulative stone diameter, number of calyces involved and stone density.

Access was obtained with a single puncture in more than 95 percent of patients in the EGA group. Patients undergoing EGA had shorter fluoroscopy time (3.2 min vs. 16.8 min; p < 0.001) and needed fewer accesses (1.03 vs. 1.22; p = 0.002). Fluoroscopy time was longer for FGA as compared with EGA after adjusting for body mass index, staghorn stones and number of accesses (p < 0.001). No significant differences were noted between groups in change in hemoglobin, blood transfusion rate, operative time and intraoperative complications. Procedures were aborted due to bleeding more commonly in the FGA group compared with the EGA group (8 vs. 0 percent; p = 0.02). Two (3.2 percent) of the EGA group had a secondary procedure for stone management, compared with 12 (12.5 percent) of the FGA group.

We conclude that EGA is safe and effective and leads to decreased fluoroscopy time, decreased need for multiple accesses, decreased risk of early termination of the procedure and decreased the number of secondary procedures. Most important, it places the ability to secure percutaneous renal access in the hands of any urologist facile with flexible ureteroscopy.

For references, please email the editor.
Update on Corporeal Excavation: A Technique for Implanting Penile Prostheses in Men with Fibrotic Corpora

Drogo Montague, MD, Kenneth Angermeier, MD, and Hadley Wood, MD

Men who have erectile dysfunction (ED) after ischemic priapism present with corpora where the cavernous smooth muscle has undergone necrosis and been replaced by scar tissue. Men who have had a previously infected penile prosthesis removed also may present with severe scarring of their corporeal smooth muscle. No treatment for ED works in these men other than penile prosthesis implantation, yet this surgery is very difficult and sometimes impossible for a variety of reasons.

In 2006, we described a new technique for penile prosthesis implantation in these individuals, which we call corporeal excavation. Using extended corporotomies, we develop by sharp dissection a plane of dissection between the inner surface of the tunica albuginea and the fibrotic smooth muscle core. This is continued until the fibrotic core has been removed (Figure 1), leaving an empty (excavated) corpus cavernosum (Figure 2). The inflatable penile prosthesis cylinders are laid in place; primary closure of the tunica albuginea over the cylinders is then easily accomplished.

The original paper reported on our experience with the procedure in nine patients. Our experience has now extended to 21 patients, 11 of whom had priapism and 10 of whom presented after removal of an infected penile prosthesis. Satisfactory outcomes were obtained in all 21 patients.

Figure 1. Seven cm core of resected fibrotic smooth muscle.

Figure 2. Excavated corpus cavernosum. Note transverse inner fibers of tunica albuginea.
Surgical Technique to Utilize Renal Allografts with Capsular Procurement Damage to Expand the Kidney Donor Pool

Charles S. Modlin Jr., MD, and Stuart Flechner, MD

Introduction

There are approximately 100,000 patients on the kidney transplant waiting list in the U.S., and that number is rapidly increasing. Not everyone in need of a kidney transplant has a potential living donor. The growing number of patients without living donor options in need of kidney transplants underscores the importance of minimizing the discard rate of deceased donor kidneys available for transplantation.

The number of kidneys recovered but not used for transplantation increased from 14.9 percent to 16.6 percent in 2007. This growing discrepancy is likely to have a larger impact on African-American and other racial/ethnic minority populations. The disparity is more pronounced due to several factors, including the relative lack of living donors in those populations, and a greater need for kidney transplantation in African-American populations due to increased incidence of kidney disease.

In the March/April 2012 issue of the *Journal of the National Medical Association*, we described surgical techniques to reduce discard rates of kidneys from renal allografts with significant capsular defects that were incurred during procurement.

Utilization of extended-criteria donors and kidneys procured after cardiac death, is one way to expand the donor pool. Using pediatric en bloc and dual kidneys are examples of expanded-criteria donor kidneys. In addition, selective use of kidneys with significant capsular damage helps optimal utilization of this scarce resource.

CASE 1: Vicryl Mesh Repair

Case 1 involved a 49-year-old male admitted for a deceased donor transplant. He is a Jehovah’s Witness and as such, expressed his wish not to undergo a blood transfusion. Cleveland Clinic was offered an appropriately matched renal allograft from a transplant program in a neighboring state, which had refused to use the allograft due to multiple arteries.

Upon inspection of the allograft, it was noted that two-thirds of the renal surface was devoid of capsule. The kidney capsule was repaired at Cleveland Clinic by covering the surface of the denuded renal allograft with Vicryl mesh, which acted as a replacement of the capsule (Figure 1). The mesh was trimmed to match the size of the defect and the edges sutured to the capsule with 3-0 chromic catgut. Evicel® (Ethicon, Sommerville, N.J.), a fibrin sealant, was applied over the mesh (Figure 2) to provide further reinforcement. The kidney was then successfully transplanted.

With the release of clamps and beginning of perfusion, there was no bleeding from the renal parenchyma. The postoperative recovery was uneventful and the patient was discharged on Day 5. More than two years later, the graft continues to function well.

CASE 2: DEXON Mesh Repair

A 63-year-old African-American female who had been waiting five years for a deceased donor kidney transplant was admitted to Cleveland Clinic to receive a kidney from a 54-year-old deceased donor. During the bench preparation of the kidney, a relatively large separation of the thin renal capsule on the posterior surface was noted, likely from a subcapsular hematoma.

Upon revascularization, persistent bleeding occurred from the posterior surface of the renal allograft. In addition, the capsule had shredded, exposing large areas of renal parenchyma. Several attempts to control the bleeding with electrocautery, argon beam laser and direct pressure were unsuccessful, and more than two units of blood loss was measured.

Figure 1. Capsular detected surgically covered with coated VICRYL PLUS antibacterial (polyglactin 910) sutures ©Ethicon, Inc. 2009, a Johnson & Johnson Company.

Figure 2. EVICEL fibrin sealant (human) approved for general hemostasis in surgery was then applied over the mesh to provide further reinforcement.
As a rescue attempt to salvage the allograft, the posterior renal surface was covered with Floseal and a 6-cm sheet of DEXON™ (polyglycolic acid) Mesh (Covidien, Mansfield, Mass.), which was sutured closed at the level of the renal hilum with several 3-0 Vicryl sutures. The kidney was further wrapped with a laparotomy sponge, and hand pressure was applied for 30 minutes. After removal of the sponge, the renal surface remained hemostatic.

Discussion: Capsular Injury

Capsular injury of renal allografts most often occurs during procurement of the donor allograft. At times the capsular injury may not be obvious until it becomes evident with the development of brisk subcapsular hematoma following implantation, when vascular clamps are released and renal allograft blood flow is re-established.

Capsular injury can also occur during live donor nephrectomy. The injury can occur during extraction of the kidney by endo catch bag. Minor capsular disruption of the allograft is unlikely to have any major impact on graft function and complication rates. However, capsular disruption, when significant, may lead to nonacceptance and discard of the allograft by the transplant surgeon, given the potential risk of major intraoperative and/or postoperative hemorrhage. Reinforcing the denuded capsule with Vicryl mesh with or without Evicel fibrin sealant, if additionally needed, is a technique that can be utilized by the transplant surgeon in order to salvage the renal allograft and ultimately increase the available pool of kidney allografts for transplantation.

For references, please email the editor.

A Novel Model to Study Mechanisms Underlying Antibody-Mediated Rejection of Renal Allografts

Robert Fairchild, PhD

The use of current immunosuppressive strategies has markedly decreased the incidence of T cell-mediated acute rejection in transplant patients. In contrast, the detected incidence of antibody-mediated graft rejection in solid organ recipients is increasing.

Acute humoral rejection (AHR) occurs in approximately 7 percent of renal transplant patients and is frequently observed in cardiac and lung graft recipients. Donor-specific antibodies can directly mediate injury to graft endothelium as well as promote the development of graft tissue fibrosis and vasculopathy. Recent recognition of the high incidence of AHR has generated considerable interest in defining mechanisms by which donor-reactive anti-class I MHC and anti-class II MHC antibodies mediate graft tissue injury. A major problem that has confronted investigation of these mechanisms is the absence of appropriate animal transplant models.

As with human transplant patients, the alloimmune response to skin and organ allografts in nonimmunosuppressed recipients is primarily mediated by T cells with little or no contribution by donor-reactive antibody. Prior sensitization of organ allograft recipients by transplanting donor skin grafts prior to the organ transplant results in the generation of memory CD4 and CD8 T cells as well as donor-reactive antibody, and it is difficult to discern the specific effects of the antibody in the rejection of the organ allograft. As an alternative approach to the use of presensitized recipients, donor-reactive polyclonal or monoclonal antibodies can be infused in organ allograft recipients. However, drawbacks to this approach include the sudden administration of a bolus of the antibody and any limitations on the time period during which the antibody is given to the recipient.

We recently observed marked increases in serum levels of donor-reactive antibody induced in complete MHC-disparate heart and kidney allografts in murine recipients lacking expression of the chemokine receptor CCR5. These dysregulated antibody responses in CCR5−/− recipients appear more quickly and have titers 15- to 50-fold higher than those observed in wild-type C57BL/6 recipients. The consequence of these antibody responses is acute humoral rejection of the grafts accompanied by intense C4d/C3d deposition in the capillaries and large vessels of the allograft (Figure). The histopathology of the response looks identical to that observed during antibody-mediated rejection of renal grafts in human transplant patients.

An advantage of this model is that the antibody-induced pathology in the renal allograft can be followed from the beginning of the donor-reactive antibody response that is first detectable in the recipient serum about five days after the transplant, and thereafter when the donor-reactive antibody titers rapidly increase. In addition, we have now established a colony of CCR5−/− mice that is further genetically manipulated so that all B cells express the human CD20 protein, allowing depletion of the B cells at any time before or after the transplant by administration of the anti-human CD20 antibody currently used to treat antibody-mediated rejection in clinical transplantation. Studies of serum from heart and renal transplant patients experiencing AHR indicate that antibodies to both donor
class I and class II HLA antigens can cause graft injury, but that injury mediated by class II MHC-specific antibodies is characterized by more intense infiltration of neutrophils and other inflammatory components in the allograft.

We have recently used the CCR5-/- mouse model to investigate the pathology induced by antibodies reactive to donor class I MHC molecules by testing the rejection of heart allografts transgenically expressing a single class I MHC disparity in the CCR5-/- recipients. Whereas the single class I MHC disparate allografts survived longer than 60 days in wild-type recipients, all CCR5-/- recipients rejected the allografts within 14 days, and this rejection was mediated by antibody reactive to the donor class I MHC disparity.

During the course of these studies, we observed that the antibody-mediated rejection of the single class I MHC disparate grafts was accompanied by the high-level expression of three novel markers in the graft: perforin, myeloperoxidase and CCL5 mRNA. Since this rejection occurs in the absence of T cell-mediated rejection mechanisms, these studies indicate that the donor-reactive antibody directly induces the expression of these three biomarkers.

These studies raise questions about the antibody-mediated mechanisms inducing the expression of these molecules that have effector functions in innate and adaptive immune responses. We now approach these questions by using immunohistochemical staining of rejecting grafts to identify the sources of the perforin, myeloperoxidase and CCL5. In addition, we are generating CCR5-/- mice that cannot express these molecules in order to test the molecules’ role in antibody-mediated rejection of heart and kidney allografts. Finally, we have developed an approach to detect the presence of these molecules in the urine of renal allograft recipients during antibody-mediated graft rejection and are applying this noninvasive detection strategy to human kidney transplant patients.

A major problem in solid organ transplantation continues to be the development of interstitial graft fibrosis and occlusive vasculopathy that occurs as a consequence of chronic injury imposed on the graft. Either donor-reactive T cell or antibody responses or both can initiate this pathology, but mechanisms underlying the initiation and progression of this pathology are poorly understood. We have used our CCR5-/- mice expressing the human CD20 protein on B cells to investigate this problem. Administration of the anti-human CD20 antibody to renal allograft recipients when the donor-reactive antibody first becomes detectable in the serum (e.g., Day 5 post-transplantation) results in the long-term survival of the renal allografts, but beginning about Day 50 the grafts begin to develop interstitial fibrosis and vasculopathy that further increase with time. Using this approach, we have begun to dissect the molecular and cellular mechanisms underlying the donor-reactive antibody induction of this pathology.

We have developed a novel model that allows more precise analysis of the mechanisms and consequences of donor-reactive antibody-mediated rejection of renal allografts. This model is being used to discern differences in the pathologies induced by antibodies specific for various donor MHC molecules, in the development of pathology resulting from chronic injury, and in the development of novel approaches to detect and monitor acute and chronic graft pathologies resulting from such antibodies.

For references, please email the editor.
When we are contemplating a treatment plan for a patient, especially in oncological cases, we always like to maximize radicality and functional reserve and minimize invasiveness. These challenges can be overcome even for a patient in a very unusual situation - concomitant tumors in one native and the transplanted kidney - after detailed preoperative planning and close collaboration of the staff who will perform the laparoscopic and open techniques.

Our patient was a 50-year-old man who presented to us 14 years after kidney transplantation with a stable renal graft function, who was incidentally found to have a right native renal mass during an annual ultrasonographic follow-up. Further images revealed not only a 7.5-cm mass in the atrophic right kidney (Figure 1), but also a 2.8-cm mostly endophytic enhancing mass in his transplanted kidney (Figure 2). He strongly preferred preserving a functioning graft.

We followed his kidney transplant skin incision but went into the peritoneal cavity instead, and, using a GelPort® (Applied Medical, Rancho Santa Margarita, Calif.) device as platform for laparoendoscopic single-site (LESS) surgery, we successfully executed the right radical nephrectomy and dissected out the perihilar and pericaval lymph nodes. The specimens were removed intact through the LESS wound.

In order to maximize the patient’s postoperative graft function, we used the same wound but changed to an open approach. First, we freed up the upstream and downstream parts of the right iliac artery, without dissecting into the original arterial anastomosis sites for potential temporary clamping. Then we performed a nonclamping partial transplant nephrectomy by circumferentially excising the tumor from its periphery down to its base, under the guidance of an intraoperative ultrasonography, and achieved hemostasis through applying thrombin using an absorbable gelatin matrix hemostatic agent and direct compression at the excision plane.

Intimate bedside-to-bench interactions and cooperation along with modern genetic testing enabled doctors to pinpoint the origin of the tumors as de novo or metastatic and helped further follow-up and therapeutic planning in this unusual case.
After removal of the partial nephrectomy specimen, remaining bleeder at the excision bed were suture-ligated; then standard renorrhaphy was done. Postoperatively, the patient had an uneventful recovery, and his renal function remained the same as its preoperative value (1.5 mg/dL).

The next clinically important and academically intriguing question was: Were they both de novo tumors or was one metastatic? If both were de novo, surgical excision would be potentially curative for stage 1 renal cell carcinoma (RCC) with good five-year survival around 85 percent. On the other hand, one tumor being metastatic would indicate a systemic stage 4 disease with five-year estimated survival at approximately 5 percent. Microscopic pathological pictures from both tumors revealed clear cell RCC, with Fuhrman nuclear grade 3, but none of 11 lymph nodes showed any metastasis. Standard clinical routines did not solve the puzzle. In order to solve it, detailed communications and discussions took place among the clinicians and the laboratory experts.

Genetic identity testing with several probes of short tandem repeats (STRs) was done for cells from either tumor, historical donor cells from the tissue bank and the recipient’s buccal mucosa. The results were clear: Both tumors had the STR patterns of the recipient among the probes tested (Figure 3). The tumor in the renal graft was a metastasis from the native kidney - most likely via hematogenous metastasis because all the lymph nodes were negative.

Several months later, the patient was found to have bony and pulmonary metastasis. He was started with weekly temsirolimus therapy. It has been more than two years since the tumors were first found. He is in good performance status, with recent image studies showing partial remission of those metastatic lesions. His immunosuppression has been decreased to 10 mg of prednisolone per day (along with weekly temsirolimus), and he still enjoys stable renal graft function (serum creatinine 1.6 mg/dL).

For references, please email the editor.
Kidney and Pancreas Transplant Program Biorepository Poised to Promote Research

Emilio Poggio, MD

Kidney transplantation is the treatment of choice for patients suffering from end-stage renal disease because it provides better quantity and quality of life than other means of renal replacement therapy. Despite tremendous advances in the medical and surgical management of these patients, the short- and long-term successes of this procedure are still not ideal, and therefore the research and clinical transplant communities are engaged in a constant effort to further identify and understand the underlying biological mechanisms that preclude the desired outcomes. Advances in the scientific field are constant but slow, and there is an increasing interest in studying new noninvasive biomarkers that will help the clinician predict or prevent poor outcomes, providing new opportunities for early interventions or development of new therapeutic strategies.

Novel biomarkers and techniques are rapidly emerging in the field of basic kidney transplantation research; however, translation into human research is lacking. Some of these biomarkers have been studied and are close to routine clinical implementation, but the vast majority are at different stages of development and are not yet available.

Furthermore, these new biomarkers are still to be measured and validated in large cohorts of kidney transplant recipients. The advent of new techniques to study the pathogenesis of immune-mediated injury, such as organ transplant rejection, will require repetitive tissue sampling in a large cohort of patients to assess their potential clinical application to patient care.

There is a need to systematically collect and store samples in biobanks/biorepositories for future investigations. State-of-the-art infrastructure, professional expertise and a large number of transplant recipients from whom samples can be obtained are necessary to accomplish this. These components are present at Cleveland Clinic; therefore, the Renal Transplant Program has established this needed biorepository, which we have named the Renal Transplant BioBank.

A large number of human samples and state-of-the-art laboratories are needed to test and validate these biomarkers.

The goal of the Renal Transplant BioBank is to establish a patient-linked biologic sample repository (blood, urine and graft histology) for use in future research studies related to metabolic, inflammatory and immunologic markers relevant to organ transplantation. However, the first step is to build up the repository, which is labor-intensive and costly. In collaboration with the Lerner Research Institute, the Kidney and Pancreas Transplant Program, with financial support provided by the Novick Center for Clinical and Translational Research in the Glickman Urological & Kidney Institute, has initiated the collection and storage of biospecimens (blood, urine, biopsy tissue) from kidney and pancreas transplant recipients who receive an organ at Cleveland Clinic. The collection is initiated after the patient has provided informed consent.

These biospecimens are intended to be used in the future to develop and test novel biomarkers that will eventually translate into better patient care. This is one of the most relevant endeavors in the research arena of the program due to its potential future application. This is a unique initiative that would be possible in only a few medical centers in the United States. Finally, the experience gained from this endeavor will be the basis for future expansion of this type of biorepository to other areas in kidney disease.

Key Points:

There is a need to systematically collect and store samples in biobanks/biorepositories for future investigations. State-of-the-art infrastructure, professional expertise and a large number of transplant recipients from whom samples can be obtained are necessary to accomplish this. These components are present at Cleveland Clinic; therefore, the Renal Transplant Program has established this needed biorepository, which we have named the Renal Transplant BioBank.

A large number of human samples and state-of-the-art laboratories are needed to test and validate these biomarkers.
The Impact of Community Health on Outcomes for Kidney Transplant Recipients in the United States

Jesse D. Schold, PhD

There are many factors that ultimately contribute to individuals’ overall health. These factors include biology and genetics, environmental conditions, behavior, and psychosocial conditions. Access to and the quality of healthcare delivery also influence health.

For the benefit of both research and clinical care, it is critical to understand the sources of risk for patients in order to develop effective interventions. The primary aim of this study was to evaluate the association of community health indicators with outcomes for kidney transplant recipients in the United States.

We conducted a retrospective observational cohort study using multivariable Cox proportional hazard models to analyze the independent association of community risk factors with recipient outcomes. We evaluated kidney transplant recipients in the United States using the National Scientific Registry of Transplant Recipients. We then merged these data with health indicators compiled from several national databases and the Centers for Disease Control and Prevention, including National Center for Health Statistics, Behavioral Risk Factor Surveillance System and National Center for Chronic Disease Prevention and Health Promotion. The final study population was 100,164 living and deceased donor adult (18+ years) kidney transplant recipients, transplanted between 2004 and 2010.

The primary results of the study demonstrated that multiple health indicators from recipients’ community residence were independently associated with outcomes including low birth weights, preventable hospitalizations, inactivity rates, and smoking and obesity prevalence. Recipients in highest-risk counties were more likely to be African-American (AOR = 1.59, 1.51-1.68), younger (ages 18-39; AOR = 1.46,1.32-1.60), with lower educational attainment (less than high school; AOR = 1.84, 1.62-2.08) and have public insurance as a primary payer (AOR = 1.55,1.47-1.64). In addition, the proportion of recipients from higher-risk counties varied dramatically by center and region.

Importantly, there was an independent graded effect between health indicators and post-transplant mortality and graft loss, including notable hazard associated with highest-risk counties (Figure, Adjusted Hazard Ratio = 1.26, 1.13-1.40).

Figure. Adjusted relative risk of graft loss and death for kidney transplant recipients based on community risk level

In conclusion, in a national cohort of patients undergoing complex medical procedures, health indicators from patients’ communities are strong independent predictors of all-cause mortality. Findings highlight the importance of community conditions for risk stratification of patients and development of individualized treatment protocols. Findings also demonstrate that standard risk adjustment does not capture important factors that may impact unbiased performance evaluations of transplant centers. Future study of the specific mechanisms by which these community risk factors are associated with diminished outcomes is critical for developing interventions to improve patient outcomes.

For references, please email the editor.

A version of this article appeared in Arch Surg. 2012 Jun;147(6):520-526.
Proteomics of Male Gametes in Relation to Oxidative Stress

Ashok Agarwal, PhD, and Rakesh Sharma, PhD

Approximately 15 percent of all couples are infertile. Fifty percent of infertile couples fail to achieve a successful pregnancy due to male factor infertility. An elevated level of seminal reactive oxygen species (ROS) is detected in 40 to 88 percent of infertile men; however, there is a shortage of information about the exact mechanisms that generate or protect against ROS.

The deleterious effects of ROS have been described throughout the human body. ROS can induce apoptosis and lipid peroxidation, as well as protein and DNA damage. Oxidative stress (OS) results when the antioxidant reserves are overwhelmed due to excessive ROS formation. The inherent intracellular and extracellular antioxidant capacity determines the susceptibility of spermatozoa to OS. Proteomic techniques, such as two-dimensional differential in-gel electrophoresis (2D-DIGE), polyacrylamide gel electrophoresis (2D-PAGE) coupled with mass spectrometry (MS), have allowed for the identification of numerous sperm-specific proteins affected by OS-related changes in male infertility.

Researchers in the Glickman Urological & Kidney Institute at Cleveland Clinic have examined the relative abundance of proteins in variety of patient populations, such as those presenting with ROS positive (ROS+) and ROS negative (ROS-) semen samples as well as those with normal and abnormal sperm count and morphology. In a novel study, the differential protein expression was compared in spermatozoa obtained from semen samples with high ROS levels and those with low ROS levels utilizing 2D-DIGE and MS. Semen samples from 20 donors and 32 infertile men were divided into two groups: ROS+ and ROS-. From each group, spermatozoa were labeled with Cy3/Cy5 fluorescent CyDye™ fluorors (GE Healthcare). Duplicate 2D-DIGE gels figure (gel 1 and gel 2) were run to provide more reliable data and allow for statistical analysis. Image analysis was performed using DeCyder™ software (GE Healthcare). Protein spots exhibiting at least a 1.5-fold difference in intensity were excised from the preparatory gel and identified by liquid chromatography mass spectrometry analysis.

In another study we examined the differential expression of proteins in the seminal plasma of men based on sperm count and morphology utilizing in-solution digestion-based proteomic analysis. Samples were categorized as normal sperm count and normal morphology (NN); normal sperm count and abnormal morphology (NA); oligozoospermia and normal morphology (ON); and oligozoospermia and abnormal morphology (OA). Proteomic analysis by LC-MS/MS technique was done to identify the differential expression of proteins.

Key Points:

Utilizing proteomic tools and bioinformatic analysis can help scientists understand the proteomic spectrum of the sperm and identify a subset of the sperm proteome, that may be implicated in the genesis of OS.

Similarly, we can identify proteins that are over- or underexpressed in the seminal plasma of men with poor sperm quality. The distinct presence of some proteins can there

In the first study, a total of 1,343 protein spots in gel 1 and 1,265 spots in gel 2 were detected. The majority of protein spots had similar expression, with 31 spots differentially expressed. Six spots were significantly decreased and 25 increased in the ROS- sample compared with the ROS+ sample. Significantly different expression of protective proteins against OS was found in ROS- compared with ROS+ samples (Table).
Comparative proteomics of the NN, NA, ON and OA samples revealed a total of 33, 34, 29 and 29 proteins, respectively, with prolactin-induced protein and semenogelin II precursor being the common proteins identified in all groups.

Twenty proteins were identified in the four groups. Compared with the NN group, four proteins 1) acidic epididymal glycoprotein–like isoform 1 precursor, 2) mucin 6, gastric 3) orosomucoid 1 precursor and 4) ubiquitin and ribosomal protein S27a precursor were identified in the NA group. Alpha-2-glycoprotein 1, zinc and clustrin isoform 1 were unique to the ON group; and cystatin C precursor, semenogelin I isoform b preprotein and transferrin were common to the OA group. Ten of these identified proteins were common to either the NA, ON or the OA groups.

Advances in proteomic approaches can recognize proteins involved in particular sperm processes such as motility, capacitation, acrosome reaction and fertilization. Using proteomic tools, we can study spermatozoa in different functional states — immature vs. mature, uncapacitated vs. capacitated, normal vs. defective, and low sperm count vs. high sperm count — all of which are key components of male reproductive potential.

The findings from our study augment the proteome spectrum of human sperm and identify a subset of the human sperm proteome that may be implicated in the genesis of oxidative stress. Differences in the levels of protein expression in the ROS+ and ROS− groups may be related to 1) the effect of OS on genes inducing or inhibiting particular protein synthesis, 2) the inherent differences in the expression of protective sperm proteins in fertile vs. infertile men, 3) the OS-inducing oxidation of amino acid residue side chains resulting in formation of carbonyl groups, 4) the fragmentation of the polypeptide chain, and 5) the formation of protein–protein cross-linked aggregates. Overall, these differences may explain the role of OS in the pathology of male infertility. Moreover, several of these proteins may serve as molecular biomarkers to show the differences between normal sperm and those with high oxidant burden. Adequate amounts of antioxidants can protect the spermatozoa from damage whereas low amounts may render the spermatozoa vulnerable to ROS attack.

Similarly the upregulation or down regulation of specific proteins that are altered in the NA, OA or ON groups can explain the differences seen in these subjects compared with the group showing normal sperm count and normal sperm morphology.

### Table: Identification of proteins obtained from 2D-DIGE analysis

<table>
<thead>
<tr>
<th>Protein</th>
<th>Difference</th>
<th>Role/Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-Kinase Anchor Protein 4 Isoform 1</td>
<td>Increased in ROS+</td>
<td>Unknown, may be oxidized</td>
</tr>
<tr>
<td>Heat Shock Protein HSP 90-Beta</td>
<td>Increased in ROS+</td>
<td>Stress protein</td>
</tr>
<tr>
<td>Endoplasmn Precursor</td>
<td>Increased in ROS+</td>
<td>Stress protein</td>
</tr>
<tr>
<td>Lactotransferrin Isoform 2</td>
<td>Decreased in ROS+</td>
<td>Antioxidant role</td>
</tr>
<tr>
<td>Lactotransferrin Isoform 1 Precursor</td>
<td>Decreased in ROS+</td>
<td>Antioxidant role</td>
</tr>
<tr>
<td>Peroxiredoxin-1</td>
<td>Decreased in ROS+</td>
<td>Antioxidant role</td>
</tr>
<tr>
<td>Superoxide Dismutase [Mn], Mitochondrial isoform A Precursor</td>
<td>Decreased in ROS+</td>
<td>Antioxidant role</td>
</tr>
<tr>
<td>Glyceraldehyde-3-Phosphate Dehydrogenase</td>
<td>Decreased in ROS+</td>
<td>Production of energy, NADH and glutathione</td>
</tr>
</tbody>
</table>

Figure 2: Gene ontology (GO) enrichment analysis for distribution of biological and cellular processes revealed that the majority of the proteins identified originate either in an extracellular or a cytoplasmic region. These may be involved in stress response or defense response, or acute inflammatory response are present in the subjects presenting with normal sperm count and morphology but may be altered or absent in other abnormal groups. Utilizing powerful proteomic tools and GO enrichment annotations can help understand the molecular pathways that may be responsible for various etiologies of male infertility.
Nanoparticles for Drug Delivery to the Testicle

Devon C Snow-Lisy, PhD, Vinod Labhasetwar, PhD, and Edmund S. Sabanegh Jr., MD

There are many drawbacks to conventional oral and intravenous therapies for the treatment of testicular disorders. Pharmaceuticals may fail to cross the immune-privileged blood-testis barrier, preventing sufficiently high intratesticular concentrations for therapeutic activity. As a result, the high systemic concentrations and the frequent dosing required for certain drugs limit their clinical applicability and increase the potential for side effects and toxicity. Currently available targeted therapies for testicular disorders are inadequate. Ultrasound-mediated transcutaneous drug delivery (i.e., sonophoresis) is limited to small molecules that would likely penetrate the skin in lesser amounts under passive conditions. Direct injection to the testis could lead to trauma to and disruption of the blood-testis barrier, potentially inducing fibrosis and anti-sperm antibody formation.

Nanoparticles are submicroscopic particles with dimensions of approximately 100 nanometers. A variety of methods and polymers is used to make nanoparticles, conferring unique properties to the final formulation. Nanoparticles can be customized to deliver selected encapsulated substances, to target specific tissues and to release these substances over time. Nanoparticles can facilitate sustained delivery of drugs that, like superoxide dismutase, an antioxidant enzyme, would normally be rapidly cleared from circulation, while reducing systemic toxicities via targeting to privileged sites.

Various testicular pathologies would benefit from targeted therapeutics, one of which is testicular torsion (Table). Testicular torsion is a urologic emergency that, despite prompt surgical detorsion, can result in testicular atrophy and infertility. The mechanism of damage in testicular torsion is an ischemic-reperfusion injury, with damage induced by excessive reactive oxygen species (ROS) during the reperfusion phase. One of human spermatozoan primary antioxidant defenses against ROS-mediated damage is superoxide dismutase, an enzyme that inactivates the superoxide anion (O2-). The exogenous delivery of superoxide dismutase to counteract the effects of excess

Key Points:

We hypothesized that superoxide dismutase-loaded nanoparticles injected intra-arterially will allow atraumatic nanoparticle penetration of the blood-testis barrier in a testicular torsion model.

In an animal model of testicular torsion, uptake was noted within the testicular tissue, and nanoparticles were noted to have crossed the blood-testis barrier.

Figure 1. Nanoparticle uptake by Sertoli cells in culture.

Nanoparticles were coincubated with testicular Sertoli cells for two and 24 hours. Using flow cytometry, the uptake of nanoparticles by Sertoli cells was significantly above controls (plain media, media with fluorescent dye) and demonstrated a dose- and time-dependent uptake.

Figure 2. Nanoparticle uptake by testicular tissue via fluorescence microscopy

Nanoparticles are labeled with coumarin dye, which fluoresces green and is noted to be within the testicular parenchyma and not within the blood vessel (asterisk). All tissue is counterstained with bisbenzimide, a compound that fluoresces blue when in contact with DNA.
Testicular pathologies that could benefit from targeted therapeutics with nanoparticles

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Current Treatment</th>
<th>Downsides of Current Therapy</th>
<th>Potential Applications of Nanotechnology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicular cancer</td>
<td>• Orchiectomy +/- surveillance radiation RPLND chemotherapy</td>
<td>• Pulmonary fibrosis</td>
<td>• Imaging of metastatic disease</td>
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<td></td>
<td></td>
<td>• Hematologic toxicity</td>
<td>• Targeted delivery of chemotherapy</td>
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<td></td>
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<td>• Gastrointestinal toxicity</td>
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<tr>
<td></td>
<td></td>
<td>• Secondary malignancies</td>
<td></td>
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<tr>
<td>Infertility</td>
<td>• Adoption, IVF or IUI</td>
<td>• Invasive, expensive and increased risk of multiple gestations</td>
<td>• Leydig (testosterone-producing) cell stimulation with LH analog or sustained release of testosterone locally</td>
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<td></td>
<td>• GnRH, hCG or recombinant FSH</td>
<td>• Difficult dosing regimen</td>
<td>• Gene therapy</td>
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<td></td>
<td>• Oral antioxidants, vitamins, antibiotics, surgery</td>
<td></td>
<td>• Targeted delivery of antioxidants/antibiotics</td>
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<tr>
<td>Testicular torsion</td>
<td>Detorsion or orchiectomy</td>
<td>Testicular atrophy/infertility</td>
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<tr>
<td>Prostate cancer</td>
<td>• Hormones</td>
<td></td>
<td>Targeted LH antagonist or steroid synthesis inhibitors</td>
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<td></td>
<td>• Hormone antagonists</td>
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<td></td>
<td>• Anti-androgens surgery</td>
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<tr>
<td>Orchialgia</td>
<td>• NSAIDs</td>
<td>Poor efficacy</td>
<td>Targeted and sustained release of NSAIDs, narcotics or anesthetics</td>
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<tr>
<td></td>
<td>• Tricyclic antidepressants, narcotics, anesthetics</td>
<td>• Medication side effects</td>
<td></td>
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<tr>
<td></td>
<td>• Surgery</td>
<td></td>
<td></td>
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<tr>
<td>Infectious diseases</td>
<td>• HAART</td>
<td>Resistance</td>
<td>• Targeted and sustained release with drug crossing the blood-testis barrier</td>
</tr>
<tr>
<td></td>
<td>• Antibiotics</td>
<td>Secondary infections</td>
<td>• Treatment with combination therapy of antibiotic and analgesic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poor efficacy</td>
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</table>

RPLND: retroperitoneal lymph node dissection; IVF: in-vitro fertilization; IUI: intrauterine insemination; GnRH: gonadotropin releasing hormone; hCG: human chorionic gonadotropin; FSH: follicle stimulating hormone; LH: luteinizing hormone; NSAIDs: nonsteroidal anti-inflammatory drug; HAART: highly active retroviral therapy.

ROS, while theoretically beneficial, has been ineffective via traditional intravenous delivery because of its short half-life of approximately six minutes in circulation.

In our initial pilot study, we hypothesized that superoxide dismutase-loaded nanoparticles injected intra-arterially will allow atraumatic nanoparticle penetration of the blood-testis barrier in a testicular torsion model. In this project, nanoparticles were formulated with polyactic co-glycolide (PLGA), a polymer that is biodegradable and biocompatible and has been used in a host of FDA-approved devices and implants. A fluorescent marker, 6-coumarin, is incorporated into nanoparticles, allowing for histological imaging and quantification of tissue uptake of these nanoparticles. In cell culture, nanoparticles were taken up in a concentration- and time-dependent manner by Sertoli cells, the nursemaid cells of the testicle (Figure 1).

In an animal model of testicular torsion, male Sprague Dawley® (Charles River, Wilmington, Mass.) rats were anesthetized and their aortas directly injected at the level of the testicular artery. After four hours, the animals were sacrificed and the testicles analyzed for uptake using histologic imaging and high-performance liquid chromatography. A significant increase in uptake was noted with intra-arterial injection of nanoparticles (3.6 ± 0.99 µg nanoparticles/gram testicle) as compared with intravenous injection (0.62 ± 0.19 µg nanoparticles/gram testicle). Uptake was noted within the testicular tissue, and nanoparticles were noted to have crossed the blood-testis barrier (Figure 2).

Despite prompt surgical detorsion, many patients with testicular torsion experience testicular atrophy and subfertility. Further work is ongoing to determine the effect of superoxide dismutase-loaded nanoparticles on mitigating the effects of testicular torsion in the rat model. It is our hypothesis that superoxide dismutase-loaded nanoparticles could mitigate the damage after testicular torsion, thereby extending the window for testicular salvage. These nanoparticles could also be utilized to treat subfertile/infertile men with elevated ROS in the semen, potentially avoiding assisted reproductive technologies. This current model will serve as a proof of concept for targeted nanoparticle-mediated drug delivery to the testis, allowing further investigation into the many potential applications (Table).

The development of novel nanoparticle drug delivery systems for the testicular microenvironment is the subject of a collaborative research effort between the Departments of Urology and Biomedical Engineering at Cleveland Clinic.
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Urology & Kidney Disease News is written for physicians and should be relied upon for medical education purposes only. It does not provide a complete overview of the topics covered and should not replace the independent judgment of a physician about the appropriateness or risks of a procedure for a given patient.

Eric A. Klein, MD
Chairman
Glickman Urological & Kidney Institute

J. Stephen Jones, MD, FACS
Chief, Surgical Operations, Fairview Hospital
Medical Editor

Sankar Navaneethan, MD
Glickman Urological & Kidney Institute
Nephrology Section Editor

Kimberley Sirk
Managing Editor
sirkk@ccf.org

Irwin Krieger
Graphic Designer

Cleveland Clinic
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9500 Euclid Avenue, Cleveland, OH 44195

The Glickman Urological & Kidney Institute is a world leader in treating complex urologic and kidney conditions in adults and children. Our physicians have pioneered medical advances including partial nephrectomy, laparoscopic, robotic and single-port urologic surgery, and research on the biological kidney, while serving tens of thousands of patients annually. The Glickman Urological & Kidney Institute is one of 25 institutes at Cleveland Clinic, a not-for-profit academic medical center ranked among the nation’s top hospitals (U.S. News & World Report), where nearly 3,000 physicians in 120 specialties collaborate to give every patient the best outcome and experience.
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Resources for Physicians

Referring Physician Center and Hotline
Cleveland Clinic’s Referring Physician Center has established a 24/7 hotline – 855.REFER.123 (855.733.3712) – to streamline access to our array of medical services. Contact the Referring Physician Hotline for information on our clinical specialties and services, to schedule and confirm patient appointments, for assistance in resolving service-related issues, and to connect with Cleveland Clinic specialists.

Physician Directory
View all Cleveland Clinic staff online at clevelandclinic.org/staff.

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• To arrange for a critical care transfer, call 216.448.7000 or 866.547.1467 (see clevelandclinic.org/criticalcaretransport).

• For STEMI (ST elevated myocardial infarction), acute stroke, ICH (intracerebral hemorrhage), SAH (subarachnoid hemorrhage), or aortic syndrome transfers, call 877.379.0026 (2633).

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Clinical Trials
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Referring Physicians Hotline
855.REFER.123 (855.733.3712)

Hospital Transfers
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On the Web at: clevelandclinic.org/Refer123
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Same-Day Appointments
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About Cleveland Clinic
Cleveland Clinic is an integrated healthcare delivery system with local, national and international reach. At Cleveland Clinic, 2,800 physicians represent 120 medical specialties and subspecialties. We are a main campus, 18 family health centers, eight community hospitals, Cleveland Clinic Florida, the Cleveland Clinic Lou Ruvo Center for Brain Health in Las Vegas, Cleveland Clinic Canada, Sheikh Khalifa Medical City, and Cleveland Clinic Abu Dhabi.

In 2012, Cleveland Clinic was ranked one of America’s top 6 hospitals in U.S. News & World Report’s annual “America’s Best Hospitals” survey. The survey ranks Cleveland Clinic among the nation’s top 10 hospitals in 14 specialty areas, and the top hospital in three of those areas.
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