

REVIEW

Biomarkers of acute kidney injury in anesthesia, intensive care and major surgery: from the bench to clinical research to clinical practice

E. MOORE¹, R. BELLOMO², A. NICHOL¹

¹Australian and New Zealand Intensive Care Research Centre, Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia; ²Department of Intensive Care, Austin Hospital, Melbourne, Australia

ABSTRACT

Acute kidney injury (AKI) is common after major surgery and reportedly occurs in approximately 36% of ICU patients (RIFLE Risk/Injury/Failure categories). It is associated with increased mortality, greater cost, and prolonged Intensive Care Unit (ICU) and hospital stay, despite attempts to develop therapies to prevent or attenuate AKI, which have had limited success. One major reason for this lack of success may be the result of delayed implementation due to the inability to detect AKI early. Traditional biomarkers of AKI (creatinine and urea) do not detect injury early enough. Thus, it is a priority to find reliable, early biomarkers that predict subsequent AKI. Innovative technologies such as functional genomics and proteomics have facilitated detection of several promising early biomarkers of AKI, such as neutrophil gelatinase-associated lipocalin (NGAL), cystatin C (CyC), liver-type fatty acid binding protein (L-FABP), interleukin-18 (IL-18), and kidney injury molecule-1 (KIM-1). These biomarkers have many potential applications during anesthesia and in the ICU. They can be used to evaluate the effect of new techniques and therapies on kidney function, as safety markers to monitor toxicity and as measures of treatment effect. For example, NGAL and cystatin C have been used in a safety monitoring trial of hydroxyethylstarch therapy and to detect AKI early, during or immediately after cardiac surgery. Clinical use beyond research settings is rapidly expanding. (*Minerva Anestesiologica* 2010;76:425-40).

Key words: Kidney failure - Creatinine - Urea - Biological markers - Lipocalins - Cystatin C - Fatty acid-binding proteins - Interleukin-18.

AKI is common with major surgery and critical illness

Acute kidney injury (AKI) is the consensus term now used to describe the continuum of the condition previously called acute renal failure. AKI as classified by the RIFLE criteria¹ (acronym for Risk, Injury, Failure, Loss and Endstage) has been reported to occur in approximately 36% of critically ill patients and is common after major

surgery such as open heart surgery.^{2, 3} Although the Acute Kidney Injury Network (AKIN) criteria, based on the RIFLE classification system are being used increasingly and are presumed to improve sensitivity in diagnosing AKI, they have not been shown to improve the ability to predict outcomes.^{4, 5} The RIFLE criteria have been extensively used and validated to classify renal function in several populations with studies cumulatively involving >250 000 subjects.⁶ Furthermore, a

recent study found RIFLE to be more robust, with a higher detection rate of AKI than AKIN in the first 48 hours post-ICU admission.⁷ Therefore, we favor the RIFLE classification for AKI.

AKI is independently associated with an increased risk of death⁶ and with prolonged length of stay.^{8, 9} Severe cases require costly treatment, can result in prolonged kidney dysfunction, and escalate the human and financial costs of care. Therefore, it seems desirable to detect AKI as early as possible in order to develop or implement potentially protective therapy.

Why therapies have been unsuccessful

To date, attempts to develop therapies to prevent or attenuate AKI have failed to show consistently protective effects. The use of diuretics has not proven to be of benefit. Fenoldopam, a vasodilator, has shown promise in certain populations¹⁰ but not in others;^{11, 12} natriuretic peptides may be of use in major surgery but have also not been effective in other situations.^{13, 14} The capacity of N-acetyl cysteine to prevent AKI after radiocontrast has been tested with unconvincing results,¹⁵⁻¹⁷ and the benefits of perioperative IV sodium bicarbonate infusion in cardiac surgical patients¹⁸ are yet to be confirmed. The most widely accepted treatment to prevent or treat AKI (although untested in controlled trials) remains prompt fluid resuscitation of circulatory volume and appropriate use of inotropes/vasopressors to maintain adequate cardiac output and perfusion pressure.¹⁹⁻²¹ Beyond these measures, cases unresponsive to fluid resuscitation in the presence of hyperkalemia, metabolic acidosis or fluid overload commonly receive renal replacement therapy/dialysis to support the kidneys.

There are several reasons why no reproducibly and consistently effective treatment for AKI has been found. First, AKI can occur as a result of multiple causes and disease processes. Whereas certain treatments may benefit subgroups, heterogeneity of disease makes finding one treatment for all types of AKI unlikely. Second, understanding of the pathogenesis of AKI is limited. An incomplete knowledge of the mechanisms involved has resulted in difficulties in developing logical approaches to prevention or treatment. Third, interventions are implemented too late. This delay

is due to our reliance on conventional biomarkers (creatinine, urea, urine output) to diagnose AKI. Such biomarkers either do not detect injury in real-time and become abnormal many hours later in the course of injury (creatinine or urea) or lack specificity (urine output). To draw a parallel with the treatment for acute myocardial infarction, one can easily imagine how difficult it would be to show the benefits of thrombolysis or stenting in the absence of troponin, electrocardiogram changes or the presence of chest pain to allow early and specific diagnosis within minutes instead of hours or a day later. By analogy, if we could detect AKI early, we would treat it more rapidly and should have a better chance of preventing or reducing injury, as is the case for several other acute syndromes in medicine.

The limitations of traditional biomarkers

The traditional clinical biomarkers for the detection of AKI are creatinine, urea, and urine output. All have serious limitations as early detectors of AKI. Creatinine is the product of the breakdown of creatine to phosphocreatine in skeletal muscle and of the subsequent liver metabolism of creatine to form creatinine.²² It is produced and released into plasma at a fairly constant rate and is filtered by the glomerulus. A small amount is also secreted into the urine. Creatinine is not reabsorbed in the tubules or metabolized by the kidney. If filtering of creatinine is deficient, blood levels rise with an inverse relationship with GFR. Unfortunately, there are several limitations to serum creatinine (SCr) as a biomarker of AKI.²² First, its release varies with age, gender, diet, muscle mass, drugs, and vigorous exercise. Second, secretion accounts for 10-40% of creatinine clearance,²² which could mask a decrease in GFR. Third, the accuracy of SCr assays can be reduced by artifact. Fourth, creatinine becomes abnormal only when more than 50% of GFR is lost and may require up to 24 hours before sufficient increases in blood concentration are detectable.

Urea is a water-soluble, low molecular weight by-product of protein metabolism. Its level is also inversely related to GFR, but several factors affect its production and clearance, limiting its reliability in estimating GFR. Urea production is incon-

TABLE I.—*Characteristics, function and significance of NGAL, cystatin C and L-FABP.*

Biomarker	Molecular weight	Origin	Normal concentration		Physiological function	Significance of rise in level	
			Urine	Plasma/Serum			
NGAL	21 kDa	Urine NGAL - mostly local synthesis in kidney (distal nephron) in response to injury - secreted into urine <i>Circulating NGAL</i> - synthesised systemically in response to renal injury → filtered by glomerulus → uptake by proximal tubule epithelia → pooled and little secreted into urine <i>Neutrophils/macrophages</i> may be a third source of renal NGAL - under investigation	Adults	1.0-20.0 ng/mL	70-105 ng/mL	<i>Bacteriostatic effect</i> - binds to iron-carrying molecules (siderophores) which are synthesised by specific bacteria to gather iron. By doing this, NGAL reduces bacterial growth <i>Antioxidant effect</i> - binds to "human siderophores" to transport iron into target cells thus stopping free and reactive iron from producing oxygen radicals which can cause oxidative stress and cell injury <i>Scavenges intracellular iron</i> for extracellular export (hypothesis) <i>Acts as a growth factor</i> - regulates cell proliferation, apoptosis and differentiation (supported by increasing evidence)	Tubular stress/injury. There is an earlier rise in urine than serum
			Children	1.0-20.0 ng/mL	30-80 ng/mL		
Cystatin C	13.3 kDa	Produced at a constant rate by nucleated cells → filtered by the glomerulus → almost completely reabsorbed and catabolised (but not secreted) in the proximal tubules		<0.1 mg/g Cr ²⁴	<1-1.5 mg/L	Potent inhibitor of lysosomal proteases (inhibits breakdown of lysosomal protein) and extracellular inhibitor of cysteine proteases (prevents breakdown of extracellular protein)	Change in GFR is reflected in changes in serum and urine levels of cystatin C - acts as a marker of GFR
L-FABP	14.3 kDa	Expressed in the liver, intestine, pancreas, lung stomach, and kidneys NoiriDoi09. Production in the liver seems to determine blood levels. Renal L-FABP is found in cytoplasm of the proximal tubules	Children (CPB) Adults (CIN)	<10 ng/mg Cr/L Portilla <i>et al.</i> ²⁷ 5-20 µg/g Cr ²⁵ Nakamura <i>et al.</i> ²⁵	60-110 ng/mL ²⁷	Renal L-FABP helps maintain low levels of free FAs in the cytoplasm by a) binding to them and transporting them to cell components to accelerate FA metabolism and by b) binding to free FAs and being excreted from the proximal tubule cells into urine for elimination ²⁶	Tubular stress/injury, which results in an accumulation of free FAs in the proximal tubules ²³ There is an earlier rise in urine than serum

stant, and values can be altered by changes in circulatory volume, protein intake, gastrointestinal bleeding, among other parameters. Rate of renal clearance of urea is not constant; 40-50% of filtered urea may be reabsorbed in the tubules.²² Consequently, urea is a poor measure of GFR, requires time to accumulate, does not reflect real-time changes in GFR and delays diagnosis.

Urine output is measured routinely in operating rooms and ICUs with indwelling catheters. A trend in urine output is a crude gauge of kidney function and may be a more sensitive indicator of changes in renal hemodynamics than a measure of solute clearance.¹ Nonetheless, many patients with AKI do not have oliguria, and many patients with oliguria in the operating room or ICU do

TABLE II.—Performance of NGAL in diagnosis of AKI in adults: studies with AUC-ROC analysis.

Year	Authors	Setting	Age	SexF %	Existing renal disease excluded	% AKI	AKI Definition (↑ in SCr)	Timing (AKI)	Sample size
<i>NGAL</i>									
2009	Liangos <i>et al.</i> ³⁸	CSA	68	28	* †	13	≥50%	72 h	103
2009	Tuladhar <i>et al.</i> ³⁹	CSA	67	30	Y	18	>44.2 μmol/L	48 h	50
2009	Han <i>et al.</i> ³⁷	CSA	NA	NA	NA	40	≥26.5 μmol/L	72 h	90
2009	Haase-Fielitz <i>et al.</i> ³⁶	CSA	70	39	Y	23	>50%	5 days	100
2009	Haase-Fielitz <i>et al.</i> ³⁵	CSA	70	39	* ‡	39	>25%	5 days	100
2009	Haase <i>et al.</i> ³⁴	CSA	70	39	* ‡	46	>50% or >26.5 μmol/L or ¥	48 h	100
2008	Xin <i>et al.</i> ⁴⁰	CSA	38	56	N	27	>50% or ≥26.5 μmol/L or ¥	48 h	33
2008	Wagener G. <i>et al.</i> ⁴⁵	CSA	63	34	§	20	≥50% or >0 26.5 μmol/L	48 h	426
2008	Koyner <i>et al.</i> ²⁴	CSA	65	29	Y	47	≥25% or RRT	72 h	72
2006	Wagener G. <i>et al.</i> ⁴¹	CSA	68	35	*	20	≥50%	10 days	81
2009	Makris <i>et al.</i> ⁴³	Trauma	NA	19	Y	NA	NA	5 days	31
2008	Ling W. <i>et al.</i> ⁴²	CIN	67	40	N	NA	≥25% or > 44.2 μmol/L	72 h	40
2008	Nickolas <i>et al.</i> ⁴⁴	ED	60	49	*	5	≥50% or 25%↓ in eGFR	Hosp stay	635

CSA: cardiac surgery associated AKI; CIN: contrast induced nephropathy; ED: emergency department; Y: yes; N: no; NA: not available or not applicable; RRT: renal replacement therapy; Hosp stay: hospital stay; Quantikine® - R&D systems. *: dialysis, †: transplant, ‡: SCr >300 μmol/L; §: endstage renal disease; ¥: urine output <0.5 mL/kg/h for >6 h.

not develop AKI. Finally, many drugs used in the operating room or ICU (*i.e.*, diuretics and vaso-pressors) act as additional confounders.

In response to these problems, innovative technologies such as functional genomics and proteomics have facilitated the detection of several potential earlier biomarkers of AKI. Some of the most promising biomarkers include neutrophil gelatinase-associated lipocalin (NGAL), cystatin C (CyC), and liver-type fatty acid binding protein (L-FABP), which have now been evaluated in several populations.

NGAL

The expression of NGAL in early, acute tubular injury was identified using functional genomics. NGAL is a measure of tubular stress (Table I); its concentration increases dramatically in response to tubular injury and precedes rises in SCr by >24 hours.²⁸ NGAL normally exerts protective bacteriostatic and antioxidant effects involving iron transport and is thought to act as an iron scav-

enger and growth factor^{29, 30} (Table I). The proposed role of NGAL and its twin molecule, hepcidin (a “master regulator” of iron²⁹) are summarized in Figure 1.

NGAL has been intensely investigated in recent years, predominantly in adult cardiac surgery. ELISA techniques have been used to measure NGAL; however, accurate and sensitive point of care tests that reduce costs and the potential for measurement error are now available^{32, 33} (Tables II-IV).

Cardiac surgery – children

In a landmark study, diagnosis of AKI by SCr occurred 1-3 days after surgery, whereas plasma and urine NGAL levels were powerful independent predictors of AKI within 2 hours of surgery (Table IV). Subsequent studies confirmed these findings^{27, 32, 33} (Table IV). Moreover, plasma NGAL predicted duration of AKI, length of stay and mortality.

Cardiac surgery – adults

The findings in children were broadly confirmed

Instrument	Time from procedure/admission (h)	Cutoff		Urine		Plasma/Serum		AUC-ROC	
		Urine	Plasma	Sensitivity %	Specificity %	Sensitivity %	Specificity %	Urine %	Plasma %
Quantikine®	2	166 ng/mg		67	11			50	
ELISA	2	393 ng/mL	426 ng/mL	93	78	80	67	96	85
NA	0	NA		NA	NA			59	
NA	3	NA		NA	NA			65	
Triage®Biosite	ICU/6hrs		150 ng/mL			79	78		80
	24		90 ng/mL			91	76		87
Triage®Biosite	ICU/6hrs		145 ng/mL			68	64		67
Triage®Biosite	ICU	>150 ng/mL		73	74			77	
ELISA kit	2	250 µg/L		71	73			88	
	2	250 µg/mmol		81	78			93	
ELISA kit	0	23.5 ng/mL		31	81			57	
	3	18.1 ng/mL		38	78			60	
	18	15.6 ng/mL		39	78			61	
ELISA	ICU	300 ng/mg		67	62	NA	NA	71	53
	ICU+6hrs	300 ng/mg		34	86	NA	NA	70	46
Immunoblot	3	213 ng/mL		69	65			74	
	18	213 ng/mL		73	78			80	
ELISA	0	25 ng/mL		91	95			98	
ELISA kit	24	9.85 ng/mL		77	70			73	
Immunoblot	0	130 µg/gCr		90	100			95	

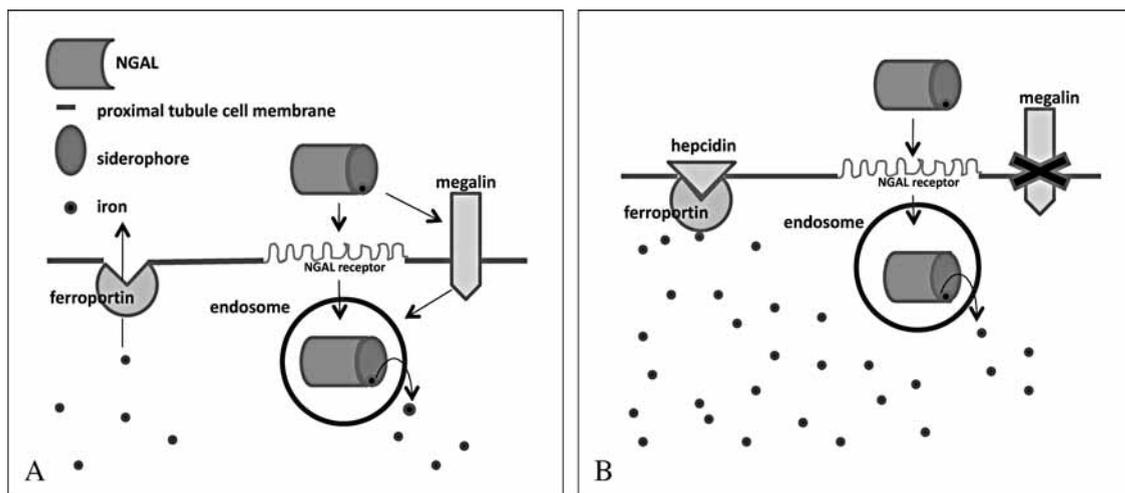


Figure 1.—A) Proposed model of NGAL and hepcidin-mediated iron trafficking in the proximal tubule. NGAL bound to siderophore and iron, delivers iron to cells via megalin and NGAL receptors. In the endosome, NGAL releases iron, which promotes expression of iron-dependent genes (*e.g.*, ferritin), stimulates epithelial proliferation and reduces renal damage. Iron moves out of cells *via* the ferroportin pathway.²⁸ Diagram modified from Schmidt-Ott *et al.* 2007.³⁰ B) Model representing potential mechanisms and pathways present in renal injury. Local and systemic NGAL expression increases. Tubular injury results in disruption to megalin receptor function, reduced NGAL-mediated intracellular iron uptake, and subsequent increase in urine and serum NGAL concentration. With inflammation, hepcidin is up-regulated and binds to ferroportin, which is internalized, endocytosed and degraded. Absence of the ferroportin pathway for iron removal promotes the accumulation of intracellular iron, which reduces extracellular free reactive iron, thereby, reducing oxidative stress. The association of higher hepcidin levels with those that do not develop AKI vs. those that develop AKI after cardiopulmonary bypass surgery is consistent with this proposed mechanism.³¹

TABLE III.—Performance of Cystatin C in diagnosis of AKI in adults: studies with AUC-ROC analysis.

Year	Authors	Setting	Age	SexF %	Existing renal disease excluded	% AKI	AKI Definition (↑ in SCr)	Timing (AKI)	Sample size
<i>Cystatin C</i>									
2009	Liangos <i>et al.</i> ³⁸	CSA	68	28	* †	13	≥50%	72 h	103
2009	Haase-Fielitz <i>et al.</i> ³⁶	CSA	70	39	N	23	≥50%	5 days	100
			70	40	Y	23	≥50%	5 days	73
2009	Haase <i>et al.</i> ³⁴	CSA	70	39	* †	46	≥50% or >26.5 μmol/L or §	48 h	100
2008	Koyner <i>et al.</i> ²⁴	CSA	65	29	Y	47	≥25% or RRT	72 h	72
2007	Ling <i>et al.</i> ⁴⁸	Tplant-liver	47	10	NA	NA	GFR<80 mL/min/1.73 m ²	1 wk	30
2004	Daniel <i>et al.</i> ⁴⁷	Tplant-ren'	40	35	NA	27	Inulin Cl <90 mL/min	NA	60
2005	Villa <i>et al.</i> ⁴⁹	Crit ill	54	32	Y	50	Cr Cl <80 mL/min/1.73 m ²	NA	50
2004	Ahlstrom <i>et al.</i> ⁴⁶	Crit ill	55	32	N	27	≥50%	ICU stay	202

CSA: cardiac surgery associated AKI; Tplant-liver/ren': post-liver or renal transplant; Crit ill: critically ill; NA: not available or not applicable; Inulin Cl: inulin clearance; Cr Cl: creatinine clearance; Y: yes; N:no. *: dialysis; †: transplant; ‡: SCr >300 μmol/L; §: urine output <0.5 mL/kg/h for >6 h. BNII automat and PENIA - Dade Behring. ELISA kit-CyC - Biovondor LLC.

TABLE IV.—Performance of biomarkers in diagnosis of AKI in children: studies with AUC-ROC analysis.

Year	Authors	Setting	Age	SexF %	Existing renal disease excluded	% AKI	AKI Definition (↑ in SCr)	Timing (AKI)	Sample size
<i>NGAL</i>									
2008	Portilla <i>et al.</i> ²⁷	CSA	3.5	48	Y	52	≥50%	5 days	40
2008	Bennett <i>et al.</i> ³²	CSA	4	48	Y	51	≥50%	3 days	196
2007	Dent <i>et al.</i> ³³	CSA	4	47	Y	37	≥50%	3 days	120
2005	Mishra <i>et al.</i> ⁵⁰	CSA	4	37	Y	28	≥50%	3 days	71
2008	Wheeler <i>et al.</i> ⁵³	SIRS/SS	3	35	Y	15	>176.82 μmol/L or *	7 days	143
2007	Hirsch <i>et al.</i> ⁵²	CIN	7	43	Y	12	≥50%	24 h	91
<i>Cystatin C</i>									
2007	Herrero-Morin <i>et al.</i> ⁵¹	Crit ill	2.9	44	Y	56	Cr Cl <80 mL/min/1.73m ²	ICU stay	25
<i>L-FABP</i>									
2008	Portilla <i>et al.</i> ²⁷	CSA	3.5	48	Y	50	≥50%	5 days	16

CSA: cardiac surgery associated AKI; CIN: contrast induced nephropathy; Crit ill: critically ill; SIRS/SS: systemic inflammatory response syndrome/Septic shock; NA: not available or not applicable; Cr Cl: creatinine clearance; Y: yes; N: no; Wblot: Western blot. *: urea >100 mg/dL. Triage® - Biosite, ARCHITECT® - Abbott.

in adults, albeit with somewhat diminished accuracy^{34, 36, 45} (Table II), with an AUC of 0.77-0.96^{34, 36, 39, 40} (Table II). Moreover, plasma NGAL was an independent predictor of AKI duration and severity,³⁴ of length of ICU stay,³⁴ and of the need for renal replacement therapy, and of hospital death^{35, 36} (Table V). The predictive value of NGAL post cardiac surgery was stronger with a strict definition of AKI (>50% *versus* >25% increase in

SCr) and increased with progressive severity of AKI (Table II).³⁵

Contrast-induced nephropathy (CIN)

In children with congenital heart disease, Hirsch *et al.*⁵² found that both serum and urine NGAL were excellent predictors for AKI at 2 hours after contrast (Table IV). In adults with normal SCr,⁵⁸ NGAL levels were significantly higher in urine at

Instrument	Time from procedure/admission (h)	Cutoff		Urine		Plasma/Serum		AUC-ROC	
		Urine	Plasma	Sensitivity %	Specificity %	Sensitivity %	Specificity %	Urine %	Plasma %
PENIA	2	192 ng/mg		42	86			50	
Nephelometry	ICU/6 h		>1.1 mg/L			77	86		83
	24		>1.2 mg/L			91	64		84
Nephelometry	ICU/6 h		>1.1 mg/L			75	89		78
	24		>1.2 mg/L			86	80		84
Nephelometry	ICU		>1.1 mg/L			74	67		76
ELISA kit-CyC	ICU	0.35 mg/g		58	72	NA	NA	69	62
	ICU+6 h	0.11 mg/g		45	84	NA	NA	72	63
NA	day 1,4,7		1.57 mg/L			85	85		94
BNII automat	~6.7 mths post		1.18 mg/L			72	80		NA
	~6.7 mths post		1.52 mg/L			60	87		NA
Nephelometry	NA		NA			NA	NA		93
BNII automat	24		NA			NA	NA		89

Instrument	Time from procedure/admission (h)	Cutoff		Urine		Plasma/Serum		AUC-ROC	
		Urine	Plasma	Sensitivity %	Specificity %	Sensitivity %	Specificity %	Urine %	Plasma %
ELISA	4	100 ng/mgCr		100	100			100	
ARCHITECT®	2	100 ng/mL		82	90			95	
Triage®	2		150 ng/mL			84	94		96
ELISA & Wblot	2	25 µg/L	25 µg/L	100	98	70	94	100	91
	2	50 µg/L		100	98			100	
ELISA	day 1		139 ng/mL			86	39		68
ELISA	2	100 ng/mL	100 ng/ml	73	100	73	98	92	91
	6	100 ng/mL	100 ng/ml	90	99	73	100	97	95
Nephelometry	1-6days		0.6 mg/L			85	63		85
ELISA & Wblot	4	486 ng/mgCr		71	68			81	

2 hours and in serum 4 hours post contrast in patients who developed CIN, whereas SCr was significantly higher at 48 hours. Consistent with this, Ling *et al.*⁴² found urine NGAL performed well in the early diagnosis of CIN (Table II).

Critically ill

Wheeler⁵³ found a significant difference in serum NGAL between children who were healthy,

critically ill with SIRS, and critically ill with septic shock (medians 80, 108 and 303 ng/mL, respectively). Furthermore, NGAL was significantly elevated in those with AKI compared to those without. In addition, Zappitelli *et al.*⁵⁵ found that there was a progressive increase in urine NGAL concentration with worsening pRIFLEmax and that urine NGAL was a good diagnostic marker for persistent AKI (Table VI). In critically ill adults,⁵⁴

TABLE V.—Performance of biomarkers in prognosis of outcomes: studies with AUC-ROC analysis.

Year	Authors	Setting	Age	SexF %	Existing renal disease excluded	% AKI	AKI Definition (↑ in SCr)	Timing (AKI)	Sample size
<i>NGAL</i>									
2009	Haase-Fielitz <i>et al.</i> ³⁶	CSA	70	39	*	23	>50%	5 days	100
2009	Siew <i>et al.</i> ⁵⁴	Crit ill	53	58	† ‡ §	14	≥50% or ¥	24 h	451
						22	≥50% or ¥	48 h	451
2007	Zappitelli <i>et al.</i> ⁵⁵	Crit ill	-6	46	§	74	≥50%	14 days	140
<i>Cystatin C</i>									
2004	Herget-Rosenthal <i>et al.</i> ⁵⁶	Risk for AKI	67	36	N	52	≥50%	NA	85
2004	Herget-Rosenthal <i>et al.</i> ⁵⁷	ATN	69	36	N	All	ATN	NA	73
2004	Ahlstrom <i>et al.</i> ⁴⁶	Crit ill	55	32	N	27	≥50%	24 h	202

CSA: cardiac surgery associated AKI; Crit ill: critically ill; ATN: acute tubular necrosis; Y: yes; N: no; BNII Automat - Dade Behring; NA: not applicable; R minus 1/2: 1 or 2 days before SCr R-criteria (RIFLE) was fulfilled; RRT: renal replacement therapy; AKI 24/48 h: sustained AKI within 24/48 h; AKI next 48 h: persistent AKI in the next 48 h; H death: in-hospital mortality. *: SCr >300 µmol/L; †: dialysis; ‡: transplant; §: endstage renal disease; ¥: >26.5 µmol/L.

the median urine NGAL at enrolment was significantly higher in those who developed AKI within 48 hours.

Kidney transplant

In a multicenter study, urine NGAL measured on the day of transplant predicted delayed graft function and dialysis (AUC 0.9).⁵⁹ In addition, Kusaka *et al.*⁶⁰ found that a decrease in NGAL predicted organ recovery before a decrease in SCr or recovery of urine output.

NGAL and chronic kidney disease (CKD)

NGAL in serum and urine appears to reflect the presence, severity^{61, 62} and progression⁶³ of CKD. NGAL seems to be a better predictor of GFR than SCr (and than CyC when GFR <30 mL/min^{62, 64}) in patients with CKD.⁶¹

Urine NGAL was also strongly predictive of AKI in children with diarrhea-associated hemolytic uremic syndrome,⁶⁵ in multitrauma⁴³ and in emergency department⁴⁴ patients (Table II).

A recent systematic review and meta-analysis of NGAL studies using standardized data sheets sent to authors, confirmed the value of NGAL as an early predictor of AKI across settings. Urine and plasma/serum NGAL performed similarly well, and the performance of NGAL improved with standardized laboratory platforms *versus* research-based assays (cutoff >150 ng/mL). NGAL

level had prognostic value for renal replacement therapy and mortality.⁶⁶

NGAL is now the most promising novel renal biomarker^{39, 50, 52} in urine and plasma. The cut-off values for NGAL range widely, with higher values used for adults *versus* children (effect of age and co-morbidities) and for cardiac surgical studies *versus* CIN (lower magnitude of injury in CIN). Therefore, it seems that each clinical setting would require the establishment of a “normal” range and cutoff value. As a general rule, however, a concentration >150 ng/mL can identify patients at high risk for AKI, and a level >350 ng/mL, those at high risk for renal replacement therapy.

If we compare the performance of NGAL in diagnosing AKI to widely used markers like troponin I, which has carried an AUC in the range of 0.7-0.8 for diagnosis of acute myocardial infarction,^{39, 67} its performance would seem sufficient for clinical adoption and inclusion into an early diagnostic panel for AKI. Clinical use of NGAL beyond confirmation of its utility is rapidly expanding.^{18, 68-70}

Cystatin C

CyC is a low molecular weight cysteine protease inhibitor (Table I, Figure 2). Its serum level is determined by glomerular filtration, in contrast to NGAL, which responds to stimuli and is a measure of tubular stress (Table I, Figure 2). Therefore,

Instrument	Time from procedure/admission (h)	Cutoff		Urine		Plasma/Serum		AUC-ROC		Outcome
		Urine	Plasma	Sensitivity %	Specificity %	Sensitivity %	Specificity %	Urine %	Plasma %	
Triage® Biosite ELISA kit	NA		340 ng/mL			75	100		83	RRT
ELISA kit	day 1	NA		NA	NA			70		AKI 24 hrs
ELISA kit	day 1	NA		NA	NA			66		AKI 48 hrs
ELISA	NA	0.2 ng/mg		78	67			79		AKI next 48hrs
Nephelometry	R minus 2					53	82		69	RRT
Nephelometry	R minus 1					76	93		75	RRT
Nephelometry BNII automat	NA	1 g/molCr		92	83			92		RRT
	NA					75	50		62	H death

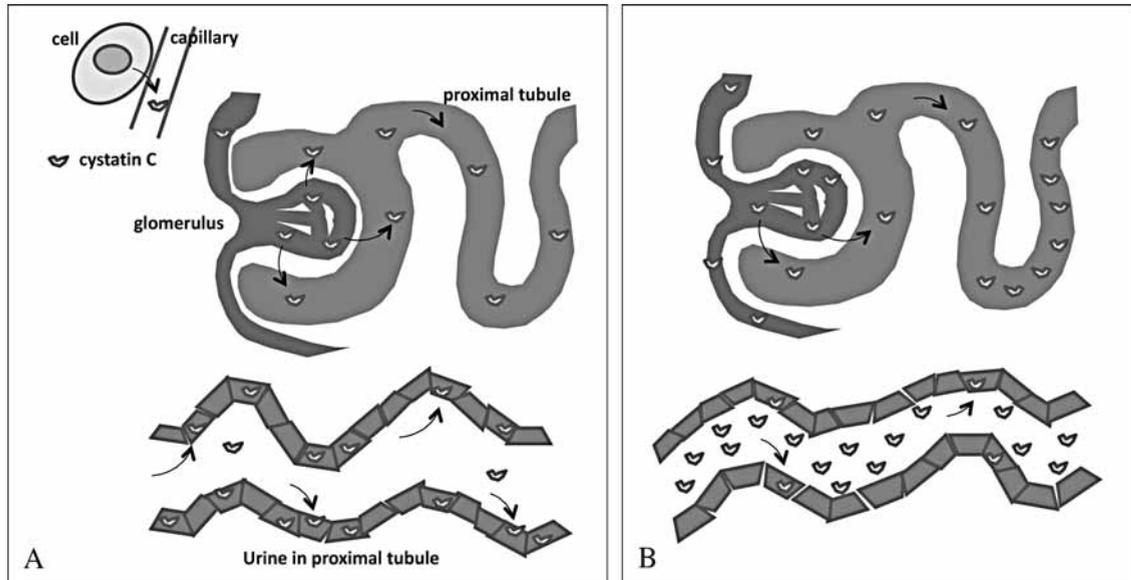


Figure 2.—A) Cystatin C is produced at a constant rate by nucleated cells. Under normal conditions, cystatin C is filtered by the glomerulus, reabsorbed by the proximal tubule and completely catabolized. Therefore, its concentration in urine is negligible, and in blood is low. B) With tubular injury, there is less filtration of cystatin C at the glomerulus due to proteinuria/ blockage/cell damage, therefore, the concentration of cystatin C in the blood increases. Less reabsorption of cystatin C by the proximal tubule due to injury also leads to increased concentration of cystatin C in the urine.

changes in serum and urine levels of CyC reflect changes in GFR. Given that its levels are not significantly affected by age, gender, race, muscle mass, infection, liver disease or inflammatory disease and that it is not secreted by the tubules, CyC is probably a better measure of glomerular function than SCr.^{49, 71}

Comparison with SCr

In a systematic review of 24 studies, Laterza *et al.*⁷² found CyC superior to SCr in detecting “impaired GFR” (0.95 *vs* 0.91, P=0.003). Apart from two unfavorable results,^{46, 73} the superiority of CyC over SCr as a diagnostic marker for AKI is supported by several studies of ICU patients.^{49, 51.}

^{56, 57, 74} Using the combined results of 11 datasets, Royakkers *et al.*⁷⁵ found the diagnostic accuracy for GFR determination of CyC superior to that of SCr: AUC 0.93 vs SCr 0.84, and CyC is now often used in this role.^{68, 76} The use of CyC as a biomarker for AKI has been investigated in several settings.

Cardiac surgery

In adults, despite an inconclusive result from Heise *et al.*,⁷⁷ other studies found that CyC predicted AKI early before SCr but that it was not superior to NGAL (Table II-III).^{24, 36} Notably, after excluding those with preoperative renal impairment, Haase-Fielitz *et al.* found that the predictive performance of CyC for AKI was reduced from 0.83 to AUC 0.78, whereas that of NGAL remained the same (Table II-III).³⁶ In this setting, the predictive value of CyC appears to be partly as a marker of chronic renal injury rather than acute injury,³⁴ and, as such, it may be useful as a complementary marker to NGAL. CyC displayed considerable prognostic value in Haase-Fielitz' study; in this study, serum CyC and plasma NGAL were independent predictors of AKI and excellent predictors of the need for renal replacement therapy and of hospital death.³⁶ In addition, Haase *et al.*³⁴ found that CyC was an independent predictor of severity and duration of AKI after adult cardiac surgery.

Post transplant

After kidney transplant, CyC predicted delayed graft function, but the prediction was relatively late^{78, 79} (at three days). In contrast, after liver transplants, Hei *et al.*⁸⁰ found that postoperative CyC predicted AKI earlier and more accurately than SCr (within 24 h) and that preoperative serum CyC also predicted postoperative AKI. Ling *et al.*⁴⁸ confirmed these findings (Table III).

Post contrast

Rickli *et al.*⁸¹ found that, after contrast application, serum CyC levels increased before SCr. Furthermore, Kimmel *et al.*⁸² found that serum CyC reflected contrast-induced changes better than SCr. Another study⁸³ found that urine CyC rose significantly at 8 hours ($P < 0.05$) and at 24 h

($P < 0.01$) after contrast, with no change in creatinine.

Thus, CyC is an earlier and more accurate marker of AKI than SCr, but it is generally preceded by NGAL in detecting AKI; elevated preoperative CyC before liver transplant predicts postoperative AKI; CyC is more sensitive to contrast-induced changes than SCr; and CyC is a better marker of chronic renal impairment and its effect on outcomes,^{34, 80} which accounts for part of its diagnostic value. As both rise sequentially, CyC could complement NGAL, which can lose diagnostic accuracy in the presence of co-morbidities. Given that automated, standardized immunonephelometric assays are commercially available and provide results in minutes, CyC represents a feasible and promising biomarker for AKI. Its inclusion in a sequential AKI diagnostic panel with NGAL appears logical.

L-FABP

L-FABP is expressed in various organs including liver and kidney.²⁶ Its function in the kidney is presumed to be the same as that in the liver: cellular uptake of fatty acids (FAs) from plasma and promotion of intracellular FA metabolism. Free FAs are easily oxidized, leading to oxidative stress that can induce cellular injury. Through its involvement in regulation of FA metabolism, L-FABP may inhibit the accumulation of intracellular FAs (Table I, Figure 3), thereby preventing oxidation of free FAs.⁸⁴ L-FABP may be an important cellular antioxidant during oxidative stress.

L-FABP can be filtered *via* glomeruli and reabsorbed in the proximal tubule cells due to its small size, which could partly explain the increase of L-FABP in proximal tubular cell injury. However, an experimental study²³ revealed that renal L-FABP expression was up-regulated and that urinary L-FABP excretion was accelerated by accumulation of free FAs. Renal L-FABP may help maintain low levels of free FAs in the cytoplasm by facilitating their intracellular metabolism and their excretion in urine (Table I, Figure 3).²⁶ This is consistent with the early and exponential rise in urine L-FABP compared with a later and more modest rise in serum L-FABP in contrast-induced nephropathy in mice⁸⁵ and post-cardiac surgery in

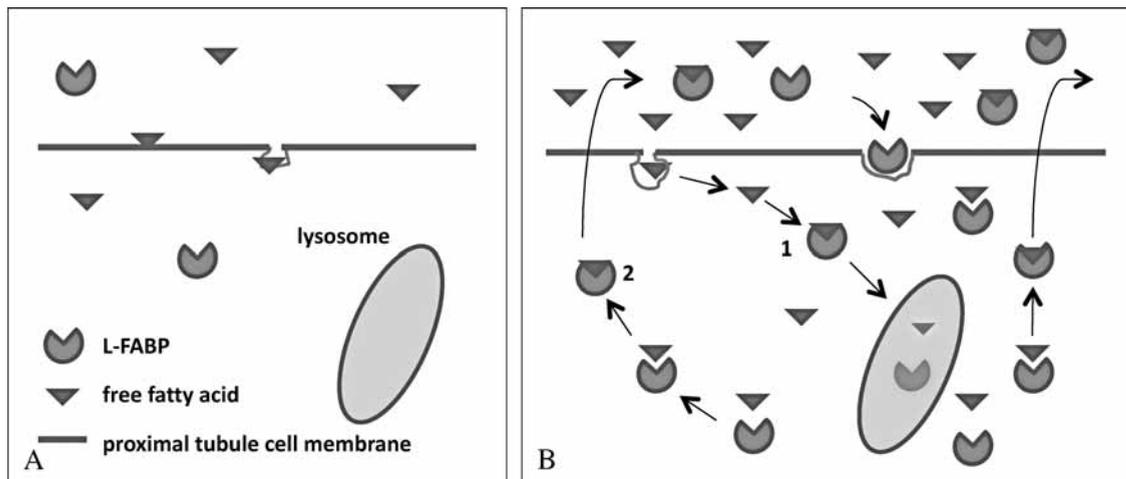


Figure 3.—A) Under normal conditions, the urinary L-FABP concentration is low. B) Proposed model of renal L-FABP up-regulation in the presence of fatty acid accumulation in proximal tubule cells after injury. Renal L-FABP and reabsorbed circulating L-FABP, filtered by the glomerulus, maintain low levels of Free Fatty Acids (FFAs) in the cytoplasm by 1) binding FFAs and transporting FFAs to lysosomes to accelerate FA metabolism and by 2) binding FFAs and removing FFAs from the cell for elimination *via* urine.

children.²⁷ ELISA techniques have been used to measure L-FABP levels; however, a urine dipstick kit has been developed and requires evaluation.²⁶

In an experimental study, urine L-FABP showed great potential for early and accurate detection of histological and functional decline in both nephrotoxin-induced and ischemia-reperfusion injury in mice.⁸⁶ Dose-response to injury was well-reflected in L-FABP levels in this study; severity of histological injury increased with ischemia time and cisplatin dose and correlated well with L-FABP levels. Urine L-FABP increased after 1 h, even in mice subjected to only 5 min of ischemia.⁸⁶

In a clinical study,²⁵ 13 of 66 patients had significantly elevated urine L-FABP before non-emergency angiogram; later, all 13 showed contrast-induced nephropathy, whereas no patient with low urine L-FABP showed signs of nephropathy. Furthermore, pre-contrast SCr showed no difference between the AKI/no AKI groups. Urine L-FABP appears to be a more sensitive predictor of AKI than SCr and could serve as a clinical predictor of contrast-induced nephropathy. In a further study, higher urine L-FABP levels differentiated patients with septic shock from those with severe sepsis, from those with AKI, and from healthy controls.⁸⁷ Of the septic shock patients; urine L-FABP levels in survivors were reduced by treatment. Non-survivors had higher urine levels with

a smaller reduction after treatment compared with survivors. Thus, L-FABP may be useful in treatment evaluation. Urine L-FABP can predict AKI in pediatric cardiopulmonary bypass surgery²⁷ with an AUC at 4 h post-surgery of 0.81 (Table IV).

Urine L-FABP shows promise as an early, accurate biomarker of AKI; however, it appears to rise later than NGAL (pediatric bypass surgery, 4 h L-FABP *vs* 2 h NGAL). The predictive ability of L-FABP for AKI requires further clinical confirmation in different patient populations.

Interleukin-18 (IL-18)

IL-18 is a proinflammatory cytokine and a powerful mediator of ischemia-induced AKI in animal models. It is induced and cleaved in the proximal tubule and is detected in urine following experimental AKI.^{88, 89} In a cross-sectional study, IL-18 levels were significantly greater in patients with established AKI but not in those with urinary tract infections, pre-renal azotemia or CKD.⁹⁰ The AUC for the diagnosis of established AKI (acute tubular necrosis) was 0.95. Consistent with this finding, the AUC of IL-18 as an early predictor of AKI in patients after kidney transplant, in patients after pediatric cardiac surgery, and in those with Acute Respiratory Distress Syndrome^{59, 91, 92} showed good performance (0.70-0.9), with the

strongest predictive value post transplant. However, in critically ill children, IL-18 performed weakly.⁹³ Haase *et al.* concluded that IL-18 may be a non-specific marker of inflammation but that it did not predict AKI post cardiac surgery.⁹⁴ In general, IL-18 has displayed low sensitivity and high specificity. There have been weak positive results for the prognostic ability of urine IL-18: at 4 hours after cardiac surgery, IL-18 weakly correlated with number of days with AKI,⁹² and in non-septic critically ill children, IL-18 predicted severity of AKI and mortality.⁹³ Furthermore, IL-18 predicts mortality in critically ill adults.⁹¹ IL-18 is specific to ischemic AKI but may also be a non-specific marker of inflammation and has shown inconsistent results. Its inclusion in urinary panels requires further evaluation.

Kidney injury molecule-1 (KIM-1)

KIM-1 is a transmembrane glycoprotein that is not expressed in normal kidneys but that is up-regulated in proximal tubular cells after ischemic or nephrotoxic injury. The ectodomain segment of KIM-1 is shed and is detected in urine.^{95, 96} In a cross-sectional study,⁹⁷ KIM-1 was markedly induced in proximal tubules in biopsies from patients with established AKI (largely ischemic), and it differentiated ischemic AKI from pre-renal azotemia and CKD. In another cross-sectional study⁹⁸ of hospitalized patients, the AUC for KIM-1 for differentiating those with AKI from controls was 0.9. A further case-control study⁹⁸ found an AUC of 0.83 for KIM-1 for the diagnosis of AKI at 12 hours post-cardiopulmonary bypass. In a recent prospective study of 90 adults undergoing cardiac surgery, urinary KIM-1, N-acetyl- β -D-glucosaminidase (NAG - a lysosomal glucosidase abundant in tubular cells which is excreted in the urine when proximal tubule cells are damaged), and NGAL were measured.³⁷ The AUC for KIM-1 to predict AKI immediately post-surgery (0.68), although low, was higher than those for NAG and NGAL. Combining the three biomarkers enhanced the sensitivity of early detection of postoperative AKI, and AUCs became 0.75 and 0.78. Furthermore, in a study that examined the relationship between KIM-1 and a composite end-point (dialysis or death) in hospitalized

patients, there was a suggestion that elevated urinary KIM-1 levels are associated with adverse outcomes in hospitalized patients who develop AKI.⁹⁹ The strength of KIM-1 appears to be detection of existing AKI. Its inclusion in a urinary AKI panel requires further investigation.

Conclusions

Given the heterogeneity of AKI and the settings in which it occurs, it is likely that diagnosis and classification of AKI will not be possible using one biomarker alone, and a panel of biomarkers comparable to the panel of cardiac enzymes used to diagnose and assess severity of acute myocardial infarction will be required. Current key renal biomarkers are NGAL and CyC, which 1) show great promise and utility, 2) have commercially available assays to provide immediate results, and 3) assess complementary aspects of renal injury (NGAL - tubular stress; CyC - GFR). In terms of sequence post-cardiac surgery, NGAL and L-FABP have higher predictive accuracy for AKI in urine and/or plasma early, at 2-4 h, which later wanes; in contrast, the predictive accuracy of CyC, IL-18 and KIM-1 increases at 12-24 h.¹⁰⁰ Such information can be used to include appropriate biomarkers in sequential predictive panels, which would open the door to a whole new area of research and, perhaps, interventions.

Novel renal biomarkers can be used to evaluate the effect of new techniques and therapies on kidney function and to provide safety markers for monitoring toxicity and AKI associated with established treatments.^{18, 69, 101} To this end, NGAL, L-FABP and CyC have superior sensitivity and detect AKI earlier than SCr, enhancing the ability to demonstrate benefits and to justify the implementation of therapies or kidney protective techniques in evaluation studies.

Optimum perioperative hemodynamic management and measures such as preoperative hydration for high risk patients could be more effectively explored. In patients undergoing liver transplantation or major surgery, in which AKI is common,^{48, 102} early detection of AKI with novel biomarkers has great potential. Novel protective therapies or those that have previously been difficult to evaluate or administer in time to prevent or

ameliorate AKI (*e.g.*, N-acetyl cysteine, bicarbonate or fenoldopam) could be more appropriately assessed or administered in a targeted manner or, in patients receiving nephrotoxins (such as calcineurin inhibitors or aminoglycosides), renal injury could be detected much earlier and drug therapy adjusted. The potential benefit and mechanisms of volatile anesthetics for kidney protection¹⁰³⁻¹⁰⁶ could also be investigated using novel biomarkers. The above information is highly relevant to the anesthesiologist; if early point-of-care biomarker measurements (*e.g.*, NGAL) were performed during or immediately after major surgery, this would allow timely implementation and evaluation of potential protective therapies, either intra-operatively or on ICU admission, targeted to those at high risk of AKI. Indeed, the consideration of pre- to postoperative changes in NGAL concentration could also aid medical decision-making, which might lead to improved outcomes. Furthermore, if NGAL reflected the likelihood for timely discharge, it may be useful in surgical case and ICU bed planning.

References

- Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P, and the ADQI workgroup. 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-R12.
- Bagshaw SM, George C, Dinu I, Bellomo R. A multi-centre evaluation of the RIFLE criteria for early acute kidney injury in critically ill patients. *Nephrol Dial Transplant* 2008;23:1203-10.
- Ostermann M, Chang RWS. Acute kidney injury in the intensive care unit according to RIFLE. *Crit Care Med* 2007;35:1837-43.
- Bagshaw SM, George C, Bellomo R, Committe ADM, Bagshaw SM, George C *et al*. A comparison of the RIFLE and AKIN criteria for acute kidney injury in critically ill patients. *Nephrol Dial Transplant* 2008;23:1569-74.
- Lopes JA, Fernandes P, Jorge S, Goncalves S, Alvarez A, Costa e Silva Z *et al*. Acute kidney injury in intensive care unit patients: a comparison between the RIFLE and the Acute Kidney Injury Network classifications. *Crit Care* 2008;12:R110.
- Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: A systematic review. *Kidney Int* 2008;73:538-46.
- Joannidis M, Metnitz B, Bauer P, Schusterschitz N, Moreno R, Druml W *et al*. Acute kidney injury in critically ill patients classified by AKIN *versus* RIFLE using the SAPS 3 database. *Intensive Care Med* 2009;35:1692-702.
- 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;16:3365-70.
- Moore EM, Simpson JA, Tobin AE, Santamaria JD. Preoperative eGFR and RIFLE-classified postoperative AKI predict length of stay post coronary bypass surgery in an Australian setting. *Anaesth Intensive Care* 2010 [In press].
- Landoni G, Biondi-Zoccai GG, Tumlin JA, Bove T, De Luca M, Calabro MG *et al*. Beneficial impact of fenoldopam in critically ill patients with or at risk for acute renal failure: a meta-analysis of randomized clinical trials. *Am J Kidney Dis* 2007;49:56-68.
- Allaqaband S, Tumuluri R, Malik AM, Gupta A, Volkert P, Shalev Y *et al*. Prospective randomized study of N-acetylcysteine, fenoldopam, and saline for prevention of radiocontrast-induced nephropathy. *Catheter Cardiovasc Interv* 2002;57:279-83.
- Stone GW, McCullough PA, Tumlin JA, Lepor NE, Madyoon H, Murray P *et al*. Fenoldopam mesylate for the prevention of contrast-induced nephropathy: a randomized controlled trial. *JAMA* 2003;290:2284-91.
- Nigwekar SU, Hix JK, Nigwekar SU, Hix JK. The role of natriuretic peptide administration in cardiovascular surgery-associated renal dysfunction: a systematic review and meta-analysis of randomized controlled trials. *J Cardiothorac Vasc Anesth* 2009;23:151-60.
- Nigwekar SU, Navaneethan SD, Parikh CR, Hix JK, Nigwekar SU, Navaneethan SD *et al*. Atrial natriuretic peptide for management of acute kidney injury: a systematic review and meta-analysis. *Clin J Am Soc Nephrol* 2009;4:261-72.
- Gonzales DA, Norsworthy KJ, Kern SJ, Banks S, Steving PC, Star RA *et al*. A meta-analysis of N-acetylcysteine in contrast-induced nephrotoxicity: unsupervised clustering to resolve heterogeneity. *BMC Med* 2007;5:32.
- Nigwekar SU, Kandula P, Nigwekar SU, Kandula P. N-acetylcysteine in cardiovascular-surgery-associated renal failure: a meta-analysis. *Ann Thorac Surg* 2009;87:139-47.
- Tepel M, van der Giet M, Schwarzfeld C, Laufer U, Liermann D, Zidek W. Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine. *N Engl J Med* 2000;343:180-4.
- Haase M, Haase-Fielitz A, Bellomo R, Devarajan P, Story D, Matalanis G *et al*. Sodium bicarbonate to prevent increases in serum creatinine after cardiac surgery: a pilot double-blind, randomized controlled trial. *Crit Care Med* 2009;37:39-47.
- Brienza N, Giglio MT, Marucci M, Fiore T, Brienza N, Giglio MT *et al*. Does perioperative hemodynamic optimization protect renal function in surgical patients? A meta-analytic study. *Crit Care Med* 2009;37:2079-90.
- Joannidis M. Medical therapy of acute kidney injury. *Acta Clinica Belgica - Supplementum* 2007;(2):353-6.
- Mahon P, Shorten G. Perioperative acute renal failure. *Curr Opin Anaesthesiol* 2006;19:332-8.
- Bagshaw SM, Gibney RTN. Conventional markers of kidney function. *Crit Care Med* 2008;36(4 Suppl):S152-8.
- Kamijo A, Sugaya T, Hikawa A, Okada M, Okumura F, Yamanouchi M *et al*. Urinary excretion of fatty acid-binding protein reflects stress overload on the proximal tubules. *Am J Pathol* 2004;165:1243-55.
- Koynert JL, Bennett MR, Worcester EM, Ma Q, Raman J, Jeevanandam V *et al*. Urinary cystatin C as an early biomarker of acute kidney injury following adult cardiothoracic surgery. *Kidney Int* 2008;74:1059-69.
- Nakamura T, Sugaya T, Node K, Ueda Y, Koide H. Urinary excretion of liver-type fatty acid-binding protein in contrast medium-induced nephropathy. *Am J Kidney Dis* 2006;47:439-44.
- Noiri E, Doi K, Negishi K, Tanaka T, Hamasaki Y, Fujita T *et al*. Urinary fatty acid-binding protein 1: an early predictive biomarker of kidney injury. *Am J Physiol Renal Physiol* 2009;296:F669-79.
- Portilla D, Dent C, Sugaya T, Nagothu KK, Kundi I, Moore P *et al*. Liver fatty acid-binding protein as a biomarker of acute kidney injury after cardiac surgery. *Kidney Int* 2008;73:465-72.

28. Schmidt-Ott KM, Mori K, Kalandadze A, Li J-Y, Paragas N, Nicholas T *et al*. Neutrophil gelatinase-associated lipocalin-mediated iron traffic in kidney epithelia. *Curr Opin Nephrol Hypertens* 2006;15:442-9.
29. Kell DB. Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. *BMC Medical Genomics* 2009;2(2).
30. Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Devarajan P *et al*. Dual action of neutrophil gelatinase-associated lipocalin. *J Am Soc Nephrol* 2007;18:407-13.
31. Ho J, Lucy M, Krokhn O, Hayglass K, Pascoe E, Darroch G *et al*. Mass spectrometry-based proteomic analysis of urine in acute kidney injury following cardiopulmonary bypass: a nested case-control study. *Am J Kidney Dis* 2009;53:584-95.
32. Bennett M, Dent CL, Ma Q, Dastrala S, Grenier F, Workman R *et al*. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. *Clin J Am Soc Nephrol* 2008;3:665-73.
33. Dent CL, Ma Q, Dastrala S, Bennett M, Mitsnefes MM, Barasch J *et al*. Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: a prospective uncontrolled cohort study. *Crit Care* 2007;11:R127.
34. Haase M, Bellomo R, Devarajan P, Ma Q, Bennett MR, Mockel M *et al*. Novel biomarkers early predict the severity of acute kidney injury after cardiac surgery in adults. *Ann Thorac Surg* 2009;88:124-30.
35. Haase-Fielitz A, Bellomo R, Devarajan P, Bennett M, Story D, Matalanis G *et al*. The predictive performance of plasma neutrophil gelatinase-associated lipocalin (NGAL) increases with grade of acute kidney injury. *Nephrol Dial Transplant* 2009;24:3349-54.
36. Haase-Fielitz A, Bellomo R, Devarajan P, Story D, Matalanis G, Dragun D *et al*. Novel and conventional serum biomarkers predicting acute kidney injury in adult cardiac surgery—a prospective cohort study. *Crit Care Med* 2009;37:553-60.
37. Han WK, Wagener G, Zhu Y, Wang S, Lee HT. Urinary biomarkers in the early detection of acute kidney injury after cardiac surgery. *Clin J Am Soc Nephrol* 2009;4:873-82.
38. Liangos O, Tighiouart H, Perianayagam MC, Kolyada A, Han WK, Wald R *et al*. Comparative analysis of urinary biomarkers for early detection of acute kidney injury following cardiopulmonary bypass. *Biomarkers* 2009;14:423-31.
39. Tuladhar SM, Puntmann VO, Soni M, Punjabi PP, Bogle RG. Rapid detection of acute kidney injury by plasma and urinary neutrophil gelatinase-associated lipocalin after cardiopulmonary bypass. *J Cardiovasc Pharmacol* 2009;53:261-6.
40. Xin C, Yulong X, Yu C, Changchun C, Feng Z, Xinwei M. Urine neutrophil gelatinase-associated lipocalin and interleukin-18 predict acute kidney injury after cardiac surgery. *Ren Fail* 2008;30:904-13.
41. Wagener G, Jan M, Kim M, Mori K, Barasch JM, Sladen RN *et al*. Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery. *Anesthesiology* 2006;105:485-91.
42. Ling W, Zhaohui N, Ben H, Leyi G, Jianping L, Huili D *et al*. Urinary IL-18 and NGAL as early predictive biomarkers in contrast-induced nephropathy after coronary angiography. *Nephron* 2008;108:c176-81.
43. Makris K, Markou N, Evodia E, Dimopoulou E, Drakopoulos I, Ntetsika K *et al*. Urinary neutrophil gelatinase-associated lipocalin (NGAL) as an early marker of acute kidney injury in critically ill multiple trauma patients. *Clin Chem Lab Med* 2009;47:79-82.
44. Nickolas TL, O'Rourke MJ, Yang J, Sise ME, Canetta PA, Barasch N *et al*. Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury. *Ann Intern Med* 2008;148:810-9.
45. Wagener G, Gubitosa G, Wang S, Borregaard N, Kim M, Lee HT. Urinary neutrophil gelatinase-associated lipocalin and acute kidney injury after cardiac surgery. *Am J Kidney Dis* 2008;52:425-33.
46. Ahlstrom A, Tallgren M, Peltonen S, Pettila V. Evolution and predictive power of serum cystatin C in acute renal failure. *Clin Nephrol* 2004;62:344-50.
47. Daniel J-P, Chantrel F, Offner M, Moulin B, Hannedouche T. Comparison of cystatin C, creatinine and creatinine clearance vs. GFR for detection of renal failure in renal transplant patients. *Ren Fail* 2004;26:253-7.
48. Ling Q, Xu X, Li JJ, Chen J, Shen JW, Zheng SS. Alternative definition of acute kidney injury following liver transplantation: based on serum creatinine and cystatin C levels. *Transplant Proc* 2007;39:3257-60.
49. Villa P, Jimenez M, Soriano M-C, Manzanares J, Casanovas P. Serum cystatin C concentration as a marker of acute renal dysfunction in critically ill patients. *Crit Care* 2005;9:R139-43.
50. Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C *et al*. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. *Lancet* 2005;365:1231-8.
51. Herrero-Morin JD, Malaga S, Fernandez N, Rey C, Dieguez MA, Solis G *et al*. Cystatin C and beta2-microglobulin: markers of glomerular filtration in critically ill children. *Crit Care* 2007;11:R59.
52. Hirsch R, Dent C, Pfrim H, Allen J, Beekman RH 3rd, Ma Q *et al*. NGAL is an early predictive biomarker of contrast-induced nephropathy in children. *Pediatr Nephrol* 2007;22:2089-95.
53. Wheeler DS, Devarajan P, Ma Q, Harmon K, Monaco M, Cvijanovich N *et al*. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. *Crit Care Med* 2008;36:1297-303.
54. Siew ED, Ware LB, Gebretsadik T, Shintani A, Moons KGM, Wickersham N *et al*. Urine neutrophil gelatinase-associated lipocalin moderately predicts acute kidney injury in critically ill adults. *J Am Soc Nephrol* 2009;20:1823-32.
55. Zappitelli M, Washburn KK, Arikan AA, Loftis L, Ma Q, Devarajan P *et al*. Urine neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in critically ill children: a prospective cohort study. *Critical Care* 2007;11:R84.
56. Herget-Rosenthal S, Marggraf G, Husing J, Goring F, Pietruck F, Janssen O *et al*. Early detection of acute renal failure by serum cystatin C. *Kidney Int* 2004;66:1115-22.
57. Herget-Rosenthal S, Poppen D, Husing J, Marggraf G, Pietruck F, Jakob H-G *et al*. Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis. *Clin Chem* 2004;50:552-8.
58. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, Malyszko JS, Dobrzycki S. Neutrophil gelatinase-associated lipocalin (NGAL) correlations with cystatin C, serum creatinine and eGFR in patients with normal serum creatinine undergoing coronary angiography. *Nephrol Dial Transplant* 2007;22:295-6.
59. Parikh CR, Jani A, Mishra J, Ma Q, Kelly C, Barasch J *et al*. Urine NGAL and IL-18 are predictive biomarkers for delayed graft function following kidney transplantation. *Am J Transplant* 2006;6:1639-45.
60. Kusaka M, Kuroyanagi Y, Mori T, Nagaoka K, Sasaki H, Maruyama T *et al*. Serum neutrophil gelatinase-associated lipocalin as a predictor of organ recovery from delayed graft function after kidney transplantation from donors after cardiac death. *Cell Transplant* 2008;17:129-34.
61. Bolignano D, Lacquaniti A, Coppolino G, Campo S, Arena A, Buemi M. Neutrophil gelatinase-associated lipocalin reflects the severity of renal impairment in subjects affected by chronic kidney disease. *Kidney Blood Press Res* 2008;31:255-8.

62. Mitsnefes MM, Kathman TS, Mishra J, Kartal J, Khoury PR, Nickolas TL *et al*. Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in children with chronic kidney disease. *Pediatr Nephrol* 2007;22:101-8.
63. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, Fazio MR *et al*. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. *Clin J Am Soc Nephrol* 2009;4:337-44.
64. Goldstein SL, Devarajan P. Progression from acute kidney injury to chronic kidney disease: a pediatric perspective. *Adv Chronic Kidney Dis* 2008;15:278-83.
65. Trachtman H, Christen E, Cnaan A, Patrick J, Mai V, Mishra J *et al*. Urinary neutrophil gelatinase-associated lipocalin in D+HUS: a novel marker of renal injury. *Pediatr Nephrol* 2006;21:989-94.
66. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A, Ngal Meta-analysis Investigator Group. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis* 2009;54:1012-24.
67. Ilva T, Eriksson S, Lund J, Porela P, Mustonen H, Pettersson K *et al*. Improved early risk stratification and diagnosis of myocardial infarction, using a novel troponin I assay concept. *Eur J Clin Invest* 2005;35:112-6.
68. Boldt J, Brosch C, Ducke M, Papsdorf M, Lehmann A. Influence of volume therapy with a modern hydroxyethylstarch preparation on kidney function in cardiac surgery patients with compromised renal function: a comparison with human albumin. *Crit Care Med* 2007;35:2740-6.
69. Capuano F, Goracci M, Luciani R, Gentile G, Roscitano A, Benedetto U *et al*. Neutrophil gelatinase-associated lipocalin levels after use of mini-cardiopulmonary bypass system. *Interact Cardiovasc Thorac Surg* 2009;9:797-801.
70. Wagener G, Gubitosa G, Wang S, Borregaard N, Kim M, Lee HT. A comparison of urinary neutrophil gelatinase-associated lipocalin in patients undergoing on- versus off-pump coronary artery bypass graft surgery. *J Cardiothorac Vasc Anesth* 2009;23:195-9.
71. Dharnidharka VR, Kwon C, Stevens G, 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;40:221-6.
72. Laterza OF, Price CP, Scott MG, Laterza OF, Price CP, Scott MG. Cystatin C: an improved estimator of glomerular filtration rate? *Clin Chem* 2002;48:699-707.
73. Mazul-Sunko B, Zarkovic N, Vrkic N, Antoljak N, Bekavac Beslin M, Nikolic Heitzler V *et al*. Proatrial natriuretic peptide (1-98), but not cystatin C, is predictive for occurrence of acute renal insufficiency in critically ill septic patients. *Nephron* 2004;97:c103-7.
74. Delanaye P, Lambermont B, Chapelle JP, Gielen J, Gerard P, Rorive G *et al*. Plasma cystatin C for the estimation of glomerular filtration rate in intensive care units. *Intensive Care Med* 2004;30:980-3.
75. Royackers AANM, van Suijlen JDE, Hofstra LS, Kuiper MA, Bouman CSC, Spronk PE *et al*. Serum cystatin C-A useful endogenous marker of renal function in intensive care unit patients at risk for or with acute renal failure? *Curr Med Chem* 2007;14:2314-7.
76. Kaartinen K, Syrjanen J, Porsti I, Hurme M, Harmoinen A, Pasternack A *et al*. Inflammatory markers and the progression of IgA glomerulonephritis. *Nephrol Dial Transplant* 2008;23:1285-90.
77. Heise D, Waeschle RM, Schlobohm J, Wessels J, Quintel M. Utility of cystatin C for assessment of renal function after cardiac surgery. *Nephron* 2009;112:c107-14.
78. Lebkowska U, Malyszko J, Lebkowska A, Koc-Zorawska E, Lebkowski W, Malyszko JS *et al*. Neutrophil gelatinase-associated lipocalin and cystatin C could predict renal outcome in patients undergoing kidney allograft transplantation: a prospective study. *Transplant Proc* 2009;41:154-7.
79. Poge U, Gerhardt T, Bokenkamp A, Stoffel-Wagner B, Klehr HU, Sauerbruch T *et al*. Time course of low molecular weight proteins in the early kidney transplantation period—influence of corticosteroids. *Nephrol Dial Transplant* 2004;19:2858-63.
80. Hei Z-q, Li X-y, Shen N, Pang H-y, Zhou S-l, Guan J-q. Prognostic values of serum cystatin C and beta2 microglobulin, urinary beta2 microglobulin and N-acetyl-beta-D-glucosaminidase in early acute renal failure after liver transplantation. *Chin Med J (Engl)* 2008;121:1251-6.
81. Rickli H, Benou K, Ammann P, Fehr T, Brunner-La Rocca HP, Petridis H *et al*. Time course of serial cystatin C levels in comparison with serum creatinine after application of radiocontrast media. *Clin Nephrol* 2004;61:98-102.
82. Kimmel M, Butscheid M, Brenner S, Kuhlmann U, Klotz U, Alscher DM *et al*. Improved estimation of glomerular filtration rate by serum cystatin C in preventing contrast induced nephropathy by N-acetylcysteine or zinc — preliminary results. *Nephrol Dial Transplant* 2008;23:1241-5.
83. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, Malyszko JS, Pawlak K, Mysliwiec M *et al*. Could neutrophil-gelatinase-associated lipocalin and cystatin C predict the development of contrast-induced nephropathy after percutaneous coronary interventions in patients with stable angina and normal serum creatinine values? *Kidney Blood Press Res* 2007;30:408-15.
84. Kamiyo-Ikemori A, Sugaya T, Kimura K. Urinary fatty acid binding protein in renal disease. *Clin Chim Acta* 2006;374:1-7.
85. Negishi K, Noiri E, Maeda R, Portilla D, Sugaya T, Fujita T. Renal L-type fatty acid-binding protein mediates the bezafibrate reduction of cisplatin-induced acute kidney injury. *Kidney Int* 2008;73:1374-84.
86. Negishi K, Noiri E, Doi K, Maeda-Mamiya R, Sugaya T, Portilla D *et al*. Monitoring of urinary L-type fatty acid-binding protein predicts histological severity of acute kidney injury. *Am J Pathol* 2009;174:1154-9.
87. Nakamura T, Sugaya T, Koide H. Urinary liver-type fatty acid-binding protein in septic shock: effect of polymyxin B-immobilized fiber hemoperfusion. *Shock* 2009;31:454-9.
88. Melnikov VY, Ecder T, Fantuzzi G, Siegmund B, Lucia MS, Dinarello CA *et al*. Impaired IL-18 processing protects caspase-1-deficient mice from ischemic acute renal failure. *J Clin Invest* 2001;107:1145-52.
89. Melnikov VY, Faubel S, Siegmund B, Lucia MS, Ljubanovic D, Edelstein CL *et al*. Neutrophil-independent mechanisms of caspase-1- and IL-18-mediated ischemic acute tubular necrosis in mice. *J Clin Invest* 2002;110:1083-91.
90. Parikh CR, Jani A, Melnikov VY, Faubel S, Edelstein CL, Parikh CR *et al*. Urinary interleukin-18 is a marker of human acute tubular necrosis. *Am J Kidney Dis* 2004;43:405-14.
91. Parikh CR, Abraham E, Ancukiewicz M, Edelstein CL, Parikh CR, Abraham E *et al*. 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;16:3046-52.
92. Parikh CR, Mishra J, Thiessen-Philbrook H, Dursun B, Ma Q, Kelly C *et al*. Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery. *Kidney Int* 2006;70:199-203.
93. Washburn KK, Zappitelli M, Arikian AA, Loftis L, Yalavarthy R, Parikh CR *et al*. Urinary interleukin-18 is an acute kidney injury biomarker in critically ill children. *Nephrol Dial Transplant* 2008;23:566-72.
94. Haase M, Bellomo R, Story D, Davenport P, Haase-Fielitz A. Urinary interleukin-18 does not predict acute kidney injury after adult cardiac surgery: a prospective observational cohort study. *Crit Care* 2008;12:R96.
95. Bagshaw SM, Langenberg C, Haase M, Wan L, May CN, Bellomo R *et al*. Urinary biomarkers in septic acute kidney injury. *Intensive Care Med* 2007;33:1285-96.

96. Zhang Z, Humphreys BD, Bonventre JV, Zhang Z, Humphreys BD, Bonventre JV. Shedding of the urinary biomarker kidney injury molecule-1 (KIM-1) is regulated by MAP kinases and juxtamembrane region. *J Am Soc Nephrol* 2007;18:2704-14.
97. Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV, Han WK *et al*. Kidney Injury Molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney Int* 2002;62:237-44.
98. Han WK, Waikar SS, Johnson A, Betensky RA, Dent CL, Devarajan P *et al*. Urinary biomarkers in the early diagnosis of acute kidney injury. *Kidney Int* 2008;73:863-9.
99. Liangos O, Perianayagam MC, Vaidya VS, Han WK, Wald R, Tighiouart H *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;18:904-12.
100. Thurman JM, Parikh CR. Peeking into the black box: new biomarkers for acute kidney injury. *Kidney Int* 2008;73:379-81.
101. Wagener G, Gubitosa G, Wang S, Borregaard N, Kim M, Lee HT. Increased incidence of acute kidney injury with aprotinin use during cardiac surgery detected with urinary NGAL. *Am J Nephrol* 2008;28:576-82.
102. Biancifiore G, Davis CL, Biancifiore G, Davis CL. Renal dysfunction in the perioperative liver transplant period. *Curr Opin Organ Transplant* 2008;13:291-7.
103. De Hert SG, Turani F, Mathur S, Stowe DF, De Hert SG, Turani F *et al*. Cardioprotection with volatile anesthetics: mechanisms and clinical implications. *Anesth Analg* 2005;100:1584-93.
104. Julier K, da Silva R, Garcia C, Bestmann L, Frascarolo P, Zollinger A *et al*. Preconditioning by sevoflurane decreases biochemical markers for myocardial and renal dysfunction in coronary artery bypass graft surgery: a double-blinded, placebo-controlled, multicenter study. *Anesthesiology* 2003;98:1315-27.
105. Landoni G, Turi S, Bignami E, Zangrillo A. Organ protection by volatile anesthetics in non-coronary artery bypass grafting surgery. *Future Cardiol* 2009;5:589-603.
106. Pagel PS, Pagel PS. Cardioprotection by volatile anesthetics: established scientific principle or lingering clinical uncertainty? *J Cardiothorac Vasc Anesth* 2009;23:589-93.

Conflicts of interest.—Elizabeth Moore: None; Rinaldo Bellomo has acted as paid consultant to Abbott Diagnostics and Biosite, Inverness. Both companies are involved in the development of NGAL assays to be applied in clinical practice; Alistair Nichol: None.

Received on November 5, 2009 - Accepted on March 1, 2010.

Corresponding author: R. Bellomo, Director of Intensive Care Research, Austin Hospital, Studley Road, Heidelberg, Victoria 3084, Australia. E-mail: rinaldo.bellomo@austin.org.au