Biomarkers in cardiorenal syndromes

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Cardiac and renal diseases often coexist and patients with cardiac and renal failure have high morbidity and mortality. Cardiorenal syndromes (CRSs) are disorders of the heart and kidneys whereby dysfunction in one organ may induce dysfunction in the other organ. Five subtypes of CRSs have been defined by the Acute Dialysis Quality Initiative Consensus Group. There is a need for early detection and monitoring of patients with CRSs. Biomarkers play a key role in the diagnosis and monitoring of acute myocardial infarction, chronic heart failure, and chronic kidney disease. In recent years, new biomarkers have been identified that may play a role in the early diagnosis of acute kidney injury. Herein, we review the use of serum and urine biomarkers in the diagnosis and management of CRSs. The established cardiac and renal biomarkers such as the cardiac troponins, natriuretic peptides, urine albumin, and creatinine, as well as the new renal biomarkers cystatin C and neutrophil gelatinase–associated lipocalin are reviewed in detail. The recent advances in assay methods, clinical studies, and recommendations in clinical guidelines are discussed. With advances in biomarker research, in future, perhaps a multimarker approach will become feasible to stratify the diagnosis of CRS for individualized treatment and prognosis. (Translational Research 2014;164:122–134)

Abbreviations: ACR = albumin-to-creatinine ratio; ACSs = acute coronary syndromes; ADHF = acute decompensated heart failure; AKI = acute kidney injury; AUROC = area under the receiver-operator curve; CKD = chronic kidney disease; CKD-EPI = chronic kidney disease epidemiology collaborations; CRSs = cardiorenal syndromes; cTn = cardiac troponin; CV = coefficient of variation; ESRD = end-stage renal disease; eGFR = estimated glomerular filtration rate; HF = heart failure; IDMS = isotope dilution mass spectroscopy; KDIGO = kidney disease: improving global outcomes; MDRD = modification of diet in renal disease; MI = myocardial infarction; NACB = National Academy of Clinical Biochemistry; NKDEP = National Kidney Disease Education Program; NGAL = neutrophil gelatinase–associated lipocalin; RCV = reference change value; URL = upper reference limit

INTRODUCTION

The cardiorenal syndromes (CRSs) are a heterogeneous group of conditions comprising both cardiac and renal dysfunction, whereby dysfunction in one organ may induce dysfunction in the other organ[1] (Table I). Type 1 CRS (acute CRS) is characterized by an acute cardiac event resulting in acute renal deterioration. The acute cardiac event commonly includes acute coronary syndrome (ACS), acute decompensated heart failure (HF), cardiogenic shock, and cardiac surgery.[2] The acute cardiac insult results in reduced cardiac output, which leads to reduced renal perfusion pressure, increased renal vascular resistance, and reduced glomerular filtration rate (GFR).[2] Type 2 CRS (chronic CRS) is characterized by chronic heart disease resulting in renal disease. Approximately 50% of patients with chronic HF have chronic kidney disease (CKD), and CKD is associated with high mortality in patients

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with HF.[3] Type 3 CRS (acute renocardiac syndrome) is characterized by an acute worsening of renal function, which causes acute cardiac dysfunction such as arrhythmia, HF, or ischemia. The development of acute kidney injury (AKI) is the primary event.[3] Type 4 CRS (chronic renocardiac syndrome) is characterized by CKD leading to decreased cardiac function, ventricular hypertrophy, diastolic dysfunction, and increased risk of adverse cardiovascular events. Patients with CKD have an increased risk of cardiovascular mortality, with cardiovascular causes representing up to 50% of all deaths in patients with CKD.[4] Type 5 CRS or secondary CRS is characterized by systemic conditions leading to simultaneous injury and dysfunction of the heart and kidney. Chronic inflammatory conditions such as systemic lupus erythematosus, vasculitis, amyloidosis, and diabetes mellitus can affect both the kidney and the heart.[5] In the acute setting, sepsis is the most common condition causing type 5 CRS.[5] In the chronic setting, diabetes mellitus is the most common condition causing simultaneous cardiac and renal dysfunction.[5]

The 5 subtypes reflect the primary and secondary pathophysiology, time frame, and simultaneous cardiac and renal dysfunction. The classification is based on clinical presentation alone and often it is not easy to distinguish between acute and chronic disease. The Acute Dialysis Quality Initiative working group recognized that many patients may populate or move between different subtypes during the course of the disease.[1] The different subtypes create new definitions of disease to identify diagnostic biomarkers, identify patients at risk, and develop strategies to prevent and manage CRS. Although it is recognized that biomarkers play an important role in diagnosis of acute and chronic HF, as well as acute and chronic renal disease, biomarkers have not yet been integrated into the diagnosis of the various CRS.[1] Further studies are needed to identify whether the biomarkers can be used to classify CRS, to risk stratify patients, and as treatment targets to monitor the efficacy of treatment. Although multiple clinical guidelines exist to manage acute and chronic heart disease, as well as acute and CKD, there are no guidelines for the management of the various CRS. Currently, early risk recognition with careful monitoring using biomarkers appears essential to developing treatment and prevention strategies. For example, the measurement of procalcitonin may play a role in acute type 5 CRS by early identification of acute sepsis. In chronic type 5 CRS, the measurement of urine albumin plays a role in the early identification of renal disease.

Cardiac and renal diseases often coexist and patients with cardiac and renal failure have high morbidity and mortality;[1] hence, there is a need for early detection and monitoring of patients with cardiac and renal diseases. The pathophysiology of CRS involves complex multiple interactions between the heart and the kidney[2] (Fig 1). Biomarkers play a key role in the diagnosis and monitoring of acute myocardial infarction (MI), chronic HF, and CKD. In recent years, new biomarkers have been identified that may play a role in the early diagnosis of AKI. Herein, we review the role of cardiac and renal biomarkers in the diagnosis and management of CRS.

CARDIAC BIOMARKERS

Cardiac troponin. An acute cardiac ischemic event is often the primary event in type 1 CRS. ACS also features in types 3 and 5 CRSs, where an MI may be triggered by AKI in type 3 CRS and by sepsis in type 5 CRS. The cardiac troponins (cTns) have a central role in the diagnosis of ACS: in the Third Universal Definition of MI, the criteria for diagnosis of MI is a rise or fall in cTn value with at least one value greater than the 99th percentile upper reference limit (URL) plus at least one evidence of myocardial ischemia, such as symptoms of ischemia, ST segment-T wave changes or new left bundle branch block, new pathologic Q waves, imaging evidence of new loss of viable myocardium or new regional wall motion abnormality, or angiographic identification of an intracoronary thrombus.[6]

CTns T (cTnT) and I (cTnI) are cardiac-specific components of the contractile apparatus of muscle. The release of cTn is specific for myocardial necrosis but cannot differentiate between ischemic and nonischemic myocardial necrosis; thus, elevations of cTn can occur in the absence of ischemic heart disease such as arrhythmias, myocarditis, hypertrophic cardiomyopathy, aortic dissection, pulmonary embolism, stroke, trauma, extreme exertion, sepsis, acute respiratory failure, and renal failure.[7] Patients with CKD without cardiac disease have raised plasma cTn, with cTnT levels higher than cTnI levels in patients with end-stage renal disease (ESRD).[8] The increased cTn concentration in patients with CKD is thought to be because of subclinical myocardial injury[9] and the measurement of cTn has been recommended for prognosis of mortality in patients with ESRD.[10] This reflects the close relationship between cardiac and renal dysfunction in the pathophysiology of CRS.

Troponin is present in the blood of patients as a heterogeneous mix of free post-translationally modified, degraded, and truncated forms, as well as in complexes of cTnI-cTnC and cTnT-cTnI-cTnC.[11] Commercial immunoassays use antibodies that detect all major circulating forms of cTn.[12] Although there is only 1 cTnT assay on the market, there are many cTnI assays available. The cTnI assays are not standardized and there are substantial intermethod differences.[13] Hemolysis,[14]

Table I. Five subtypes of the cardiorenal syndromes

Type	Syndrome	Definition	Biomarkers
1	Acute CRS	Acute worsening of cardiac function, such as acute coronary syndrome, acute decompensated HF, cardiogenic shock and cardiac surgery, leading to renal dysfunction	Cardiac troponin, B-type natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP), creatinine, cystatin C, NGAL
2	Chronic CRS	Chronic abnormalities in cardiac function, that is, HF, leading to renal dysfunction	BNP and NT-proBNP, creatinine, cystatin C, albuminuria
3	Acute renocardiac syndrome	Acute worsening of renal function, that is, acute kidney injury causing cardiac dysfunction	Creatinine, cystatin C, NGAL, cardiac troponin, and BNP and NT-proBNP
4	Chronic renocardiac syndrome	Chronic abnormalities in renal function, that is, chronic kidney disease leading to cardiac dysfunction	Creatinine, cystatin C, albuminuria, and BNP and NT-proBNP
5	Secondary cardiorenal syndromes	Systemic conditions such as sepsis, systemic lupus erythematosus, vasculitis, amyloidosis, and diabetes mellitus, causing simultaneous dysfunction of the heart and kidneys	C-reactive protein, procalcitonin, creatinine, albuminuria, cystatin C, NGAL, cardiac troponin, and BNP and NT-proBNP

Abbreviations: CRS, cardiorenal syndrome; HF, heart failure; NGAL, neutrophil gelatinase–associated lipocalin.

and rarely, heterophilic antibodies,[15] or macrotroponin[16] may cause false-positive or false-negative results in cTn immunoassays. Because of the central role of cTns in the diagnosis of MI, the statement of the joint European Society of Cardiology/American College of Cardiology Foundation/American Heart Association/World Health Federation (ESC/ACCF/AHA/WHF) Task Force recommended that cTn assays are independently validated to have optimal precision, that is, coefficient of variation (CV) of ≤10% at the 99th percentile URL.[6] A scorecard of commercially available cTn assays designating whether current commercial assays are guideline acceptable is available.[17]

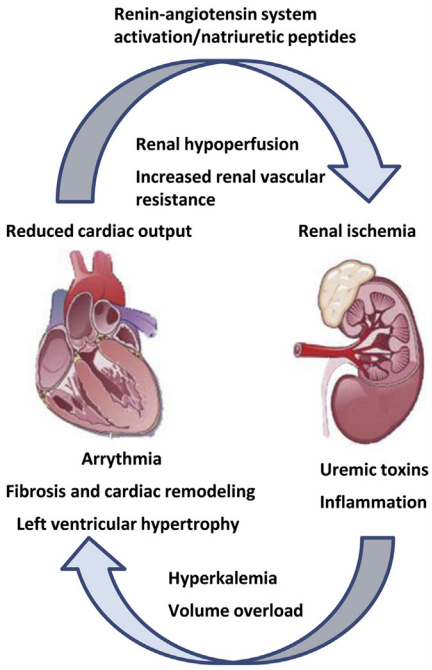
Recently, advances in technology have led to the development of high-sensitivity cTn assays that can measure cTn in the concentration range of a few nanograms per liter.[18] High-sensitivity cTn assays measure the URL with a CV of <10% and detect cTn in at least 50% of apparently healthy individuals.[18] Commercially available cTn assays have been further defined by generations, depending on the percent of measurable normal values less than the 99th pcercentile,[17] with third generation high-sensitivity cTn assays able to measure more than 95% of normal values less than the 99th percentile.[17] The 99th percentile URL greatly depends on the reference population chosen,[19] and appears to be higher for males compared with females,[20] thus gender-specific cutoffs may be necessary. The new high-sensitivity cTn assays appear to detect cTn release at an earlier time point,[21] leading to the suggestion of a 1-hour rule-in and rule-out of acute MI using high-sensitivity cTnT.[21] The joint ESC/ACCF/AHA/WHF Task Force recommends that blood is drawn for cTn measurement using high-sensitivity assays, at first assessment and 3–6 hours later.[22]

The ability of high-sensitivity cTn assays to detect cTn in healthy individuals has allowed the calculation of the biological variation for cTn. Studies in healthy volunteers using high-sensitivity cTnI assays gave a 1-hour reference change value of around 46%;[23,24] thus, it appears that a change in cTn values of around 50% would be required to diagnose MI using high-sensitivity assays.[25] Besides diagnosis of acute MI, high-sensitivity cTn assays, have been shown to be useful for predicting coronary heart disease, HF, and cardiovascular mortality in general populations,[26,27] and this raises the possibility that cTn may be used in the future as a cardiovascular disease risk assessment tool in asymptomatic individuals.[28]

Natriuretic peptides. Acute HF is a key feature of types 1, 3, and 5 CRSs, and chronic HF is a key feature of types 2 and 4 CRSs. The natriuretic peptides B-type natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP) are widely recognized to be the "gold standard" biomarker for the detection of cardiac dysfunction.[29] In the Breathing Not Properly trial of 1586 patients presenting to the emergency department with shortness of breath, BNP levels had a higher diagnostic accuracy (81.6%) than clinical judgment (74%) in diagnosing HF.[30] The 2013 ACCF/AHA guideline for the management of HF recommends the measurement of BNP or NT-proBNP in the diagnosis and prognosis of HF.[31]

Volume expansion or pressure overload results in ventricular wall stress and the synthesis of pre-proBNP in the ventricular myocardium, which is cleaved to

Markers of myocardial cell damage	cTnI, cTnT
Markers of myocardial strain	BNP, NT-proBNP, midregion pro-atrial natriuretic peptide (MR-proANP), midregion pro-adrenomedullin (MR-proADM), copeptin
Markers of fibrosis and remodelling	Soluble ST2, galectin-3, matrix metalloproteinases (MMPs), collagen propeptides
Markers of atherosclerosis and plaque rupture	Pregnancy-associated plasma protein A (PAPP-A), placental growth factor (PlGF), lipoprotein associated phospholipase A2 (Lp-PLA2), Lp(a), oxidized LDL, growth differentiation factor-15 (GDF-15),
Markers of ischemia	Ischemia-modified albumin (IMA), heart fatty acid binding protein (HFABP)
Inflammatory markers	hsCRP
Renal markers	Cystatin C, NGAL, creatinine, urinary albumin/creatinine ratio
Genetic polymorphisms	9p21.3, PCSK9
MicroRNAs	Mir-1, mir-133, mir-499



Functional markers of kidney injury	**Damage markers of kidney injury**
Estimated glomerular filatration rate (eGFR) using •Creatinine •Cystatin C or •Both creatinine and cystatin C	Albuminuria
	Cystatin C
	Neutrophil gelatinase associated lipocalin (NGAL)
	Kidney injury molecule-1 (KIM-1)
	Interleukin-18 (IL-18)
RIFLE criteria •Risk •Injury •Failure •Loss •End stage renal disease	Liver-type fatty acid binding protein (L-FABP)
	N-acetyl-beta-D-glucosaminidase (NAG)
	Beta-2 microglobulin
	Retinol-binding protein-4 (RBP-4)
AKIN criteria •AKIN-1 •AKIN-2 •AKIN-3	Glutathione-S-transferase (GST)
	Netrin-1
	Clusterin
	Osteopontin

Fig 1. Pathophysiology and biomarkers in cardiorenal syndromes. The picture in the middle shows the complex pathophysiological interactions between the heart and the kidney; where dysfunction in one organ may induce dysfunction in the other. The left panel shows the potential components of a multimarker panel for cardiovascular risk profiling. The right panel shows the new diagnostic approach with both functional and injury criteria for AKI. Potential new AKI damage markers are listed on the right. AKI, acute kidney injury.

proBNP (1-108).[32] ProBNP (1-108), a weakly active form, is then processed into BNP, the active form, and NT-proBNP, the inactive form.[32] Both BNP and NT-proBNP levels increase with age and are higher in women than men.[33] There is an inverse relationship between body mass index and both BNP and NT-proBNP.[34] Besides congestive HF, other conditions, such as heart disease, atrial fibrillation, myocarditis, pulmonary hypertension, pulmonary embolism, sepsis, critical illness, and hyperthyroidism, can also cause elevated BNP and NT-proBNP levels.[34]

Plasma concentrations of BNP and NT-proBNP are increased in patients with impaired kidney function at a threshold of estimated GFR (eGFR) of 60 mL/min.[35] There is a stronger inverse correlation between NT-proBNP and GFR than for BNP.[36] However, studies have shown that NT-proBNP testing is valuable for patients with CKD with suspected HF, irrespective of renal function.[37] BNP and NT-proBNP have also been shown to predict cardiovascular mortality in patients with ESRD,[38,39] and risk of progression to ESRD in patients with mild to moderate CKD.[40,41] The predictive value of cardiac biomarkers in patients with CKD and ESRD reflects the pathophysiological interactions between the heart and the kidney in CRS.

There are many commercial sandwich-type immunoassays for the measurement of both BNP and NT-proBNP, with a lack of standardization of currently available assays. Intermethod differences are higher for BNP than NT-proBNP assays.[42] However, BNP and NT-proBNP levels are generally highly correlated, with similar diagnostic and prognostic accuracies.[43] In the laboratory, NT-proBNP appears more stable than BNP.[44] Another advantage of NT-proBNP is that serum, heparinized plasma, and EDTA plasma all can be used, whereas for BNP, only EDTA plasma should be used.[44]

The cutoffs for BNP are $<$100 pg/mL for unlikely HF, and $>$400 pg/mL for likely HF.[29] For NT-proBNP, the

cutoffs are <400 pg/mL unlikely HF, and >2000 pg/mL likely HF.[29] The International Collaborative for NT-proBNP study defined age appropriate cutoffs for ruling in HF: 450 pg/mL for <50 years, 900 pg/mL for 50–75 years, and 1800 pg/mL for >75 years.[34] For patients with CKD with GFR <60 mL/min, a cutoff of 200 pg/mL for BNP and 1200 pg/mL for NT-proBNP has been proposed.[35]

Both BNP and NT-proBNP have consistently high intraindividual biological variations.[45] The week-to-week reference change value for BNP in healthy and stable HF patients is approximately 71% and for NT-proBNP 47%,[46] therefore, although patients who demonstrate clinical improvement have decreasing concentrations of BNP or NT-proBNP, they may not exceed the biological variation.[46] Thus, the 2013 ACCF/AHA guideline for the management of HF concluded that the usefulness of BNP- or NT-proBNP-guided therapy by serial measurement is not well established.[31]

BNP and NT-proBNP levels have been shown to predict death and cardiovascular events in the general population.[47] Future applications of BNP and NT-proBNP levels may include assessment of patients with valvular heart disease, patients on chemotherapy at risk for drug-induced cardiotoxicity, and for preoperative evaluations, as well as screening of asymptomatic individuals for heart disease, for example, preparticipation screening of athletes.[48]

Other cardiac biomarkers. Besides the troponins and natriuretic peptides, other biomarkers have been evaluated as multimarker panels for cardiovascular risk profiling. The components of a multimarker panel include markers of myocardial strain, ischemia, inflammation, fibrosis, and atherosclerosis (Fig 1).[48] In particular, the markers of myocardial fibrosis soluble ST2 and galectin-3 are mentioned in the 2013 ACCF/AHA Heart Failure Guideline as prognostic markers and additive to natriuretic peptides in their prognostic value.[31] The long-term intraindividual variations of soluble ST2 (11%) and galectin-3 (20%) were lower than that of BNP (50%) and NT-proBNP (33%),[49] suggesting that soluble ST2 and galectin-3 may be more useful in monitoring of HF than the natriuretic peptides. Renal markers, such as microalbuminuria, have also been suggested as part of the multimarker panels for cardiovascular risk profiling,[48] reflecting the intimate pathophysiological relationship between the cardiovascular and renal systems.

RENAL BIOMARKERS

Albuminuria. Proteinuria has long been known to be a marker of renal damage, and quantification of proteinuria or albuminuria is widely used for identifying and monitoring patients with CKD. Albumin is the predominant protein in urine in renal damage, and measurement of the albumin-to-creatinine ratio (ACR) in first morning or random spot urine is generally recommended for annual screening of patients with type 2 diabetes mellitus for CKD.[50] The Kidney Disease: Improving Global Outcomes (KDIGO) 2012 Clinical Practice Guideline for the Evaluation and Management of CKD proposed albuminuria as an additional biomarker to classify stages of CKD.[51] Thus albuminuria is an important biomarker in types 2 and 4 CRSs, and chronic type 5 CRS secondary to diabetes mellitus.

The urinary ACR varies with age, sex, and ethnicity, increasing with age, and higher for females compared with males.[52] Increased albumin excretion in the urine occurs with exercise, fever, and posture.[50] The measurement units and cutoff values for albuminuria are varied, with some reporting as "mg albumin/g creatinine" and others as "mg albumin/mmol creatinine." Normal albuminuria is generally defined as <30 mcg albumin/mg of creatinine, high albuminuria 30–300 mcg albumin/mg of creatinine, and very high albuminuria >300 mcg albumin/mg of creatinine.[50,51] The International Diabetes Foundation and National Institute of Clinical Excellence guidelines recommend an ACR threshold of 2.5 mg/mmol for males and 3.5 mg/mmol for females.[53,54]

Urinary albumin is routinely measured in diagnostic laboratories using turbidimetric, nephlometric, or immunometric assays with limits of detection between 2 and 10 mg/L.[50] Calorimetric test strips or dipstick tests for urine protein have a limit of detection of around 150 mg/L and are unable to detect low amounts of albumin in the urine.[50] However, qualitative or semiquantitative screening test strips for low amounts of albumin in urine (also known as microalbuminuria) have been developed which have a limit of detection between 20 and 50 mg/L.[50] The National Academy of Clinical Biochemistry Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus recommend that semiquantitative or qualitative screening test strips should have a sensitivity of >95% to be useful for screening.[55] Additionally, the KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of CKD has recommended that the term microalbuminuria should not be used to avoid confusion.[51] The intraindividual variation for urine ACR was approximately 30%, and was lower in first morning urine compared with random spot urine.[56] The National Academy of Clinical Biochemistry Guidelines recommend that the analytical CV of methods to measure albuminuria should be <15%.[55]

Albuminuria not only reflects glomerular damage, it is also thought to be a marker of generalized endothelial dysfunction,[57] reflecting the pathophysiology of the

Table II. RIFLE and AKIN criteria for classification of AKI

Criteria	Serum creatinine criteria	Urine output criteria
RIFLE		
Risk	Serum creatinine increase by 1.5-fold or GFR decrease by >25% from baseline	<0.5 mL/kg/h for 6 h
Injury	Serum creatinine increase by 2.0-fold or GFR decrease >50% from baseline	<0.5 mL/kg/h for 12 h
Failure	Serum creatinine increase to 3.0-fold or GFR decrease >75% from baseline or serum creatinine ≥4 mg/dL with an acute increase of at least 0.5 mg/dL	<0.3 mL/kg/h for 24 h or anuria for 12 h
Loss	Loss of renal function requiring renal replacement therapy for at least 4 wk	
End-stage renal failure	Loss of renal function requiring renal replacement therapy for at least 3 mo	
AKIN		
Stage 1	Serum creatinine increase ≥0.3 mg/dL in ≤48 h or increase by 1.5–2.0-fold from baseline in ≤48 h	<0.5 mL/kg/h for 6 h
Stage 2	Serum creatinine increase by >2.0–3.0-fold from baseline in ≤48 h	<0.5 mL/kg/h for 12 h
Stage 3	Serum creatinine increase >3.0-fold from baseline in ≤48 h or serum creatinine ≥4 mg/dL with an acute increase of at least 0.5 mg/dL in ≤48 h or need for renal replacement therapy	<0.3 mL/kg/h for 24 h or anuria for 12 h or need for renal replacement therapy

Abbreviations: AKI, acute kidney injury; GFR, glomerular filtration rate.
To convert the values for serum creatinine in mg/dL to μmol/L, multiply by 88.4.

interactions between the cardiovascular and renal systems in types 2 and 4 CRSs. Higher amounts of albuminuria are associated with increased mortality,[58] cardiovascular disease,[57] and stroke.[59] Urine albumin at concentrations ≥30 mg/g creatinine are considered a continuous risk marker for cardiovascular events.[54] Albuminuria may be reduced by renin-angiotensin-aldosterone system inhibitors, and reduction in albuminuria has been proposed as a therapeutic target.[60]

Creatinine. Serum creatinine is the most widely used laboratory test of kidney function, and is used to derive the eGFR as an indicator of kidney function. CKD, a key feature of CRS types 2 and 4, is manifest as a GFR of <60 mL/min/1.73 m^2 [51]. Both the Risk, Injury, Failure, Loss, End-stage (RIFLE)[61] and the Acute Kidney Injury Network (AKIN)[62] criteria (Table II) use a decline in serum creatinine to define AKI, which is a key event in CRS types 1, 3, and 5. Thus, the measurement of serum creatinine levels is essential in the diagnosis of all 5 types of CRSs.

Creatinine is a small molecular weight (113 Da) molecule, formed by the nonenzymatic dehydration of creatine in muscle.[63] It is freely filtered by the glomerulus and not reabsorbed by the renal tubules; however, tubular secretion of creatinine accounts for 10%–20% of its excretion.[63] Creatinine increases proportionally to muscle mass, and hence varies with age, sex, ethnic group, and extreme diets.[64] The standard method of measurement of creatinine uses the Jaffé reaction, in which creatinine reacts with alkaline picrate to form a red-orange complex, is subject to interference by non-creatinine chromogens such acetoacetate, pyruvate, ketoacid and protein, and reducing agents such as glucose, ascorbate, and urate.[63] Current commercial assays widely use the kinetic alkaline picrate method, which takes advantage of the differential rate of color development, allowing a separation of creatinine from interferences.[63] Enzymatic methods based on creatinine iminohydrolase or creatininase are more expensive and used by fewer laboratories.[63] In 2008, the National Kidney Disease Education Program launched the Creatinine Standardization Program to reduce interlaboratory variability in creatinine assay calibration.[70] Most laboratories now use a creatinine assay with a calibration that is traceable to an isotope dilution mass spectroscopy reference method, thus creatinine measurements from different laboratories using both the Jaffe and the enzymatic methods are comparable.[63]

Several serum creatinine-based equations have been developed to estimate GFR, such as the Cockcroft and Gault,[65] modification of diet in renal disease (MDRD),[66,67] and Chronic Kidney Disease Epidemiology Collaborations (CKD-EPI) equations (Table III).[68] The equations for calculating GFR are based on the principle that creatinine is proportional to muscle mass, which can be estimated from an individual's age, sex, and weight.[69] Therefore, in the elderly, malnourished patients with muscle wasting disorders and amputees, GFR may be overestimated.[69] The MDRD equation underestimates GFR at lower creatinine

Table III. Equations for estimating glomerular filtration rate

Creatine-based equations	
Cockcroft and Gault[65]	[140 − age (y) × body weight (kg)]/[72 × serum creatinine (mg/dL)] × 0.85 if female
Simplified MDRD[66]	$186.3 \times [\text{Serum creatinine (mg/dL)}]^{-1.154} \times [\text{age (y)}]^{-0.203} \times 0.742$ if female × 1.210 if African American
Simplified MDRD traceable to IDMS[67]	$1.75 \times [\text{Serum creatinine (mg/dL)}]^{-1.154} \times [\text{age (y)}]^{-0.203} \times 0.742$ if female × 1.212 if African American
CKD-EPI (IDMS traceable)[68]	$141 \times \min[[\text{serum creatinine (mg/dL)}]/k, 1]^{\alpha} \times \max[[\text{serum creatinine (mg/dL)}]/k, 1]^{-1.209} \times 0.993^{\text{age (y)}} \times 1.018$ if female × 1.159 if African American, where k is 0.7 for females and 0.9 for males and α is −0.329 for females and −0.411 for males [min indicates the minimum of serum creatinine/k or 1 and max indicates the maximum of serum creatinine/k or 1]
Cystatin C-based equations	
CKD-EPI (cystatin C)[95]	$133 \times \min[[\text{serum cystatin C (mg/L)}]/0.8, 1]^{-0.449} \times \max[[\text{serum cystatin C (mg/L)}]/0.8, 1]^{-1.328} \times 0.996^{\text{age}} \times 0.932$ if female
Stevens et al[96]	$127 \times [\text{Serum cystatin C (mg/L)}]^{-1.17} \times \text{age}^{-0.13} \times (0.91$ if female) × (1.06 if black)
Creatinine and cystatin C-based equations	
CKD-EPI (creatinine-cystatin C)[95]	$135 \times \min(\text{Scr}/k, 1)^{-a} \times \max(\text{Scr}/k, 1)^{-0.601} \times \min(\text{Scys}/0.8, 1)^{-0.375} \times \max(\text{Scys}/0.8, 1)^{-0.711} \times 0.995^{\text{age}}$ [0.969 if female] [1.08 if black] Scr is serum creatinine (mg/dL) and Scys is serum cystatin C (mg/L) k is 0.7 for females and 0.9 for males a is −0.248 for females and −0.207 for males

Abbreviations: CKD-EPI, Chronic Kidney Disease Epidemiology Collaborations; GFR, glomerular filtration rate; IDMS, isotope dilution mass spectroscopy; MDRD, modification of diet in renal disease.
To convert the values for serum creatinine in mg/dL to μmol/L, multiply by 88.4.

concentrations, whereas the Cockcroft-Gault and Schwartz equations overestimate GFR at lower creatinine concentrations.[69]

The National Kidney Disease Education Program recommends reporting eGFR using an MDRD equation modified for standardized creatinine, with eGFR values above 60 mL/min reported as >60 mL/min.[70] The CKD-EPI equation has been reproducibly shown to be more accurate than the MDRD equation for estimating eGFR, especially at eGFR levels above 60 mL/min.[71] In addition, the CKD-EPI equation was shown to improve prediction of mortality and adverse outcomes.[72,73] Hence, the KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of CKD recommends reporting eGFR using the 2009 CKD-EPI creatinine equation.[51] However, using the CKD-EPI equation leads to a lower estimated prevalence of CKD with reclassification to higher CKD stages.[74]

The intraindividual variation for creatinine is 4.7% for healthy individuals, whereas the interindividual variation is 14.4%.[75] The small biological variation implies that very small changes in GFR can be identified; hence creatinine is a sensitive and reliable marker for monitoring renal function in chronic renal disease.[76] However, because the equations for deriving eGFR are based on an equilibrium between creatinine production and creatinine excretion, creatinine is not as useful in AKI.[76]

New biomarkers of AKI. The use of variables that reflect decline in kidney function, such as urine output and serum creatinine in the definition of AKI, has impeded early identification of AKI and mortality of patients with AKI has remained high.[1] Encouragingly, in recent years, several biomarkers of early structural kidney damage have been identified that may identify early AKI before a significant increase in serum creatinine level.[77] The most notable are cystatin C, neutrophil gelatinase–associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), interleukin-18 (IL-18), and liver-type fatty acid binding protein[77] (Fig 1). Other candidates include beta-2-microglobulin, *N*-acetyl-beta-D-glucosaminidase, glutathione-*S*-transferase, netrin-1, clusterin, and osteopontin[78] (Fig 1). Among these biomarkers, reliable and automated assay methods are commercially available only for cystatin C and NGAL,[77] therefore only cystatin C and NGAL will be discussed in detail here.

In a consensus conference of the 10th Acute Dialysis Quality Initiative, a new injury criteria incorporating the new damage biomarkers for diagnosis of AKI was proposed.[78] Under this new diagnostic approach, AKI can be defined by abnormal levels of kidney injury biomarkers even in the absence of oliguria or elevated serum creatinine, thus defining a new spectrum of AKI including (1) loss of function without damage, (2) damage without loss of function, and (3) damage with loss of function.[78] Several studies have shown that a combination of injury biomarkers predicts AKI better than single biomarkers,[79-81] thus increasing severity of kidney damage is suggested by increasing biomarker positivity.[78] Although there is optimism that these new biomarkers may enable early diagnosis of AKI, and thus reduce mortality associated with AKI, validation of results in large multicentre studies and in multiple different etiologies is still needed.[77]

Cystatin C. Cystatin C is a 13-kDa cysteine proteinase inhibitor produced by all nucleated cells.[82] It is freely filtered by the glomerulus, and reabsorbed but not secreted by the renal tubules,[82] hence cystatin C clearance cannot be calculated. A major advantage of cystatin C over creatinine is that it is not influenced by changes in muscle mass,[82] therefore cystatin C is more accurate for estimating GFR in patients with extremes of body mass, including infants and elderly.[82] However, cystatin C concentration is influenced by age, sex, body mass index, smoking status, raised C-reactive protein, abnormal thyroid function, certain cancers, and use of corticosteroids.[83]

Cystatin is distributed in the extracellular space and has a half-life of 1.5 hours (compared with 4 hours for creatinine).[82] Therefore, after kidney injury, cystatin C concentration increases earlier than creatinine concentration, enabling earlier identification of AKI.[82] The ability of cystatin C to detect AKI has been demonstrated with conflicting results.[84-87] In a recent meta-analysis, serum cystatin C appeared to be a good biomarker of AKI with an area under the receiver-operator curve (AUROC) of 0.87, whereas urinary cystatin C had a lower AUROC of 0.67.[88] This may be because urinary concentrations of cystatin C are very low (0.03–0.18 mg/L) compared with serum cystatin C concentrations (0.54–1.21 mg/L).[89-91]

Cystatin C is measured using liquid agglutination of latex particles coated with polyclonal antibodies against cystatin C.[92] There is no reference method available and significant interassay variation has been reported.[93] The intraindividual variation for cystatin C is 8.6% for healthy individuals and the interindividual variation for cystatin C is 15.1%.[75] For cystatin C, interindividual variance accounted for 25% and intraindividual variance accounted for 75% of the biological variability,[94] whereas interindividual variance accounted for 93% and intraindividual variance accounted for only 7% of serum creatinine biological variation.[94] This suggests that cystatin C is potentially a better marker for detecting impaired renal function than serum creatinine, but creatinine is still the preferred marker for serial monitoring of renal function in individuals.[75,94]

Equations to estimate GFR using cystatin C alone, or in combination with creatinine (Table III), have been shown to be superior to creatinine-based equations for estimating GFR[95,96] and predicting progression to ESRD.[97] Cystatin C has also been shown to demonstrate stronger associations than creatinine with cardiovascular disease, HF, ESRD, and mortality.[98-101] Thus, the ESC recommends the use of cystatin C in the prognosis of patients with non-ST segment elevation MI.[29]

The 2012 KDIGO CKD guidelines suggest that cystatin C is used as a confirmatory test in circumstances when creatinine-based eGFR is less accurate.[51] Cystatin C is suggested to be measured in adults who do not have other markers of kidney damage, with eGFR of 45–59 mL/min/1.73 m^2 using creatinine-based equations, to confirm the diagnosis of CKD using a cystatin C-based or creatinine and cystatin C-based eGFR equation.[51] Although cystatin C-based equations have higher correlation with measured GFR and stronger association with adverse outcomes, the use of cystatin C for screening at a population level is not recommended by the KDIGO guidelines because of a lack of evidence of cost effectiveness.[51]

Neutrophil gelatinase–associated lipocalin. NGAL is a 25-kDa protein of the lipocalin superfamily, originally isolated from human neutrophils.[102] Although NGAL has been compared, as a biomarker of kidney injury,[103] to cTns in the heart, it is not specific for the kidney and is also produced by other tissues.[102] It exists as a 25-kDA monomer, a 45-kDa disulfide-linked homodimer, or 135-kDa heterodimer covalently conjugated with gelatinase (matrix metalloproteinase 9).[104] The monomer is predominantly released by renal tubular cells, whereas the homodimer is predominantly synthesized by neutrophils.[102] NGAL protein increased in the plasma and urine 24–48 hours preceding serum creatinine increase.[105] However, NGAL may also be produced by neutrophils in urinary tract infections, and urinary white blood cell counts were found to be significantly correlated with NGAL.[106]

Many studies have shown a role for NGAL as an early diagnostic marker for AKI.[107] The first prospective clinical studies in children who underwent cardiac surgery showed an AUROC of >0.9, for both urine and plasma NGAL.[108,109] These studies used enzyme-linked immunosorbent assays,[108,109] which are not for routine clinical use. A whole-blood point-of-care competitive immunoassay (Triage NGAL test; Biosite) was later developed, which showed an AUROC of 0.96 (sensitivity, 0.84; specificity, 0.94) for predicting AKI with a cutoff of 150 ng/mL in children undergoing cardiac surgery.[110] More recently, an automated commercial method using chemiluminescent microparticle immunoassay on the ARCHITECT platform (Abbott Diagnostics) was developed to measure urine NGAL.[111] This assay also performed well as an early predictive marker of AKI with an AUROC of 0.96 in children who underwent cardiac surgery.[112] An enhanced turbidimetric immunoassay for measuring urine and plasma NGAL on automated chemistry analyzers has also been developed (NGAL test; BioPorto Diagnostics).[113]

NGAL has also been evaluated as an early predictive biomarker of AKI in adults undergoing cardiac surgery,[114,115] contrast-induced nephropathy,[116,117] with delayed renal graft function,[118,119] in the intensive

care[120,121] and emergency setting.[122] In a meta-analysis, the performance of urinary NGAL as a predictor of AKI was superior to that of plasma NGAL, with an AUROC of 0.84 for urine compared with an AUROC of 0.77 for plasma.[107] The performance of NGAL was better in children than in adults,[107] perhaps because of the presence of comorbidities in adults, such as pre-existing kidney disease. The reference intervals for NGAL are gender and age dependent.[123] The biological variations of NGAL and NGAL-to-creatinine ratio were 84% and 81% respectively, for first morning urine samples, suggesting that a 2-fold increase in NGAL concentrations is required to confirm AKI.[124]

Although NGAL appears to be a promising biomarker for AKI in most studies, there are many limitations that limit its use in current clinical practice, such as a lack of consensus on whether serum or urine NGAL should be measured, and if urine, whether NGAL-to-creatinine ratios should be used.[125] There are no recommendations yet available on diagnosis thresholds and quality specifications of the NGAL assay.[125] Current commercial immunoassays are not able to distinguish between the monomer, homodimer, or heterodimer forms of NGAL.[126] Therefore, further work still needs to be done to resolve the analytical issues before large-scale prospective clinical trials can be performed to determine the diagnostic utility of NGAL as a biomarker of AKI.

Kidney injury molecule 1. KIM-1 is a type 1 transmembrane glycoprotein with an immunoglobulin and mucin domain.[127] It is upregulated in response to ischemic or nephrotoxic injury and expressed at high levels on the apical membranes of proximal tubules in the kidney.[127] KIM-1 is thought to function as a phosphatidylserine receptor that confers on epithelial cells the ability to phagocytose apoptotic cells.[127] The ectodomain of KIM-1 is shed from proximal tubules and can be detected in urine by immunoassay.[127] KIM-1 was first demonstrated to be a urinary biomarker for AKI in 2002.[128] Subsequently, many more clinical studies have been performed in different settings of AKI, and in combination with other potential AKI biomarkers.[129] A recent meta-analysis estimated the sensitivity of urinary KIM-1 to be 74% and specificity 86%.[129] KIM-1 has been approved by the US Food and Drug Administration as an AKI biomarker for preclinical drug development.[130]

CONCLUSIONS

Although there has been significant progress in the recent decade in biomarker discovery for the diagnosis and prognosis of CRS, further work needs to be done before translation of the newer biomarkers into clinical utility. A clinically useful biomarker is one that is easily measured at low cost with a low turnaround time, and can provide information in addition to clinical assessment to influence medical decision. The cTns, BNP, NT-proBNP, and creatinine have proven to be essential in the detection of cardiac and renal disease. Newer biomarkers of AKI such as cystatin C, NGAL, and KIM-1, need further well-designed, adequately powered clinical studies that pay attention to the assay method. For the acute CRS (types 1, 3, and acute type 5), the development of high-sensitivity cTn assays can be expected to increase the sensitivity of early identification of acute ischemic cardiac events. In addition, the development of new AKI biomarkers that can detect AKI more rapidly than current definitions can be expected to improve the management of acute CRS involving AKI. For the chronic CRS (types 2, 4, and chronic type 5 CRSs), further research into the pathophysiology of the interactions between the heart and the kidney may identify new biomarkers to improve early detection of cardiac and renal dysfunction. Examples of such potential biomarkers include markers of endothelial dysfunction such as pentraxin-3 and asymmetric dimethylarginine.[131] Rapid progress in cardiac and renal biomarker research can be expected with the identification of numerous potential candidates. In future, perhaps a multimarker approach will become feasible to stratify the diagnosis of CRS for individualized treatment and prognosis.

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