

Technology Insight: biomarker development in acute kidney injury—what can we anticipate?

Bruce A Molitoris*, Vyacheslav Y Melnikov, Mark D Okusa and Jonathan Himmelfarb

SUMMARY

Early diagnosis has been the 'Achilles heel' of acute kidney injury (AKI) that has prevented successful implementation of treatment strategies. To date, pharmacological intervention has been largely unsuccessful or equivocal, and morbidity and mortality associated with AKI have remained unacceptably high. Despite their well-known limitations, the most widely used biomarkers for the early diagnosis of AKI are serum creatinine, blood urea nitrogen and urine output. Development of new biomarkers is imperative. A variety of methods have been employed to discover new biomarkers of AKI, including transcriptomics, proteomics, gene arrays, lipidomics and imaging technologies. Clinical trials are underway to establish the validity of the biomarkers discovered using these techniques. This Review summarizes the importance of biomarkers of AKI, from their discovery to clinical practice, from the current perspective and that of what to expect in the future. Great strides forward are being made in breaking down important barriers to the successful prevention and treatment of this devastating disorder.

KEYWORDS acute renal failure, acute tubular necrosis, glomerular filtration rate, prerenal azotemia, renal function

REVIEW CRITERIA

The PubMed database was the main source of literature reviewed during preparation of this manuscript.

CME

BA Molitoris is Professor of Medicine and Director of the Division of Nephrology and VY Melnikov is a Fellow in the Division of Nephrology, in the Department of Medicine and the Indiana Center for Biologic Microscopy, Indiana University School of Medicine, Indianapolis, IN, MD Okusa is John C Buchanan Distinguished Professor of Medicine in the Division of Nephrology, and Director of the Center for Immunity, Inflammation and Regenerative Medicine, in the Department of Medicine, University of Virginia, Charlottesville, VA, and J Himmelfarb is Director of the Division of Nephrology in the Department of Medicine, Center for Clinical and Translational Research, Maine Medical Center, Portland, ME, USA.

Correspondence

*Division of Nephrology, 950 West Walnut Street, R2-202 Indianapolis, IN 46202, USA
bmolitor@iupui.edu

Received 11 October 2007 Accepted 4 December 2007 Published online 29 January 2008

www.nature.com/clinicalpractice
doi:10.1038/ncpneph0723

Medscape Continuing Medical Education online

Medscape, LLC is pleased to provide online continuing medical education (CME) for this journal article, allowing clinicians the opportunity to earn CME credit. Medscape, LLC is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide CME for physicians. Medscape, LLC designates this educational activity for a maximum of 1.0 AMA PRA Category 1 Credits™. Physicians should only claim credit commensurate with the extent of their participation in the activity. All other clinicians completing this activity will be issued a certificate of participation. To receive credit, please go to <http://www.medscape.com/cme/ncp> and complete the post-test.

Learning objectives

Upon completion of this activity, participants should be able to:

- 1 List characteristics of a good biomarker.
- 2 Specify clinical risk factors for acute kidney injury (AKI) after cardiac surgery.
- 3 Describe limitations of biomarkers for the surveillance of AKI.
- 4 Identify new biomarkers that may help predict outcomes of AKI.

Competing interests

The authors declared no competing interests. Charles P Vega, the CME questions author, declared that he has served as an advisor or consultant to Novartis, Inc.

INTRODUCTION

Acute kidney injury (AKI) is a devastating clinical disease associated with unacceptably high mortality rates.¹ With its incidence increasing at a rapid rate,^{2–4} AKI remains a diagnostic and therapeutic dilemma.^{1,2,5} Recent attention has focused on understanding the epidemiology of AKI and on translating basic pathophysiologic understanding into clinical advances. Central to this translational effort is the need to develop 'biomarkers' that will improve the precision of the early diagnosis of AKI and facilitate effective therapeutic intervention. A number of epidemiologic studies have identified clinical and laboratory parameters that have advanced our understanding of the at-risk population and of the overall importance of AKI severity as it relates to clinical outcomes. The pioneering study by Thakar *et al.* identified clinical risk factors for patients undergoing cardiovascular surgery who developed AKI

requiring renal replacement therapies.⁶ In this high-risk population, prevention therapies could be used to reduce the incidence of AKI.

The Acute Dialysis Quality Initiative developed a set of criteria for defining AKI, based upon serum creatinine and urine output, called the RIFLE (Risk, Injury, Failure, Loss, and End-stage) criteria.⁷ Pioneering epidemiologic studies that used the RIFLE criteria have shown conclusively that the morbidity and mortality associated with AKI are correlated with the severity of kidney injury as defined by elevations in serum creatinine level.^{1,8} These data are important as they indicate that prevention of AKI or attenuation of the extent of injury could result in marked decreases in morbidity, mortality and the associated costs of AKI. The studies re-emphasize the need for biomarkers that are sensitive, specific and quantitative in facilitating the early diagnosis of AKI. The numerous potential biomarkers that have been identified in preclinical studies are only now beginning to be tested in clinical validation studies. This Review will, therefore, propose a framework for the validation of biomarkers that affect therapeutic decisions. Specifically, we describe the utility of biomarkers for the identification of patients at risk of developing AKI, the surveillance of early events leading to or preceding AKI, and the diagnosis and prognostication of AKI. The importance of this endeavor cannot be overemphasized, as both primary prevention and secondary therapeutic decisions depend on this information.

WHAT IS A BIOMARKER?

The development of new biomedical technologies is rapidly advancing, creating potentially novel methods to diagnose and monitor disease states. A more prominent role for biomarkers is proposed in assessing overall disease risk, in early non-invasive screening and detection, in disease stratification and prognostication, and in assessing response to therapeutic interventions ('theragnostics'). Novel biomarkers also have considerable potential utility for research and clinical purposes.

The term biomarker (shortened from 'biological marker') was introduced in 1989 as a Medical Subject Heading (MeSH) term; biomarkers are defined as "measurable and quantifiable biological parameters (e.g. specific enzyme concentration, specific hormone concentration, specific gene phenotype distribution in a population, presence of biological substances)

which serve as indices for health- and physiology-related assessments, including disease risk, psychiatric disorders, environmental exposure and its effects, disease diagnosis, metabolic processes, substance abuse, pregnancy, cell line development, epidemiologic studies, etc."⁹ Generally speaking, a biomarker is a measurable indication of a specific biologic state that is relevant to a specific disease process. In 2001, an NIH working group standardized the definition of a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."¹⁰ The FDA uses the term 'biomarker' to describe any measurable diagnostic indicator that is used to assess the risk or presence of disease.¹¹

The concept of biomarkers is related to, but distinct from, that of disease risk factors or intermediate and surrogate end points. Preferred definitions and a conceptual framework for biomarkers and surrogate end points have been defined by a consensus working group, and a basic glossary of related terminology is provided in Box 1.¹⁰ Biomarkers can sometimes serve as surrogate end points; for example, to evaluate response to therapy in clinical trials in lieu of clinically meaningful 'hard' outcomes such as morbidity and mortality. The validity of surrogate end points in clinical trials is, however, often questioned, due to risks of residual confounding.¹² As such, the threshold for surrogate end point validity is often set higher than that for biomarker validity. It is recommended that a surrogate end point should explain more than 50% of the effect of the exposure (intervention) on the outcome of interest.^{9,13}

UTILITY OF BIOMARKERS

Desirable performance characteristics for biomarkers have been well described. A perfect biomarker is easily measurable, accurate, reproducible, cost-effective and easy to interpret by the clinician. Measures of the biomarker should be widely separable in patients with the disease state compared with individuals who are in good health. The biomarker should be acceptable to the patient, and applicable to men and women over a range of different ages. The biomarker should be detectable early in the disease process to allow diagnosis and effective therapy to take place before permanent sequelae occur.^{9,14} The desirable properties of a biomarker will vary according to the intended use.

Box 1 Biomarkers: a basic glossary.

Biological marker (biomarker): a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention

Type 0 biomarker: a marker of the natural history of a disease that correlates longitudinally with known clinical indices

Type 1 biomarker: a marker that captures the effects of a therapeutic intervention in accordance with its mechanism of action

Surrogate end point (type 2 biomarker): a marker that is intended to substitute for a clinical end point; a surrogate end point is expected to predict clinical benefit (or harm or lack of benefit) on the basis of epidemiological, therapeutic, pathophysiological, or other scientific evidence

Risk factor: a risk factor is associated with a disease because it is in the causal pathway leading to the disease

Risk marker: a risk marker is associated with a disease (statistically) but need not be causally linked; it can be a measure of the disease process itself

Clinical end point: a characteristic or variable that reflects how a patient feels, functions, or survives

Intermediate (nonultimate) end point: a true clinical end point (a symptom or measure of function, such as symptoms of angina frequency or exercise tolerance) but not the ultimate end point of the disease, such as survival or the rate of other serious and irreversible morbid events

Validation of a biomarker (assay or method validation): a process for assessing performance characteristics (i.e. sensitivity, specificity, and reproducibility) of a biomarker measurement or an assay technique

Qualification of a biomarker (clinical validation): the evidentiary process linking a biomarker to disease biology or clinical outcome

Evaluation of a biomarker: a process of linking biomarkers to outcomes, often with a view to establishing surrogate status

Permission obtained from Nature Publishing Group
© Biomarkers Definitions Working Group (2001) *Clin Pharmacol Ther* 69: 89–95

There is growing awareness of the importance of rigorous analysis and systematic testing of prototypical biomarkers. Proper design of validation studies for candidate biomarkers is crucial, but has often been lacking in biomarker research.^{15,16} As an example, it is no longer sufficient for a novel biomarker simply to be associated with risk in a study population, such

as patients with AKI. To be useful, the biomarker should independently provide information about risk or prognosis that is additive to that provided by conventional clinical factors, and/or the biomarker should account for a large proportion of the risk associated with the disease. Biomarker assays should be reproducible. If the biomarker is to be used as a diagnostic test, it should be sensitive and specific; that is, it should have high predictive value. The generally accepted measure of performance is the receiver operating characteristic (ROC) curve, which compares test sensitivity to 1 – test specificity for all possible cutoff values. The area under the ROC curve (also known as the C statistic), is the proportion of pairs for which the model assigns greater probability to patients who will experience the event than to individuals who will not.

When viewed from the perspective of ROC curves (as opposed to risk ratios), current biomarkers for many clinical conditions unfortunately often add only marginal predictive power to that provided by conventional clinical factors in assessing risk. In this regard, it is worth examining data on biomarkers of cardiovascular risk, one of the better-studied areas of biomarker development. Wang *et al.* assessed the performance of 10 biomarkers for predicting first major cardiovascular events and death in the Framingham population and found a marginal increase in the C statistic (from 0.795 to 0.816), even when adding the five most powerful biomarkers to a model based on conventional risk factors.¹⁷ Honda and co-workers evaluated the C statistics for serum albumin, C-reactive protein, interleukin (IL)-6 and fetuin-A in an end-stage renal disease population and found that these biomarkers had marginal utility for predicting cardiovascular events or mortality.¹⁸ At present, therefore, available biomarkers of cardiovascular risk—although associated with hazard for adverse outcomes in large populations—have limited utility in the management of individual patients.

An ideal biomarker for AKI would be inexpensive, easy and rapid to quantify, repeatable, capable of determining the severity of injury, unique to the kidney, would increase early in the course of injury, and would have a high degree of sensitivity and specificity. Sensitivity, specificity and ROC curves are commonly used to measure the discrimination of a biomarker. ‘Discrimination’ refers to the ability of a biomarker to distinguish ‘case’ from ‘non-case’.

An additional consideration in evaluating biomarker utility is that sensitivity, specificity and the C statistic are not directly dependent on the relative frequency or pre-test probability of the disease. As such, appropriate interpretation of a biomarker requires a Bayesian approach in which pre-test probability of disease is integrated with biomarker test results to estimate the post-test probability of disease.^{19,20} This combination yields a predictive value for a positive or negative test. Nomograms that use Bayesian analysis to generate post-test probability of disease or outcomes are readily available.²¹ The positive predictive value of a biomarker falls as the prevalence (or pre-test probability) of the disease or outcome falls, so that a biomarker validated in a population with a high prevalence of a disease might not perform as well in populations in which the disease is less prevalent. The lower the prevalence of disease or outcome in a population, the higher the rate of false-positive results; the higher the disease prevalence, the higher the rate of false-negative results.

In addition to having good discrimination, an ideal biomarker will also exhibit a high level of calibration. ‘Calibration’ refers to the capacity of a biomarker to predict risk in sub-groups of the population relative to the actual observed risk.^{22,23} Calibration is frequently assessed using the Hosmer–Lemeshow goodness-of-fit statistic.²⁴ In well-calibrated models, there are only small differences between predicted and actual risks, resulting in Hosmer–Lemeshow statistics that are small in absolute value with nonsignificant probability values. Risk prediction algorithms that uniformly overestimate or underestimate risk can be recalibrated; for example, recalibration of the Framingham Coronary Heart Disease risk score for a Chinese cohort substantially improved risk prediction.²⁵

USE OF MULTIPLE BIOMARKERS

AKI is a complex syndrome with multiple diverse etiologies, and often occurs in the setting of systemic diseases such as sepsis. Sepsis is a syndrome that presents enormous challenges for biomarker development (Figure 1).²⁶ The ability to predict risk in such complex clinical circumstances might be enhanced by strategies that employ multiple simultaneous biomarkers of risk,²⁷ or by integration of biomarkers with other risk estimates.²⁸ For example, cardiovascular risk biomarkers, and sensitive markers of inflammation, myocyte necrosis, vascular

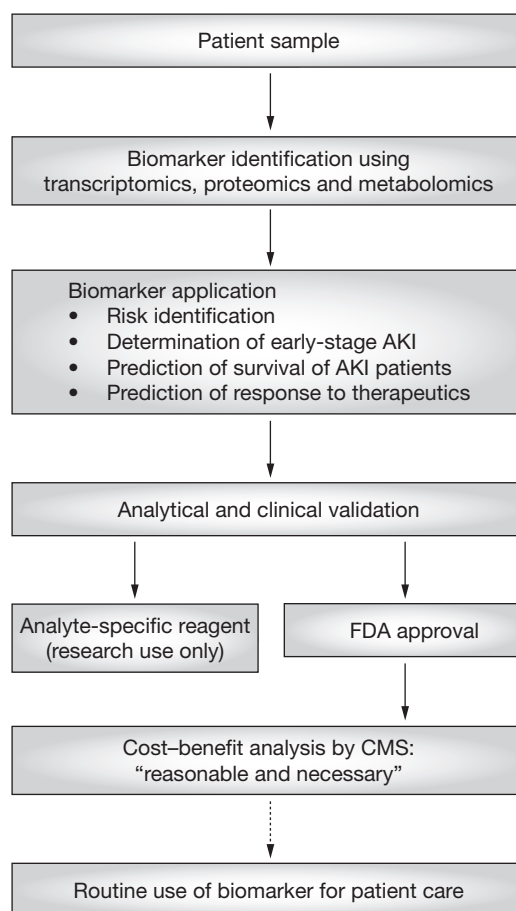


Figure 1 Chronology of biomarker development. After identification, a biomarker is evaluated for a particular clinical indication. Both analytical and clinical validation are necessary before FDA evaluation in the US (not necessary for research-only purposes). Following FDA approval, the CMS determines if the biomarker is “reasonable and necessary” for improvement of patient care and, therefore, reimbursable. Abbreviations: AKI, acute kidney injury; CMS, Center for Medicaid and Medicare Services. Permission obtained from Nature Publishing Group © Ludwig JA and Weinstein JN (2005) *Nat Rev Cancer* 5: 845–856

damage, platelet activation, and hemodynamic stress, have all been suggested to be of benefit in assessing risk for acute coronary syndrome. Some, but not all, studies have indicated that combining different pathogenic components of risk pathways adds utility to risk assessment.²⁷ Similarly, it has been suggested that multimarker approaches enhance risk prediction in oncology,²⁹ including in diffuse large B cell lymphomas³⁰ and glioblastoma multiforme.³¹ Most RNA-based studies employ multigene fingerprints on molecular patterns. Pattern-based analyses have been

associated with differences in breast cancer survival rates.^{32–35} One strategy—combining multimarkers, nonparametric data analysis, and integration of biomarker data with clinical variables (i.e. age, diastolic blood pressure, serum albumin, and method of vascular access)—augmented the capacity of plasma cytokine levels to predict cardiovascular outcomes in dialysis patients.³⁶ This type of approach might well be effective to define risk and prognosis in AKI.

THE LONG AND UNCERTAIN PATH TO BIOMARKER UTILITY

Despite intensified interest and investment in biomarker development in recent years, surprisingly few biomarkers are used in clinical practice.³⁷ Moreover, the rate of introduction of valid biomarkers into clinical medicine is falling over time rather than increasing. Since 1998, the rate of introduction of new protein analytes that have received FDA approval has fallen to an average of one biomarker per year.³⁸ This drop has led many observers to conclude that the potential clinical utility of biomarkers has yet to be realized.^{39,40} Even those biomarkers that are FDA-approved are rarely used in standard clinical practice. For example, of the more than 20 FDA-approved cancer biomarkers, only two have been incorporated into clinical practice guidelines for the Tumor, Node, Metastasis (TNM) Staging System. There is frequently a gap in the progress of biomarker development between discovery (candidate biomarkers are often discovered during investigator-initiated, government-funded research) and the more distal stages of clinical validation (often facilitated by manufacturers of commercial *in vitro* diagnostics). Weaknesses have been identified in the middle section of the biomarker pipeline, a stage at which development is often not funded specifically; as a consequence, knowledge gaps often occur at this point.³⁷

Despite much recent attention in biomarker development being directed towards high throughput ‘-omic’ technologies (e.g. genomics, proteomics and metabolomics), and towards *in silico* analyses of databases, advances in basic biologic understanding of multiple disease processes have not led to a considerable increase in the number of biomarkers approved by the FDA. An additional challenge for these high throughput methodologies is the lack of technology standardization. Further, most attempts at biomarker validation have been made using

relatively small retrospectively-collected datasets. Few prospective studies have validated initial findings in diverse patients in multiple institutions, or done so using well-established standardization of all steps in the biomarker development process.⁴¹ Finally, the requisite multivariate statistical approaches are prone to overfitting, particularly when more flexible, non-linear algorithms are used. The problem of overfitting data is particularly relevant when multimarker patterns are being evaluated.⁴² Biomarker identification and testing with high throughput technologies also faces enormous statistical issues related to multiple comparisons, particularly when the search for a biomarker is ‘unbiased’ (e.g. when genome-wide comparisons are made between cases and controls).⁴³ Concerns about false-positive findings are particularly relevant when biomarker testing is performed in a population with a low expected rate of disease prevalence, a phenomena which has been referred to as the ‘incidentalome’.⁴⁴

Studies employing information-rich patterns in which individual analytes are not identified are particularly problematic due to issues of technology standardization. An example is the use of surface enhanced laser desorption/ionization (SELDI)-proteomic profiles of disease, where few of the ‘unidentified flying particles’ that are components of published patterns have been identified, and data reproducibility has been problematic.^{38,45–47} Ultimately, the extreme nature of human biologic variability, coupled with stochastic uncertainties in many disease processes, contributes to tremendous observed heterogeneity in clinical outcomes. These formidable challenges to fulfillment of the promise of biomarkers are frequently underestimated during the early discovery phase. To be clinically useful, a biomarker must be relevant to the individual patient, not just to a population of patients with the same disease process.

PHASES OF AKI FOR WHICH SPECIFIC BIOMARKERS ARE NEEDED

For the purpose of this Review, we discuss biomarkers of AKI within the framework of the following four distinct phases: risk identification; surveillance; diagnosis; and prognosis. Although this is an arbitrary classification, and some biomarkers might be useful in several disease phases, it provides a pathophysiologic and clinical framework that is a useful basis for therapeutic decisions. We have also grouped potential

Table 1 Clinical phases of acute kidney injury and types of biomarkers that are useful in each phase.

Class of biomarker	Risk identification	Surveillance	Diagnosis	Prognosis
Clinical	Congestive heart failure Liver disease Diabetes mellitus CKD Sepsis	↓ Blood pressure ↓ Urine output	Urine output	Renal replacement therapy
Functional	↑ Serum creatinine ↓ GFR ↓ Renal blood flow	GFR Renal blood flow	Cystatin C Fractional excretion: sodium GFR Fractional excretion: urea	GFR
Structural	Proteinuria?	NAG NHE3	KIM-1 NHE3 NAG GST	KIM-1
Inflammatory	Reactive oxygen species? FGF23? C-reactive protein? Pentraxins?	NGAL IL-18	NGAL IL-18	–

Abbreviations: CKD, chronic kidney disease; FGF23, fibroblast growth factor 23; GFR, glomerular filtration rate; GST, glutathione S-transferase; IL-18, interleukin 18; KIM-1, kidney injury molecule 1; NAG, N-acetyl-β-(D)-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; NHE3, sodium hydrogen exchanger 3.

biomarkers into four broad categories, again for clinical reasons; these categories are clinical, functional, structural and inflammatory biomarkers. These groupings according to AKI phase and biomarker type are shown in Table 1, which includes some examples of known and potential biomarkers. Note that different categories can be complementary, such that utilization of all information will yield improved approaches. The ability to combine different biomarkers from different categories, and even within the same category, will be important for the development of specific diagnostic panels, which is currently underway. For example, the combination of a functional biomarker such as glomerular filtration rate (GFR) and a structural biomarker such as sodium hydrogen exchanger isoform 3 (NHE3) could have synergistic effects. Both biomarkers are altered in prerenal azotemia and acute tubular necrosis, but the change in GFR would be rapidly reversible, and NHE3 urinary levels lower, in prerenal azotemia. As such, prerenal azotemia and acute tubular necrosis could be rapidly differentiated.

Identifying patients at risk of developing AKI

Epidemiologic studies have identified several clinical and functional factors associated with increased risk and incidence of AKI. Thakar *et al.*⁶ and Palomba *et al.*⁴⁸ identified several common clinical parameters that are associated with an

increased incidence of AKI following cardiac surgery, including chronic kidney disease, congestive heart failure, combined coronary artery bypass grafting and valve surgery, and prolonged cardiopulmonary bypass pump time. Particularly notable was the association of increasing AKI risk and incidence with increasing patient baseline serum creatinine level. These data imply that the likelihood of developing AKI increases as pre-existing chronic kidney disease worsens.^{6,49} Unfortunately, the data are insufficiently quantitative and prospective studies for validation are required. Furthermore, as the functional data were based on serum creatinine levels and not on a true measure of GFR, it is not possible to accurately determine the effect of reduced GFR on risk of developing AKI. The clinical importance of the differences in data when serum creatinine level is used instead of a true measure of GFR will be delineated in detail later.

A validated quantitative structural or inflammatory marker for AKI risk stratification has not yet been identified. Many potential biomarkers can be proposed (e.g. inflammation, generation of reactive oxygen species, and endothelial dysfunction) as they are known to be associated with chronic kidney disease. It is essential to establish a repository for urine and blood samples collected in all prospective studies from patients at high risk of developing AKI. This information would allow comparison of

individuals who do and do not develop AKI, and stratification according to clinical parameters. Stratification of patients into risk categories is clinically important as it defines which patients require, or would benefit most from, the highest level of surveillance for development of AKI. Gene polymorphisms could be another means of determining risk. Data indicate that polymorphisms in the promoters of the genes encoding the cytokines TWF-2 and IL-10 are related to poor outcomes in patients with AKI who require dialysis.⁵⁰ Further study of gene polymorphisms—‘genetic epidemiology’—might generate new leads.⁵¹

A method to identify patients who will benefit most from intensive surveillance is essential because limited resources hamper our ability to follow all patients closely. Detecting high-risk patients also aids their care; for example, agents known to enhance the risk of AKI can be discontinued, the hemodynamic state of the patient can be optimized, and preventative therapies (e.g. during radiocontrast administration) can be used to improve outcomes. Preventative therapies for AKI itself are being developed. Their use in patients with a known risk factor such as cardiovascular surgery will have to be evaluated and justified on the basis of a risk–benefit ratio and a cost–savings benefit (Box 1).

Surveillance phase

Monitoring patients to detect the early events that warn of impending AKI has been a very challenging area of clinical investigation. As kidney injury, for the most part, lacks clinical signs or symptoms, the physician is left with biochemical tests that are nonspecific, insensitive and often not ordered in a timely fashion. Decreased urine output and blood pressure (Table 1) have been associated with development of both prerenal azotemia and acute injury. The use of insensitive markers of AKI (i.e. urine output, blood urea nitrogen and serum creatinine) has been blamed for the failure of therapeutic trials in AKI despite promising preclinical studies.^{52,53}

Serum creatinine level has traditionally been used as a surveillance marker of AKI. Recent prospective data indicate that increases in serum creatinine level of as little as 25.5 $\mu\text{mol/l}$ (0.3 mg/dl) portend a worse prognosis, and that patient outcomes worsen as the concentration of serum creatinine rises above this point.⁵⁴ Unfortunately, many factors that are independent of changes in renal function affect plasma

creatinine, making it a relatively poor biomarker of GFR in AKI. Creatinine concentration is an insensitive indicator of baseline GFR and of alterations in GFR under physiologic conditions. As AKI is a non-steady-state condition, the increasing serum creatinine level during AKI cannot be used to estimate GFR using standard formulas. The rate of generation of creatinine in serum varies with age, race, state of hydration, muscle mass, metabolic state and muscle injury. Creatinine excretion is principally a result of glomerular filtration; however, tubular secretion is responsible for creatinine excretion to an increasing relative degree as GFR decreases. Further, some drugs and organic compounds block creatinine excretion. Finally, the increase in serum creatinine level after the onset of AKI is slow, and can be retarded further by enhancing the hydration status of the patient. This latter phenomenon often masks reductions in GFR in postsurgical patients who are given large volumes of fluid for blood pressure control. For example, mortality rates increased in one study of post-cardiovascular surgery patients as creatinine levels decreased by more than 25.5 $\mu\text{mol/l}$ (0.3 mg/dl) from baseline.⁵⁴

Also important is the fact that by the time a change in serum creatinine concentration has been observed, a critical therapeutic window might have been missed. Cystatin C concentration has been shown to be superior to serum creatinine level for prediction of the occurrence of AKI in patients with sepsis.⁵⁵ Finally, it is standard practice to measure serum creatinine levels on a daily basis, meaning that AKI occurring today might not be detected until tomorrow’s blood work results are reviewed. The delay in diagnosis that is attributed to inadequate sampling can be easily remedied.

These shortcomings in the utility of serum creatinine level as a biomarker of AKI have hampered clinical trials, and led to the genesis of the Acute Dialysis Quality Initiative and the Acute Kidney Injury Network. These organizations represent the efforts of workgroups that seek to develop consensus and evidence-based statements in the field of AKI. The Acute Dialysis Quality Initiative developed a set of criteria, based upon serum creatinine and urine output, that has recently been modified by the Acute Kidney Injury Network.⁵⁶ These criteria are standards for diagnosing and classifying AKI, and have become the subject of early validation studies in selected groups of patients.^{57–59}

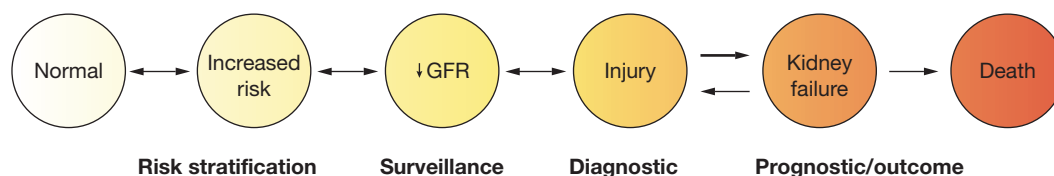


Figure 2 Association between clinical stages of acute kidney injury and phases of biomarker utility. Increasing severity of the clinical stages of acute kidney injury is indicated by increasing color intensity. Phases of potential biomarker utility are aligned with their most relevant clinical stages. Abbreviation: GFR, glomerular filtration rate. Permission obtained from BioMed Central Ltd © Mehta RL *et al.* (2007) *Crit Care* **11**: R31

Figure 2 indicates how the phases of AKI for which biomarkers are required can be related to a working clinical model of the disorder. Data accumulated using the RIFLE classification system have repeatedly shown that the severity of kidney injury, as determined by serum creatinine level, correlates with patient mortality.^{1,8} Unfortunately, this classification system does not allow stratification of early-stage AKI patients by severity of injury in a timeframe that is useful to the clinician for therapeutic purposes. Also, the data on the association between change in serum creatinine concentration and mortality do not prove cause and effect. Until an intervention is shown to reduce both the magnitude of changes in serum creatinine level and mortality, the validity of creatinine as an end point will continue to be questioned.

Biomarkers developed for diagnostic purposes are now in early clinical studies (see next section). Of these, three might be useful for patient surveillance, as their concentrations increase very early in the course of AKI; unfortunately, the specificity of these markers in studies of adults is not high. Early elevation of the urine levels of neutrophil gelatinase-associated lipocalin (NGAL) and IL-18, and of NGAL in serum, have been observed in clinical studies.^{60–63} These markers are hallmarks of inflammation, which might be one of the first signs of systemic or local kidney injury. Another early-stage indicator of altered renal function or kidney injury is urinary NHE3, which has been found at increased levels in both prerenal azotemia and AKI, and, therefore, has potential as a surveillance marker.⁶⁴ The lack of overlap between urinary levels of NHE3 in patients with prerenal azotemia and in those with AKI could provide a means of differentiating between these two common (and commonly overlapping) disease states. As NHE3 is expressed in the proximal tubule brush border membrane,

increased levels in urine provide information about the site of injury.

Diagnostic biomarkers

The use of validated biomarkers is essential for prompt diagnosis of AKI and could facilitate early initiation of specific interventions that ameliorate tissue injury and reduce mortality. Traditional methods that are used clinically to diagnose AKI—such as monitoring changes in serum creatinine level and creatinine clearance, urinalysis, ultrasound imaging of the kidney and urinary tract, and renal biopsy—each have limitations that preclude accurate and early diagnosis. For example, a diagnostic dilemma often results when attempting to differentiate prerenal azotemia from intrarenal causes of AKI in patients with sepsis, volume depletion or reduced effective arterial volume status, and in those using drugs such as non-steroidal anti-inflammatory agents. Whereas prerenal AKI involves adaptive functional alterations in GFR and is responsive to increased kidney perfusion, intrarenal AKI is secondary to acute cellular injury (e.g. acute tubular necrosis)⁶⁵ and is a ‘volume unresponsive’ condition. Despite being traditionally categorized as distinct entities, prerenal AKI and renal AKI represent a continuum of response to renal perfusion that ranges from responsiveness to unresponsiveness. Distinguishing between these two conditions is currently accomplished through the use of maneuvers that increase the volume status of the patient; subsequent reduction of serum creatinine level indicates prerenal AKI.^{66–68} This distinction is critically important as the appropriate therapy for each condition is different. It is obviously preferable not to subject patients with AKI to an expensive and potentially harmful intervention if the underlying disease process is secondary to reduced renal blood flow, which can be effectively corrected by other means such as volume expansion.

The lack of sensitivity of currently available biomarkers, and the assumption that a graded increase in levels of urinary or serum indicators can be used to quantify the severity of tubular injury, underscores the theoretical need for novel diagnostic biomarkers. The underlying premise is that the discovery and validation of biomarkers for AKI will improve the early diagnosis of tubular injury (e.g. in a manner analogous to the use of troponin I for the diagnosis of acute myocardial infarction), thereby facilitating timely therapeutic intervention. New, sensitive biomarkers should also help to differentiate between etiologies of AKI (e.g. prerenal AKI, AKI due to ischemia or toxins, glomerulonephritis and allergic interstitial nephritis), predict the severity of AKI (which will aid prognosis and determination of when to initiate renal replacement therapy), monitor the effectiveness of therapy, and determine the site of injury so that therapies can be targeted and the severity of injury predicted.^{69,70}

Several important candidate biomarkers are entering clinical validation studies. Nevertheless, a panel of other, newer biomarkers will probably be needed in the future; no single biomarker is likely to have the characteristics necessary to fulfill the purposes outlined above. Potential new biomarkers identified in preclinical models include exosomal fetuin-A⁷¹ and renal L-type fatty acid-binding protein.⁷² A biomarker panel would consist of up to 20 biomarkers that can be assessed simultaneously from a small sample volume. As the timing of diagnosis is crucial to early initiation of therapy, it will be necessary to identify biomarkers that vary over the time course of injury. The level of some biomarkers rises soon after injury and declines rapidly, whereas the converse is true for others. For example, in a study of children who had cardiopulmonary bypass surgery, the concentration of NGAL increased 25-fold within 2 h of surgery and began to drop after 6 h, whereas the level of urinary IL-18 increased 4–6 h after surgery and persisted for up to 48 h postoperatively.⁷³ So, combining markers might be useful for the detection of AKI, as are creatine phosphokinase and lactate dehydrogenase for the diagnosis of acute myocardial infarction.

Prognostic biomarkers

The ability to predict death and/or the need for dialysis early in the course of AKI is greatly important. Data indicate that the severity of

kidney injury, as determined by serum creatinine level^{1,8} or measured GFR at diagnosis,⁵³ can predict outcomes; only measured GFR can be obtained early enough to be useful for outcomes prediction, however. Having a functional biomarker, such as GFR, and a structural biomarker that can be used to quantify the extent of injury early in the course of AKI, such as kidney injury molecule 1 (KIM-1), might also lead to more rapid and appropriate initiation of renal replacement therapy. Several clinical risk factors that are present at the point of initial consultation have been documented in the prospective Project to Improve Care in Acute Renal Disease (PICARD) study.⁵ These factors include advanced age, hepatic failure, sepsis and thrombocytopenia. The combination of urinary *N*-acetyl- β -(D)-glucosaminidase activity and KIM-1 was used to predict clinical outcomes in one study of AKI.⁷⁴ Patients were divided into quartiles and associations between the two biomarkers and APACHE II score, multiple organ failure score, dialysis requirement, hospital death and maximal serum creatinine level were detected. Levy *et al.*⁷⁵ showed that improvement in organ function within the first 24 h of diagnosis of sepsis was predictive of better outcomes. As such, the extent of renal injury, determined using either a functional or structural test early in the course of AKI, could be the variable that is most predictive of mortality or of the need for dialysis. Differentiating between patients who would benefit from early initiation of renal replacement therapy and those from whom support should be withheld or withdrawn is an important clinical issue for which few objective data are available on which to base decisions. This important area is not receiving adequate attention at present.

CONCLUSIONS

Extensive work needs to be done if biomarkers are to be successfully incorporated into clinical practice for AKI. There is an urgent need to continue discovery, characterization and validation of novel biomarkers. The current repository constitutes a fraction of the serum and urinary biomarkers that could be discovered using advanced molecular and analytical methods to study genes (transcriptomics),^{76,77} proteins (proteomics)^{78,79} and metabolites (metabolomics).⁸⁰ Biomarkers of AKI need to provide early evidence of risk and injury, and

differentiate between types of injury (e.g. that caused by nephrotoxins, ischemia, sepsis and interstitial nephritis). Biomarkers must have the capacity to discriminate between different forms of AKI in both elderly and pediatric populations, as well as in patients with a variety of comorbidities. It is particularly important for biomarkers to have the capacity to differentiate between prerenal azotemia and cellular injury, as the therapies for these two conditions differ considerably. Sophisticated analytical algorithms will be needed in order to predict specific etiologies of AKI on the basis of patterns of urinary biomarker abundance. An artificial neural network was recently employed for this purpose in glomerular diseases.⁸¹

Biomarkers for AKI must be validated in well-designed, cost-effective and adequately powered clinical trials, and compared with a standardized definition of AKI. There is growing consensus that RIFLE and the AKI score provide the long-sought consistent definition of AKI.^{56–58} A collaborative network of centers for the study of AKI is needed so that clinical information can accumulate and repositories of biomarkers can be maintained; independent biomarker studies could be prohibitively expensive. Biomarkers will need to be identified using assays that are simple, rapid and reproducible, such that timely and cell-specific clinical intervention is possible.

KEY POINTS

- A biomarker is a measurable indication of a specific biologic state that is relevant to a specific disease process
- A perfect biomarker is easily measurable, accurate, reproducible, cost-effective, easy to interpret and provides useful clinical information
- A biomarker of acute kidney injury (AKI) should independently provide information that is additive to that provided by conventional clinical factors and/or the biomarker should account for a large proportion of the risk associated with AKI
- Biomarkers would be most useful in AKI for identification of at-risk individuals, surveillance of events that precede the condition, diagnosis and prognosis
- Numerous potential biomarkers of AKI identified in preclinical investigations are now beginning to be tested in clinical validation studies

References

- 1 Hoste EA *et al.* (2006) RIFLE criteria for acute kidney injury are associated with hospital mortality in critically ill patients: a cohort analysis. *Crit Care* **10**: R73
- 2 Xue JL *et al.* (2006) Incidence and mortality of acute renal failure in Medicare beneficiaries, 1992 to 2001. *J Am Soc Nephrol* **17**: 1135–1142
- 3 Xue JL *et al.* (2007) Longitudinal study of racial and ethnic differences in developing end-stage renal disease among aged Medicare beneficiaries. *J Am Soc Nephrol* **18**: 1299–1306
- 4 Hsu CY *et al.* (2007) Community-based incidence of acute renal failure. *Kidney Int* **72**: 208–212
- 5 Chertow GM *et al.* (2006) Mortality after acute renal failure: models for prognostic stratification and risk adjustment. *Kidney Int* **70**: 1120–1126
- 6 Thakar CV *et al.* (2005) A clinical score to predict acute renal failure after cardiac surgery. *J Am Soc Nephrol* **16**: 162–168
- 7 Bellomo R *et al.* (2004) 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* **8**: R204–R212
- 8 Uchino S *et al.* (2006) Pulmonary artery catheter versus pulse contour analysis: a prospective epidemiological study. *Crit Care* **10**: R174
- 9 Vasan RS (2006) Biomarkers of cardiovascular disease: molecular basis and practical considerations. *Circulation* **113**: 2335–2362
- 10 Biomarkers Definitions Working Group (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* **69**: 89–95
- 11 Gutman S and Kessler LG (2006) The US Food and Drug Administration perspective on cancer biomarker development. *Nat Rev Cancer* **6**: 565–571
- 12 De Gruttola VG *et al.* (2001) Considerations in the evaluation of surrogate endpoints in clinical trials: summary of a National Institutes of Health workshop. *Control Clin Trials* **22**: 485–502
- 13 Freedman LS *et al.* (1992) Statistical validation of intermediate endpoints for chronic diseases. *Stat Med* **11**: 167–178
- 14 Roberts MA *et al.* (2006) Cardiovascular biomarkers in CKD: pathophysiology and implications for clinical management of cardiac disease. *Am J Kidney Dis* **48**: 341–360
- 15 Sargent D and Allegra C (2002) Issues in clinical trial design for tumor marker studies. *Semin Oncol* **29**: 222–230
- 16 Gion M *et al.* (1999) A guide for reviewing submitted manuscripts (and indications for the design of translational research studies on biomarkers). *Int J Biol Markers* **14**: 123–133
- 17 Wang TJ *et al.* (2006) Multiple biomarkers for the prediction of first major cardiovascular events and death. *N Engl J Med* **355**: 2631–2639
- 18 Honda H *et al.* (2006) Serum albumin, C-reactive protein, interleukin 6, and fetuin A as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD. *Am J Kidney Dis* **47**: 139–148
- 19 Diamond GA *et al.* (1995) Prior restraint: a Bayesian perspective on the optimization of technology utilization for diagnosis of coronary artery disease. *Am J Cardiol* **76**: 82–86
- 20 Diamond GA and Kaul S (2004) Prior convictions: Bayesian approaches to the analysis and interpretation of clinical megatrials. *J Am Coll Cardiol* **43**: 1929–1939

- 21 Fagan TJ (1975) Letter: nomogram for Bayes theorem. *N Engl J Med* **293**: 257
- 22 Justice AC *et al.* (1999) Assessing the generalizability of prognostic information. *Ann Intern Med* **130**: 515–524
- 23 van Houwelingen HC (2000) Validation, calibration, revision and combination of prognostic survival models. *Stat Med* **19**: 3401–3415
- 24 Lemeshow S and Hosmer DW Jr (1982) A review of goodness of fit statistics for use in the development of logistic regression models. *Am J Epidemiol* **115**: 92–106
- 25 Liu J *et al.* (2004) Predictive value for the Chinese population of the Framingham CHD risk assessment tool compared with the Chinese Multi-Provincial Cohort Study. *JAMA* **291**: 2591–2599
- 26 Ludwig JA and Weinstein JN (2005) Biomarkers in cancer staging, prognosis and treatment selection. *Nat Rev Cancer* **5**: 845–856
- 27 Morrow DA and Braunwald E (2003) Future of biomarkers in acute coronary syndromes: moving toward a multimarker strategy. *Circulation* **108**: 250–252
- 28 Koenig W (2007) Cardiovascular biomarkers: added value with an integrated approach? *Circulation* **116**: 3–5
- 29 Hanash S (2003) Disease proteomics. *Nature* **422**: 226–232
- 30 Alizadeh AA *et al.* (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* **403**: 503–511
- 31 Liang Y *et al.* (2005) Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. *Proc Natl Acad Sci USA* **102**: 5814–5819
- 32 Perou CM *et al.* (2000) Molecular portraits of human breast tumours. *Nature* **406**: 747–752
- 33 Sorlie T *et al.* (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* **98**: 10869–10874
- 34 Sotiriou C *et al.* (2003) Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proc Natl Acad Sci USA* **100**: 10393–10398
- 35 van't Veer LJ *et al.* (2002) Gene expression profiling predicts clinical outcome of breast cancer. *Nature* **415**: 530–536
- 36 Knickerbocker T *et al.* (2007) An integrated approach to prognosis using protein microarrays and nonparametric methods. *Mol Syst Biol* **3**: 123
- 37 Rifai N *et al.* (2006) Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat Biotechnol* **24**: 971–983
- 38 Anderson NL (2005) The roles of multiple proteomic platforms in a pipeline for new diagnostics. *Mol Cell Proteomics* **4**: 1441–1444
- 39 Dalton WS and Friend SH (2006) Cancer biomarkers—an invitation to the table. *Science* **312**: 1165–1168
- 40 Wilson JF (2006) The rocky road to useful cancer biomarkers. *Ann Intern Med* **144**: 945–948
- 41 Hammond ME and Taube SE (2002) Issues and barriers to development of clinically useful tumor markers: a development pathway proposal. *Semin Oncol* **29**: 213–221
- 42 Ransohoff DF (2004) Rules of evidence for cancer molecular-marker discovery and validation. *Nat Rev Cancer* **4**: 309–314
- 43 Hunter DJ and Kraft P (2007) Drinking from the fire hose—statistical issues in genomewide association studies. *N Engl J Med* **357**: 436–439
- 44 Kohane IS *et al.* (2006) The incidentalome: a threat to genomic medicine. *JAMA* **296**: 212–215
- 45 Petricoin EF *et al.* (2002) Use of proteomic patterns in serum to identify ovarian cancer. *Lancet* **359**: 572–577
- 46 Baggerly KA *et al.* (2005) Signal in noise: evaluating reported reproducibility of serum proteomic tests for ovarian cancer. *J Natl Cancer Inst* **97**: 307–309
- 47 Ransohoff DF (2005) Lessons from controversy: ovarian cancer screening and serum proteomics. *J Natl Cancer Inst* **97**: 315–319
- 48 Palomba H *et al.* (2007) Acute kidney injury prediction following elective cardiac surgery: AKICS score. *Kidney Int* **72**: 624–631
- 49 Thakar CV *et al.* (2005) Influence of renal dysfunction on mortality after cardiac surgery: modifying effect of preoperative renal function. *Kidney Int* **67**: 1112–1119
- 50 Jaber BL *et al.* (2004) Cytokine gene promoter polymorphisms and mortality in acute renal failure. *Cytokine* **25**: 212–219
- 51 Jaber BL *et al.* (2005) Polymorphism of host response genes: implications in the pathogenesis and treatment of acute renal failure. *Kidney Int* **67**: 14–33
- 52 Hirschberg R *et al.* (1999) Multicenter clinical trial of recombinant human insulin-like growth factor I in patients with acute renal failure. *Kidney Int* **55**: 2423–2432
- 53 Allgren RL *et al.* (1997) Anaritide in acute tubular necrosis. Auriculin Anaritide Acute Renal Failure Study Group. *N Engl J Med* **336**: 828–834
- 54 Lassnigg A *et al.* (2004) Minimal changes of serum creatinine predict prognosis in patients after cardiothoracic surgery: a prospective cohort study. *J Am Soc Nephrol* **15**: 1597–1605
- 55 Herget-Rosenthal S *et al.* (2004) Early detection of acute renal failure by serum cystatin C. *Kidney Int* **66**: 1115–1122
- 56 Mehta RL *et al.* (2007) Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care* **11**: R31
- 57 Akcan-Arikan A *et al.* (2007) Modified RIFLE criteria in critically ill children with acute kidney injury. *Kidney Int* **71**: 1028–1035
- 58 Cruz DN *et al.* (2007) North East Italian Prospective Hospital Renal Outcome Survey on Acute Kidney Injury (NEiPHROS-AKI): targeting the problem with the RIFLE Criteria. *Clin J Am Soc Nephrol* **2**: 418–425
- 59 Kuitunen A *et al.* (2006) Acute renal failure after cardiac surgery: evaluation of the RIFLE classification. *Ann Thorac Surg* **81**: 542–546
- 60 Mishra J *et al.* (2005) Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. *Lancet* **365**: 1231–1238
- 61 Wagener G *et al.* (2006) Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery. *Anesthesiology* **105**: 485–491
- 62 Bachorzewska-Gajewska H *et al.* (2006) Neutrophil-gelatinase-associated lipocalin and renal function after percutaneous coronary interventions. *Am J Nephrol* **26**: 287–292
- 63 Parikh CR *et al.* (2005) Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the intensive care unit. *J Am Soc Nephrol* **16**: 3046–3052
- 64 du Cheyron D *et al.* (2003) 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* **42**: 497–506

- 65 Thadhani R *et al.* (1996) Acute renal failure. *N Engl J Med* **334**: 1448–1460
- 66 Miller TR *et al.* (1978) Urinary diagnostic indices in acute renal failure: a prospective study. *Ann Intern Med* **89**: 47–50
- 67 Espinel CH and Gregory AW (1980) Differential diagnosis of acute renal failure. *Clin Nephrol* **13**: 73–77
- 68 Esson ML and Schrier RW (2002) Diagnosis and treatment of acute tubular necrosis. *Ann Int Med* **137**: 744–752
- 69 Nguyen MT and Devarajan P (2007) Biomarkers for the early detection of acute kidney injury. *Pediatr Nephrol* [doi:10.1007/s00467-007-0470-x]
- 70 Hewitt SM *et al.* (2004) Discovery of protein biomarkers for renal diseases. *J Am Soc Nephrol* **15**: 1677–1689
- 71 Zhou H *et al.* (2006) Exosomal fetuin-A identified by proteomics: a novel urinary biomarker for detecting acute kidney injury. *Kidney Int* **70**: 1847–1857
- 72 Yamamoto T *et al.* (2007) Renal L-type fatty acid binding protein in acute ischemic injury. *J Am Soc Nephrol* **18**: 2894–2902
- 73 Parikh CR *et al.* (2006) Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery. *Kidney Int* **70**: 199–203
- 74 Liangos O *et al.* (2007) Urinary N-acetyl- β -D-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. *J Am Soc Nephrol* **18**: 904–912
- 75 Levy H *et al.* (2005) Steroid use in PROWESS severe sepsis patients treated with drotrecogin alfa (activated). *Crit Care* **9**: R502–R507
- 76 Supavekin S *et al.* (2003) Differential gene expression following early renal ischemia/reperfusion. *Kidney Int* **63**: 1714–1724
- 77 Yoshida T *et al.* (2002) Global analysis of gene expression in renal ischemia-reperfusion in the mouse. *Biochem Biophys Res Commun* **291**: 787–794
- 78 Holly MK *et al.* (2006) Biomarker and drug-target discovery using proteomics in a new rat model of sepsis-induced acute renal failure. *Kidney Int* **70**: 496–506
- 79 Nguyen MT *et al.* (2005) Early prediction of acute renal injury using urinary proteomics. *Am J Nephrol* **25**: 318–326
- 80 Portilla D *et al.* (2006) Metabolomic study of cisplatin-induced nephrotoxicity. *Kidney Int* **69**: 2194–2204
- 81 Varghese SA *et al.* (2007) Urine biomarkers predict the cause of glomerular disease. *J Am Soc Nephrol* **18**: 913–922

Acknowledgments

The authors acknowledge that portions of this manuscript were derived from a meeting held by the Acute Kidney Injury Network in Vancouver, BC, Canada, 11–14 September 2006. This work was supported by NIH grants DK56223, DK62324 and DK58413 to MDO, and by DK53465 and DK61594, and VA Merit Review, to BAM. Charles P Vega, University of California, Irvine, CA, is the author of and is solely responsible for the content of the learning objectives, questions and answers of the Medscape-accredited continuing medical education activity associated with this article.

Competing interests

The authors declared no competing interests.