

NEPHROLOGY

Rounds®

Biomarkers for Early Detection of Acute Kidney Injury

By WON K. HAN, MD

For the past 30 years, there have been no major improvements in the mortality rate of hospitalized patients with severe acute kidney injury (AKI), despite advances in supportive care. One key reason is because a change in the serum creatinine (SCr), which has been the standard metric for detection and progression of AKI, is not sufficiently sensitive for an early diagnosis of AKI. The absence of a more sensitive biomarker has impaired progress in this field and has had a detrimental effect on the design and outcome of AKI clinical trials.

Recently, several proteins emerged as sensitive and specific biomarkers with a capacity to be used in the detection of early kidney injury and grading of injury severity. This issue of *Nephrology Rounds* reviews four potentially tandem AKI biomarkers that have shown promise in recent human studies: neutrophil gelatinase associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), interleukin-18 (IL-18), and cystatin C (Cys-C).

Acute kidney injury

AKI, previously referred to as acute renal failure, is a heterogeneous entity associated with various clinical presentations, treatments, and procedures. In fact, AKI presents a continuum of morbidity that can vary from subclinical injury, in which serum creatinine changes minimally, to severe oliguric renal dysfunction associated with tubular necrosis and failure of the kidney to function. It is often found in the setting of multiple organ failure and sepsis, and is an important cause of morbidity and mortality in hospitalized patients. The incidence of hospital-acquired AKI varies from 5% in patients with normal renal function to 25% in intensive care unit (ICU) patients.¹⁻³ Mortality rates of patients with postoperative AKI range from 50%-70% among ICU patients who require renal replacement therapy.⁴⁻⁷ Various combinations of ischemic insult and nephrotoxic injury to the kidney are the main causes of hospital-acquired AKI in severely ill patients. Within the last 30 years, the mortality rate of patients with severe AKI in the ICU has not decreased significantly despite advances in supportive care, including continuous renal replacement therapy.⁸ Within the last decade, many potential therapeutic agents have been tried, but with little success.

Defining acute kidney injury

More than 30 definitions for the diagnosis of AKI are found in published studies, most of which are based on SCr values. It is postulated that introducing therapy early in the disease process would reduce the mortality rate associated with AKI. However, the lack of a standard definition for AKI has had a detrimental effect on AKI clinical trials. In 2002, the Acute Dialysis Quality Initiative (ADQI) workshop proposed consensus recommendations based on the RIFLE (risk, injury, failure, loss, and end-stage renal disease [ESRD]) criteria as the uniform standard for diagnosis and classification of AKI.⁹ In 2005, the Acute Kidney Injury Network (AKIN) proposed the term "acute kidney injury" to reflect the entire spectrum of acute renal failure; further, they suggested the following working definition for AKI: a process that causes an abrupt (within 48 hours) reduction in kidney function, defined as an increase in SCr (≥ 0.3 mg/dL or a 50% increase) or a reduction in urine output (documented oliguria of <0.5 mL/kg/hr for >6 hrs). Recent studies have confirmed that relatively small increases in SCr (0.2-0.3 mg/dL) are associated with significant worsening of patient outcomes.^{10,11} In addition, new AKIN staging criteria incorporated an absolute increase in SCr ≥ 0.3 mg/dL and removed the loss and ESRD categories from the staging system (Table 1).¹²

Detecting AKI in a timely fashion with the current AKIN staging criteria is a challenge because it is entirely based on an increase in SCr or a decrease in urine output. Changes in SCr are insensitive for early diagnosis of AKI, since damage to renal tubules does not necessarily result in changes of kidney function parameters, such as SCr. In cases of more extensive tubular injury, there is a time lag between the injury and an increase in SCr.

Urgent need for early biomarkers in the management of AKI

Traditional blood (creatinine, blood urea nitrogen [BUN]) and urinary markers of kidney injury (urinary casts, fractional excretion of sodium) do not allow for early detection of AKI.¹³ The

AS PRESENTED IN THE ROUNDS OF
THE NEPHROLOGY DIVISION OF
BRIGHAM AND WOMEN'S HOSPITAL
BOSTON, MASSACHUSETTS



BRIGHAM AND
WOMEN'S HOSPITAL



HARVARD
MEDICAL SCHOOL
TEACHING AFFILIATE

Co-Editors

Joseph V. Bonventre, M.D., Ph.D.,
(Division Director)

Barry M. Brenner, M.D., F.R.C.P.,
(Director Emeritus)

Nephrology Division Brigham and Women's Hospital

Reza Abdi, M.D.
M. Javeed Ansari, M.D.
Jessamyn Bagley, Ph.D.
Sangeeta Bhattia, M.D., Ph.D.
Joseph V. Bonventre, M.D., Ph.D.
Barry M. Brenner, M.D.
Anil K. Chandraker, M.B., M.R.C.P.
David M. Charytan, M.D.
Mary Choi, M.D.
Kenneth B. Christopher, M.D.
Gary C. Curhan, M.D., Sc.D.
Bradley M. Denker, M.D.
Jeremy Duffield, M.D., Ph.D.
John P. Forman, M.D.
Markus H. Frank, M.D.
Indira Gulena, M.D.
Dirk M. Hentschel, M.D.
Andreas Herrlich, M.D., Ph.D.
Li-Li Hsiao, M.D., Ph.D.
Benjamin D. Humphreys, M.D., Ph.D.
John J. Iacomini, Ph.D.
Takaharu Ichimura, Ph.D.
Vicki Rubin Kelley, Ph.D.
Julie Lin, M.D., M.P.H.
Edgar L. Milford, M.D.
David B. Mount, M.D.
Nader Najafian, M.D.
Jagdeep Obhrai, M.D.
Shona Pendse, M.D.
Martin R. Pollak, M.D.
Mohamed H. Sayegh, M.D.
Julian L. Seifter, M.D.
Jagesh V. Shah, Ph.D.
Alice M. Sheridan, M.D.
Ajay K. Singh, M.B., M.R.C.P. (U.K.)
Theodore I. Steinman, M.D.
Chun-Ming Sung, M.D.
Eric N. Taylor, M.D.
Chaorui Tian, M.D., Ph.D.
John K. Tucker, M.D.
Vishal Vaidya, Ph.D.
Sushrut S. Waikar, M.D.
Wolfgang C. Winkelmayer, M.D., Sc.D.
Xueli Yuan, M.D., Ph.D.
Kambiz Zandi-Nejad, M.D.
Jing Zhou, M.D., Ph.D.

Brigham and Women's Hospital

Website: www.brighamandwomens.org/renal

The editorial content of *Nephrology Rounds* is determined solely by the Nephrology Division of Brigham and Women's Hospital.

**Nephrology Rounds is approved
by the Harvard Medical School
Department of Continuing Education
to offer continuing education credit**

Table 1: AKIN staging criteria for AKI		
Stage	Creatinine criteria	Urine output criteria
1	Increase in SCr of ≥ 0.3 mg/dL or increase in SCr of 1.5- to 2.0-fold from baseline	< 0.5 mL/kg/h for 6 hours
2	Increase in SCr of 2.0- to 3.0-fold from baseline	< 0.5 mL/kg/h for 12 hours
3	Increase SCr to > 3.0 -fold from baseline or SCr > 4 mg/dL with an acute rise of ≥ 0.5 mg/dL	< 0.3 mL/kg/h $\times 24$ hours or anuria for 12 hours
Stages removed from RIFLE criteria in AKIN stages		
Loss	Persistent acute renal failure = complete loss of kidney function > 4 weeks	
ESRD	End-stage renal disease (> 3 months)	

AKIN: acute kidney injury network; SCr: serum creatinine; RIFLE: risk, injury, failure, loss, ESRD

change in SCr does not discriminate the time and type of renal insult nor the site and extent of glomerular or tubular injury. Previous animal studies clearly demonstrated that treatment should be initiated well before the rise of SCr and very early after the insult.¹⁴⁻¹⁸ Therefore, the absence of sensitive and specific biomarkers for the early detection of AKI impairs progress in the diagnosis and treatment of patients with AKI and has a detrimental effect on the design and, possibly, the outcomes of clinical trials. Many potential therapeutic agents, such as atrial natriuretic peptide, insulin-like growth factor-1, and endothelin receptor antagonist,¹⁹⁻²² have been tried, but have had little success. The lack of reliable biomarkers for early injury detection leads to a delay in the introduction of treatment until well into the course of renal disease. At present, there is no single intervention or sequence of clinical interventions that will significantly improve renal function after the onset of acute tubular injury or necrosis. Dialysis remains the only Food and Drug Administration (FDA)-approved treatment option for established AKI.

The identification of sensitive and specific serum and/or urinary markers that correlate with renal injury would have great practical importance for managing patients with AKI. Such markers would allow earlier detection of renal injury, provide prognostic information on the course of renal impairment, provide a rationale for the selection of patients for clinical studies, guide the timing of therapy, and assess the response to therapy. An ideal biomarker of AKI would be a molecule that:

- increases in the urine or blood within minutes to hours after a renal insult
- remains elevated as long as the injury is present
- correlates quantitatively with the extent of injury
- decreases as renal recovery takes place.

New biomarkers under evaluation in humans

A biomarker can be any parameter found in a patient that can be quantified. The best biomarkers are obtained non-invasively, are easy to measure, and can be detected in fluids easily obtained at the bedside or in an outpatient setting. Many urinary proteins and biochemical markers have been evaluated as noninvasive indicators of renal injury (Table 2).²³⁻²⁵

Changes in excretion of specific markers in the urine have been proposed as reflecting injury to specific regions of the nephron. For example, high-molecular-weight proteinuria is proposed as an indication of glomerular injury;²⁶⁻²⁹ low-molecular-weight proteinuria,^{30,31} brush border antigens,^{30,32-34} urinary enzymes,^{30,35-44} and other urinary proteins⁴⁵⁻⁵³ are proposed to indicate damage to the proximal convoluted tubule. As a result, insults to specific areas of the nephron, as might occur with a particular nephrotoxin, may be associated with different urinary excretion patterns of these markers. However, attempts to use these markers in screening patients for renal injury and identifying the site of kidney injury have been disappointing; this is due to a lack of sufficient validation, the lack of standardized assays, instability in urine, and the changes in pattern specificity of urinary marker excretion with advancing renal dysfunction.

Recently, several protein biomarkers emerged through the application of functional genomics and proteomics to human and animal models of AKI, and proved to be promising biomarkers as noninvasive indicators of AKI.

Neutrophil gelatinase-associated lipocalin

NGAL is a member of the lipocalin superfamily; members of this family are thought to transport a variety of ligands within a β -barreled calyx.⁵⁴ The human form of NGAL was originally identified as a 25-kDa protein covalently bound to gelatinase from human neutrophils and found to exhibit marked upregulation in early postischemic mouse and rat kidneys.⁵⁵ The expression of NGAL protein is predominantly detected in proliferating nuclear antigen-positive proximal tubule cells.

NGAL has been implicated as an early predictive urinary biomarker of ischemic AKI in pediatric and adult patients after cardiac surgery.^{56,57} Mishra et al⁵⁶ conducted a prospective study in 71 children undergoing cardiopulmonary bypass (CPB) where AKI was defined as an increase of 50% in SCr. Nearly one-third (20 of 71) of the children developed AKI; urinary NGAL was increased within 2 hours of CPB and preceded an increase in SCr by 1-3 days. The area under the receiver operating characteristic curve (AUC-ROC) was 0.99 at 2 hours and 1.00 at 4 hours following CPB. None of the 20 children with AKI progressed to severe AKI or received renal replacement therapy. In a cohort of 140 children who were critically ill and mechanically ventilated, urinary NGAL levels were elevated in the AKI group 2 days before and after a 50% rise in SCr with an AUC-ROC of 0.78.⁵⁸ Urinary NGAL also was evaluated in adult patients who underwent cardiac surgery. In a single-center prospective study with a cohort of 81 patients, 16 patients developed AKI, and 5 required renal replacement therapy. Urinary NGAL levels were increased within 1 hour after surgery and revealed an AUC-ROC of 0.74 at 3 hours and 0.8 at 18 hours.⁵⁷

Urinary NGAL also demonstrated good prediction for the development of delayed graft dysfunction and renal replacement therapy in a small prospective, multicenter study of pediatric and adult kidney transplantation.⁵⁹ The AUC-ROC was 0.9 on the day following kidney transplantation, which indicates an excellent predictive biomarker. Furthermore, NGAL was associated with predicting AKI following contrast dye-induced AKI;⁶⁰ however, NGAL measurements may be influenced by settings of infection with inflammatory conditions. A large multicenter study has been initiated to further define the role of NGAL as a marker for AKI using a rapid assay by Biosite Inc. (San Diego, CA).

Table 2: Urinary biomarkers for kidney injury in humans	
Biomarker	Site of renal injury
Low-molecular-weight proteins	
α 1-microglobulin	Proximal tubule
β 2-microglobulin	Proximal tubule
Retinol binding protein	Proximal tubule
High-molecular-weight proteins	
Albumin	Glomerular
Immunoglobulin	Glomerular
Transferrin	Glomerular
Brush border antigens	
Adenosine deaminase binding protein	Proximal tubule
Carbonic anhydrase	Proximal tubule
Other tubular antigen	Proximal tubule
Urinary enzymes	
N-acetyl- β -D-glucosaminidase	Proximal tubule
Alanine aminopeptidase	Proximal tubule
Alkaline phosphatase	Proximal tubule > distal tubule
Cathepsin B	Proximal tubule
β -glucosidase	Proximal tubule > distal tubule
γ -glutamyltransferase	Proximal tubule
α -glutathione-S-transferase	Proximal tubule
Lactate dehydrogenase	Distal tubule > proximal tubule
Others	
Cysteine-rich protein	Proximal tubule
Cytokines (IL-1, IL-6, IL-8, TNF- α)	Proximal tubule
Exosomal fetuin-A	Proximal tubule
Hepatocyte growth factor	Proximal tubule
Sodium/hydrogen exchanger isoform	Proximal tubule
Tamm-Horsfall glycoprotein	Distal tubule

IL: interleukin; TNF: tumor necrosis factor

Kidney injury molecule-1

KIM-1 is a type 1 transmembrane glycoprotein containing a novel 6-cysteine immunoglobulin-like domain plus a threonine/serine and proline-rich domain characteristic of mucin-like O-glycosylated proteins. It is undetectable in normal kidney tissue or urine, but is expressed at high levels in dedifferentiated proximal tubule epithelial cells from human and rodent kidneys after ischemic or toxic injury, and in renal cell carcinoma.⁶¹⁻⁶⁴ KIM-1 is also known as hepatitis A virus cellular receptor 1 and T-cell immunoglobulin- and mucin-domain-containing molecule 1.⁶⁵⁻⁶⁷

KIM-1 has been implicated as a urinary biomarker of AKI in pediatric and adult patients. In a small cross-sectional study, Han et al⁶² demonstrated that soluble forms of cleaved KIM-1 can be detected in the urine of patients with established AKI; elevated urinary KIM-1 levels were found within 12 hours after an initial ischemic insult and prior to the appearance of granular casts in the urine. Urinary KIM-1 was also evaluated after pediatric cardiac surgery in a case-control study,⁶⁸ using samples from the same cohort in the NGAL study previously described.⁵⁶ Urinary KIM-1 had an AUC-ROC of 0.57 at 2 hours, 0.83 at 12 hours, and 0.78 at 24 hours.

High urinary KIM-1 expression was also associated with adverse clinical outcomes in patients with established AKI. In a prospective study, a cohort of 201 patients with established AKI, Liangos et al⁶⁹ demonstrated that urinary KIM-1 and

Table 3: Promising biomarkers for AKI in humans		
Biomarker	Detection assay	Associated injury
KIM-1	ELISA/Luminex [®]	Ischemic AKI, nephrotoxins, RCC
NGAL	ELISA/Luminex [®]	Ischemic AKI, nephrotoxins, DRAF
IL-18	ELISA/Luminex [®]	AKI, DRAF
Cystatin C	Nephelometry	Reduced in GFR, proximal tubule injury

KIM-1: kidney injury molecule-1; ELISA: enzyme-linked immunosorbent assay; RCC: renal cell carcinoma; NGAL: neutrophil gelatinase associated lipocalin; DRAF: delayed renal allograft function; GFR: glomerular filtration rate

N-acetyl- β -D-glucosaminidase (NAG) were significantly associated (AUC-ROC of 0.78) with the clinical composite endpoints of death or renal replacement therapy.

KIM-1 is particularly interesting given its “kidney dominance” of expression. In a follow-up study involving a larger number of adult patients, the temporal expression patterns and the utility of urinary KIM-1 for the early detection of AKI are being studied.

Interleukin-18

IL-18 is a proinflammatory cytokine that is involved in mediating inflammation in many organs.⁷⁰⁻⁷² Inflammation plays an important role in the pathophysiology of AKI associated with ischemia, sepsis, and many nephrotoxins.⁷³ Renal IL-18 messenger ribonucleic acid (mRNA) levels are significantly upregulated in the proximal tubules following ischemia-reperfusion injury, autoimmune nephritis, and cisplatin-induced nephrotoxicity.⁷⁴ The mature form of IL-18 (18 kDa) is produced from a precursor form of IL-18 (24 kDa) after enzymatic cleavage by IL-18-converting enzyme.⁷⁵ In a cross-sectional study, Parikh et al⁷⁶ demonstrated that urinary IL-18 was significantly increased in the urine of patients with AKI and increased within 24 hours after kidney transplantation in patients with delayed allograft dysfunction. Urinary IL-18 had an AUC-ROC of 0.95 for patients with established AKI and delayed graft function. In a prospective study of 138 patients with acute respiratory distress syndrome, elevated urinary IL-18 in AKI group preceded elevation of SCr by 1-2 days with AUC-ROC of 0.73 and was an independent predictor of death in this cohort.⁷⁷

In a small prospective, multicenter study of kidney transplantations in 53 children and adults, urinary IL-18 was shown to be a good predictor for the development of delayed graft dysfunction and renal replacement therapy.⁵⁹ The AUC-ROC was 0.9 on the day following kidney transplantation for both urinary IL-18 and NGAL. Urinary IL-18 was evaluated after pediatric cardiac surgery in a case-control study, using samples from the same cohort in the NGAL study described previously.⁵⁶ Urinary IL-18 has an AUC-ROC of 0.61 at 4 hours, 0.75 at 12 hours, and 0.73 at 24 hours following CPB for prediction of AKI.⁷⁸

Comparisons among the three biomarkers (NGAL from the original study and IL-18 and KIM-1 at later dates using frozen samples) should be made with caution for several reasons. The cohorts were all children and the sample size was

small. Patients with chronic kidney disease were excluded from the cohort and there were no patients who required dialysis or who died. The heterogeneity of AKI was not well addressed; as a result, further studies are needed to address these issues.

Cystatin C

Cys-C is a nonglycosylated basic protein that is synthesized at a relatively constant rate and released into the plasma by all nucleated cells. Serum Cys-C is freely filtered by the glomerulus and catabolized in the proximal tubules.⁷⁹ A decline in renal function is associated with a rise in serum Cys-C concentrations. Several studies have demonstrated that a change in serum Cys-C is more sensitive than a change in SCr as a marker of change in glomerular filtration.⁸⁰⁻⁸² Furthermore, serum Cys-C is a stronger predictor for the risk of death and cardiovascular events in older patients.^{83,84} Herget-Rosenthal et al⁸⁵ demonstrated that urinary excretion of Cys-C in patients with nonoliguric AKI at study entry revealed higher sensitivity and specificity than α 1-microglobulin and NAG. It was also better than the severity of illness score by Liano et al,⁸⁶ in predicting the requirement for renal replacement therapy. Further, in a prospective single-center study of 85 ICU patients at high risk to develop AKI,⁸⁷ a 50% increase in serum Cys-C predicted AKI 1-2 days prior to an elevation in SCr, with AUCs of 0.97 and 0.82, respectively.

Serum Cys-C levels are known to be unaffected by gender, age, or race, with the exception of corticosteroid use, rheumatic disease, and malignant diseases;⁸⁸⁻⁹⁰ however, Knight et al⁹¹ demonstrated that age, weight, gender, C-reactive protein levels, and smoking may influence serum Cys-C concentration. Additional studies must examine the measurement of serum Cys-C in diverse patient populations to characterize the timing of increased Cys-C excretion relative to the onset of AKI, as well as the discriminatory characteristics of this potential biomarker in various kidney disease states.

Limitations of biomarkers for early detection of AKI in clinical situation

Additional studies are necessary before biomarkers may be used in routine clinical practice. Currently, these promising biomarkers have been tested in only small studies and limited clinical situations. None of these biomarkers have been systematically evaluated in the various clinical settings of AKI. Furthermore, the studies have been insufficiently powered to establish a cutoff value that is predictive of AKI. In addition, there are very limited data available, at present, about temporal expression patterns for various biomarkers including NGAL, KIM-1, IL-18, and Cys-C in the various clinical settings of AKI from the onset of renal insult.

The heterogeneity of AKI suggests that more than one marker may be necessary to obtain sufficient sensitivity and specificity for AKI screening. An analysis of multiple biomarkers may optimize early detection of AKI, as in the case of prostate cancer detection.⁹² Analyses of multiple markers may also provide insight into the pathophysiology and sites of nephron injury, since different markers may be chosen to reflect injury to different nephron segments.

Nevertheless, it would be naïve to call the result positive if one or more biomarkers are positive, since such an approach would be inefficient and increase sensitivity at the expense of specificity, or vice versa. A linear combination that would maximize the AUC-ROC for detection of AKI may be useful, but this approach remains to be validated by future studies.⁶⁸ Currently, there is no standard procedure to combine the multiple biomarkers for clinical use.

Another major challenge is the development of a rapid assay for validated biomarkers at the bedside or in a clinical laboratory for the detection of AKI. Current quantification methods include ELISA, Luminex[®]-based assay, and nephelometry, which are not fast enough for rapid assay. Recently, Biosite Inc. launched a prospective multicenter study evaluating NGAL in early and evolving stages of AKI. The study is using the Triage[®] NGAL test, which supposedly can accurately measure NGAL concentrations in blood and provide results within 15 minutes.

Finally, larger prospective studies are necessary to validate the temporal expression pattern of various biomarkers for early detection of AKI, to determine how to combine multiple biomarkers for early detection of AKI, and to discover how the temporal course relates to the onset, severity, and outcome of AKI. More information must be gathered regarding the accuracy of measurement in the presence of interfering substances and the stability of the various biomarkers in routine clinical storage conditions, including a number of freeze-thaw cycles.

Conclusions

There is a need for a new standard definition of AKI that is not based on a change in SCr. The new definition of AKI should be based on multiple biomarkers and clinical parameters, and capable of detecting initial renal injury within minutes to hours. Any potential AKI biomarker should undergo several clinical validation processes for both assay performance and diagnostic utility. Furthermore, several requirements must be met in order for AKI biomarkers to be useful in daily clinical practice. AKI biomarkers must:

- Allow for early detection of renal injury
- Identify the severity of AKI
- Provide a rationale for risk stratification in clinical studies, including the identification of patients at risk for AKI
- Guide timing of therapy
- Reflect improvement and worsening of the kidney injury
- Be amenable to quick and reliable measurement at the bedside or clinical laboratory.

When armed with new tools, it is likely that more clinical trials will lead to advanced therapies, which can be introduced earlier in the course of the disease and may be more effective in reversing or ameliorating tubular injury in AKI.

Won K. Han, MD, is an Assistant Professor in the Division of Nephrology, Thomas Jefferson University Hospital, Department of Medicine, Jefferson Medical School, Philadelphia, PA.

References

1. Chertow GM, Lee J, Kuperman CJ, et al. Guided medication dosing for inpatients with renal insufficiency. *JAMA*. 2001;286:2839-2844.
2. Liangos O, Wald R, O'Bell JW, Price L, Pereira BJ, Jaber BL. Epidemiology and outcomes of acute renal failure in hospitalized patients: a national survey. *Clin J Am Soc Nephrol*. 2006;1:43-51.
3. de Mendonca A, Vincent JL, Suter PM, et al. Acute renal failure in the ICU: risk factors and outcome evaluated by the SOFA score. *Intensive Care Med*. 2000;26:915-921.
4. Zanardo G, Michielon P, Paccagnella A, et al. Acute renal failure in the patient undergoing cardiac operation. *J Thorac Cardiovasc Surg*. 1994;107:1489-1495.
5. Novis BK, Roizen MF, Aronson S, Thisted RA. Association of preoperative risk factors with postoperative acute renal failure. *Anesth Analg*. 1994;77:143-149.
6. Spiegel DM, Ullian ME, Zerbe GO, Berl T. Determinants of survival and recovery in acute renal failure patients dialyzed in intensive-care units. *Am J Nephrol*. 1991;11:44-47.
7. Schiffel H, Lang SM, König A, Strasser T, Haider MC, Held E. Biocompatible membranes in acute renal failure: prospective case-controlled study. *Lancet*. 1994;344:570-572.
8. Thadhani R, Pascual M, Bonventre JV. Acute renal failure. *N Engl J Med*. 1996;334:1448-1460.
9. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure – definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care*. 2004;8:R204-R212.
10. Lassnigg A, Schmidlin D, Mouhieddine M, et al. Minimal changes of serum creatinine predict prognosis in patients after cardiothoracic surgery: a prospective cohort study. *J Am Soc Nephrol*. 2004;15:1597-1605.
11. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol*. 2005;16:3365-3370.
12. Mehta RL, Kellum JA, Shah SV, et al. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care*. 2007;11:R31.
13. Star RA. Treatment of acute renal failure. *Kidney Int*. 1998;54:1817-1831.
14. Lieberthal W, Sheridan AM, Valeri CR. Protective effect of atrial natriuretic factor and mannitol following renal ischemia. *Am J Physiol*. 1990;258:F1266-F1272.
15. Conger JD, Falk SA, Hammond WS. Atrial natriuretic peptide and dopamine in established acute renal failure in the rat. *Kidney Int*. 1991;40:21-28.
16. Kelly KJ, Tolkoff-Rubin NE, Rubin RH, et al. An oral platelet-activating factor antagonist, Ro-24-4736, protects the rat kidney from ischemic injury. *Am J Physiol*. 1996;271:F1061-F1067.
17. Chiao H, Kohda Y, McLeroy P, Craig L, Housini I, Star RA. Alpha-melanocyte-stimulating hormone protects against renal injury after ischemia in mice and rats. *J Clin Invest*. 1997;99:1165-1172.
18. Miller SB, Martin DR, Kissane J, Hammerman MR. Rat models for clinical use of insulin-like growth factor I in acute renal failure. *Am J Physiol*. 1994;266:F949-F956.
19. Allgren RL, Marbury TC, Rahman SN, et al. Anaritide in acute tubular necrosis. Auriaculin Anaritide Acute Renal Failure Study Group. *N Engl J Med*. 1997;336:828-834.
20. Hirschberg R, Koppole J, Lipsett P, et al. Multicenter clinical trial of recombinant human insulin-like growth factor I in patients with acute renal failure. *Kidney Int*. 1999;55:2423-2432.
21. Lewis J, Salem MM, Chertow GM, et al. Atrial natriuretic factor in oliguric acute renal failure. Anaritide Acute Renal Failure Study Group. *Am J Kidney Dis*. 2000;36:767-774.
22. Wang A, Holeslaw T, Bashore TM, et al. Exacerbation of radiocontrast nephrotoxicity by endothelin receptor antagonism. *Kidney Int*. 2000;57:1675-1680.
23. Han WK, Bonventre JV. Biologic markers for the early detection of acute kidney injury. *Curr Opin Crit Care*. 2004;10:476-482.
24. Devarajan P. Emerging biomarkers of acute kidney injury. *Contrib Nephrol*. 2007;156:203-212.
25. Vaidya VS, Ferguson MA, Bonventre JV. Biomarkers of acute kidney injury. *Annu Rev Pharmacol Toxicol*. 2008;48:463-493.
26. Jungers P, Hannedouche T, Itakura Y, Albouze G, Descamps-Latscha B, Man NK. Progression rate to end-stage renal failure in non-diabetic kidney diseases: a multivariate analysis of determinant factors. *Nephrol Dial Transplant*. 1995;10:1353-1360.
27. Bazzi C, Petrini C, Rizza V, Arrigo G, D'Amico G. A modern approach to selectivity of proteinuria and tubulointerstitial damage in nephrotic syndrome. *Kidney Int*. 2000;58:1732-1741.
28. Mackinnon B, Shakerdi L, Deighan CJ, Fox JG, O'Reilly DS, Boulton-Jones M. Urinary transferrin, high molecular weight proteinuria and the progression of renal disease. *Clin Nephrol*. 2003;59:252-258.
29. Corso A, Zappasodi P, Pascutto C, et al. Urinary proteins in multiple myeloma: correlation with clinical parameters and diagnostic implications. *Ann Hematol*. 2003;82:487-491.
30. Tolkoff-Rubin NE, Rubin RH, Bonventre JV. Noninvasive renal diagnostic studies. *Clin Lab Med*. 1988;8:507-526.
31. Bazzi C, Petrini C, Rizza V, et al. Urinary excretion of IgG and alpha(1)-microglobulin predicts clinical course better than extent of proteinuria in membranous nephropathy. *Am J Kidney Dis*. 2001;38:240-248.
32. Taniguchi N, Tanaka M, Kishihara C, et al. Determination of carbonic anhydrase C and beta 2-microglobulin by radioimmunoassay in urine of heavy-metal-exposed subjects and patients with renal tubular acidosis. *Environ Res*. 1979;20:154-161.
33. Zager RA, Johannes GA, Sharma HM. Quantitating the severity of proximal tubular brush border injury by a simple direct binding radioimmunoassay. *Am J Kidney Dis*. 1982;1:353-358.
34. Mutti A, Lucertini S, Valcavi P, et al. Urinary excretion of brush-border antigen revealed by monoclonal antibody: early indicator of toxic nephropathy. *Lancet*. 1985;26:914-917.
35. Gibey R, Dupond JL, Alber D, Leconte des Floris R, Henry JC. Predictive value of urinary N-acetyl-beta-D-glucosaminidase (NAG), alanine-aminopeptidase (AAP) and beta-2-microglobulin (beta 2M) in evaluating nephrotoxicity of gentamicin. *Clin Chim Acta*. 1981;116:25-34.
36. Stonard MD, Gore CW, Oliver GJ, Smith IK. Urinary enzymes and protein patterns as indicators of injury to different regions of the kidney. *Fundam Appl Toxicol*. 1987;9:339-351.
37. Kohli MM, Ganguly NK, Naur S, Sharma VK. Urinary excretion of renal brush border membrane enzymes in leprosy patients: effect of multidrug therapy. *Experientia*. 1996;52:127-130.
38. Donaldio C, Puccini R, Lucchesi A, Giordani R, Rizzo G. Urinary excretion of proteins and tubular enzymes in renal transplant patients. *Ren Fail*. 1998;20:707-715.
39. Nouwen EJ, De Broe ME. Human intestinal versus tissue-nonspecific alkaline phosphatase as complementary urinary markers for the proximal tubule. *Kidney Int*. 1994;47:S43-S51.
40. Olbricht CJ, Steinker M, Auch-Schwelk W, Bossaller C, Haas J, Koch KM. Effect of cyclosporin on kidney proteolytic enzymes in men and rats. *Nephrol Dial Transplant*. 1994;9:22-26.
41. Girolami JP, Bascands JL, Pecher C, et al. Renal kallikrein excretion as a distal nephrotoxicity marker during cadmium exposure in rats. *Toxicology*. 1989;55:117-129.
42. Usuda K, Kono K, Dote T, et al. Urinary biomarkers monitoring for experimental fluoride nephrotoxicity. *Arch Toxicol*. 1998;72:104-109.
43. Branten AJ, Mulder TP, Peters WH, Assmann KJ, Wetzels JF. Urinary excretion of glutathione S transferases alpha and pi in patients with proteinuria: reflection of the site of tubular injury. *Nephron*. 2000;85:120-126.
44. Boldt J, Brenner T, Lang J, Kumle B, Isgro F. Kidney-specific proteins in elderly patients undergoing cardiac surgery with cardiopulmonary bypass. *Anesth Analg*. 2003;97:1582-1589.
45. Lynn KL, Marshall RD. Excretion of Tamm-Horsfall glycoprotein in renal disease. *Clin Nephrol*. 1984;22:253-257.
46. Torffvit O, Jorgensen PE, Kamper AL, et al. Urinary excretion of Tamm-Horsfall protein and epidermal growth factor in chronic nephropathy. *Nephron*. 1998;79:167-172.
47. Taman M, Liu Y, Tolbert E, Dworkin LD. Increase urinary hepatocyte growth factor excretion in human acute renal failure. *Clin Nephrol*. 1997;48:241-245.
48. Sugimura K, Goto T, Tsuchida K, Takemoto Y, Kim T, Kishimoto T. Production and activation of hepatocyte growth factor in acute renal failure. *Ren Fail*. 2001;23:597-603.
49. du Cheyron D, Daubin C, Poggioli J, et al. Urinary measurement of Na⁺/H⁺ exchanger isoform 3 (NHE3) protein as new marker of tubule injury in critically ill patients with ARF. *Am J Kidney Dis*. 2003;42:497-506.
50. Muramatsu Y, Tsujie M, Kohda Y, et al. Early detection of cysteine rich protein 61 (CYR61, CCN1) in urine following renal ischemic reperfusion injury. *Kidney Int*. 2002;62:1601-1610.
51. Kwon O, Molitoris BA, Pescovitz M, Kelly KJ. Urinary actin, interleukin-6, and interleukin-8 may predict sustained ARF after ischemic injury in renal allografts. *Am J Kidney Dis*. 2003;41:1074-1087.
52. Mariano F, Guida G, Donati D, et al. Production of platelet-activating factor in patients with sepsis-associated acute renal failure. *Nephrol Dial Transplant*. 1999;14:1150-1157.
53. Zhou H, Pisitkun T, Aponte A, et al. Exosomal Fetuin-A identified by proteomics: a novel urinary biomarker for detecting acute kidney injury. *Kidney Int*. 2006;70:1847-1857.
54. Flower DR, North AC, Sansom CE. The lipocalin protein family: structural and sequence overview. *Biochim Biophys Acta*. 2000;1482:9-24.
55. Mishra J, Ma Q, Prada A, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *J Am Soc Nephrol*. 2003;14:2534-2543.
56. Mishra J, Dent C, Tarabishi R, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. *Lancet*. 2005;365:1231-1238.
57. Wagener G, Jan M, Kim M, et al. Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery. *Anesthesiology*. 2006;105:485-491.

58. Zappitelli M, Washburn KK, Arikan AA, et al. Urine neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in critically ill children: a prospective cohort study. *Crit Care*. 2007;11:R84.
59. Parikh CR, Jani A, Mishra J, et al. Urine NGAL and IL-18 are predictive biomarkers for delayed graft function following kidney transplantation. *Am J Transplant*. 2006;6:1639-1645.
60. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, Malyszko JS, Dobrzycki S. Neutrophil-gelatinase-associated lipocalin and renal function after percutaneous coronary interventions. *Am J Nephrol*. 2006;26:287-292.
61. Ichimura T, Bonventre JV, Bailly V, et al. Kidney Injury Molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. *J Biol Chem*. 1998;273:4135-4142.
62. Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV. Kidney Injury Molecule-1 (KIM-1): A novel biomarker for human renal proximal tubule injury. *Kidney Int*. 2002;62:237-244.
63. Ichimura T, Hung CC, Yang SA, Stevens JL, Bonventre JV. Kidney injury molecule-1: a tissue and urinary biomarker for nephrotoxicant-induced renal injury. *Am J Physiol Renal Physiol*. 2004;286:F552-F563.
64. Han WK, Alinani A, Wu CL, et al. Human kidney injury molecule-1 is a tissue and urinary tumor marker of renal cell carcinoma. *J Am Soc Nephrol*. 2005;16:1126-1134.
65. Feigelstock D, Thompson P, Mattoo P, Kaplan GG. Polymorphisms of the hepatitis A virus cellular receptor 1 in African green monkey kidney cells result in antigenic variants that do not react with protective monoclonal antibody 190/4. *J Virol*. 1998;72:6218-6222.
66. Feigelstock D, Thompson P, Mattoo P, Zhang Y, Kaplan GG. The human homolog of HAVcr-1 codes for a hepatitis A virus cellular receptor. *J Virol*. 1998;72:6621-6628.
67. Kuchroo VK, Umetsu DT, DeKruyff RH, Freeman GJ. The TIM gene family: emerging roles in immunity and disease. *Nat Rev Immunol*. 2003;3:454-462.
68. Han WK, Waikar SS, Johnson A, et al. Urinary biomarkers for detection of acute kidney injury. *Kidney Int*. 2008;73(7): 863-869.
69. Liangos O, Perianayagam MC, Vaidya VS, et al. Urinary N-acetyl-beta-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. *J Am Soc Nephrol*. 2007;18:904-912.
70. Jordan JA, Guo RF, Yun EC, et al. Role of IL-18 in acute lung inflammation. *J Immunol*. 2001;167:7060-7068.
71. Pomerantz BJ, Reznikov LL, Harken AH, Dinarello CA. Inhibition of caspase 1 reduces human myocardial ischemic dysfunction via inhibition of IL-18 and IL-1beta. *Proc Natl Acad Sci U S A*. 2001;98:2871-2876.
72. Hedtjarn M, Leverin AL, Eriksson K, Blomgren K, Mallard C, Hagberg H. Interleukin-18 involvement in hypoxic-ischemic brain injury. *J Neurosci*. 2002;22:5910-5919.
73. Bonventre JV, Weinberg JM. Recent advances in the pathophysiology of ischemic acute renal failure. *J Am Soc Nephrol*. 2003;14:2199-2210.
74. Leslie JA, Meldrum KK. The role of interleukin-18 in renal injury. *J Surg Res*. 2008;145(1):170-175.
75. Sugawara I. Interleukin-18 (IL-18) and infectious diseases, with special emphasis on diseases induced by intracellular pathogens. *Microbes Infect*. 2000;2:1257-1263.
76. Parikh CR, Jani A, Melnikov VY, Faubel S, Edelstein CL. Urinary interleukin-18 is a marker of human acute tubular necrosis. *Am J Kidney Dis*. 2004;43:405-414.
77. Parikh CR, Abraham E, Ancukiewicz M, Edelstein CL. Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the intensive care unit. *J Am Soc Nephrol*. 2005;16:3046-3052.
78. Parikh CR, Mishra J, Thiessen-Philbrook H, et al. Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery. *Kidney Int*. 2006;70:199-203.
79. Randers E, Erlandsen EJ. Serum cystatin C as an endogenous marker of the renal function—a review. *Clin Chem Lab Med*. 1999;37:389-395.
80. Coll E, Botey A, Alvarez L, et al. Serum cystatin C as a new marker for noninvasive estimation of glomerular filtration rate and as a marker for early renal impairment. *Am J Kidney Dis*. 2000;36:29-34.
81. Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am J Kidney Dis*. 2002;40:221-226.
82. Christensson A, Ekberg J, Grubb A, Ekberg H, Lindström V, Lilja H. Serum cystatin C is a more sensitive and more accurate marker of glomerular filtration rate than enzymatic measurements of creatinine in renal transplantation. *Nephron Physiol*. 2003;94(2):p19-27.
83. Sarnak MJ, Katz R, Stehman-Breen CO, et al. Cystatin C concentration as a risk factor for heart failure in older adults. *Ann Intern Med*. 2005;142:497-505.
84. Shlipak MG, Sarnak MJ, Katz R, et al. Cystatin C and the risk of death and cardiovascular events among elderly persons. *N Eng J Med*. 2005;352:2049-2060.
85. Herget-Rosenthal S, Poppen D, Husing J, et al. Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis. *Clin Chem*. 2004;50:552-558.
86. Liano F, Junco E, Pascual J, Madero R, Verde E. The spectrum of acute renal failure in the intensive care unit compared with that seen in other settings. The Madrid Acute Renal Failure Study Group. *Kidney Int Suppl*. 1998;66:S16-S24.
87. Herget-Rosenthal S, Marggraf G, Husing J, et al. Early detection of acute renal failure by serum cystatin C. *Kidney Int*. 2004;66:1115-1122.
88. Poge U, Gerhardt T, Bokenkamp A, et al. Time course of low molecular weight proteins in the early kidney transplantation period—influence of corticosteroids. *Nephrol Dial Transplant*. 2004;19:2858-2863.
89. Mange H, Liebmann P, Tanil H, et al. Cystatin C, an early indicator for incipient renal disease in rheumatoid arthritis. *Clin Chim Acta*. 2000;300:195-202.
90. Kleber M, Cybulla M, Bauchmuller K, Ihorst G, Koch B, Engelhardt M. Monitoring of renal function in cancer patients: an ongoing challenge for clinical practice. *Ann Oncol*. 2007;18:950-958.
91. Knight EL, Verhave JC, Spiegelman D, et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int*. 2004;65:1416-1421.
92. Mikolajczyk SD, Song Y, Wong JR, Matson RS, Rittenhouse HG. Are multiple markers the future of prostate cancer diagnostics? *Clin Biochem*. 2004;37:519-528.

Acknowledgements

This work was supported in part by National Institutes of Health grant 5KO8DK64075.

Upcoming Scientific Meetings

3-6 May 2008

American Society of Pediatric Nephrology (ASPEN) Annual Meeting

Toronto, Ontario, Canada

Contact: ASPN

Tel: 281-419-0052

Website: www.aspneph.com

Email: info@aspneph.com

14-17 May 2008

The American Society of Hypertension (ASH) 22nd Annual Scientific Meeting and Exposition

New Orleans Marriott

New Orleans, Louisiana

Contact: Tel. 212-696-9099

Website: www.ash-us.org

31 May - 4 June 2008

American Transplant Congress 2008

Toronto, Ontario, Canada

Contact: ATC

Website: www.atcmeeting.org

Email: atc@ahint.com

Disclosure: Dr. Han has stated that he has no disclosures to announce in association with the contents of this issue.

This activity is supported by an educational donation provided by

Amgen

©2008 Nephrology Division, Brigham and Women's Hospital, Boston, Massachusetts, which is solely responsible for the contents. The opinions expressed in this publication do not necessarily reflect those of the publisher or sponsor, but rather are those of the author based on the available scientific literature. Publisher: **SNELL Medical Communication Inc.** in cooperation with the Nephrology Division, Brigham and Women's Hospital. [®]*Nephrology Rounds* is a registered trade mark of **SNELL Medical Communication Inc.** All rights reserved. The administration of any therapies discussed or referred to in *Nephrology Rounds* should always be consistent with the recognized prescribing information as required by the FDA. **SNELL Medical Communication Inc.** is committed to the development of superior Continuing Medical Education.