Biomarkers in chronic kidney disease: a review

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Chronic kidney disease (CKD) is a major public health problem. The classification of CKD by KDOQI and KDIGO and the routine eGFR reporting have resulted in increased identification of CKD. It is important to be able to identify those at high risk of CKD progression and its associated cardiovascular disease (CVD). Proteinuria is the most sensitive marker of CKD progression in clinical practice, especially when combined with eGFR, but these have limitations. Hence, early, more sensitive, biomarkers are required. Recently, promising biomarkers have been identified for CKD progression and its associated CVD morbidity and mortality. These may be more sensitive biomarkers of kidney function, the underlying pathophysiological processes, and/or cardiovascular risk. Although there are some common pathways to CKD progression, there are many primary causes, each with its own specific pathophysiological mechanism. Hence, a panel measuring multiple biomarkers including disease-specific biomarkers may be required. Large, longitudinal observational studies are needed to validate candidate biomarkers in a broad range of populations prior to implementation into routine CKD management. Recent renal biomarkers discovered include neutrophil gelatinase-associated lipocalin, kidney injury molecule-1, and liver-type fatty acid-binding protein. Although none are ready for use in clinical practice, it is timely to review the role of such biomarkers in predicting CKD progression and/or CVD risk in CKD.

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Chronic kidney disease (CKD) is a major public health problem affecting 13.5% of the adult Australian[1] and 11% of the US population.[2] The introduction of kidney function estimating equations[3,4] and CKD classifications by the NKF KDOQI (National Kidney Foundation Kidney Disease Outcomes Quality Initiative[5] and the KDIGO (Kidney Disease: Improving Global Outcomes)[6] have highlighted the condition and facilitated its diagnosis. Hence, early identification of those likely to progress to end-stage renal disease (ESRD) has become increasingly important. Existing measures such as estimated glomerular filtration rate (eGFR) and proteinuria assist with this stratification.[7,8] However, proteinuria has limitations as a biomarker of CKD progression and response to interventions.[9] Therefore, new validated biomarkers are required for CKD progression and cardiovascular disease (CVD) risk. Proteomic studies will provide data regarding potential biomarkers in CKD[10–12] but these must be placed in clinical context.[13,14] In view of recent developments in renal biomarker discovery, it is timely to review the cross-sectional and longitudinal observational studies investigating these in CKD.

This review will first address biomarkers in CKD progression and, second, biomarkers of CVD in CKD. Biomarkers are reviewed according to the main mechanisms they reflect (see Table 1 and Figure 1), although there is some overlap. Reviewed studies are summarized in Table 2. Biomarker assay variability, reliability, and stability are beyond the scope of this review.

BIOMARKER DEVELOPMENT AND TESTING IN PATIENTS

There are several clinical studies where biomarkers are being tested. The CaNPREDDICT (Canadian Study of Prediction of Risk and Evolution to Dialysis, Death and Interim Cardiovascular Events Over Time) study is testing biomarkers 6 monthly in 2500 prevalent patients with eGFR 15–45 ml/min in ~50 centers across Canada over 36 months. The Spanish NEFRONA Project (Usefulness of imaging techniques and novel biomarkers in the prediction of cardiovascular risk in patients with CKD in Spain) is recruiting 2661 patients and 843 controls into a prospective observational study.[15] Outcomes include cardiovascular

Table 1 | Renal biomarkers according to the pathophysiological process

	Biomarker
Kidney function (GFR)	Cystatin C β-Trace protein
Tubulointerstitial injury	NGAL KIM-1 NAG L-FABP
Glomerular injury	Podocin Nephrin Podocalyxin
Endothelial dysfunction	ADMA
Oxidative stress	Ox-LDL AOPP TBARS Plasma and urinary F_2-isoprostanes MDA Protein reduced thiols TAS Protein carbonyls AGE Urinary 8-hydroxydeoxy guanosine 4-hydroxy-nonenal Antioxidant enzyme activities (e.g., superoxide dismutase, glutathione peroxidase, catalase) GGT
Inflammation	CRP and hs-CRP PTX3 sTNFrii IL-18 Tenascin TIMP-1
Fibrosis	TGF-β1
Cardiovascular dysfunction	ANP BNP and NT-proBNP cTnT Adrenomedullin
Metabolic disorders	Adiponectin FGF-23 ApoA-IV

Abbreviations: ADMA, asymmetric dimethylarginine; AGE, advanced glycation end product; ANP, atrial natriuretic peptide; AOPP, advanced oxidation protein products; ApoA-IV, apolipoprotein A-IV; BNP, brain natriuretic peptide; CRP, C-reactive protein; cTnT, cardiac troponin T; FGF-23, fibroblast growth factor-23; GFR, glomerular filtration rate; GGT, γ-glutamyltransferase; hs-CRP, high-sensitivity-CRP; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid-binding protein; MDA, malondialdehyde; NAG, *N*-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal brain natriuretic peptide; Ox-LDL, oxidized low-density lipoproteins; PTX3, pentraxin 3; sTNFrii, soluble tumor necrosis factor receptor II; TAS, total antioxidant status; TBARS, thiobarbituric acid reactive substances; TGF-β1, transforming growth factor-β1; TIMP-1, tissue inhibitor of metalloproteinases-1.

events and mortality, carotid intima-media thickness, a composite atherosclerosis score, and biomarkers.

Other studies include the MMKD (Mild to Moderate Kidney Disease) study that recruited patients in Germany, Austria, and South Tyrol[16,17] and reported a range of biomarkers.[17–22] The CRIC (Chronic Renal Insufficiency Cohort)[23,24] and the CRIB (Chronic Renal Impairment in Birmingham) study are both focusing on traditional and nontraditional cardiovascular risk factors in CKD.[25] CKD studies have been reported from Japan,[26] China,[27] and Germany (Cooperative Health Research in the Region Augsburg (KORA) Study Group).[28] Some of these have measured multiple biomarkers.

STUDY CHARACTERISTICS AND SELECTION CRITERIA

Studies were identified through a systematic search of PubMed using key terms: biomarkers, chronic kidney disease and cardiovascular disease and papers were scrutinized for additional references.

BIOMARKERS IN THE DIAGNOSIS OF CKD AND ITS PROGRESSION: KIDNEY FUNCTION

Cystatin C

Cystatin C is a small molecule (13 kDa) that is filtered and metabolized after tubular absorption.[29,30] It is a sensitive biomarker of kidney function in mild-to-moderate kidney disease.[31] In the MMKD study, serum cystatin C predicted CKD progression.[32] The 65 of 227 patients who progressed over 7 years of follow-up had higher serum cystatin C at baseline, suggesting that it is a promising biomarker of CKD progression.[32]

β-Trace protein (BTP)

BTP is a lipocalin glycoprotein[33,34] and a more sensitive indicator of glomerular filtration than serum creatinine.[35,36] It has been proposed as an indicator of reduced GFR, particularly in the 'creatinine-blind' range.[35] However, others have shown that BTP is inferior to cystatin C as an indicator of GFR.[37] In a report from the MMKD study group, BTP also provided reliable risk prediction for CKD progression.[32] Although promising, BTP requires validation in large CKD populations.

Uric acid (UA)

Although not newly discovered, UA fulfils many criteria for a validated biomarker in CKD. It is elevated in CKD and may have a role in the pathophysiology of CKD progression through endothelial dysfunction, vascular smooth muscle cell proliferation, increased interleukin-6 (IL-6) synthesis, insulin resistance, and impairment of nitric oxide production.[38] Observational data indicate a relationship between serum UA and CKD prevalence and progression.[39–41] However, not all studies have supported this contention.[19] Although allopurinol lowers UA as well as improves renal function, a larger, longer duration randomized controlled trial is required.[38]

BIOMARKERS IN THE DIAGNOSIS OF CKD AND ITS PROGRESSION: KIDNEY STRUCTURE

Proteinuria

Increased urinary albumin excretion is an established predictor of CKD progression[42] and may reflect both glomerular and tubulointerstitial injury. Analysis of data from the Nord-Trøndelag Health Study found that combining microalbuminuria with eGFR improved the ability to predict progression to ESRD.[7] However, as a surrogate

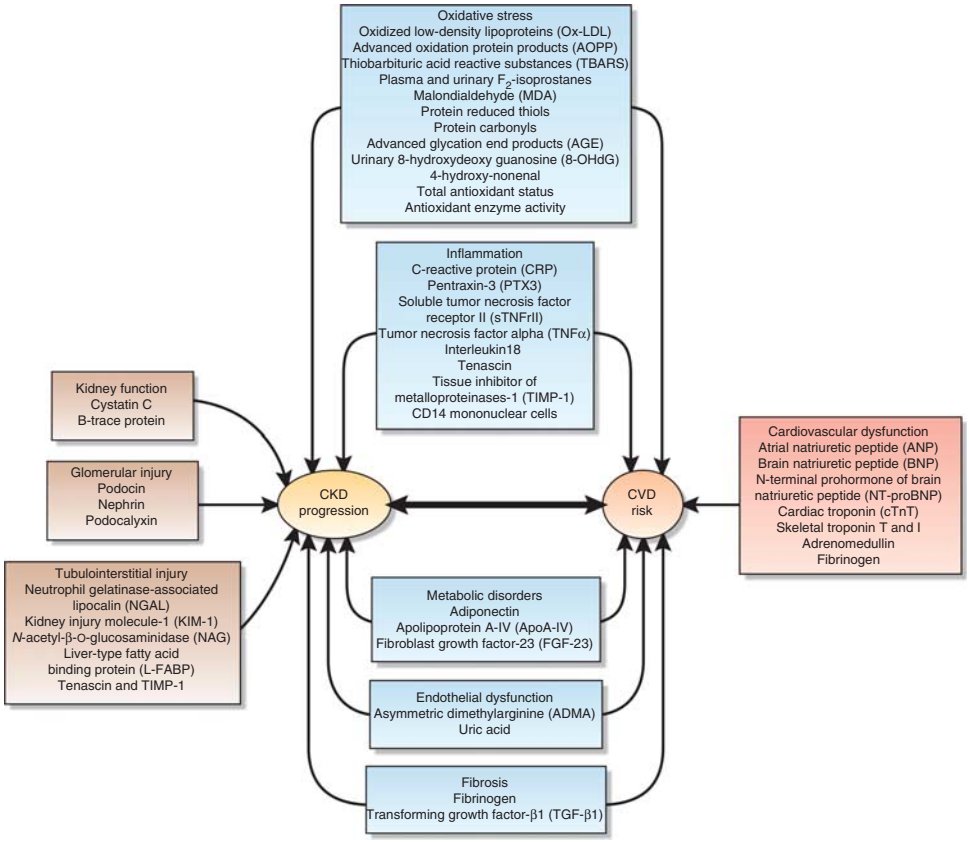

Figure 1 | Biomarkers of chronic kidney disease (CKD) progression and renal cardiovascular disease (CVD).

marker of kidney damage, proteinuria has limitations, particularly when assessing interventions in CKD that may result in change in proteinuria.[9] These limitations are based on the differing etiologies of CKD and the associated pathophysiological mechanisms responsible for the proteinuria in each case. In addition, the presence of proteinuria itself may cause further kidney damage.[43] Data from the Alberta Province laboratory registry showed that for a particular eGFR, increased proteinuria was associated with the risk of CKD progression, myocardial infarction, and mortality.[8] Screening for microalbuminuria can detect early-stage CKD and has been shown, using microsimulation modeling, to be cost effective in patients with diabetes or hypertension.[44]

Neutrophil gelatinase-associated lipocalin (NGAL)

NGAL, a ubiquitous lipocalin iron-carrying protein,[45] is highly expressed in the tubular epithelium of the distal nephron and released from tubular epithelial cells following damage such as acute kidney injury (AKI). It was originally isolated from neutrophils[46] but is also expressed in tissues such as kidney, liver, epithelial cells,[47,48] and vascular cells in atherosclerotic plaques.[49] It has multiple molecular forms in the urine, one being dimeric originating from neutrophils and the other monomeric from kidney tubular epithelial cells.[50] This difference has the potential to improve the specificity of NGAL as a renal biomarker. Serum and urinary NGAL have been identified as early biomarkers of AKI.[51–58]

AKI is increasingly recognized as a prelude to CKD.[59] In an experimental study in rats, ongoing inflammation and immune activity were found to be involved with the pathogenesis of CKD, and NGAL was upregulated, suggesting that it may be a valuable biomarker for the development of CKD after AKI.[60,61] Thus, there has been interest in NGAL as a biomarker in CKD. In addition, recent evidence suggests that NGAL may even be involved as a mediator of CKD progression.[62]In fact, the NGAL (Lipocalin 2) knockout mouse has markedly reduced renal lesions seen in CKD progression.[62] The same investigators in an associated retrospective clinical study in patients with autosomal dominant polycystic kidney disease found that an increase in urinary NGAL was especially seen in those patients who progressed more rapidly to end-stage kidney disease. Also, in kidney tissue from patients with IgA nephropathy and congenital nephron deficit, NGAL expression was increased in renal tubules.

In a cross-sectional study of 80 nondiabetic patients with CKD stages 2–4, serum NGAL was elevated, with the highest levels seen in those with the most advanced CKD.[63] Cross-sectional studies in IgA nephropathy,[64] autosomal dominant polycystic kidney disease,[65] glomerulonephritis,[66] pediatric lupus nephritis,[67] and CKD[68] have shown that urinary and serum NGAL are increased across these diseases and may

Table 2 | Studies investigating biomarkers in the progression of CKD and associated comorbidities

Authors	Biomarker	Study population	Study type	Study duration	Study outcomes
Horstrup *et al.*[96]	Tenascin and TIMP-1	Renal outpatients, Berlin, Germany (*n*=54)	Cross-sectional	NA	CKD patients had elevated serum and urinary tenascin and TIMP-1 compared with controls
Kamijo *et al.*[90]	u-L-FABP	Renal biopsy patients from St Marianna University School of Medicine (*n*=50)	Cross-sectional	NA	u-L-FABP correlated with urinary protein excretion and the degree of tubulointerstitial damage on renal biopsy
Kamijo *et al.*[91]	u-L-FABP	Nondiabetic CKD patients (*n*=48)	Prospective observational	12 months	u-L-FABP was a more sensitive biomarker of CKD progression than proteinuria. u-L-FABP increased with reduced kidney function
Lee *et al.*[158]	GGT	CARDIA study; African-American and Caucasian males and females (*n*=2478)	Prospective observational	15 years	Serum GGT predicted microalbuminuria in those with hypertension and diabetes
Tonelli *et al.*[116]	CRP and sTNFrii	CARE study participants with CKD (*n*=687)	*Post hoc* analysis of a randomized controlled trial	58 months	Increased CRP and sTNFrii at baseline predicted greater eGFR decline
Ravani *et al.*[101]	ADMA	CKD patients, Cremona, Italy (*n*=131)	Prospective observational	7 months	ADMA levels predicted development of ESRD
Fliser *et al.*[22]	ADMA	Nondiabetic CKD, Germany, Austria, and South Tyrol (*n*=227)	Prospective observational	52.8 months	ADMA levels were significant predictors of CKD progression to ESRD and doubling of serum creatinine
Brunner *et al.*[67]	uNGAL	Pediatric SLE nephritis (*n*=35)	Cross-sectional	NA	uNGAL was significantly higher in SLE patients than controls and correlated with renal disease activity
Boes *et al.*[17]	ApoA-IV	MMKD study, nondiabetic CKD (*n*=177)	Prospective observational	>7 years	Increased ApoA-IV was associated with increased CKD progression
Mitsnefes *et al.*[68]	sNGAL	Pediatric CKD stages 2–4 (*n*=45)	Cross-sectional	NA	sNGAL correlated with cystatin C and both correlated with measured GFR and eGFR. NGAL outperformed cystatin C and eGFR at lower levels of measured GFR
Ding *et al.*[64]	uNGAL and NAG	IgA nephropathy, *n*=70 (40 Lee grade III, 30 Lee grade II)	Cross-sectional	NA	uNGAL increased in IgA Lee grade II compared with controls. uNGAL was more sensitive than NAG
Ryu *et al.*[159]	GGT	Korean nondiabetic, nonhypertensive males (*n*=10,337)	Prospective observational	3.5 years	Serum GGT a possible early predictor for the development of CKD
Tong *et al.*[121]	PTX3	CKD stages 3 and 4 (*n*=71)	*Post hoc* analysis of a prospective observational	NA	PTX3 was negatively correlated with eGFR
Bolignano *et al.*[65]	uNGAL and sNGAL	ADPCKD (*n*=26)	Cross-sectional	NA	uNGAL and sNGAL were elevated in patients with ADPCKD compared with controls and both correlated with kidney function. Those with greater cyst development had higher uNGAL and sNGAL
Bolignano *et al.*[66]	uNGAL	Glomerulonephritis (proteinuria >1 g/day) (*n*=33)	Cross-sectional	NA	uNGAL higher in glomerulonephritis compared with controls and significantly correlated with serum creatinine and urinary protein excretion
Lajer *et al.*[100]	ADMA	Type 1 diabetics with CKD (*n*=397)	Prospective observational	11.3 years	Plasma ADMA levels were associated with progression of type 1 diabetic nephropathy
Saraheimo *et al.*[144]	Adiponectin	Finnish Diabetic Nephropathy study, type I diabetics (*n*=1330)	Prospective observational	5 years	Adiponectin was associated with CKD progression only in those with macroproteinuria
Targher *et al.*[160]	GGT	NHANES (*n*=13,188)	Cross-sectional	NA	Increased GTT had a strong independent relationship with CKD
Hanai *et al.*[102]	ADMA	Japanese type 2 diabetics with normoalbuminuria or microalbuminuria (*n*=225)	Prospective observational	5.2 years	Increased ADMA levels were associated with increased risk of progression of nephropathy
Teppala *et al.*[161]	GGT	NHANES (*n*=9516)	Cross-sectional	NA	No association between GGT and CKD
Bolignano *et al.*[69]	NGAL	White Europeans with CKD (*n*=96)	Prospective observational	18 months	Baseline urinary and serum NGAL were predictors of CKD progression
Nishida *et al.*[71]	Serum and urinary NGAL	Pediatric kidney disease (*n*=85)	Prospective observational	12 months	Urinary NGAL was a superior marker of CKD in children than serum NGAL
Spanaus *et al.*[21]	BNP and NT-proBNP	MMKD study, nondiabetic CKD (*n*=177)	Prospective observational	>7 years	Increased BNP and NT-proBNP both predicted CKD progression
Dieplinger *et al.*[20]	ANP and ADM	MMKD study, nondiabetic CKD (*n*=177)	Prospective observational	>7 years	Increased ANP and ADM both predicted CKD progression

Table 2 | Continued

Authors	Biomarker	Study population	Study type	Study duration	Study outcomes
Kiatchoosakun *et al.*[198]	cTnT	Stage 3 and 4 CKD (*n*=103)	Cross-sectional	NA	Increased cTnT was associated with reduced kidney function
Levin *et al.*[212]	PTH, PO_4, calcium	CaNPREDDICT study, British Columbia, Canada, eGFR <30 ml/min per 1.73 m^2 (*n*=4231)	Retrospective	4–31 months	CKD progression was associated with younger age, male gender, higher eGFR, higher BP, PO_4 and PTH levels, lower Hb levels, and higher proteinuria
Sayers *et al.*[213]	Birth weight and other biomarkers[a]	Aboriginal Childhood Cohort Study (*n*=475)	Prospective cohort	11.4 years	Birth weight was negatively correlated with BP
Takamatsu *et al.*[26]	Urinary type IV collagen and albumin	Community study, Arita, Japan (*n*=1554)	Cross-sectional	NA	Albumin creatinine ratio was associated with CKD in younger participants
Fakhrzadeh *et al.*[118]	CRP	Kahrizak Elderly Study, elderly Iranians (*n*=122)	Cross-sectional	NA	Combination of metabolic syndrome and high CRP strongly associated with CKD risk
Waanders *et al.*[77]	Urinary KIM-1	Proteinuric non-diabetic CKD (*n*=34)	*Post hoc* analysis of randomized controlled trial	6 weeks	Urinary KIM-1 increased in nondiabetic CKD and decreased with antiproteinuric therapy
Keller *et al.*[214]	Ten inflammatory and procoagulant biomarkers[b]	Cardiovascular Health Study (elderly) (*n*=4128)	Prospective cohort	7 years	Baseline serum albumin associated with rapid eGFR decline (>3 ml/min per 1.73 m^2 per year) in the elderly
Fox *et al.*[117]	CRP	Jackson Heart Study, African Americans (*n*=4320)	Cross-sectional	NA	CRP was associated with CKD
Manghat *et al.*[137]	FGF-23	South East Greater London, UK, Stage 1–4 CKD (*n*=145)	Cross-sectional	NA	FGF-23 was elevated in CKD stage 3 and correlated with diabetes, serum phosphate, and CRP
Hung *et al.*[119]	CRP polymorphisms	AASK Study, African Americans CKD (*n*=642)	Prospective observational	Range 3.5–6.5 years	CRP SNPs associated with higher CRP were not associated with CKD progression. CKD patients with the rs2808630_GG genotype had a greater risk of progression
Nielsen *et al.*[92]	u-L-FABP	Type 1 diabetics with normoalbuminuria or microalbuminuria (*n*=227)	Prospective observational	18 years	High u-L-FABP predicted development and progression of diabetic nephropathy
Tsioufis *et al.*[103]	CRP and ADMA	Untreated essential hypertensives (*n*=13,905)	Cross-sectional	NA	Elevated CRP and ADMA were associated with microalbuminuria
Kern *et al.*[84]	Urinary NAG pentosidine, AGE fluorescence	DCCT trial type 1 diabetes (*n*=91)	Nested case–control	1–9 years	Baseline urinary NAG predicted macro- and micro-albuminuria. Baseline urinary AGE predicted microalbuminuria
Spanaus *et al.*[32]	Serum BTP	MMKD study, nondiabetic CKD (*n*=177)	Prospective observational	>7 years	BTP along with serum creatinine and cystatin C were reliable predictors of CKD progression
Wu *et al.*[72]	uNGAL	Drug-induced chronic tubulointerstitial nephritis (*n*=36)	Prospective observational	6–33 months	Baseline uNGAL was predictive of renal function decline
Kanbay *et al.*[192]	FGF-23	Gensini score in mild CKD (*n*=177)	Cross-sectional	NA	FGF-23 was independently associated with Gensini score, a measure of coronary atherosclerosis
Zhou *et al.*[131]	Urinary CD14 mononuclear cells	Patients with ADPCKD (*n*=16)	Prospective observational	2 years	Baseline urinary CD14 mononuclear cells showed a statistically significant correlation with kidney volume in males
Vaidya *et al.*[79]	NAG and KIM-1	Patients with type 1 diabetes mellitus (*n*=659)	Prospective observational	2 years	Lower urinary levels of NAG and KIM-1 were associated with regression of microalbuminuria
Viau *et al.*[62]	uNGAL	Patients with ADPCKD (*n*=87)	Retrospective	6 years	Patients with higher urinary NGAL had faster progression to end-stage kidney disease

Abbreviations: AASK, African American Study of Kidney Disease and Hypertension; ADM, adrenomedullin; ADMA, asymmetric dimethylarginine; ADPCKD, autosomal dominant polycystic kidney disease; AGE, advanced glycation end product; ANP, atrial natriuretic peptide; ApoA-IV, apolipoprotein A-IV; BNP, brain natriuretic peptide; BP, blood pressure; BTP, β-trace protein; CaNPREDDICT study, Canadian Study of Prediction of Risk and Evolution to Dialysis, Death and Interim Cardiovascular Events Over Time study; CARDIA, Coronary Artery Risk Development in Young Adults; CARE, Cholesterol and Recurrent Events; CKD, chronic kidney disease, CRP, C-reactive protein; cTnT, cardiac troponin T; DCCT, Diabetes Control and Complications Trial; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; FGF-23, fibroblast growth factor-23; GGT, γ-glutamyltransferase; Hb, hemoglobin; KIM-1, kidney injury molecule-1; MMKD, Mild to Moderate Kidney Disease study; NA, not applicable; NAG, *N*-acetyl-β-o-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; NHANES, National Health and Nutrition Examination Survey; NT-proBNP, N-terminal brain natriuretic peptide; PO_4, phosphate; PTH, parathyroid hormone; PTX3, pentraxin 3; SLE, systemic lupus erythematosus; sNGAL, serum NGAL; SNP, single-nucleotide polymorphism; sTNFrii, soluble tumor necrosis factor receptor II; TIMP-1, tissue inhibitor of metalloproteinases-1; u-L-FABP, urinary liver-type fatty acid-binding protein; uNGAL, urinary NGAL.
[a]Blood pressure, total cholesterol, apolipoprotein A-1, apolipoprotein B, respiratory function, ultrasound kidney size, urinary albumin/creatinine ratio, fasting triglycerides, insulin, and glucose.
[b]Albumin, CRP, interleukin-6, intercellular adhesion molecule-1, white blood cell count, fibrinogen, factor VII, factor VIII, D-dimer, and plasmin-antiplasmin complex.

reflect disease activity and kidney function. Baseline serum and urinary NGAL were predictors of eGFR decline in a longitudinal study in 96 patients with CKD from a range of etiologies over a median follow-up period of 18.5 months.[69] Both urinary and serum NGAL levels also increased in parallel in a study of diabetic patients, suggesting that NGAL may play an important role in the pathophysiology of renal adaptation to diabetes and associated nephropathy.[70] There were significant correlations between serum and urinary NGAL and eGFR and between the levels of urinary NGAL and the degree of proteinuria in 85 pediatric patients with CKD from a variety of etiologies.[71] In a study of 36 patients with drug-induced chronic tubulointerstitial nephritis, increased urinary NGAL at baseline predicted decline in kidney function.[72] There is now strong evidence that increased urinary and serum NGAL reflect damage across a spectrum of kidney diseases[73] as well as AKI and may predict progression of CKD.[69] The availability of more specific assays that are able to detect the different forms of NGAL may increase the specificity of urinary NGAL as a biomarker in CKD.[50]

Kidney injury molecule 1 (KIM-1)

KIM-1 is a transmembrane tubular protein with uncertain function, not detectable in the normal kidney, but elevated in experimental and clinical kidney damage.[74] KIM-1 expression is significantly increased within kidney biopsy tissue collected from patients with kidney disease.[74] It is increased in the urine in AKI[75]and kidney disease,[74] and is increased in kidney transplant patients and associated with graft loss.[76] Experimental studies suggest that KIM-1 may be an indicator of AKI to CKD transition.[60] A retrospective study in proteinuric nondiabetic kidney disease patients found elevated levels of urinary KIM-1.[77] In addition, urinary KIM-1 decreased with short-term antiproteinuric therapies.[77] The diagnostic performance of KIM-1 in animal models as a predictor of drug-induced kidney injury was recently evaluated alongside more traditional serum creatinine and blood urea nitrogen and urinary *N*-acetyl-β-D-glucosaminidase (NAG),[78] where KIM-1 levels better correlated with the degree of kidney tubular histopathology, suggesting that KIM-1 measurement could facilitate elimination of potentially nephrotoxic drug candidates. Lower urinary levels of KIM-1 were associated with the regression of microalbuminuria in patients with type 1 diabetes mellitus.[79] Long-term observational studies in larger populations are required to assess urinary KIM-1 as a CKD biomarker.

N-Acetyl-β-D-glucosaminidase

NAG is predominantly a biomarker of proximal tubular damage,[80–83] but may also be a biomarker of injury to other parts of the nephron. A nested case–control study from the Diabetes Control and Compliance Trial found that baseline urinary NAG predicted both micro- and macro-albuminuria in type 1 diabetics.[84] NAG is also elevated in the urine in patients with glomerulonephritis compared with healthy controls.[85] A recent study in type 1 diabetes mellitus found that lower levels of urinary NAG were associated with the regression of microalbuminuria.[79] It has not been assessed longitudinally in CKD.

Liver-type fatty acid-binding protein (L-FABP)

L-FABP is expressed in proximal tubular cells and is a biomarker of inflammation investigated in diabetes, diabetic nephropathy, hypertension, and early CKD.[86–90] Urinary L-FABP (u-L-FABP) was correlated with the degree of tubulointerstitial damage and urinary protein excretion in a study of 50 patients with CKD.[90] In a prospective study, u-L-FABP was more sensitive than proteinuria in predicting CKD progression.[91] Baseline u-L-FABP predicted the development of micro- and macro-albuminuria, independent of recognized biomarkers, in 165 normoalbuminuric patients from a cohort of 227 type 1 diabetics.[92] In a recent intervention study, pitavastatin 1 mg per day reduced u-L-FABP in 30 CKD patients, which may reflect alleviation of tubulointerstitial damage.[93]

Tenascin and tissue inhibitor of metalloproteinases 1

Tenascin is a noncollagenous matrix protein involved in interstitial renal pathology.[94] Tissue inhibitor of metalloproteinases 1 has a role in inhibition of the degradation of matrix and is upregulated in models of kidney disease.[95] Compared with healthy controls, patients with CKD have elevated serum levels of tenascin and tissue inhibitor of metalloproteinases 1 (ref. 96). Urinary levels of these molecules were also significantly raised but did not correlate with the degree of proteinuria. At this stage, these molecules are in the preliminary stages of assessment.

Glomerular injury: urinary nephrin, podocin, and podocalyxin

Although proteinuria is a feature of glomerular damage, new biomarkers of kidney podocyte injury including urinary nephrin, podocin, and podocalyxin have been discovered.[97,98] Urinary nephrin and podocin are elevated in diabetic nephropathy and active lupus nephritis. Increased urinary podocalyxin is seen in IgA nephropathy, lupus nephritis, and post-streptoccocal glomerulonephritis.[99] Although promising, and perhaps more specific for glomerular disease, these biomarkers require more detailed assessment before reaching clinical utility.

BIOMARKERS IN THE DIAGNOSIS OF CKD AND ITS PROGRESSION: ENDOTHELIAL DYSFUNCTION

Asymmetric dimethylarginine (ADMA)

ADMA is an amino acid that inhibits nitric oxide synthase and, if present in increased quantities, results in decreased nitric oxide production. Reduced nitric oxide production is associated with endothelial dysfunction and kidney damage. ADMA has been investigated as a biomarker in ESRD, but more recently in CKD progression.[100–102] In a study investigating early CKD in type 1 diabetics, increased plasma levels of ADMA were predictive of the development and

progression of nephropathy.[102] In another study, incident CKD patients were followed prospectively where plasma ADMA was inversely correlated with GFR and was a predictor of progression to ESRD.[101] The progression of CKD was also greater in another population of nondiabetic CKD patients with elevated plasma ADMA levels.[22] In a cross-sectional study in patients with untreated hypertension, there was an association between ADMA levels and the presence of microalbuminuria and albumin/creatinine ratio.[103]

BIOMARKERS IN THE DIAGNOSIS OF CKD AND ITS PROGRESSION: CARDIOVASCULAR PEPTIDES

Natriuretic peptides

Natriuretic peptides and adrenomedullin are important for cardiovascular and kidney homeostasis and their effects include natriuresis, hypotension, and diuresis.[104,105] Natriuretic peptides have mainly been investigated as biomarkers in cardiac disease where elevated concentrations are associated with poor prognosis, degree of left ventricular dysfunction, and congestive cardiac failure.[106–111] In CKD, N-terminal pro-brain natriuretic peptide (pro-BNP), atrial natriuretic peptide, and adrenomedullin levels have been investigated in the MMKD study where high levels of each were associated with CKD progression.[20,21] In the first report from these investigators, BNP and N-terminal pro-BNP were measured in 177 nondiabetic CKD patients. Those patients that reached an end point, defined as doubling of serum creatinine or dialysis requirement, had significantly higher BNP and N-terminal pro-BNP levels. However, after adjustment for established predictors of CKD progression, only BNP was predictive of reaching the combined end point. Subsequently, these investigators measured plasma atrial natriuretic peptide and adrenomedullin in the same population and found that increased plasma concentrations also predicted CKD progression.[20] Hence, these biomarkers may prove useful in determining CKD trajectory.[21]

BIOMARKERS IN THE DIAGNOSIS OF CKD AND ITS PROGRESSION: INFLAMMATION AND FIBROSIS

There are increasing numbers of inflammatory and fibrotic markers investigated in CKD and the key ones are reviewed here.

C-reactive protein (CRP) and soluble tumor necrosis factor (TNF) receptor II

CRP is a short pentraxin and an established biomarker of inflammation in kidney disease.[112,113] TNF-α is a proinflammatory mediator, which binds to soluble TNF receptors, and plasma levels of TNF-α are increased in CKD.[114,115] Soluble TNF receptor II is one such receptor, which is also an inflammatory marker. A *post hoc* analysis of data from the CARE (Cholesterol and Recurrent Events) trial showed that increased plasma concentrations of CRP and soluble TNF receptor II at baseline were independently associated with a greater rate of kidney function decline.[116] A cross-sectional report from the Jackson Heart Study, a longitudinal observational study conducted in African Americans, showed that CRP was associated with the presence of CKD.[117] Elderly Iranians with the metabolic syndrome and high CRP levels had significantly increased risk of CKD.[118] Single-nucleotide polymorphisms of CRP associated with higher CRP levels in African Americans were not predictive of CKD progression.[119] However, CKD patients with the rs2808630_GG genotype had a greater risk of CKD progression. Increased CRP levels were associated with microalbuminuria in patients with untreated essential hypertension.[103]

Pentraxin-3 (PTX3)

PTX3 is a long pentraxin produced in response to inflammatory signals by immune cells in contrast to CRP that is produced by hepatic cells.[120] In a study of 71 stage 3 and 4 CKD patients, there was an inverse relationship between PTX3 and eGFR.[121] The investigation of this biomarker in CKD is in the very early stages.

Urinary IL-18

Urinary IL-18 is a biomarker of kidney tubular injury.[122] In a cross-sectional study, IL-18 was markedly increased in patients with established AKI from ischemia, but not in patients with AKI from urinary tract infection, CKD, nephrotic syndrome, or those with prerenal failure.[122,123] Urinary IL-18, more likely, offers prognostic information regarding severity and mortality at the time of AKI diagnosis, rather than indicating progression to CKD. However, evidence that patients with diabetic nephropathy have renal tubular cell overexpression of IL-18 may indicate that it deserves further study in the context of CKD.[124]

Transforming growth factor-β1 (TGF-β1)

TGF-β1 is a healing modulator and if secretion is increased there is accumulation of extracellular matrix and subsequent fibrosis.[125] Urinary TGF-β1 is increased in diabetic nephropathy and glomerulonephritis.[126,127] Serum TGF-β1 was associated with risk factors for kidney disease in African Americans, but not Caucasians.[128] In a cross-sectional study, urine concentrations of TGF-β1 were related to the severity of diabetic nephropathy.[129]

CD14 mononuclear cells

In autosomal dominant polycystic kidney disease, where there is great heterogeneity in disease progression, GFR is known to be a poor indicator. Measurement of CD14 in the urine correlates with total kidney volume in males, and hence may prove to be a valuable early biomarker in this disease.[130,131]

BIOMARKERS IN THE DIAGNOSIS OF CKD AND ITS PROGRESSION: METABOLIC FACTORS

Fibroblast growth factor-23 (FGF-23)

FGF-23 is a hormone mediating phosphaturia and is regulated by 1,25-dihydroxyvitamin D_3. It suppresses 1α hydroxylase activity in the proximal renal tubule.[132] Serum

concentrations of FGF-23 are elevated in early CKD and ESRD.[133,134] The cause of FGF-23 elevation in CKD is unclear[135] but, in early CKD, FGF-23 may rise before the rise in serum phosphate.[136] In a cross-sectional study of 145 patients with CKD stages 1–4, FGF-23 was found to be associated with other indicators of CKD complications such as diabetes, serum phosphate, and CRP. FGF-23 was elevated more in stage 3 than stage 1 and 2 CKD.[137] The phosphate binder sevelemer reduced FGF-23 levels in early-stage CKD patients with normal serum phosphate levels.[138]

Apolipoprotein A-IV

Apolipoprotein A-IV is a glycoprotein involved in cholesterol transport and is increased in ESRD[139] and earlier stages of CKD.[140] The MMKD study demonstrated that proteinuric nondiabetic CKD patients with elevated apolipoprotein A-IV had significantly faster CKD progression.[17] The elevation of apolipoprotein A-IV is reflective of kidney function and may result from reduced renal catabolism.[17]

Adiponectin

Adiponectin is a hormone secreted by adipocytes[141] and is elevated in kidney disease.[142,143] In the Finnish Diabetic Nephropathy study, adiponectin levels were only predictive of CKD progression in those with macroproteinuria, and not in those with initial normoalbuminuria or microalbuminuria.[144] In this context, adiponectin could not be considered a biomarker for early detection of CKD progression.

BIOMARKERS IN THE DIAGNOSIS OF CKD AND ITS PROGRESSION: OXIDATIVE STRESS

Oxidative stress refers to the *in vivo* oxidation of lipids, proteins, carbohydrates, and DNA, and it involves many biochemical pathways. Hence, there are many compounds called 'biomarkers of oxidative stress'. Cross-sectional studies have demonstrated that CKD patients have increased levels of oxidative stress biomarkers.[145–151] These studies have compared CKD patients with matched controls[145,147–149] and/or compared different CKD stages.[145–147,149,150] Biomarkers have included plasma/serum measures of oxidized low-density lipoproteins,[145] advanced oxidation protein products,[148] thiobarbituric acid reactive substances,[148] protein carbonyls,[147,151] isoprostanes,[147,150,151] protein reduced thiols,[147] total antioxidant status,[150] 4-hydroxy-nonenal,[151] superoxide dismutase[149] and glutathione peroxidise[149] activities, urinary 8-hydroxydeoxy guanosine,[149] and advanced glycation end products.[151] Advanced glycation end products are associated with the development of diabetic nephropathy.[152] Pentosidine is an advanced glycation end product, which may be present in the urine.[153] In a nested case–control study from the Diabetes Control and Compliance Trial, baseline urinary pentosidine predicted macroalbuminuria and baseline advanced glycation end product predicted microalbuminuria.[84]

Findings from studies in CKD patients include: (1) oxidized low-density lipoproteins are associated with endothelial injury[145] and inflammation,[148] (2) elevated oxidative stress occurs early in CKD,[147] and increases as CKD progresses,[150] and (3) patients with diabetic nephropathy[147,151] and proteinuria[149] have additional elevations of oxidative stress. Oxidative stress increases further on renal replacement therapy[145,146] and regresses after kidney transplantation.[154]

Of most relevance to this review is the equivocal relationship between eGFR and biomarkers of oxidative stress. Two studies reported that oxidative stress was related to kidney function,[145,150] and one reported no link.[147] This difference may relate to the biomarkers measured and highlights the complexity of oxidative stress assessment.

Future research should include the direct assessment of reactant generation at the tissue level, use of multiple biomarkers for a more comprehensive assessment of pathways involved, and longitudinal cohort studies assessing how changes in these measures are related to outcomes such as morbidity and mortality.

γ-Glutamyl transpeptidase (GGT)

Until recently, the epidemiological associations of GGT were unexplored.[155] GGT has mainly been used as a marker of liver disease and alcohol intake,[156] but it has also been touted as a biomarker of oxidative stress.[157] In the CARDIA (Coronary Artery Risk Development in Young Adults) study, those with hypertension and diabetes had an association between GGT (within the normal range) and the occurrence of microalbuminuria.[158] Ryu *et al.*[159] reported, in a cohort of nonhypertensive and nondiabetic Korean males, that GGT may be an early predictor of CKD even when allowing for confounding factors at baseline. In a cross-sectional analysis of 13,188 adults in the NHANES (National Health and Nutrition Examination Survey) 2001–2006, there was a strong independent association between elevated GGT and CKD.[160] Adjustment was undertaken for comorbidities, demographics, alcohol consumption, lipid-lowering therapy, the presence of viral hepatitis, and laboratory results. However, Teppala *et al.*[161] reported cross-sectional data from NHANES 1999–2002 showing no association between increased GGT levels and CKD when adjusted for age, gender, race, smoking, alcohol consumption, diabetes, hypertension, obesity, and serum lipids. The reasons for the differences between these studies are unclear.

DISEASE-SPECIFIC BIOMARKERS

Although elements of CKD progression are common to all underlying primary renal etiologies, there are specific pathophysiological mechanisms linked to the primary renal diseases underpinning the diagnosis of CKD. For this reason, it is unlikely that a single biomarker will be able to predict CKD progression when encompassing all primary renal diseases. Disease-specific markers may supplement more general biomarkers.

For instance, in idiopathic membranous nephropathy, 70% of patients from one study had the M-type phospholipase A_2 receptor identified in glomerular extracts, and

the presence of antibodies against this antigen supported this as important in the disease.[162] In addition- single nucleotide polymorphisms of the phospholipase A_2 receptor 1 may be involved in the etiology of idiopathic membranous nephropathy.[163,164]

BIOMARKERS OF CVD MORBIDITY AND MORTALITY IN CKD

CVD is intimately associated with CKD and its progression, and hence it is not surprising that there has been a keen interest in searching for biomarkers that might predict CVD morbidity and mortality in the CKD population. Along with traditional risk factors, nontraditional risk factors contribute to CVD in CKD. The next section reviews biomarkers that have been investigated in the context of predicting CVD morbidity and mortality in CKD. These studies are summarized in Table 3.

Cystatin C

As plasma cystatin C is an accurate biomarker of kidney function, and the latter is closely correlated with CVD risk, it follows that plasma cystatin C would also be a biomarker of CVD risk. Prospective studies have demonstrated an increased CVD risk associated with increased plasma cystatin C levels.[165] In fact, in elderly individuals without CKD, plasma cystatin C is a biomarker for the risk for CVD and mortality[166,167] and reflects coronary artery disease severity.[168] In patients with CKD, elevated plasma levels of cystatin C are associated with all-cause mortality, cardiovascular events, and incident heart failure.[169] Plasma cystatin C is, therefore, a powerful predictor of CVD and mortality in those with and without CKD.

Uric acid

Although there are some observational data linking serum UA with cardiovascular and all-cause mortality in the general population, there is little information assessing this relationship in CKD.[170] Analysis of data from the ARIC (Atherosclerosis Risk In Communities) study found that although there was an independent association between elevated serum UA and mortality, there were no significant associations for cardiovascular events and mortality in the CKD population.[170]

Natriuretic peptides

BNP was prospectively studied as a biomarker of cardiovascular events in a CKD population in Japan, where the relative risk of cardiovascular events was significantly higher in those

Table 3 | Studies investigating biomarkers in CVD morbidity and mortality in CKD

Authors	Biomarker	Study population	Study type	Study duration	Study outcomes
Tong *et al.*[121]	PTX3	CKD stages 3 and 4 (n=71)	*Post hoc* analysis of prospective cohort	NA	PTX3 was positively correlated with cardiovascular events and mortality
Goicoechea *et al.*[184]	Fibrinogen	CKD patients with eGFR <60 ml/min per 1.73 m^2 (n=128)	Prospective observational longitudinal	5.5 years	Serum fibrinogen predicted all-cause mortality in CKD stages 3–5
Weiner *et al.*[185]	Fibrinogen, albumin, triglyceride, and CRP	ARIC study participants, eGFR 60 ml/min per 1.73 m^2 (n=1678)	Prospective cohort study	108 months	Increased fibrinogen, triglyceride, CRP, decreased albumin, predicted composite events (MI, stroke, all-cause mortality)
Levin *et al.*[212]	PTH, PO_4, calcium	CaNPREDDICT study, British Columbia, Canada, eGFR <30 ml/min per 1.73 m^2 (n=4231)	Retrospective	4–31 months	Death associated with older age, lower BP, Hb, higher PO_4, PTH. Vitamin D associated with reduced death
Lajer *et al.*[100]	ADMA	Type 1 diabetics with CKD (n=397)	Prospective observational	11.3 years	Plasma ADMA levels predicted fatal and nonfatal cardiovascular events
Ravani *et al.*[101]	ADMA	CKD patients, Cremona, Italy (n=131)	Prospective observational study	27 months	ADMA levels predicted mortality
Liabeuf *et al.*[196]	sTRAIL	CKD stages 2–5 included 31% CKD stage 5D (n=130)	Prospective cohort	20 months–2.1 years	sTRAIL inversely associated with mortality
Junyent *et al.*[15]	Multiple biomarkers	NEFRONA Project Spain (n=2661)	Prospective cohort (in progress)	4 years	Cardiovascular events, mortality, surrogate vascular measures, and biomarkers (in progress)
Sakuma *et al.*[171]	BNP	Iwate-KENCO study, Ninohe, Japan (n=15,394)	Prospective observational	2.8 years	BNP a strong predictor of cardiovascular events
Nielsen *et al.*[92]	u-L-FABP	Type 1 diabetics with normoalbuminuria or microalbuminuria (n=227)	Prospective observational	18 years	High u-L-FABP predicted mortality
Seiler *et al.*[191]	FGF-23	CKD patients not on dialysis (n=149)	Prospective observational	4.8 years	Elevated FGF-23 were predictive of cardiovascular events
Rogacev *et al.*[207]	CD14++ and CD16+ monocytes	CKD patients not on dialysis (n=119)	Prospective observational	4.9 years	Presence of CD14++ and CD16+ monocytes were independently associated with cardiovascular events

Abbreviations: ADMA, asymmetric dimethylarginine; ANP, atrial natriuretic peptide; ARIC, Atherosclerosis Risk In Communities; BP, blood pressure; BNP, brain natriuretic peptide; CaNPREDDICT study, Canadian Study of Prediction of Risk and Evolution to Dialysis, Death and Interim Cardiovascular Events Over Time; CARDIA, Coronary Artery Risk Development in Young Adults; CKD, chronic kidney disease; CRP, C-reactive protein; cTnT, cardiac troponin T; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; GGT, γ-glutamyltransferase; Hb, hemoglobin; MI, myocardial infarction; NA, not applicable; NHANES, National Health and Nutrition Examination Survey; NT-proBNP, N-terminal brain natriuretic peptide; PO_4, phosphate; PTH, parathyroid hormone; PTX3, pentraxin 3; sTRAIL, soluble tumor necrosis factor-related apoptosis-inducing ligand; u-L-FABP, urinary liver-type fatty acid-binding protein.

with the highest BNP levels.[171] In children with CKD, increased levels of BNP were associated with ventricular hypertrophy with higher levels present in eccentric than concentric hypertrophy, suggesting that BNP may be predictive of abnormal cardiac geometry.[172] The natriuretic peptides have proven useful CVD biomarkers in the general population and have potential for similar use in CKD patients pending further studies.

Asymmetric dimethylarginine

ADMA is a biomarker of atherosclerosis[173] and is associated with increased blood pressure[174] and insulin resistance.[175] It is a strong independent predictor of cardiovascular and mortality risk in end-stage kidney disease,[176] but has not been as extensively investigated in predialysis CKD. In a prospective study in 131 incident CKD patients followed for a mean of 27 months, plasma ADMA levels were independently predictive of total mortality.[101] Also, in another prospective observational study of type 1 diabetics with overt nephropathy, plasma ADMA was predictive of both fatal and nonfatal cardiovascular events.[100] Further studies of this biomarker in more diverse CKD populations are required.

Fibrinogen

Serum fibrinogen is a predictor of CVD events in the general population,[177–180] and is independently predictive of cardiovascular and all-cause mortality in end-stage kidney disease.[181] This association has been demonstrated in patients with CKD.[182,183] More recent studies in patients with stage 3 and 4 CKD indicated that an elevated serum fibrinogen level was an independent predictor of all cause mortality.[184] Weiner *et al.*[185] assessed nontraditional cardiovascular risk factors including serum fibrinogen in stage 3 and 4 CKD patients in both the ARIC and Cardiovascular Health studies where increased serum fibrinogen was independently predictive of composite events that included myocardial infarction, stroke, and all-cause mortality. Serum fibrinogen may prove useful in a cardiovascular biomarker panel for identifying CKD patients at risk of CVD.

Neutrophil gelatinase-associated lipocalin

Recent interest has focused on NGAL as a biomarker of CVD as well as in AKI and CKD progression.[186] This stems from experimental[187] and clinical evidence in the nonrenal population.[188,189] However, in a recent pilot study of 46 elderly patients with heart failure and eGFR 72.3 ± 15.1 ml/min per $1.73\,m^2$, plasma NGAL levels increased in parallel with predictive indicators of CVD, with plasma NGAL levels >783 ng/ml correlating with CVD mortality after 2 years.[190] NGAL may prove to be a useful biomarker of CVD associated with CKD but studies in CKD populations are required.

Fibroblast growth factor-23

Elevated levels of FGF-23 have been shown to be predictive of mortality in ESRD and cardiovascular events in CKD patients before starting dialysis.[191] A cross-sectional study in patients with mild CKD found an independent association between increased FGF-23 and the Gensini score, a measure of total coronary atherosclerosis.[192] Additional validation of this association is required.[193]

Pentraxin-3

Only one study assessing PTX3 as a biomarker of CVD in CKD was identified, where PTX3 was associated with protein-energy wasting, inflammation, CVD, and all-cause mortality in a study of 71 patients with stage 3 and 4 CKD.[121]

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)

TRAIL is a factor associated with protection against inflammation and is involved with apoptosis[194] and atherosclerosis.[195] Liabeuf *et al.*[196] assessed soluble TRAIL in 130 patients with stage 2–5 CKD that included 31% ESRD patients. Soluble TRAIL was found to be a biomarker associated with reduced inflammation and mortality.

Troponin T and I

Troponin T and I are sensitive biomarkers of cardiac injury in the general population. Although mechanisms vary for causation, increased plasma Troponin T and I, above the cutoffs for a normal population, were independently predictive of subsequent death in ESRD patients.[197] Elevations in troponin T (cTnT) were frequent findings in patients with stage 3 and 4 CKD and elevated cTnT has been associated with reduced renal clearance.[198] Elevated cTnT in patients with CKD has also been shown to be because of reduced renal clearance in patients with congestive heart failure.[199] However, detectable cTnT is associated with reduced survival.[200] The same investigators found cTnT and cTnI elevated in CKD and that elevated cTnI was associated with increased mortality.[201] In addition, elevated levels of cTnT and cTnI are seen frequently in patients with CKD predialysis even in the absence of an acute coronary syndrome. In fact, it has been shown that elevated levels of cTnT measured in patients before starting dialysis predict asymptomatic multivessel coronary artery stenosis.[202] cTnT and cTnI were found to be equivalent in differentiating short-term prognosis in patients with CKD with non-ST-segment elevation acute coronary syndromes but this was more useful in those with more advanced CKD.[203] Thus, despite elevations in these biomarkers of CVD being seen associated with reduced renal clearance, they are of use as biomarkers of mortality risk in predialysis CKD patients.

Liver-type fatty acid-binding protein

In a prospective cohort study of 165 normoalbuminuric type 1 diabetics, u-L-FABP was predictive of all-cause mortality, independent of traditional risk factors such as gender, age, smoking status, $HbA1_c$, blood pressure, urinary albumin excretion rate, and kidney function.[92] Although this study was not performed in the CKD population, it explored progression to proteinuria and u-L-FABP may be worth

assessing as a candidate biomarker of CVD and mortality in CKD patients.

CD14 + + CD16 + monocytes

Macrophages and monocytes are recognized participants in the atherosclerotic process.[204] Subsets of monocytes have been identified based on the expression of different receptors (lipopolysaccharide CD14 and FcγIII CD16) on their surface. High counts of CD14 + + CD16 + monocytes are increased in inflammation[205] and are also associated with the development of cardiovascular events in the dialysis setting.[206] In a prospective study of 119 patients with predialysis CKD from a range of etiologies, the presence of CD14 + + and CD16 + monocytes was associated independently with cardiovascular events.[207] At this stage, these results are purely a stimulus for further investigation.

NEW BIOMARKER DEVELOPMENT

New biomarkers are usually identified from experimental studies before investigation in clinical populations. Recently, studies have investigated predictive biomarkers of acute drug-induced kidney injury.[78,208–210] For example, in a rodent study of nephrotoxin-induced injury, urinary TFF3 (trefoil factor 3) protein levels were markedly reduced, and urinary albumin increased in association with tubular injury. *In situ* hybridization of TFF3 mRNA also decreased in injured tubules. Albumin outperformed serum creatinine and blood urea nitrogen for detecting tubular injury, but TFF3 augmented the potential of blood urea nitrogen and serum creatinine to detect kidney damage.[210] The Nephrotoxicity Working Group within the Critical Path Institute, a pharmaceutical industry public–private partnership, submitted a recommendation to the US FDA (US Food and Drug Administration) and the European Medicines Agency for further study of seven urinary biomarkers of kidney injury (KIM-1, albumin, total protein, β2-microglobulin, cystatin C, clusterin, and trefoil factor 3), which qualified for use in regulatory decision-making (Table 4). Most biomarkers in the panel showed better sensitivity and specificity than blood urea nitrogen and serum creatinine, and all added complementary information.[211] Testing those not currently assessed in CKD populations may prove fruitful. The sequence of biomarker discovery and development through to clinical utility is summarized in Figure 2.

SUMMARY

To enable nephrologists to focus on CKD patients that will have a poor trajectory, we need better biomarkers that can identify this earlier. Serum creatinine, eGFR, and proteinuria are insensitive and reliance on these may result in extensive time lapse where successful interventions could be tested and applied. Some biomarkers reviewed show promise but further validation is required in larger, more diverse populations before translation into clinical practice. Of those

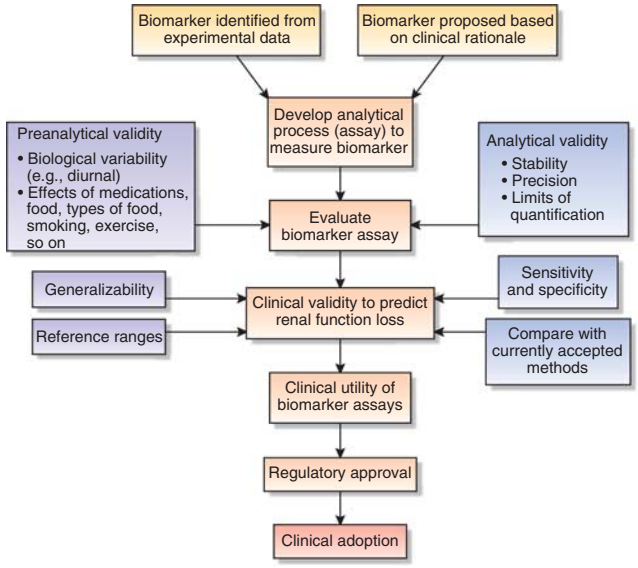

Figure 2 | Summary of biomarker discovery and development through to clinical utility.

Table 4 | Summary of claims submitted to the FDA and EMEA on biomarkers associated with nephrotoxicity

Biomarker	Qualified preclinically	Adds information to SCr and BUN[a,b]	Outperforms SCr and/or BUN[a,b,c,d]	Analytically validated assay	Widely available assay	Qualified for clinical use[e]
KIM-1	Yes	Yes[a]	Yes[a]	Yes	Pending	Yes
Albumin	Yes	Yes[a]	Yes[a]	Yes	Yes	Yes
CLU	Yes	Yes[a]	Yes[a]	Yes	Yes	Pending
TFF3	Yes	Yes[a]	No	Yes	Pending	Pending
Total protein	Yes	Yes[b]	Yes[b,c]	Yes	Yes	Yes
Cystatin C	Yes	Yes[b]	Yes[b,c]	Yes	Yes	Yes
β2-Microglobulin	Yes	Yes[b]	Yes[b,c]	Yes	Yes	Yes

Abbreviations: BUN, blood urea nitrogen; CLU, clusterin; EMEA, European Medicines Agency; FDA, Food and Drug Administration; KIM-1, kidney injury molecule-1; SCr, serum creatinine; TTF3, trefoil factor 3.
[a]Acute tubular alterations.
[b]Acute glomerular injury with acute tubular reabsorption impairment.
[c]Biomarker outperformed SCr.
[d]If an inclusion receiver operating characteristic (ROC) analysis (true positive rate (sensitivity) against the rate of false positives (1–specificity) for a continuous variable against a specific reference standard) analysis is considered, instead of an exclusion ROC analysis, cystatin C and β2-microglobulin outperform not only SCr but also BUN with respect to the prediction of histopathologically confirmed kidney injury (see Honkanen *et al.*[126] for further details of ROC analysis).
[e]Qualified for clinical use refers to a 'case-by-case' on text and not to a broad general qualification.

reviewed, NGAL has the greatest promise as a biomarker of CKD progression and cystatin C as a biomarker of kidney function, CKD progression, and cardiovascular risk. It is unlikely that a single marker will satisfy the requirement of predicting CKD progression and cardiovascular morbidity and mortality as it would be unlikely to reflect the complexities of the multiple pathophysiological processes involved in CKD progression or the underlying primary renal disease. It is more likely that a focused panel of biomarkers will be most rewarding.

In conclusion, the search for new relevant biomarkers to better stratify patients with CKD according to the risk of progression, morbidity, and mortality is underway. It is important to determine whether the newly identified biomarkers are purely associations or real biomarkers of underlying pathophysiological processes. Testing biomarkers prospectively in large, divergent populations over extended follow-up periods, and validating them against hard outcome measures such as the development of ESRD and mortality is required before translation into clinical practice. Although advances in proteomics technologies, sample conditioning, and analysis methods have greatly improved productivity and efficiency in biomarker discovery, biomarker verification and validation remains a significant, costly, and high-risk undertaking in the commercial development and deployment of novel biomarkers for CKD.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

RGF, SKV, GCG, JSC, MAC, and WEH are responsible for writing the manuscript. All authors have read and approved the final manuscript.

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