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REVIEW

Biomarkers in chronic kidney disease, from kidney function to kidney damage

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Abstract

Chronic kidney disease (CKD) typically evolves over many years, with a long latent period when the disease is clinically silent and therefore diagnosis, evaluation and treatment is based mainly on biomarkers that assess kidney function. Glomerular filtration rate (GFR) remains the ideal marker of kidney function. Unfortunately measuring GFR is time consuming and therefore GFR is usually estimated from equations that take into account endogenous filtration markers like serum creatinine (SCr) and cystatin C (CysC). Other biomarkers such as albuminuria may precede kidney function decline and have demonstrated to have strong associations with disease progression and outcomes. New potential biomarkers have arisen with the promise of detecting kidney damage prior to the currently used markers. The aim of this review is to discuss the utility of the GFR estimating equations and biomarkers in CKD and the different clinical settings where these should be applied. The CKD-Epidemiology Collaboration equation performs better than the modification of diet in renal disease equation, especially at GFR above 60 mL/min per 1.73 m^2. Equations combining CysC and SCr perform better than the equations using either CysC or SCr alone and are recommended in situations where CKD needs to be confirmed. Combining creatinine, CysC and urine albumin to creatinine ratio improves risk stratification for kidney disease progression and mortality. Kidney injury molecule and neutrophil gelatinase-associated lipocalin are considered reasonable biomarkers in urine and plasma to determine severity and prognosis of CKD.

Key words: Chronic kidney disease; Estimated glomerular filtration rate; Kidney damage; New biomarkers; MicroRNA

Core tip: Until more accurate equations are developed the chronic kidney disease (CKD) epidemiology collaboration appears to be superior to other glomerular filtration rate (GFR) estimating equations. In circumstances where CKD requires confirmation estimated GFR based on the combined creatinine-cystatin C equation is recommended. The recent advances in molecular biology have resulted in promising biomarkers for CKD detection and prognosis; however more research is needed before applying them into clinical practice.

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INTRODUCTION

Chronic kidney disease (CKD) has become a public-health problem. The definition of CKD was introduced by de National Kidney Foundation (NFK/KDOQI) in 2002 and latter adopted by the international group Kidney Disease Improving Global Outcomes (KDIGO) in 2004. The definition of CKD requires a decrease in kidney function with a glomerular filtration rate (GFR) of less than 60 mL/min per 1.73 m^2 and/or kidney damage for 3 mo or more. Kidney damage refers to pathologic abnormalities documented by biopsy or imaging, alterations in urinary sediment or proteinuria (proteinuria/creatinuria > 200 mg/g, albuminuria/creatinuria > 30 mg/g)[1].

One important aspect about of classification of CKD is that it can usually be detected with non invasive testing. CKD classification is relevant as it has been associated with outcomes such as kidney disease progression, cardiovascular disease and all cause mortality. It is also important as it can allow therapeutic interventions in earlier stages to slow disease progression reduce complications related to decreased estimated GFR (eGFR), cardiovascular (CVD) risk and improve quality of life and survival[2-4]. GFR is the most important marker of kidney function. Unfortunately GFR cannot be easily measured in most clinical or research settings (see below), and therefore estimating equations are based on filtration markers such as serum creatinine (SCr) and cystatin C (CysC). Other biomarkers such as albuminuria may precede kidney function decline and have demonstrated to have strong associations with disease progression and outcomes. New potential biomarkers have arisen with the promise of detecting kidney damage prior to the commonly used markers of kidney disease. The aim of this review is to summarize the most recent findings of most biomarkers in CKD and its implications in clinical practice.

KIDNEY FUNCTION MEASUREMENT

Kidney function estimation was commonly made using SCr concentration, blood urea nitrogen (BUN) level and urine analysis[5]. However accumulating evidence has demonstrated that these biomarkers are not optimal to detect kidney disease in early stages[6-9]. The KDIGO recommends that CKD be diagnosed, classified, and staged by GFR[10]. In clinical practice GFR is crucial for diagnosis, management, drug dosing and prognosis, in addition to its utility for research and public health[11-13]. GFR is the volume of fluid filtered from the glomerular capillaries into the Bowman's capsule per unit time[14,15]. GFR values are associated with age, sex and body surface and are 120 and 130 mL/min per 1.73 m^2 in young men and women, respectively (GFR declines with age)[16-18].

mGFR

Establishing the true GFR is difficult because the filtration process simultaneously takes place in millions of glomeruli and filtrate composition and volume change when passing through the kidney. GFR is measured (mGFR) indirectly as the clearance of filtration markers that are eliminated by the kidney only by glomerular filtration. Clearance can be measured as either plasma or urinary methods that record the clearance of endogenous or exogenous substances by the kidney[11]. As such, an ideal substance is one that is freely filtered at the glomeruli and neither secreted nor reabsorbed by the renal tubules[15,18]. Inulin is an exogenous filtration marker derived from a fructose polymer and is a physiologically inert substance and is considered an ideal substance for mGFR[19,20]. Although inulin clearance is considered the gold-standard method for mGFR[20,21], the need for continuous infusion, multiple blood samples and urine collection, make it cumbersome and expensive to measure and has led to research of alternative methods with other biomarkers[10,21-24].

Other methods for mGFR have also been validated. Soveri *et al*[24] reported that kidney excretion of 51Cr-EDTA or iothalamate, and plasma removal of 51Cr-EDTA or iohexol, using inulin clearance as reference, were sufficiently accurate (P30 > 80%) methods to measure GFR[24]. Among these iohexol is the most recent biomarker for mGFR, it is a non-ionic and non radioactive contrast agent, its molecular weight is 821 Da, has a small extra renal clearance and could be measured only as plasma clearance without the need of urine collections[25]. Some of its other advantages are low expense, wide availability, stability in biologic fluids, and rare adverse reactions when given in a small dose (5 mL of 300 mg/mL iodine)[26,27]. In addition, iohexol does not require a continuous Ⅳ infusion and can be given as an intravenous bolus injection. It can be measured by several different techniques, the most used is the high-performance liquid chromatography (HPLC). However, HPLC requires a great deal of effort which limits its usefulness in the clinical setting[28]. Capillary electrophoresis (CE) a technique in which electrophoretic separations are performed in capillary tubes and is easier and faster than HPLC[29]. Shihabi *et al*[30] demonstrated that the iohexol determination by CE correlates well with HPLC.

However all these methods still require the need of continuous infusion or bolus administration of the marker (subcutaneous or intravenous) and like inulin, their complexity limits their application in clinical practice and epidemiological studies, mostly for the length of time that the procedure entails.

Routinely, GFR is usually estimated from prediction equations which are based on endogenous serum markers like creatinine or CysC in addition to demographic variables such as age, sex and race[13,16,31]. Measured GFR is reserved for situations where eGFR may be inaccurate such as patients in non-steady state, or individuals that

possess different characteristics compared to those where the estimating equation was created such as old age, loss of muscle mass (malnutrition, amputation, paraplegia) obesity, chronic illness or in situations where precise GFR is important, like kidney definition[12,32-34].

GFR estimation

Given the limitations of creatinine as a marker of kidney function, implementation of prediction equations has been widely used to eGFR from endogenous filtration markers without the need of clearance calculation[32]. As mentioned above, SCr and CysC are the most commonly used endogenous filtration markers for eGFR.

Creatinine: SCr derives from creatine degradation with a weight of 113 Da[35]. It is freely filtered but is not reabsorbed or metabolized however a significant percentage of creatinine in the urine derives from proximal tubular secretion[16,36]. One of the requirements for utilizing estimating equations based on SCr is stable kidney function. In addition, non-GFR determinats, such as variation in production associated to dietary intake, or changes in muscle mass, variation in tubular secretion and extrarenal creatinine excretion (associated with advanced kidney disease) need to be accounted when utilizing creatinine[13,32,37,38].

Another important factor that limits the accuracy of equations is the variability in SCr measurement[39]. In a study that examined frozen samples from 554 participants, where creatinine was measured with different assays, the SCr changed on average 0.23 mg/dL. This difference can result in substantial variations in GFR estimation when the SCr concentration is relatively normal[40]. The recognition that small variations in SCr translates in significant changes in kidney function has prompted to standardize creatinine determinations throughout clinical laboratories. In 2006 a standard method was introduced as a reference and was used in combination with the isotope-dilution mass spectrometry method in order to achieve better consensus among methods[41,42].

CysC: CysC has come to light as another marker of kidney function during the past decade. However, its clinical use worldwide remains limited compared with that of SCr[43]. CysC is a non-glycosylated protein produced by all nucleated cells. CysC is freely filtered, reabsorbed and completely metabolized in tubular cells and therefore is not subjected to tubular secretion[44,45]. Compared to creatinine, CysC has a more stable rate of production with less intra variability; however CysC serum levels are also influenced by non GFR determinants, such as uncontrolled thyroid disease, corticosteroid use, age, sex, ethnicity, smoking and adipose tissue[46-48]. In a recent meta analyses, the reciprocal value of CysC was more closely related to GFR (correlation coefficient 0.82 *vs* 0.74) and higher area under de curve (0.93 *vs* 0.84)[49].

In addition, CysC predicts outcomes and the association is stronger than SCr. Shlipak *et al*[50] reported CysC level to have an important association with mortality across the GFR range, including individuals with GFR between 60 and 90 mL/min per 1.73 m^2, grouped as "preclinical kidney disease"[50]. These findings have been reproduced in other studies in older adults where CysC has been shown to be a better predictor of adverse cardiovascular and non cardiovascular outcomes compared to to SCr[51-56]. Potential explanations for these findings may be accounted by the fact that compared to SCr, CysC is not influenced by muscle mass and reflect a better marker of GFR in this population[53]. In addition, these findings have also been reproduced in the general population and CysC estimated GFR has consistently provided a stronger association with outcomes than equations based on SCr eGFR[57].

Estimating equations

Since Effersoe in 1957 developed the first equation to estimate GFR[58], more de 20 equations have been developed. Most of the equations incorporate demographic and clinical variables[39]. The most commonly used equations include Cockroft Gault (CG)[59], 4-modification of diet in renal disease (MDRD)[60,61], 2009 CKDEPI[62] and more recently the equation that combines creatinine and CysC[63]. Since the standardization of creatinine, the CG equation is barely used in clinical practice[39].

CG: The CG formula was created almost thirty years ago in order to estimate creatinine clearance. It was developed in a population of white men and therefore the equation does not take into consideration sex, race and body surface area. Until recently, CG equation was solely utilized for drug dosing however the equation has been recently compared to the widely used equations with similar findings[59,64].

MDRD equation: The MDRD equation was developed in 1999 from a study including 1628 mostly white and non diabetic patients with CKD stages 3 and 4. The original equation included 6-variables and was further abbreviated in year 2000 to a four variable equation that included age, sex, ethnicity, and SCr[60]. In 2006 it was adapted to be used with standardized creatinine[61]. The four variable equation demonstrated to have similar performance compared to the six variable equation[65]. Although the MDRD has demonstrated to have high accuracy for individuals with CKD, the equation underestimates GFR in healthy individuals resulting in false positive diagnosis of CKD in this population[66].

CKD-epidemiology collaboration equation: The CKD epidemiology collaboration (CKD-EPI) was developed in 2009 and resulted from a study that included 8250 participants and was validated in similar cohort of 3900 subjects. Compared to the MDRD coh–ort, the CKD-EPI had higher GFR (68 mL/min per 1.73 m^2 *vs* 40 mL/min per 1.73 m^2), younger

age, included diabetics, blacks and kidney transplant recipients[39,62,67]. Linear regression was employed to estimate the logarithm of measured GFR from standardized SCr concentrations, gender, race, and age. The main objective for the CKD-EPI was to develop an equation that was superior to the MDRD, especially amongst those subjects with GFR > 60 mL/min per 1.73 m^2. Indeed, the same variables were used in CKD-EPI and MDRD equations but CKD-EPI performed better in those with GFR > 60 mL/min per 1.73 m^2. In subjects with GFR > 60 mL/min per 1.73 m^2 the P30% was 88.3% (86.9%-89.7%) and 84.7% (83%-86.3%) for CKD-EPI and MDRD, respectively, while in subjects with GFR < 60 mL/min per 1.73 m^2 the P30% for CKD-EPI was 79.9% (78.1%-81.7%) and for MDRD was 77.2% (75.5%-79%). Furthermore the CKD prevalence was estimated using the CKD-EPI and MDRD Study equations among 16032 adults from the NHANES cohort. Median eGFR by CKD-EPI was almost 10 mL/min per 1.73 m^2 higher than by MDRD. As a result, the CKD-EPI resulted in a significantly lower estimated CKD prevalence than the MDRD equation in the g (11.6% *vs* 13.1%, respectively)[62].

CysC and combined CysC and creatinine equations: In order to overcome the imprecision of creatinine estimating equations, Stevens *et al*[48], developed three eGFR equations for CysC (using CysC alone, CysC with demographic factors, and CysC with SCr and demographic factors) and compared them with mGFR iothalamate and 51-EDTA in 3418 patients. The equation that included CysC with SCr yielded the most accurate GFR estimates (P30 of 89%)[48]. Segarra *et al*[68] found that CysC-based GFR equations performed better than the CKD-EPI equation in a study of 3114 hospitalized patients because creatinine generation is dependent on the presence of muscle mass and malnourishment[68]. Similarly CysC-based GFR was superior than the CKD-EPI equation in certain subgroups of patients in which SCr level may be insensitive to capture reduced kidney function such as patients with chronic liver disease, frail elders, AIDS and malignancy[69-74].

Inker *et al*[63] developed a new GFR estimating equation that was based on CysC alone or in combination with creatinine in a cohort of 5000 subjects and was further validated in a cohort of 1119 subjects with measured GFR. The authors developed two new equations involving CysC (2012 CKD-EPI cys, and 2012 CKD-EPI Cys-cr) and compared them to the 2009 CKD-EPI equation. Bias was not different between the three equations however precision and accuracy was improved with the combined CysC-cr equation. Also in subjects whose eGFRcr was of 45-59 mL/min per 1.73 m^2, the combined equation reclassified correctly 17% to a no CKD category (GFR > 60 mL/min per 1.73 m^2). The authors concluded that the combined equation performed better than equations based on either CysC or SCr and should be used in those subjects where CKD needs to be confirmed[63].

Ongoing studies include the eGFR-C study which is a prospective longitudinal cohort study of 1300 adults with stage 3 CKD that will be followed for 3 years with reference iohexol mGFR. The objective of the study is to evaluate the performance of GFR-estimating equations, including the new equations that incorporate CysC in addition to albuminuria, in order to monitor GFR progression in this populations. Data will be analyzed to assess the impact of race, proteinuria and diabetes on equation performance[75].

Equations, their performance and their implications

When we evaluate the performance of an equation we should take into account bias, precision, and accuracy. Bias has been defined as a median difference between the measured and estimating GFR, precision this is the repeatability or reproducibility of the measurement and accuracy is defined as percentage of eGFR within 30% of measured GFR. Accuracy is probably the best single measure for comparing equations because it incorporates bias and precision. The 2002 KDOQI guidelines concluded that an eGFR within 30% of an mGFR was satisfactory for clinical interpretation, and as a performance metric for accuracy, the guidelines recommended that > 90% of participants in the validation population have eGFR within 30% of the measured GFR (P30 > 90%)[76]. Although accuracy in GFR assessment has significantly improved and bias was decreased with the CKD-EPI equation, precision has not substantially improved. This imprecision is due to random error secondary to variation in non-GFR determinants and GFR measurement error, whilst bias reflects differences between the development and validation populations in measurement methods for GFR, assays for filtration markers, or the relationship of the surrogates to the non-GFR determinants of the filtration marker[13].

In one study conducted by Michels *et al*[77] that included 271 patients with a mean SCr of 1.2 mg/dL, the CG, MDRD, and CKD-EPI equations were compared with mGFR using the I-iothalamate filtration marker (median mGFR 78.2 mL/min per 1.73 m^2), to assess the agreement between equations and examine whether the agreement was influenced by other known variables such as age, weight, body mass index and level of GFR. In general this study concluded that the CKD-EPI equation gives the overall best GFR estimation however the performance was close to MDRD[77].

One of the largest studies where MDRD and CKD-EPI were compared with the aim to assess performance was performed in a population of 12898 individuals from North America, Europe and Australia. The P30 ranged from 59%-95% and was higher for the CKD-EPI than for the MDRD equation in most studies, bias varied according to level of eGFR, was smaller for the CKD-EPI than for the MDRD equation at higher eGFR, but larger at lower eGFR. Table 1 shows the performance comparison of the equations in these populations. Authors from this study concluded that equations did

Table 1 Performance comparison of creatinine-based estimated glomerular filtration rate in North America/Europe/Australia

Ref.	Country	Patients, *n*	mGFR (value mL/min × 1.73 m^2, SD)	eGFR (equation)	Results		
					[1]Bias (95%CI) mL/min × 1.73 m^2	[2]Precision (95%CI)	[3]P30 (95%CI), %
Murata *et al*[180]	United States	5238	I-Iothalamate, urine (55.9, SD 29.7)	MDRD	-4.1	ND	77.6
				CKD-EPI	-0.7		78.4
Levey *et al*[62]	United States	3896	I-Iothalamate, urine and others (68, SD 36)	MDRD	-5.5 (-5.0 to -5.9)	0.274 (0.265-0.283)[4]	80.6 (79.5-82.0)
				CKD-EPI	-2.5 (-2.1 to -2.9)	0.250 (0.241-0.259)[4]	84.1 (83.0-85.3)
Lane *et al*[181]	United States	425	I-Iothalamate, urine (50, IQR 29 to 69)	MDRD	-1.0	15.0[5]	75
				CKD-EPI	-1.7	13.8[5]	80
Michels *et al*[77]	The Netherlands	271	I-Iothalamate, urine (78.2, SD 33)	MDRD	14.6 mL/min	19.9[6]	81.2
				CKD-EPI	12.3 mL/min	12.1[6]	84.5
Tent *et al*[182]	The Netherlands	253 before donation, 253 after donation	I-Iothalamate, urine (115, SD 20) and (73, SD 13)	MDRD	-22 mL/min (20-25)	20 (14-26)[5]	73 (68-79)
				CKD-EPI	-14 mL/min (11-16)	18 (14-22)[5]	89 (85-93)
				MDRD	-15 mL/min (14-16)	12 (9-15)[5]	71 (65-76)
				CKD-EPI	-11 mL/min (9-11)	12 (10-16)[5]	89 (85-93)
Kukla *et al*[183]	United States	107 on steroid-free early post tranplantation 81 on steroid-free at 1 yr	I-Iothalamate, urine (55.5, SD 17) and (56.8, SD 17.7)	MDRD	8.23	17.9[4]	71.7
				CKD-EPI	13.30	21.1[4]	58.5
				MDRD	2.40	15.8[4]	75.0
				CKD-EPI	6.91	17.3[4]	66.7
White *et al*[184]	Canada	207	Tc-DTPA, plasma (58, SD 22)	MDRD	-7.4	14.4[5]	79 (73-84)
				CKD-EPI	-5.2	15.7[5]	84 (78- 88)
Pöge *et al*[185]	Germany	170	Tc-DTPA, plasma (39.6, IQR 11.8 to 82.9)	MDRD	4.49	10.0[6]	71.8
				CKD-EPI	8.07	10.9[6]	64.1
Jones[186]	Australia	169	Tc-DTPA, plasma (75, IQR 5 to 150)	MDRD	-3[7]	ND	81
				CKD-EPI	-1.5[7]		86
Cirillo *et al*[187]	Italy	356	Inulina, plasma (71.5, SD 36.3)	MDRD	-5.2	14.9[6]	87.4
				CKD-EPI	-0.9	13.2[6]	88.2
Eriksen *et al*[188]	Norway	1621	Iohexol, plasma (91.7, SD 14.4)	MDRD	1.3 (0.4-2.1)	18.2 (17.2-19.5)[5]	93 (91-94)
				CKD-EPI	2.9 (2.2-3.5)	15.4 (14.5-16.3)[5]	95 (94-96)
Redal-Baigorri *et al*[189]	Denmark	185	Cr-EDTA, plasma (85.1, SD 20.3)	MDRD	0.81 (IQR, -1.56 to 3.19)	16.49[6]	88.6
				CKD-EPI	1.16 (IQR, -0.76 to 3.09)	13.37[6]	89.7

[1]Computed as estimated GFR minus measured GFR. Positive numbers indicate overestimation and negative numbers indicate underestimation of measured GFR. Smaller absolute values indicate lesser bias; [2]Lower values indicate greater precision; [3]Higher values indicate greater accuracy. Among the 3 studies (14, 18, 19) that reported alternative measures of accuracy, results were consistent with P30 in all. In addition to P30, references 14, 18, and 19 reported P10; Reference 14 also reported P20; [4]Evaluated as the root mean square error for the regression of estimated GFR on measured GFR; [5]Evaluated as the IQR for the differences between estimated and measured GFR; [6]Evaluated as the SD of the differences between estimated and measured GFR; [7]Converted to raw scale by multiplying percentage of bias by measured GFR. CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; mGFR: Measure glomerular filtration rate; eGFR: Estimated glomerular filtration rate; IQR: Interquartile range; MDRD: Modification of diet in renal disease; ND: Not documented; P30: Percentage of estimated GFR values within 30% of measured GFR; Tc-DTPA: Technetium-diethylene-triamine-pentaacetate; Cr-EDTA: Chromium-ethylenediamine-tetraacetic-acid. Adapted from Earley *et al*[39].

not perform as well in regions outside North America, Europe, and Australia. In Asia and Africa, equations were less accurate (P30 ranged from 29%-94%). Equation performance can be improved by deriving local "race/ethnicity" coefficients; however, the new equations are more accurate in the Caucasian populations. The coefficients also do not seem to be generalizable beyond the local population presumably reflecting differences in SCr generation due to racial, ethnic, and regional variations in muscle mass and diet, and use of non standardized SCr[39].

Thus far the new equation CKD-EPI Cys-cr has been evaluated in diverse populations. The berlin initiative study (BIS) included 610 older adults with a mean SCr level

of 1.0 mg/dL, and mean CysC level of 1.15 mg/L. The study intended to assess the performance of the CKD-EPI Cys-cr equations compared to the mGFR by iohexol. A major finding of this study was that CysC had a stronger association with mGFR than creatinine and the best GFR estimation was derived from a combined Cys-cr equation (named BIS-2)[78]. The combined CKD-EPI Cys-cr equation performed well in Japanese and Chinese individuals[79-81]. One recent study compared the CKD-EPI Cys-cr and other four approved equations in a cohort of 788 adult Chinese patients and a Tc_DPTA mGFR of 76 mL/min per 1.73 m^2. Compared to other equations, the CKD-EPI Cys-cr had less bias, (-4.11 mL/min per 1.73 m^2) and higher accuracy (P30% of 77.03%)[80]. In a population of almost 700 kidney transplant recipients the performance of the CKD-EPI Cys-cr was superior showing less bias and better accuracy compared with 2009 CKD-EPI, using inulin mGFR as reference[82].

In addition, it is important to mention that the performance of the equations is affected not only by demographic and clinical factors but by the reference method considered as the gold standard to measure GFR in different populations[83-85].

From the epidemiological standpoint, CKD prevalence was assessed in diverse populations comparing the MDRD and CKE-EPI equation[62]. For example, the Atherosclerosis Risk in Communities Study reclassified 43.5% to a higher eGFR category compared with CKD stage 3 for MDRD[86]. The AusDiab (Australian Diabetes, Obesity and Lifestyle) study reclassified 266 participants identified as having CKD with MDRD to no CKD with CKD-EPI, decreasing the prevalence of CKD in adults > 25 year 1.9% in Australia[87]. The kidney early evaluation program included 116321 individuals where 17.5% and 2.7% were reclassified to higher or lower eGFR categories, respectively, when compared with MDRD[88].

Reclassifying subjects to a higher GFR has demonstrated to translate in a lower risk for outcomes. In a recent meta-analysis, the CKD-EPI and MDRD equations were compared with respect to CKD stage and risk prediction in a 1.1 million adults from distinct cohorts followed over seven years. Outcomes included mortality, cardiovascular mortality, and kidney failure. In this study CKD-EPI reclassified to a higher and lower estimated GFR category 24.4% and 0.6% respectively, compared with the MDRD, and when the CKD-EPI equation was used, the prevalence of CKD was reduced by 2.4 percent. Furthermore, in individuals with MDRD eGFR of 45-59 mL/min per 1.73 m^2, the CKD-EPI creatinine equation reclassified 34.7% to eGFR of 60-89 mL/min per 1.73 m^2 and 1.2% to eGFR of 30-44 mL/min per 1.73 m^2. Individuals reclassified to a higher eGFR category had 0.80, 0.73, and 0.49 lower adjusted risks for death, cardiovascular disease, mortality, respectively, than those not reclassified. Overall net reclassification favored the CKD-EPI over the MDRD for the three outcomes[86].

Rule *et al*[89] evaluated the association of CKD risk factors (urine albumin, lipid profile, uric acid, hypertension, diabetes and smoking) with eGFR based on Cr and/or CysC and compared them with iothalamate mGFR in 1150 subjects with a mean age 65 year and mean mGFR of 80 mL/min per 1.73 m^2. Authors concluded that the association between most of the risk factors was stronger for CysC than SCr and CysC was a better predictor for risk stratification and management of CKD than SCr eGFR[89].

These data demonstrates that the CKD-EPI equation is superior for GFR estimation leading to fewer false-positive diagnoses of CKD. In addition the CKD-EPI equation translates in a decreased prevalence of CKD and is associated with a more precise risk prediction for outcomes and prognosis. The KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease, based on this evidence recommends that CKD be diagnosed, classified, and staged by eGFR and suggests CKD-EPI should be utilized as the preferred equation[1].

Other endogenous biomarkers for kidney function

Blood urea nitrogen: BUN increases as GFR declines however is less valuable than the SCr since the BUN can vary independently of the GFR. The production rate of urea is not stable and increases with rich protein diets or tissue breakdown such as bleeding, muscle trauma or steroid administration. On the other hand a very low protein diet or liver failure can decrease BUN without affecting GFR[32,90].

B2-microglobulin: B2-microglobulin (B2-M) is a small molecule of 11.8 kDa and constitutes a class Ⅰ HLA, is present in all nucleated cells in the body, and has a large quantity of immune cells like lymphocytes and monocytes. It has the characteristic that it is freely filtered in the glomeruli and is reabsorbed and metabolized in the proximal tubule[91]. Levels of B2-M are elevated in kidney disease, in addition to other conditions such as malignancies, autoimmune diseases, infections and aging[92]. There is data to demonstrate that plasma B2-M is a good endogenous marker of GFR and that in the context of GFR decline the increase of serum B2-M occurs prior than SCr. B2-M has been associated with death in a cohort of 1034 elderly subjects and appeared to be superior than CysC, even after adjustment for known risk factors[93,94]. Lack of further studies in the last decade however has limited the utility of this biomarker in clinical practice.

KIDNEY DAMAGE

The kidney damage refers to pathologic abnormalities documented by biopsy or imaging, alterations in urinary sediment or proteinuria (proteinuria/creatinuria > 200 mg/g, albuminuria/creatinuria > 30 mg/g). Damage usually precedes alterations in functions. For instance it is known that albuminuria precedes the decrease in

eGFR, hence the importance to count with markers of renal damage in stages that are blind for current markers of renal function decline. In theory this could facilitate early diagnosis, guide interventions and monitor disease progression.

Albuminuria

Albumin excretion rate (AER) can be determined in 24 h urine collections or in spot collections. Increases should be confirmed in at least two of three samples, within a period from 3 to 6 mo[11]. Microalbuminuria, or incipient nephropathy, is defined as an AER of 20-200 μg/min in timed samples, or 30-300 mg/24 h in 24 h samples, however spot collections are accurate enough that they can replace 24 h collections and these are now strongly recommended by the most recent guidelines[1,95].

The corresponding values that define microalbuminuria in a urine sample are AER > 30 mg/24 h or an albumin-creatinine ratio (ACR) of 30-300 mg/g (0.3-3 mg/mmol). Higher values indicate macroalbuminuria, also called clinical nephropathy[1]. Taking these values into account the prevalence of microalbuminuria in 4101 individuals of NHANES (1999-2000) with ACR 30-300 mg/g and ACR > 300 mg/g was 7.3% and 1.7% in men and 10.4% and 0.9% in females, respectively[96].

The threshold of ACR > 30 mg/g to define kidney damage has been validated as a risk factor for adverse events in different populations. In high risk patients for CKD, the ACR > 30 mg/g is has demonstrated to be a risk factor cardiovascular (CV) death and all cause mortality, progression of kidney disease, acute kidney injury (AKI) and kidney failure[97,98]. Likewise, these findings have been reproduced in low risk cohorts. In more than 1 million participants from 21 cohorts, ACR > 30 mg/g and ACR > 300 mg/g were associated with higher risk for death (HR of 1.6 and 2, respectively). Moreover the risk for CV mortality was two-fold higher with ACR > 30 mg/g compared to those with ACR of 5 mg/g and this risk persisted after adjustment for GFR and other known risk factors. This risk also applies to ACR levels < 30 mg/g. In study of Waheed *et al*[99], ACR of 10 mg/g compared to 5 mg/g was associated with all cause mortality. This however may not necessarily reflect kidney damage and may be a marker of endothelial dysfunction.

On the basis of the linear association of albuminuria with progression of CKD, end stage renal disease (ESRD), and all cause of mortality independent of eGRF, albuminuria staging has been added in the 2012 KDIGO guidelines.

Combination of biomarkers

Combining albuminuria with eGFR improves the prediction of CKD progression. This was demonstrated in the Nord-Trondelag Health (HUNT-2) study that included 65589 participants, where albuminuria and eGFR independently predicted kidney disease progression and the combination of both markers was superior to predict those subjects at highest risk for ESRD development[100]. In a large prospective cohort involving more than 26000 subjects, the authors evaluated whether combining eGFR creatinine, CysC, and urine ACR could improve risk prediction when compared with eGFR alone. In this cohort the adjusted mortality risk was six fold higher in patients with CKD identified by all three markers and was also three fold higher in patients with CKD defined by both eGFR Cys-cr, compared to those with CKD defined by eGFR creatinine alone. The risk for CKD progression to kidney failure was higher among patients with CKD defined by all three markers. The authors concluded that adding CysC to SCr and ACR was superior for prediction for kidney disease progression and death[101].

New biomarkers for kidney damage

Although albuminuria is a powerful biomarker, it may occur after the damage has occurred or may not be present in other types of kidney damage such as tubulointerstitial disease and hypertensive kidney disease. This has led to the search for new biomarkers that are also non-invasive and could better correlate with the etiology of the kidney disease. Moreover; early identification of patients with CKD could allow implementing early interventions to reduce CVD or CKD progression. In the next few paragraphs we describe the most promising biomarkers in CKD (Table 2) and its utility (Table 3).

Kidney injury molecule

Kidney injury molecule (KIM-1) is a transmembrane protein is a type 1 transmembrane protein whose expression has been upregulated after kidne injury[102,103]. KIM-1 is an early biomarker for proximal tubular damage since it is expressed in the urine during the first 12 h of the tubular injury[104]. Experimental and clinical studies have demonstrated high KIM-1 expression in areas of fibrosis and inflammation. In murine models with polycystic kidney disease, KIM 1 is highly expressed in renal tubules, it associates with interstitial fibrosis in human allografts and in type 1 diabetes mellitus regression of microalbuminuria has been associated with lower urinary levels of KIM-1[105-108].

Persistent expression of KIM-1 has been associated to inflammation characterized by high monocyte chemoattractant protein-1 (MCP-1) levels[109]. In contrast, in experimental models, mice with mutant KIM-1 are protected from fibrosis and had lower inflammatory markers[110]. In a retrospective analysis of 107 diabetic type 1 with CKD stages 1-3 (AER > 500 mg/24 h) followed for 5-15 years, 63% of those subjects with higher KIM-1 levels (> 97 pg/mL) progressed to ESRD whereas only 20% of patients with lower levels progressed. In addition baseline plasma KIM-1 levels correlated with rate of eGFR decline after adjustment for baseline urinary albumin-to-creatinine ratio, eGFR, and Hb1Ac[111]. KIM-1 may represent a promising marker for the future. Larger

Table 2 Novel biomarkers in chronic kidney disease

Biomarker source	Ref.	Population/type of study	Commentaries
u-LFABP Urinary	Nielsen *et al*[190]	227 newly diagnosed type 1 diabetic patients/longitudinal	Baseline u-LFABP levels predicted development of microalbuminuria (HR = 2.3, 95%CI: 1.1-4.6), and predicted mortality (HR = 3.0, 95%CI: 1.3-7.0)
NAG Urinary	Kern *et al*[191]	87 type 1 diabetics with microalbuminuria and 174 controls/ longitudinal	Baseline NAG independently predicted microalbuminuria (OR = 1.86, $P < 0.001$) and macroalbuminuria (OR = 2.26, $P < 0.001$) but risk was attenuated in multivariate models
CTGF Urinary	Nguyen *et al*[192]	318 type 1 diabetic patients and 29 control subjects/cross sectional	U-CGTF was significantly higher in diabetic nephropathy than micro o normoalbuminuria. U-CGTF correlated with albuminuria and GFR
IL-18 Kidney tissue	Miyauchi *et al*[193]	12 type 2 diabetes with overt nephropathy and 7 patients with MCD/ cross sectional	IL-18 expression in tubular cells was observed highly observed (83%) in patients with diabetes but only observed in 14.3% of MCD
ApoA-Ⅳ Plasma	Boes *et al*[194]	177 non-diabetic patients with mild to modetare renal CKD/longitudinal	Baseline ApoA-Ⅳ was a significant predictor of disease progression (HR = 1.062, 95%CI: 1.018-1.108) and patients with level above the median had significantly faster progression compared with patients with level below median ($P < 0.0001$)
CD14 mononuclear cells Urinary	Zhou *et al*[195]	16 patients with autosomal dominat polycystic kidney disease/longitudinal	Baseline urinary CD14 mononuclear cells correlated with 2 yr change in total kidney volume in males
NGAL	Bolignano *et al*[121]	33 patients with glomerulonephritis and proteinuria > 1 g per day/cross sectional	u-NGAL was higher in glomerulonephritis compared with controls and significantly correlated with serum creatinine and urinary protein excretion
Urinary	Smith *et al*[124]	158 patients with CKD stages 3 and 4/longitudinal	u-NCR was associated with a higher risk of death and initiation of renal replacement therapy
Urinary	Bolignano *et al*[125]	96 white patients with CKD/ longitudinal	Baseline urinary and serum NGAL were predictors of CKD progression
Urinary/serum	Shen *et al*[119]	92 patients with chronic glomerulonephritis CKD stage 2-4, and 20 control subjects/longitudinal	s-NGAL levels were higher compared to controls and negatively correlated with the eGFR Patients with sNGAL level > 246 ng/mL had a poor 2 yr renal survival compared with the control group
Serum	Bhavsar *et al*[123]	286 participants from the ARIC and 143 matched controls/longitudinal	Higher quiartiles of NGAL (but no KIM-1) were associated with incident CKD
KIM-1 Serum	Krolewski *et al*[111]	107 diabetic type 1 with CKD 1-3 (AER > 500 mg/24 h)/longitudinal	Baseline plasma KIM-1 levels correlated with rate of eGFR decline KIM-1 levels (> 97 pg/mL) correlated with progression to ESRD
Urinary	Peters *et al*[109]	65 patients with Proteinuric IgAN and 65 control subjects/longitudinal	In patients with IgAN uKIM-1 excretion was significantly higher than controls uKIM-1 is independently predictor of ESRD
FGF-23	Nakano *et al*[134]	738 Japanese patients with CKD stages 1-5/longitudinal	Levels of FGF-23 associated with kidney function decline or initiation renal replacement therapy
Serum	Fliser *et al*[137]	227 non diabetic patients with CKD stages 1-4/longitudinal	FGF-23 was an independent predictor of CKD progression
	Lee *et al*[138]	380 patients with type 2 diabetes/ longitudinal	Levels of FGF-23 was associated with increased risk of ESRD and was a significant risk factor for all cause mortality

u-LFABP: Liver-type fatty acid-binding protein; NAG: N-Acetyl-b-O-glucosaminidase; CTGF: Connective tissue growth factor; IL-18: Interleukin-18; ApoA-Ⅳ: Apolipoprotein A-Ⅳ; NGAL: Neutrophil gelatinase associated lipocalin; MCD: Minimal change disease; ARIC: Atherosclerosis Risk In communities; IgAN: IgA nephropathy; u-NCR: u-NGAL to creatinine ratio; eGFR: Estimated glomerular filtration rate; FGF-23: Fibroblast growth factor 23; CKD: Chronic kidney disease; KIM-1: Kidney injury molecule; AER: Albumin excretion rate; GFR: Glomerular filtration rate; U-CGTF: Urinary-connective tissue growth factor; u-NGAL: Urinary-NGAL; s-NGAL: Serum-NGAL; ESRD: End stage renal disease.

Table 3 Utility of new biomarkers in chronic kidney disease

Biomarker	Origin	Outcome assessed
Urinary liver-type fatty acid-binding protein	Proximal tubule	Diabetic Nephropathy: Microalbuminuria and mortality
Urinary N-Acetyl-b-O-glucosaminidase	Proximal tubule	Diabetic Nephropathy: Albuminuria
Urinary connective tissue growth factor	Proximal tubule	Diabetic Nephropathy: Glomerular filtration rate decline
Interleukin-18	Tubulointerstitial	Diabetic Nephropathy: Albuminuria
Apolipoprotein A-Ⅳ	Intestinal enterocytes	CKD: CKD Progression
Urinary CD14 mononuclear cells		Polycystic kidney disease: Kidney volume
Neutrophil gelatinase associated lipocalin	Proximal and distal tubule	Glomerulonephritis: GFR and proteinuria CKD: CKD progression, renal replacement therapy and mortality
Kidney injury molecule-1	Proximal tubule	CKD: CKD progression and renal replacement therapy
Fibroblast growth factor-23	Osteocytes and osteoblasts	Diabetic Nephropathy and others CKD: CKD progression and mortality
Urinary retinol binding protein 4	Proximal tubule	Congenital or acquired tubular dysfunction: Proximal tubule dysfunction

CKD: Chronic kidney disease.

studies however are still warranted before KIM-1 could be applied routinely in clinical practice.

Neutrophil gelatinase-associated lipocalin: Neutrophil gelatinase-associated lipocalin (NGAL) is a lipocalin iron-carrying protein of 25 kDa and is part of the well-defined super family of proteins called lipocalins, is expressed by tubular renal epithelial cells following tubulointerstitial injury[112-114]. NGAL has been an established marker for acute kidney injury however its role in CKD is less studied[115-119]. In patients with IgA nephropathy urinary NGAL level was higher compared to controls and was also associated with disease severity[120]. In patients with glomerular proteinuria above 1 g/24 h and in patients with polycystic kidney disease, NGAL levels were higher compared to controls and significantly correlated to SCr[121,122]. NGAL has also been associated to incident CKD progression in adults. In a community based population of 286 subjects, NGAL was evaluated as an independent risk factor for incident CKD. Those in the highest quartile of NGAL had a higher risk for incident CKD, effect that was attenuated after adjustment for creatinuria and albuminuria[123]. In a cohort of 158 adults with stage 3 or 4 CKD, urinary NGAL to creatinine ratio was associated with mortality and renal replacement therapy and this risk was independent of kidney and CV risk factors[124]. Similar results were found in a cohort of 96 CKD patients followed for 18.5 mo where plasma and urinary NGAL predicted CKD progression after adjustment for eGFR[125].

Thus far there is evidence to support that NGAL levels either in plasma or urine can predict kidney disease progression independent of GFR, however the data is limited by the number of participants and larger studies are needed before establishing this biomarker in clinical practice.

Fibroblast growth factor 23: Fibroblast growth factor 23 (FGF-23) is 32-kDa phosphaturic protein secreted by bone osteocytes. Among its functions is to promote phosphate excretion, decrease calcitriol production and suppress parathyroid hormone[126-128]. In CKD the increase of FGF-23 level precedes the decline in vitamin 1,25-$(OH)_2$ vitamin D3 and the increase of PTH level. Although FGF-23 is higher in patients with moderate to severe CKD, there is data to support that the rise of FGF-23 occurs earlier in the disease. In the past decade several studies have found an association between high FGF-23 levels, kidney disease progression and mortality in subjects with CKD[129-132]. In a cohort of 227 non diabetic patients with CKD followed for more than 4 years, FGF-23 was an independent risk factor for kidney disease progression. Likewise Semba *et al*[133] in 701 healthy women (mean eGFR 60 mL/min × 1.73 m^2), and Nakano *et al*[134] in 738 Japanese patients with CKD stages 1-5 (mean eGFR 35 mL/min × 1.73 m^2) reported that increasing levels of FGF-23 associated with decline in kidney function or initiation renal replacement therapy after a follow-up of 2 and 4.4 years, respectively. In addition, in patients undergoing renal replacement therapy, elevated FGF-23 levels have been associated with CV outcomes such as left ventricular hypertrophy and increased risk of mortality[133-138]. It is important to mention that this association has been independent of phosphate levels and CKD stage.

Asymmetric dimethylarginine: Asymmetric dimethylarginine (ADMA) is an aminoacid of 202 Da, it is normally synthesized intracellularly and eliminated through the urine. One of its adverse effects is the inhibition of the nitric oxide synthases and this mechanism has been associated to adverse cardiovascular side effects[139,140]. As kidney function deteriorates ADMA levels increase and this has been associated to kidney parenchymal damage through the decrease in dimethylarginine-dimethylamino-hydrolase[141,142]. ADMA has been associated to CKD progression. In the diabetic and non diabetic population, ADMA levels are higher as GFR declines and are associated with rapid kidney function decline[143,144]. In a recent study of 164 CKD patients followed for one year, elevated ADMA and markers of oxidative stress were strong predictors of progression in patients with CKD stages 3-4[145]. Moreover, ADMA has been associated to death and CV events in the CKD population[146,147]. Some authors had considered ADMA to be the "missing link" between cardiovascular disease and CKD[139]. Whether counteracting the effects of ADMA in CKD should be explored as a strategy to prevent cardiorenal complications would need to be confirmed in larger studies.

MCP-1: MCP-1 belongs to the group of inflammatory chemokines[148,149]. Expression of MCP-1 is up regulated in kidney diseases that have a sustained inflammatory response, such as in diabetic nephropathy and lupus nephritis[150,151]. Studies have demonstrated glomerular and tubular kidney cells release MCP-1 in response to high glucose levels and urine levels of MCP-1 are increased in diabetic nephropathy[152,153]. Likewise MCP-1 levels in urine are over expressed in active lupus nephritis[151-154]. Emerging evidence suggest that MCP-1 has a significant role in the pathogenesis of many kidney diseases and urinary MCP-1 is a promising biomarker with diagnostic and prognostic implications[155-157].

Urine retinol-binding protein 4: Urine retinol-binding protein 4 (uRBP4) is a 21 KDa protein derived of plasma RBP4 (pRBP4), is an integrant of the lipocalin family and is produced mainly in the liver but also in the adipose tissue where it performs as an adipokine that has been linked to insulin resistance and obesity[158,159]. Unlike other biomarkers such as NGAL and KIM-1, uRBP4 is currently the most sensitive functional biomarker of proximal tubule. pRBP4 is filtered at the glomerulus and completely reabsorbed in the proximal tubule. In addition, it is known that variation levels of pRBP4 (secondary to nutrition, vitamin A levels, liver disease and infection) have small effect on uRBP 4 as a

biomarker[160]. Sensitivity for uRBP4 however decreases as kidney function declines due to false positives that occur in the presence of glomerular disease[161]. This marker was been useful in several diseases related with proximal tubule dysfunction, either hereditary, such as Fanconi syndrome, dent type 1 syndrome and lowe syndrome[162], or acquired conditions that directly affect proximal tubule such as drug toxicity in human immunodeficiency virus, cadmium toxicity, plasma cell dyscrasias, AKI diagnosis and other renal tubulointerstitial diseases[163]. Amer *et al*[164] assessed the prognostic value in renal transplantation of a panel of urinary proteins in 221 patients at 1 year post transplant and reported that patients with glomerular lesions had higher albuminuria than patients with normal histology, and in patients with tubulointerstitial disease, uRBP4 has over expressed. In addition, uRBP4 was a risk factor for long term allograft loss and this risk was independent of kidney biopsy histology and albuminuria[164].

Future directions

Advances in technology during the last decade have enlightened our knowledge regarding genetic regulatory pathways. A fast growing arena are the microRNAs (miRNAs), the current number of miRNAs in humans are estimated to be between 700 and 1000, and they have been implicated in several physiological events as well pathologic process, including kidney disease[165]. miRNA have selective expression by different organs, and the kidney expresses mostly miRNA 192, 194, 204, 215 and 216 which have been implicated in proliferation, migration and structure of renal cells[166,167]. Little changes in these molecules have implications in kidney function, for instance it is know that deletion of the miRNA 30 family decreases renal cells, affects blood pressure and develop vascular damage and extensive fibrosis[168]. Other miRNAs are related with diverse pathophysiologic process, miRNA 155 is associated to blood pressure control through down regulation of type 1 angiotensin Ⅱ receptor[169,170], miRNA 192 and 200 families are related to fibrotic damage in diabetic nephropathy manly by regulation of transforming growth factor beta[171], miRNA 15, 17 and 31 are associated with cystogenesis in polycystic kidney disease[172], and finally miRNA 142, 155 and 223 are increased in acute rejection related to activation of epithelial cells and blood mononuclear cells[173], and can discriminate between acute humoral rejection and cellular rejection[174]. MiRNA expression pathways have also been evaluated as diagnostic biomarkers in other pathologies. In a study of lupus nephritis patients miRNA 27 and 192 in urine could identified in renal biopsies of lupus patients with nephritis[175]. The knowledge of miRNA in health and disease remains with several questions concerning its regulation, production and specific target. In addition most studies have measured miRNA in tissue and therefore become cumbersome to measure in clinical practice. Studies evaluating its utility in plasma and urine are urgently needed. Nonetheless this is a rapidly growing field and future research may provide a better understanding of the pathophysiology in kidney disease and may reveal potential diagnosis and therapeutic options.

Not only in the area of proteomics (NGAL, KIM-1, *etc.*) and transcriptomics (miRNAs) have the kidney markers evolved, the latest piece added to the puzzle corresponds to metabolomics, and as it name points out, is the measure of end products of basic metabolic molecules. These end products could improve the utility of other type of biomarkers[176]. Currently, metabolomics in kidney disease have mainly been studied in uremia, renal cell carcinoma, glomerulonephritis, diabetes mellitus, polycystic kidney disease and drug related nephrotoxicity. For instance in patients with drug related nephrotoxicity, end products from amino acids and simple sugars increase in urine before tissular changes become apparent. The latter has been described with antibiotics[177], and immunosupression therapy, for example, the increase of metabolomic end products during the first month after cyclosporine predicts kidney damage[178]. Similarly metabolomics has been associated to several metabolic profiles (mainly amino acids, derivatives of sugar and phospholipids) that could be useful in the diagnosis and prognosis of different types of renal disease as diabetic nephropathy, IgA nephropathy and other glomerulonephritis, in addition to diagnosis, metabolomics offers a promising future in the area of pharmaco-metabolomics, which could lead to personalized therapeutic targets[179]. At this point metabolomics main limitation is related to problems with specificity and technical variability and is not ready to be implemented in clinical practice.

CONCLUSION

During the last century, SCr has been the most used biomarker to screen and diagnose kidney disease. SCr however has several limitations and should be utilized only in estimating equations. The CKD-EPI is more generalizable and performs better than the MDRD estimating equation, especially in the healthy population. More recently the GFR estimating equation that combines SCr and CysC has demonstrated to be superior than equations that use either SCr or CysC alone, and is recommended in specific conditions, such as when confirmation of CKD is required. Albuminuria remains one of the strongest risk factors for outcomes and the combination of SCr, CysC and urinary albumin to creatinine ratio improves risk stratification predicts CKD progression and mortality.

In the last decade several other promising biomarkers have emerged. However, although these biomarkers are highly sensitive and specific and have allowed an earlier diagnosis of kidney disease with promising results; none of them have been validated to make clinical decisions upon their positivity. These biomarkers should have the potential to indicate injury type or the specific site of harm. It is improbable however that one biomarker would be sufficient to guide intervention upon their result. Larger and long term studies are warranted before applying these biomarkers in clinical practice. The CKD

Biomarkers Consortium has 15 ongoing studies with the aim to develop and validate novel biomarkers for CKD. In the meantime current biomarkers in CKD should be cautiously implemented acknowledging its strengths and limitations.

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