

Normalisation of urinary biomarkers to creatinine for clinical practice and research – when and why

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ABSTRACT Acute kidney injury (AKI) and chronic kidney disease (CKD) are major health problems. Urinary biomarkers have both diagnostic and prognostic utility in AKI and CKD. However, how biomarker excretion rates should be reported, especially whether they should be normalised to urinary creatinine concentration (uCr), is controversial. Some studies suggest that normalisation to uCr may be inappropriate at times, as urinary creatinine excretion rate may vary greatly, depending on the situation. Notably, recent studies suggest that while normalisation of values to uCr may be valid for the evaluation of CKD and prediction of AKI sequelae and occurrences, it could be inappropriate for the diagnosis of AKI, or in the presence of certain acute kidney disease states.

Keywords: biomarkers, creatinine, glomerular filtration rate, renal failure, urine

INTRODUCTION

Kidney disease and end-stage kidney failure are major health problems in Singapore today. The number of dialysis patients has increased dramatically from 1999 to 2008, with the number of incident patients increasing by almost 43.6%, and prevalent patients, by 69.5%.⁽¹⁾ The effects of chronic kidney disease (CKD), however, can be greatly mitigated by early detection, which allows for the execution of targeted and comprehensive therapy to stop or retard kidney disease progression.⁽²⁾ While acute kidney injury (AKI) is a known risk factor for CKD progression, current methods of diagnosis, such as the measurement of serum creatinine, are unsatisfactory due to their delay in the diagnosis of kidney disease.⁽³⁾ As serum creatinine is affected by filtration function, its rise reflects a significant, and often permanent, loss of functioning nephrons. Clinical management and research of kidney disease is impeded by the lack of earlier and specific markers of AKI and CKD. However, several novel urinary biomarkers have been found to be potential diagnostic and prognostic tools for AKI and CKD. In current practice, the most commonly used biomarker for the diagnosis and prognostication of AKI and CKD is protein (and/or albumin). Although a properly collected 24-hour urine assay for albumin is the reference standard for the identification and classification of kidney disease, a spot urine sample is usually collected for convenience and accuracy. The time of urine collection, urine concentration, and urine flow rate affect the concentration of the biomarkers, and assays obtained are often normalised to urinary creatinine concentration (uCr) to account for these differences. Similarly, other markers of kidney function in health and disease may be normalised (e.g. electrolytes).⁽⁴⁾ One problem, however, is the inconsistency and validity of applying normalisation.⁽⁵⁾ This impedes the comparison of biomarkers between different trials and studies, and makes it difficult to decide how they can be used in clinical practice.⁽⁶⁾ This problem is significant, as intraindividual variations differ greatly in urinary biomarkers

such as urinary neutrophil gelatinase-associated lipocalin (NGAL) when using absolute concentrations for measurement, compared to concentrations normalised to uCr.^(7,8) Potential biomarkers secreted at low amounts into urine could also have been missed due to the lack of an agreed or best method.⁽⁵⁾

Many novel urinary biomarkers have been identified for use in early and accurate diagnosis of AKI and prediction of clinical outcomes.⁽⁹⁾ A recent study by Chan et al showed how a panel of novel biomarkers of kidney injury offers additional prediction of glomerular filtration rate (GFR) decline in CKD progression.⁽¹⁰⁾ Examples of urinary biomarkers studied include kidney injury molecule-1 (KIM-1), NGAL, interleukin 18 (IL-18), and liver-type fatty acid-binding protein (L-FABP). Many of the studies involving these urinary biomarkers often normalise biomarker concentrations to uCr. In studies on potential biomarkers for the detection of AKI, tubular enzymuria,⁽¹¹⁻¹³⁾ NGAL,⁽¹⁴⁻¹⁶⁾ KIM-1⁽¹⁷⁾ and IL-18⁽¹⁸⁾ were normalised to uCr to control for variations in urinary flow rates. Normalisation is also commonly used in studies regarding CKD or progression of kidney disease, such as studies involving tissue inhibitor of metalloproteinase 1,⁽¹⁹⁾ L-FABP,^(20,21) NGAL,⁽²²⁾ N-acetyl-β-D-glucosaminidase and KIM-1.⁽²³⁾

Urinary electrolytes, such as sodium, calcium, phosphate, urea and uric acid, are also clinically helpful. For example, fractional excretion of sodium (FE_{Na}) is widely used to differentiate prerenal disease from acute tubular necrosis when investigating the cause of AKI.⁽²⁴⁾ Likewise, due to the difficulties in measuring 24-hour urinary electrolyte excretion,⁽²⁵⁾ studies have sought to use spot urine samples, which works via normalising urinary electrolyte concentrations to uCr.⁽²⁶⁻³¹⁾ This is supported by moderate to strong linear relationships found between normalised values of spot samples and 24-hour excretion values of certain electrolytes (e.g. sodium,^(28,32,33) potassium,⁽²⁸⁾ urea,⁽³⁴⁾ calcium,^(35,36) phosphate).⁽³⁰⁾

The question that remains is whether reporting urinary biomarker concentrations as a ratio to uCr is valid in the

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examination of biomarkers as diagnostic and prognostic tools for different renal pathologies. In the following sections, we examine spot urine biomarker assays, and the evidence for and against normalisation of biomarker concentrations to uCr.

CREATININE NORMALISATION

As water reabsorption in kidneys affect urinary solute concentrations, urinary biomarker concentrations are frequently reported as a ratio to uCr.⁽³⁷⁾ The reporting of albuminuria as an albumin/creatinine ratio,⁽³⁷⁾ for example, is an accepted method of accounting for albumin concentration variation arising from different pathologies.⁽⁶⁾ The underlying assumption for this approach is that urinary creatinine excretion rate (uCER) is constant between different individuals, within an individual over time, or that biomarker excretion has a linear relationship with uCER across individuals.⁽³⁷⁾ This may not necessarily be true. Variations in uCER between individuals occur because of differences in age, sex, race,⁽³⁸⁾ diurnal creatinine production, physical activity, diet, emotional stress, muscle mass and disease state;⁽³⁹⁾ for example, patients with CKD have a lower uCER than those without CKD.⁽³⁹⁾ uCER may also decline as kidney disease progresses, due to an increase in extrarenal degradation of creatinine.⁽⁴⁰⁾ Thus, unless one takes the biomarker excretion rate to be linearly related to uCER (which may be acceptable in certain groups of patients, such as older males),⁽⁷⁾ and consequently uCr differences in uCER would bias the normalised urinary biomarker value, even if the actual biomarker excretion rate is unchanged.⁽³⁷⁾ Some studies suggest that uCER vary even within individuals, with intraindividual coefficients of variation (CVs) estimates ranging from 10.5% to 14.4%.⁽⁴¹⁾ Other studies also suggest that creatinine excretion shows diurnal, hour-to-hour, and day-to-day variation.⁽⁴¹⁾ However, other studies suggest that compared to absolute values, using urine biomarker values as a ratio to creatinine (in the case of NGAL) reduces intraindividual CVs.^(8,9)

DISEASE STATES AND ACUITY

Many of the novel biomarkers of kidney injury are being studied in AKI syndromes, and there is a lot of interest in the appropriateness and validity of applying uCr normalisation. A recent study by Waikar et al showed that KIM-1 excretion and uCER are affected differently when an acute disease state is present,⁽³⁷⁾ hence making creatinine normalisation inappropriate. This seems reasonable, as uCER in AKI is a dynamic process affected by glomerular filtration and tubular secretion, while urinary biomarker excretion reflects different functional and structural consequences of damage.⁽⁴²⁾

When severe AKI is present, uCER decreases in proportion to the magnitude of the decrease in GFR, hence abruptly increasing normalised biomarker levels, despite constant production and excretion rates of the biomarker. Such amplifications are also significant, though less pronounced, in less severe cases of AKI.⁽³⁷⁾ A recent study has also shown that normalising values to uCr resulted in poorer diagnosis of AKI on admission to the intensive care unit, as compared to absolute concentration.⁽⁶⁾ In this study, the area under curve (AUC) of the receiver operating characteristic curve for predicting AKI for normalised concentrations were lower

than the AUCs for absolute concentration for all biomarkers except for KIM-1.⁽⁶⁾ This was attributed to the fact that in evolving AKI, uCER decreases as GFR decreases, but increases as plasma creatinine increases, hence causing uCER to asymptote toward the original rate before GFR decreases.⁽⁶⁾ The decrease in uCER in AKI also affects the interpretation of urinary electrolyte indices such as FE_{Na} . As renal failure progresses, the decrease in uCr causes FE_{Na} to increase even without a change in urinary sodium, hence complicating the interpretation of the index.^(43,44)

There are also marked differences in uCER in patients who have undergone kidney transplantation, with extrapolated uCER ranging from under 300 mg/day in a patient with delayed graft function to more than 2,100 mg/day in a patient with prompt graft function.⁽³⁷⁾ This could be significant since several studies involving potential biomarkers for predicting graft loss or delayed graft function, such as NGAL, IL-18, and KIM-1, often normalise biomarker excretion rates to uCr.⁽⁴⁵⁾ In one study involving the predictive value of KIM-1 for graft loss, creatinine clearance was found to decrease significantly with increasing KIM-1 excretion, and was also found to be an independent predictor of graft loss.⁽⁴⁶⁾

The question as to when and how we should apply normalisation remains. The studies examined so far show that the validity of normalisation to uCr depends very much on the aim of the research (outcome measure), the type of biomarker considered, and the clinical presentations of the patients.

IMMEDIATE DIAGNOSIS OF AKI VS. PREDICTION OF SUBSEQUENT AKI

For the reasons discussed above, the immediate diagnosis of AKI on admission to an intensive care unit is probably best done using absolute concentrations rather than normalised values. However, for the prediction of death, dialysis, or subsequent development of AKI, normalised concentrations may be preferred.⁽⁶⁾ With AKI determined using AKIN48 (Acute Kidney Injury Network score at 48 hours) and RIFLE24 (sustained AKI is defined using the Risk, Injury, Failure, Loss, ESRD criteria, occurring for a duration of 24 hours at any time within 7 days), the prediction of subsequent AKI is more accurate using normalised concentrations than absolute concentrations or excretion rates. The study also showed that normalised concentrations best predicted hard outcomes such as mortality, and the need for dialysis or renal replacement therapy.⁽⁶⁾ It is worth considering, however, that the above phenomenon may be due in part to the decreased uCER caused by reduced GFR, which may result in signal amplification of the biomarkers. Thus, while reporting normalised values alone could be clinically useful as an amplified signal, this could mask the mechanism of biomarker signal increase.⁽⁶⁾

DISEASE ACUITY AND TYPE OF BIOMARKER

The validity of normalisation in AKI and CKD probably differs, with normalisation of values probably more appropriate in chronic rather than acute kidney conditions. It is common for clinicians to normalise urinary excretion of biomarkers to uCr in evaluating kidney injury or disease in chronic conditions,

such as microalbuminuria in diabetes mellitus and proteinuria in nephrotic syndromes, as an estimate of 24-hour urine excretion.^(47,48) It is reported that using absolute values instead of normalised ones lead to falsely low biomarker concentration interpretations in chronic states of reduced GFR, and that spot assessments of biomarkers of CKD normalised to uCr have been shown to be more effective than timed collections in situations such as assessments of microalbuminuria and proteinuria.⁽⁴²⁾ However, as previously discussed, the same cannot be said for acute disease states due to the different effects of acute disease states on biomarker excretion and uCER.

However, despite this simplistic dichotomy, it is clear that clinical research methodology is more complex. One important research question is the utility of urinary biomarkers of AKI in patients with CKD.⁽⁴⁹⁾ Patients with CKD may have ongoing kidney injury. Other concerns also demand our attention. Although proteinuria remains the most important predictor for CKD progression (as an indirect marker of glomerular and tubular injury), do novel biomarkers of AKI (alone or in a panel) provide additional predictive information? In such studies, how should we analyse the information? If uCr normalisation is applied, then it stands to reason that it is assumed that the kidney injury process in CKD is a constant process, which may not be the case. Moreover, it is unlikely that urinary biomarker excretion rates can be normalised and expressed as estimates of 24-hour concentrations. Urinary biomarker assays are often limited by the stability of the biomarker, so most studies would only have spot urine assays. Timed urine collections risk the degradation of biomarkers, as urine often contains proteases. Furthermore, other characteristics of novel urinary biomarkers may impact the applicability of normalisation.

Deciding on the method of analysis is easier if the regular physiology and pathophysiology of the biomarker are known, such as the timing of its appearance, its association with the degree and cause of the injury, the duration of its appearance, disappearance, and the concentrations attained, and the corresponding anatomical injury. Potential biomarkers also have to be considered in the type of evaluation offered.^(50,51) Kidney injury assessments may be made via measurements of kidney function, oxidative stress, cellular and structural injury, immune responses, and fibrosis. Clearly, a panel of biomarkers is attractive in that it is able to provide a more holistic assessment of kidney injury, be it AKI or CKD; however, the research methodology, analysis, interpretation, and clinical application will be challenging.

PATIENT PRESENTATION

Different patient populations and presentations may require different approaches in studying urinary biomarkers. Patients with well-defined potential insults may be easier to study in terms of defining baseline kidney function and the timing of the collection of urine specimens. Examples include patients undergoing open-heart surgery,⁽⁵²⁾ and patients undergoing imaging study using intravenous contrast.⁽⁵³⁾ Other patient populations may be more difficult to study, such as previously healthy patients seen in the

emergency room, who may have a serum creatinine rise of only 26.5 $\mu\text{mol/L}$ (0.3 mg/dL) from baseline (which is stage 1 AKI according to the AKIN criteria).⁽⁵⁴⁾ However, the assessment would be difficult if there is no prior serum creatinine measurement, or if creatinine elevation is a result of volume depletion. Moreover, these patients usually have an indeterminate time of kidney injury. Urinary biomarkers that can help predict immediate AKI or AKI sequelae (e.g. need for dialysis or death) will help improve decisions on admissions.

SUMMARY

Recent evidence suggests that the use of novel urinary biomarkers of kidney injury in the diagnosis and study of acute kidney injury syndromes should probably not be normalised to uCr. However, the best and most appropriate method remains elusive. It is advisable that studies report both absolute and normalised values. Researchers and clinicians need to carefully interpret the findings in the context of the biomarker assayed, the clinical presentation of patients, and the clinical outcomes studied.

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