Biomarkers of Renal Function

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Biomarkers of Renal Function: Towards Clinical Actionability

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This review provides an overview of the clinical value of the most relevant renal biomarkers, focusing on two main clinical conditions: acute kidney injury and chronic kidney disease. We categorize biomarkers according to their actionability, in terms of a documented response to treatment in relation to outcomes. Furthermore, we introduce a new category of renal biomarkers, metabolic biomarkers, and underscore their capacity to be highly actionable.

Medical professionals are probably familiar with Galen's (129–201 AD) doctrine on using black bile, yellow bile, phlegm, and blood to assess a patient's health, or the tasting of a patient's urine to diagnose diabetes mellitus, which was first described in 1675.[1] It took until 1957 for the term "biological marker" to be introduced.[2] The National Institutes of Health (NIH) defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[3] According to the NIH definition, a biomarker can be used as a *diagnostic tool* for the identification of patients with a disease or abnormal condition, as an indicator of *disease prognosis*, or as a tool for *monitoring* of clinical response to an intervention. Hence, biomarkers can support the clinical management of patients in several ways. Furthermore, biomarkers can be of value in research by serving as surrogates of eventual clinical outcomes or as criterion for early enrollment in clinical trials.

In order to validate the performance of biomarkers, available studies mainly focus on parameters such as specificity, sensitivity, robustness, and reproducibility. While we underline the importance of these quality indicators, one important indicator is virtually lacking in the available literature: "actionability." The term "actionable biomarker" has been used in heart failure and rheumatology,[4,5] and can be defined as "the extent to which a biomarker can be acted upon to improve clinical management."

Recent advances in (high-throughput) laboratory technologies have helped generate an expanding list of potential biomarkers and panels of biomarkers related to kidney (dys-)function. The number of patients suffering from chronic kidney disease (CKD) is substantial: in the general population, the prevalence of CKD at any stage is estimated at 3.31–17.3%.[6] End-Stage Kidney Disease (ESKD), defined by the World Health Organization (WHO) as the requirement for life-saving dialysis or kidney transplantation, is estimated at 1.4 million patients worldwide. The incidence of ESKD is growing each year by ~8%, driven by aging populations, hypertension, and the increasing prevalence of type 2 diabetes mellitus (http://www.who.int/). The alarming demographic trends render the detection, monitoring, and prediction of kidney disease increasingly relevant. The availability of specific biomarkers permits recognition of kidney damage separately from changes in kidney function. Therefore, biomarkers are often categorized as damage biomarkers, functional biomarkers, risk factors, and tools for risk prediction. In line with the general trend in the biomarker literature, however, only a few papers allow assessment of clinical utility and actionability. As a consequence, the impact of renal biomarkers on patient management in clinical practice is currently limited.

To add to routine clinical practice, a biomarker should provide additional actionable information compared to standard methods. In this review we summarize the current knowledge on the actionability of current and novel biomarkers in two main clinical conditions: acute kidney injury (AKI) and chronic kidney disease (CKD). We categorize the literature by its focus on diagnosis, prognosis, and monitoring response to pharmacological and nonpharmacological interventions.

METHODS

The number of publications on novel candidate renal biomarkers in the setting of AKI or CKD is increasing steadily. Therefore, any list of renal biomarkers is probably incomplete by its time of publication. As many high-quality reviews and meta-analysis are available already, and as the aim of the current review is to review the actionability of current and novel biomarkers, we searched for reviews, systematic reviews, and meta-analyses and focused on actionability.

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Search strategy

In January 2017, a search was performed in PubMed using the following search terms: "biomarker," "marker," "biological marker," "functional marker," "urinary marker," "serum marker," or "urinary marker"; and "renal damage, "renal function," "chronic kidney disease," "acute kidney injury," or "kidney function." This search resulted in 6,499 hits, of which only the 423 reviews and 78 systematic reviews and meta-analyses published in the last 5 years were retained. The 5-year time period was chosen to include the most recent meta-analyses and systematic reviews, which in turn compile studies from a much longer time frame. Articles primarily regarding kidney transplantation and articles in pediatrics and animal studies were excluded. Three authors (S.H.B., R.S.H., G.N.) examined the references independently and 48 references were selected as being relevant by at least two authors (26 meta-analyses and 11 reviews). From these meta-analyses and reviews we selected 11 clinical trials to illustrate the best available evidence regarding the actionability of renal biomarkers.

Quality assessment

We used the PRISMA checklist to appraise the quality of reporting of the selected systematic reviews and meta-analyses (http://www.prisma-statement.org/). This checklist is currently the standard for investigators to report their findings and consists of 27 items. Each checklist item was assigned "yes" for compliance or "no" for noncompliance. Discrepancies were decided by consensus agreement.

Below we summarize the current knowledge on biomarkers and their actionability in the setting of AKI and CKD. Both clinical conditions will be briefly introduced with a clinical scenario and three clinical questions to illustrate the settings where biomarkers can potentially add to clinical management, namely, diagnosis, prognosis, and monitoring response to treatment.

Acute kidney injury

Clinical scenario. A 73-year-old male is admitted to the ICU with pneumococcal sepsis. His past medical history is remarkable for hypertension and chronic pulmonary obstruction disease (COPD) Gold stadium II. He has a 30 pack-year history of smoking cigarettes. On admission he is intubated and hypotensive on vasopressors.

This patient is at risk for AKI, and biomarkers have the potential to support the clinical management of this patient in several ways:

- Diagnosis: Which biomarkers should be used for the detection of AKI?
- Monitoring: Which biomarkers can assess response to treatment?
- Prognosis: Which biomarkers predict AKI-related outcome in terms of need for dialysis or AKI-associated death?

Diagnosis: Detection of AKI. AKI is currently diagnosed using the Kidney Disease Improving Global Outcomes (KDIGO) criteria, a consensus-based definition based on functional biomarkers, namely, serum creatinine (sCr) level and urine output-based criteria.[7] However, in the early stages of AKI, sCr may still be normal, since there may not have been sufficient time for creatinine to accumulate. Therefore, urinary and serum damage biomarkers have been widely studied. Our search strategy resulted in 11 meta-analyses on the accuracy of damage biomarkers to detect AKI. The main findings of these meta-analyses are summarized in **Table 1**.[8–18] Some meta-analyses evaluated more than one biomarker: five analyses evaluated urinary neutrophil gelatinase-associated lipocalin (NGAL), five plasma NGAL, two serum cystatin C, one urinary cystatin C, two urinary kidney injury molecule-1 (KIM-1), three urinary interleukin (IL-18), two urinary liver-type fatty acid-binding protein (L-FABP), and one urinary tissue inhibitor of metalloproteinase-2 (TIMP-2) multiplied by insulin-like growth factor binding protein 7 (IGFBP7). A schematic overview of renal biomarkers, organized by tissue source and renal cell type is provided in **Supplemental Figure 1**. Alge and Arthur[19] gave a clear overview of the mechanistic relevance and function of renal biomarkers. After kidney injury, intrarenal NGAL production is dramatically upregulated in the thick ascending limb and the collecting duct. Plasma NGAL also increases as a result of increased hepatic production, and NGAL is filtered by the glomerulus and taken up by the proximal tubule. KIM-1 is a transmembrane protein that contains extracellular mucin and Ig domains. Basal expression of KIM-1 is low in the normal kidney. However, it is upregulated in proximal tubular epithelial cells after injury. IL-18 is a proinflammatory cytokine, which is upregulated in the kidney upon renal damage. L-FABP is a renoprotective protein and is localized predominantly in the proximal tubule. In addition to promoting the metabolism of long-chain and very-long-chain fatty acids, L-FABP also has antioxidant properties. TIMP-2 and IGFBP7 induce G_1 cell cycle arrest after an insult, which prevents ensuing cell death. TIMP-2 is secreted by the ureteric bud, and it has been proposed that injured tubular epithelial cells secrete IGFBP7. Finally, functional tubular markers may be of importance to estimate kidney damage. It has been proposed that, for example, the tubular handling of phosphate decreases with age, and the tubular maximum reabsorption capacity (TmP-GFR) may as such reflect tubular function. The prognostic significance of reduced functional tubular markers such as the TmP-GFR, however, remains unknown.

Quality of reporting of the selected meta-analyses was moderate to good and ranged from a score of 15/27 to 27/27 on the PRISMA checklist. Pooled sensitivity, specificity, and area under the receiver operating curve (AuROC) for almost all analyses were $\geq$0.70, except for the specificity of serum NGAL in two meta-analyses regarding patients with sepsis,[17,18] the specificity of TIMP-2 $\times$ IGFBP7 in one meta-analysis,[15] the sensitivity of urinary IL-18 in two meta-analyses,[12,13] and the AuROC of urinary IL-18, urinary cystatin C, as well as serum cystatin C in one meta-analysis.[11] Neither the ideal cutoff point, nor the optimal timing, was reported for any of the studied biomarkers. Furthermore, all meta-analyses were limited due to considerable heterogeneity between the included studies. Hjortrup *et al.* even aborted their plans of conducting a meta-analysis of the value of NGAL to predict AKI in patients with sepsis, because of the heterogeneity in included studies.[10] They stated "The results of the included studies varied greatly, as did those of studies in general intensive care unit (ICU) patients only. Put another way, the results ranged from a predictive value equivalent to flipping a coin to NGAL being an excellent early marker of AKI." Finally, none of the included studies in the meta-analyses focused on the actionability of the biomarker of interest by showing how the results of biomarker testing should be used to guide clinical management. This illustrates that the currently available data are not sufficient to conclude that individual biomarkers should be used routinely for early detection of AKI.

Other analyses investigated biomarker panels. The rationale behind panels of biomarkers over single biomarkers would be that a combination of biomarkers may be less influenced by the underlying disease state. For instance, underlying infections may influence NGAL concentrations. This may have resulted in less diagnostic accuracy of NGAL in the two meta-analyses in patients with sepsis[17,18] compared to the meta-analysis in patients after cardiac surgery.[9] However, if NGAL levels were elevated along with elevations of KIM-1 and L-FABP, the diagnostic likelihood could be enhanced. In order to assess the quality of research on biomarker combinations in the setting of early AKI diagnosis, Meisner *et al.* conducted a systematic review with a focus on the statistical methods of the included articles.[20] They found that each of the included articles was susceptible to at least one source of bias. Furthermore, in six out of seven cases the AuROC decreased, varying from 14–35%, when they applied the published results to TRIBE-AKI data (Translational Research Investigating Biomarker Endpoint of AKI; one of the most carefully conducted cohort studies for early detection of AKI after major cardiac surgery). Again, none of the included studies investigated the potential benefit of biomarker-guided clinical interventions.

A step towards clinical implementation of the use of a biomarker combination was made in 2014, when the US Food and Drug

Table 1 Quality of reporting and summary results of meta-analyses on the performance of biomarkers to diagnose AKI

Biomarker	PRISMA checklist	Studies, (patients)	Age	Setting	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	Pooled AuROC (95% CI)
Urinary NGAL							
Hjortrup, 2013[10]	23/27	11 (2,875)	All	ICU	[a]	[a]	[a]
Zhang, 2016[18]	26/27	12 (1,263)	Adults	Sepsis	0.80 (0.77-0.83)	0.80 (0.77-0.83)	0.90
Haase-Fielitz, 2014[9]	15/27	20 (3,869)	All	Cardiac surgery	0.74	0.75	0.82
Haase-Fielitz, 2014[9]	15/27	14 (5,347)	All	Critically ill	0.73	0.84	0.80
Ho, 2015[11]	27/27	16 (2,906)	Adults	Cardiac surgery			0.72 (0.66-0.79)
Plasma NGAL							
Haase-Fielitz, 2014[9]	15/27	10 (3,194)	All	Cardiac surgery	0.70	0.85	0.83
Haase-Fielitz, 2014[9]	15/27	9 (3,154)	All	Critically ill	0.84	0.73	0.79
Zhang, 2016[18]	26/27	6 (433)	Adults	Sepsis	0.83 (0.77-0.88)	0.57 (0.54-0.61)	0.86
Kim, 2016[17]	26/27	6 (1,072)	All	Sepsis	0.88 (0.82-0.92)	0.47 (0.37-0.58)	
Ho, 2015[11]	27/27	6 (2,428)	Adults	Cardiac surgery			0.71 (0.64-0.77)
Serum cystatin C							
Yong, 2017[16]	26/27	30 (4,247)	Adults	All	0.82 (0.75-0.87)	0.82 (0.78-0.86)	0.89
Ho, 2015[11]	27/27	3 (594)	Adults	Cardiac surgery			0.69 (0.63-0.74)
Urinary cystatin C							
Ho, 2015[11]	27/27	3 (276)	Adults	Cardiac surgery			0.63 (0.37-0.89)
Urinary KIM-1							
Shao, 2014[14]	27/27	11 (2,979)	All	All	74.0 (61.0-84.0)	86.0 (74.0-93.0)	0.86 (0.83-0.89)
Ho, 2015[11]	27/27	6 (1,774)	Adults	Cardiac surgery			0.72 (0.59-0.84)
Urinary IL-18							
Lin, 2015[12]	24/27	11 (2,796)	All	All	0.51 (0.46-0.55)	0.79 (0.77-0.80)	0.77
Liu, 2013[13]	26/27	23 (4,512)	All	All	0.58 (0.52-0.64)	0.75 (0.69-0.80)	0.70 (0.66-0.74)
Ho, 2015[11]	27/27	5 (1,625)	Adults	Cardiac surgery			0.66 (0.56-0.76)
Urinary L-FABP							
Susantitaphong, 2013[8]	22/27	7 (687)	All	All	0.75 (0.60-0.85)	0.78 (0.62-0.88)	
Ho, 2015[11]	27/27	6 (1,700)	Adults	Cardiac surgery			0.72 (0.60-0.85)
Urinary TIMP-2 x IGFB7							
Su, 2017[15]	23/27	10 (1,709)	Adults	All	0.84 (0.80-0.88)	0.57 (0.55-0.60)	0.88

AuROC, area under receiver operating curve.
[a]Meta-analysis not conducted because of heterogeneity of included studies.

Administration (FDA) approved marketing of NephroCheck (Astute Medical, San Diego, CA).[21] NephroCheck multiplies the urinary concentrations of TIMP-2 and IGFBP7 and divides this product by 1,000 to report a single test result with units of $(ng/mL)^2/1{,}000$ to assess the risk of developing severe AKI (defined as KDIGO stage 2 or 3) within 12 h after testing. The FDA's review included two clinical studies to evaluate the test's safety and effectiveness. With a cutoff of 0.3 $(ng/ml)^2/1{,}000$, NephroCheck accurately detected 92% of AKI patients in one study and 76% in the other.[22,23] In both studies, NephroCheck incorrectly gave a positive result in about half of patients without AKI. TIMP-2 and IGFBP7 individually identified patients with early AKI with significantly greater accuracy than NGAL, KIM-1, IL-18, L-FABP, or cystatin C. The combination of the two markers performed best.[24]

In 2016, the Acute Kidney Injury Advisory Group of the American Society of Nephrology published recommendations on the use of NephroCheck in the clinical setting.[25] They state that use of TIMP-2 × IGFBP7 is appropriate in patients ≥21 years old, who are admitted to the ICU and have undergone cardiac bypass or other major high-risk surgery, or have sepsis, or have one other risk factor for AKI. In this setting, a positive test implies a 27% absolute risk for KDIGO stage 2 or 3 AKI within 12 h and should prompt consideration of nephrology consultation and preventive strategies such as avoiding nephrotoxins, optimizing volume status and hemodynamics, and close monitoring of urine output. Unapproved uses of NephroCheck and limitations include: low-risk patients in the hospital and emergency department, daily or serial measurements, proteinuria (urinary albumin >125 mg/dL interferes

Table 2 Quality of reporting and summary results of meta-analyses on the performance of biomarkers to predict AKI-related outcome

Biomarker	PRISMA checklist	Studies, (patients)	Age	Setting	Outcome (95% CI)
Serum cystatin C					
Feng, 2014[30]	26/27	6 (2,332)	All	All	OR dialysis requirement 2.34 (1.46-3.75) OR death 4.40 (1.58-12.22)
Urinary IL-18 Liu, 2013[13]	26/27	—	All	All	[a]
Urinary L-FABP					
Susantitaphong, 2013[8]	22/27	3 (436) 3 (561)	All	All	Sensitivity dialysis requirement 0.69 (0.35-0.91) Specificity dialysis requirement 0.43 (0.03-0.95) Sensitivity in-hospital death 0.93 (0.66-0.99) Specificity in-hospital death 0.79 (0.27-0.97)

[a]Meta-analysis not conducted because of insufficient data.

with the NephroCheck result and >3,000 mg/dL invalidates it) and bilirubinuria (urine bilirubin concentrations >7.2 g/dL interfere with the result).[25] Our search strategy yielded one meta-analysis on TIMP-2 × IGFBP7 of good quality (**Table 1**).[15] The summary AuROC and sensitivity of the 10 included studies were good (0.99 and 0.84, respectively), but specificity was relatively poor (0.57). Furthermore, it has not been demonstrated that NephroCheck-guided earlier start of supportive measures improves patients' outcome, and therefore future clinical trials are needed before routine measurements can be implemented in daily practice. Until now, to our knowledge, there are no registered ongoing trials investigating optimal NephroCheck-stratified patient management.

Monitoring: Response to treatment in AKI. A biomarker suitable to monitor response to treatment should reflect the efficacy or lack of efficacy of specific interventions and the change in biomarker level during effective treatment should be an adequate surrogate for clinical improvement. Currently, recovery of renal function is assessed with the functional markers sCr and creatinine-based estimated glomerular filtration rate (eGFR).[7] However, creatinine-based estimates of GFR will underestimate the true GFR during recovery of kidney function, because of a lag time in the decline of sCr concentration. Despite the known limitations of sCr, other biomarkers to monitor response to treatment in AKI are not widely investigated. Our search strategy yielded no meta-analyses or systematic reviews on the value of serial measurements of novel biomarkers to monitor response to treatment. This may well be due to the fact that treatment of AKI is mainly supportive and no therapeutic options have been shown to be effective.[7]

Prognosis: Prediction of AKI-related outcome. A prognostic biomarker is most meaningful when the results of testing are clinically actionable and changes in the biomarker level reflect changes in prognosis. Serum creatinine fulfills both criteria since the event "AKI" itself, based on sCr-derived criteria, is strongly associated with an increased risk of CKD, endstage renal disease (ESRD) and mortality, and a decline reflects improved prognosis.[26,27] However, individual long-term follow-up studies have identified a subgroup of patients without AKI as defined by sCr, but with elevated biomarkers of renal damage, who are at increased risk of adverse outcomes.[28,29] This suggests that novel biomarkers of AKI may provide additional prognostic information beyond that offered by sCr. Our search strategy resulted in only three meta-analyses studying the prognostic value of novel biomarkers on AKI-related outcome.[8,13,30] These meta-analyses investigated serum cystatin C, urinary IL-18, and urinary L-FABP, respectively (**Table 2**). The studies' performance to predict dialysis requirement or in-hospital death were either poor (urinary L-FABP),[8] acceptable (serum cystatin C),[30] or could not be determined due to insufficient data for pooling studies (urinary IL-18).[13]

All meta-analyses included studies wherein the biomarker was measured prior to the onset of AKI, since the included studies aimed to validate the biomarker as an early AKI diagnostic. Furthermore, none of the meta-analyses included studies reporting on the added value of novel biomarkers to traditional baseline prognostic variables, such as sCr or eGFR.

In addition to these meta-analyses, Schaub and Parikh[31] recently provided a clear overview of individual studies investigating the association of novel biomarkers with short- and long-term outcomes in different clinical settings of patients with AKI. They conclude that there is extensive evidence showing that biomarkers are related to important patient outcomes, such as renal replacement therapy and death. They underscore the importance of enrolling patients with elevated biomarkers in future clinical trials to investigate the possible benefit of biomarker-guided therapy. Most of the studies included in this review also collected biomarker specimens prior to the diagnosis of AKI. In contrast, below we highlight three clinical studies in patients with established AKI, wherein the added value of biomarkers to baseline models with sCr was investigated.

Hall *et al.*[32] measured urinary concentrations of NGAL, KIM-1, and IL-18 and determined fractional excretion of sodium, fractional excretion of urea, and microscopy score for casts and tubular cells in a heterogeneous group of 249 hospitalized patients on the first day of meeting AKI criteria. There was an approximate 3-fold increase in adjusted risk for worsened AKI stage from enrollment to peak sCr or in-hospital death for upper vs. lower values of NGAL, KIM-1, and IL-18 and microscopy score. The net reclassification index (quantifies how well a new model reclassifies subjects to the observed outcome) improved after adding these biomarkers to a baseline clinical assessment (age ≥65 years, body mass index, male gender, non-Caucasian race, baseline eGFR, surgery before AKI, diabetes, and hypertension).[32] Another prospective study in patients admitted for acute decompensated heart failure also showed that urinary NGAL and urinary IL-18 predicted AKI progression and improved risk reclassification compared with the clinical model (including SCr-based eGFR) alone.[33] In a third prospective cohort of cardiac surgery patients with stage I AKI, urinary IL-18 combined with percentage change in sCr or urinary KIM-1 had the best discriminative ability to identify patients at high risk for progressing to more advanced AKI or death within 30 days.[34]

Although these studies illustrate that there are novel biomarkers that provide prognostic information additional to sCr and eGFR, none of the studies has shown that these biomarkers are actionable, i.e., that therapy induced changes in the level of the specific biomarker in patients with established AKI reflect changes in outcome.

Table 3 Clinical value of biomarkers in AKI based on systematic reviews and meta-analyses, authority approval, and estimated assay costs

	Clinical value (color indicates performance, +/- indicates level of evidence)					
Biomarker	Diagnosis	Monitoring	Prognosis	Actionability	Approved for clinical use	Estimated assay costs ($/sample)
uNGAL	+++	-	-	-	EMA	$19.62
uCysC	++	-	-	-	EMA/FDA	$18.94
uKIM-1	+++	-	-	-	-	$19.72
uIL-18	+++	-	-	-	-	$17.65
uL-FABP	++	-	+	-	-	$24.38
sNGAL	+++	-	-	-	EMA	$19.62
sCysC	+++	-	++	-	EMA/FDA	$18.94
TIMP-2 x IGFBP7	+	-	-	-	EMA/FDA	$85.00

The green or red colors are indicative of good (green) or poor (red) performance of biomarkers in different settings of AKI based on systematic reviews and meta-analyses and whether there are mixed results (purple) or no data was found (gray). We use + or – to indicate the level of evidence found in literature. – indicates no meta-analyses have been found for this biomarker on their diagnostic, monitoring or prognostic value, + indicates one meta-analysis with a PRISMA-score <25, ++ indicates one meta-analysis with a PRISMA-score ≥25, and +++ indicates multiple meta-analyses with PRISMA-scores ≥25. The "approved" column indicates whether a biomarker is approved by EMA and/or FDA for clinical use. Prices are indicative and expressed in US dollars, based on the cheapest human ELISA kit, per triplicate measurement. Prices are a gross underestimation of the true costs, because personnel expenses and costs for good laboratory practice setup etc. are not included in this estimation.

Conclusions and considerations regarding the clinical value of biomarkers in AKI

In **Table 3**, we put available data in the perspective of clinical applicability by providing an overview of the diagnostic, monitoring, and prognostic value of biomarkers, as well as the European Medicines Agency (EMA) and/or FDA approval status and an estimation of the costs. Based on this table, urinary NGAL, urinary KIM-1, urinary L-FABP, and serum cystatin C perform reasonably in the early diagnosis of AKI across study populations. Combining biomarkers has the potential to give more accurate results; however, methodological issues may lead to bias in the development of biomarker combinations. TIMP-2 × IGFBP7 shows promising results; however, specificity is relatively poor and evidence that NephroCheck-guided earlier start of supportive measures improve patient outcome is not available. On the contrary, the added value of NephroCheck for future research is evident. Future intervention trials in AKI may consider using NephroCheck as a criterion for early enrollment, since a delay in the recognition of AKI (resulting from the use of sCr level as entry criterion) may have attenuated the effect of various interventions in prior studies.

When appraising the clinical value of candidate biomarkers for AKI there are some issues to consider. First, it is difficult to compare the value of novel biomarkers with those of sCr, because sCr is almost always used in the reference standard (e.g., the AKI criteria as formulated by KDIGO). Second, it is also difficult to compare the value of novel biomarkers in separate studies, due to the heterogeneity between studies in terms of clinical setting, age, laboratory assays, timing of measurement, AKI criteria used, etc. And third, the most important issue, in sharp contrast to the total number of studies focused on establishing their accuracy to detect renal damage, there are no controlled studies on the impact of biomarker levels on decision-making, hence their actionability.

Chronic kidney disease

Clinical scenario. A 69-year-old woman is seen at the outpatient clinic. She was diagnosed with type 2 diabetes 4 years ago. Other medical problems include obesity and hypothyroidism. She is seen for routine follow-up and is noted to have a blood pressure of 168/105 mmHg.

This patient is at risk for developing chronic kidney disease. Biomarkers can support the clinical management of this patient in several ways:

- Diagnosis: Which biomarkers should be used for the detection of CKD?
- Monitoring: Which biomarkers can assess response to treatment in patients with CKD?
- Prognosis: Which biomarkers predict the onset of CKD and/or related outcome in terms of need for renal replacement therapy, cardiovascular complications, and death?

Diagnosis: Detection of CKD. CKD is defined as GFR <60 mL/min/1.73 m^2 or the presence of ≥1 marker(s) of kidney damage (albumin to creatinine ratio (ACR) ≥30 mg/g, urinary sediment abnormality, electrolyte or other abnormality due to tubular disorder, abnormalities on histology, structural abnormalities detected by imaging, or history of kidney transplantation), or both, of at least 3 months duration.[35] The CKD Prognosis Consortium conducted several meta-analyses, providing a solid basis for including eGFR and ACR measures to establish a CKD diagnosis, by showing that eGFR <60 ml/min/1.73 m^2 and increased albuminuria (ACR >30 mg/g or dipstick >trace) are consistently associated with an increased risk for progressive renal function loss and ESRD across different populations (see Prognosis).[36–38] However, eGFR and albuminuria also have limitations. A limitation for the use of albuminuria to diagnose CKD is that urinary albumin levels are highly variable, and not all types of kidney disease lead to albuminuria.[39] Furthermore, by the time a change is observed in sCr (and hence, eGFR), a critical therapeutic window may have been missed, because increased sCr levels reflect a substantial loss of functioning nephrons, and so earlier detection may seem warranted.[40] In this regard, several reviews reported on the peptidomic CKD marker panel CKD273 as a marker for early renal damage, preceding changes in eGFR and in ACR[41–44] and predicting renal function loss. This panel was identified during a comparison of healthy control patients and patients with various biopsy-proven renal diseases.[45] The CKD273 panel was better correlated to percentage change in eGFR in 522 patients during a follow-up of 3 years than percentage change in albuminuria. Furthermore, the CKD273 panel identified 75% of the rapid progressors, whereas urinary albumin identified

Table 4 Quality of reporting and summary results of meta-analyses on the performance of biomarkers to monitor response to treatment in CKD

					Outcome (when applicable with 95% CI)		
Biomarker	PRISMA checklist	Studies, (patients)	Setting	Biomarker change	Pooled HR doubling sCr, ESRD, death	Pooled RR ESRD	Treatment effect ratio on ESRD[a]
Proteinuria							
Inker, 2014[49]	24/27	32 (9,008)	CKD	50% reduction	0.74 (0.67-0.82)		
Lambers Heerspink, 2015[48]	26/27	21 (78,342)	All	30% reduction		0.76 (0.66-0.89)	
Jun, 2015[50]	26/27	7 (17,740)	All	Any change			0.82 (0.59-1.16)
sCr					**Treatment effect ratio on ESRD[a]**		
Jun, 2015[50]	26/27	20 (95,457)	All	Doubling of sCr	0.98 (0.85-1.14)		
sCr-based eGFR					**Pooled HR doubling sCr, ESRD, death**		
Lambers Heerspink, 2014[51]	26/27	37 (9,488)	CKD	30% reduction	9.6 (7.3-12.6)		
				40% reduction	20.3 (14.1-29.3)		

HR, hazard ratio; RR, relative risk; ESRD, endstage renal disease; BP, blood pressure.
[a]Treatment effect ratio explained in the text.

65% of the rapid progressors. Net reclassification index analysis suggested that the urinary CKD273 panel improved the detection of rapid progressors by 30% compared with the use of albuminuria alone.[46] Of note, the CKD273 risk classifier is currently applied in an ongoing trial that not only aims to confirm its ability to predict development of microalbuminuria in normoalbuminuric patients with type 2 diabetes mellitus, but also aims to determine whether early initiation of treatment with an aldosterone antagonist can reduce the risk of transition to microalbuminuria in designated "high-risk" individuals. This is a rare example of a biomarker-directed therapy trial, that explicitly attempts to evaluate its actionability in a clinically relevant setting.[47]

Monitoring: Response to treatment in CKD. The treatment objectives of all patients with CKD, regardless of the cause, include the prevention of cardiovascular events and a reduction in the rate of progression of renal function loss towards ESRD and other complications, such as anemia, mineral bone disorder, hyperkalemia, and metabolic acidosis. These objectives require pharmacological, as well as nonpharmacological measures (e.g., lifestyle and nutritional) interventions, preferably well aligned.[35] Hence, we searched for biomarkers to assess the response to pharmacological as well as nonpharmacological interventions, but almost exclusively found studies focusing on pharmacological interventions assessed with the classical biomarkers sCr and proteinuria.[48–51] The majority of these included studies were studies of diabetic or hypertensive kidney disease and tested renin angiotensin system blockade.

The results of our search strategy are summarized in **Table 4**.

Inker *et al*. showed that a drug-induced early reduction in proteinuria is consistently associated with slower progression of kidney disease and this association was stronger when baseline proteinuria was higher.[49] Similar results were found by Heerspink *et al*. and Jun *et al*.[48,50] Jun *et al*. assessed the correlation between drug-induced changes in sCr and proteinuria and ESRD and determined the treatment effect ratio (TER).[50] TER was defined as the ratio of the treatment effects on ESRD and the effects on the change in surrogate outcomes. TERs close to 1 indicate greater agreement between ESRD and the surrogate, and these ratios were pooled across interventions. The TER for sCr was excellent 0.98 (0.85–1.14) and for proteinuria was good 0.82 (0.59–1.16). These results demonstrate the actionability of proteinuria and sCr as a marker to monitor for subsequent clinical outcomes, and they support the use of change in proteinuria and sCr to inform CKD prognosis in clinical practice.

Prognosis: Prediction of CKD-related outcome. We subsequently searched for meta-analyses focusing on the prediction of CKD-related outcome by renal biomarkers. Our search strategy resulted in three meta-analyses evaluating the usefulness of potential biomarkers for prediction of ESRD as individual outcome[36,52,53] (**Table 5**) in general and high-risk populations and nine meta-analyses evaluating the usefulness of potential biomarkers for prediction of CKD-related outcome (e.g., mortality, ESRD) in patients with established CKD[37,38,52–58] (**Table 6**).

In the literature, the need for biomarkers that exclusively identify those patients who are most at risk of progressive renal function loss is emphasized, because of the assumption that better assessment of prognosis guides clinical management and early treatment could slow, stop, or possibly even reverse progression towards ESRD.[35] It is well established that decreasing eGFR and increasing albuminuria predict progressive renal function loss across different populations.[35] The CKD diagnostic framework encompasses different categories of eGFR and albuminuria (stages) in order to identify people who will go on to have poor renal outcomes.[35] However, people within the same CKD stage can have very different absolute risks for adverse renal outcome, and there is substantial overlap between the categories.[59] It has also been suggested that an eGFR-based definition of CKD with a threshold of <60 ml/min/1.73 m^2 defines a considerable number of people who will never progress to symptomatic renal disease as CKD patients.[60]

Shlipak *et al*. showed that eGFR on the basis of serum cystatin C provides stronger associations for ESRD (as well as for death from any cause and death from cardiovascular causes) than eGFR on the basis of creatinine.[52] Indeed, the 2012 KDIDGO CKD guideline suggests the use of cystatin C-based eGFR to validate the diagnosis of CKD in patients who are considered to have CKD solely on the basis of a creatinine-based eGFR of 45–59 ml/min/1.73 m^2, without albuminuria or other markers of renal damage.[35] The subgroup of patients which is reclassified to a cystatin C-based eGFR of 60 ml/min/1.73 m^2 or more has a substantially lower risk of death.[52] In contrast, Inker *et al*. showed that the filtration markers serum β-trace protein and β-2 microglobulin do not provide

Table 5 Quality of reporting and summary results of meta-analyses on the performance of biomarkers to predict ESRD in general and high-risk populations

Biomarker	PRISMA checklist	Studies, (patients)	Setting	Reference test	Outcome (when applicable with 95% CI)	
sCr-based eGFR					**Pooled HR ESRD**	
Gansevoort, 2011[36]	26/27	9 (845,125)	General population	eGFR 60, 45 and 15 (vs. 95)	3.69 (2.36-5.76), 29.3 (19.5-44.1), 454.9 (112.4-1840.2)	
Cystatin C based eGFR					**Net reclassification index ESRD**	
Shlipak, 2013[52]	21/27	2 (37,872)	General population	sCr-based eGFR	0.10 (0.00-0.21)	
ß-2 microglobulin based eGFR					**Difference in c-statistic ESRD**	**Net reclassification index ESRD**
Inker, 2017[53]	21/27	3 (17,903)	General population or high risk	sCr-based eGFR	0.005 (0.001-0.009)	0.011 (-0.019, 0.040)
Serum ß-trace protein based eGFR					**Difference in c-statistic ESRD**	**Net reclassification index ESRD**
Inker, 2017[53]	21/27	3 (17,903)	General population or high risk	sCr- based eGFR	-0.001 (-0.003, 0.000)	0.020 (-0.012,0.053)
Albuminuria					**Pooled HR ESRD**	
Gansevoort, 2011[36]	26/27	9 (845,125)	General population	ACR 30, 300 and 1000 mg/g (vs. 5)	4.87 (2.30-10.3), 13.4 (5.49-32.7), 28.4 (14.9-54.2)	

HR, hazard ratio; ESRD, endstage renal disease.

substantial additional information to traditional prediction models including sCr based eGFR and albuminuria.[53]

In line with the results in general and high-risk populations, Matsushita *et al*. showed that ACR is one of the strongest predictors of cardiovascular mortality and, although to a lesser extent than ACR, eGFR also improves cardiovascular risk prediction in patients with established CKD.[38] The change in C-statistic by incorporating eGFR and/or ACR in prediction models was similar or superior to the contributions of most of the individual traditional risk factors including blood pressure, lipids, and smoking. Another meta-analysis, including 721,357 participants with CKD stages 3–5 showed that an original four-variable kidney failure risk equation (age, sex, eGFR, ACR) achieved excellent discrimination (ability to differentiate those who developed kidney failure from those who did not); overall C statistic, 0.90 (95% confidence interval (CI) 0.89–0.92) at 2 years and 0.88 (95% CI 0.86–0.90) at 5 years. Calibration (the difference between observed and predicted risk) was adequate in North American cohorts; however, in some non-North American cohorts the addition of a calibration factor was necessary.[58]

The studies summarized in almost all other meta-analyses in patients with established CKD generally did not study the value of the particular biomarker *in addition* to clinical models with eGFR and ACR, but predominantly used traditional regression models to show that the association between a candidate biomarker and outcome persisted after adjustment for clinical factors. Elevated cardiac troponin level was associated with a higher risk (~2–4-fold) for all-cause mortality and cardiovascular death among CKD patients without suspected ACS.[55,56] One meta-analysis evaluated the value of urinary NGAL and urinary KIM-1 in predicting CKD stage 3, ESRD, and overall mortality. Only the predictive value of uNGAL for ESRD was supported by level A evidence (relative risk (RR) 1.40, 95% CI 1.21–1.61), with the level of evidence for other findings being insufficient to recommend their utility in practice.[54] Although not a meta-analysis, systematic review, or review, a recent study by Hsu *et al*. is of interest since it addressed the additional prognostic value of biomarkers KIM-1, NGAL, and L-FABP compared to eGFR and ACR. Strikingly, none of these biomarkers improved the already high (0.89) C-statistic for the basic clinical model including age, sex, race, clinical center, ACR, eGFR, diabetes mellitus, cardiovascular disease, systolic blood pressure, body mass index, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker use, and education.[61]

Thus, overall, no biomarker so far seems able to outperform sCr-based eGFR or albuminuria, except for serum cystatin C-based eGFR. And again, none of the studies has shown that results of testing of other biomarkers are actionable and that changes in the level of the specific biomarker in patients with established CKD reflect changes in prognosis.

Conclusions and considerations regarding the clinical value of biomarkers in CKD

In **Table 7**, we put available data in the perspective of clinical applicability by providing an overview of the diagnostic, monitoring, and prognostic value of biomarkers in CKD, as well as the EMA and/or FDA approval status and an estimation of the costs. Based on this table, there are no well-validated biomarkers that outperform sCr-based eGFR and proteinuria, except for serum cystatin C-based eGFR in predicting ESRD and mortality. Most promising is the biomarker panel CKD273, especially because of an ongoing trial testing the concept of biomarker-guided therapy initiation (PRIORITY).[47]

TOWARDS BETTER CLINICAL APPLICABILITY: ROLE OF METABOLIC MARKERS

Based on the evidence summarized above, unfortunately the clinical actionability of novel biomarkers is not supported by empirical studies. This may well reflect the general difficulties in generating prospective trials, in terms of funding, organization, and time. It has been argued that especially in nephrology there is a paucity of trials.[62] Yet biomarkers can potentially be highly useful when properly selected and tested. To this purpose, we propose to slightly broaden the concept of biomarkers, as outlined below.

Table 6 Quality of reporting and summary results of meta-analyses on the performance of biomarkers to predict CKD-related outcome in patients with established CKD

Biomarker	PRISMA checklist	Studies, (patients)	Setting	Biomarker aspect	Outcome (when applicable with 95% CI)	
sCr-based eGFR					**Pooled HR ESRD**	**Pooled HR overall mortality**
Astor, 2011[37]	21/27	14 (21,688)	CKD	Reduction in eGFR of 15 (below an eGFR of 45)	6.24 (4.84-8.05)	1.47 (1.22-1.79)
					C-statistic difference cardiovascular mortality	
Matsushita, 2015[38]	21/27	15 (24,777)	CKD	Omitting eGFR from full model	-0.0079 (-0.0123, -0.0036)	
Albuminuria					**Pooled HR overall mortality**	**Pooled HR ESRD**
Astor, 2011[37]	21/27	14 (21,688)	CKD	Eight-fold higher ACR	1.40 (1.27-1.55)	3.04 (2.27-4.08)
					C-statistic difference cardiovascular mortality	
Matsushita, 2015[38]	21/27	15 (24,777)	CKD	Omitting ACR from full model	-0.0141 (-0.0193,-0.0088)	
Cystatin C based eGFR					**Net reclassification index overall mortality**	**Net reclassification index ESRD**
Shlipak, 2013[52]	21/27	5 (2,960)	CKD	sCR-based eGFR	0.21 (0.17-0.26)	0.03 (-0.03-0.08)
ß-2 microglobulin based eGFR					**Net reclassification index overall mortality**	**Net reclassification index ESRD**
Inker, 2017[53]	21/27	3 (5,415)	CKD	sCR-based eGFR	0.010 (-0.006-0.026)	0.098 (0.028-0.168)
Serum ß-trace protein based eGFR					**Net reclassification index overall mortality**	**Net reclassification index ESRD**
Inker, 2017[53]	21/27	3 (5,415)	CKD	sCr-based eGFR	0.003 (-0.018-0.024)	0.014 (-0.049-0.076)
Urinary NGAL					**Pooled RR overall mortality**	
Zhou, 2016[54]	26/27	3 (411)	CKD	1 SD increase	1.10 (1.03-1.18)	
Urinary KIM-1					**Pooled RR ESRD**	
Zhou, 2016[54]	25/27	3 (931)	T1DM, T2DM, CKD	1 SD increase	1.13 (0.96-1.33)	
CRP					**Pooled HR overall mortality**	**Pooled HR cardiovascular mortality**
Li, 2015[55]	23/27	20 (17,085)	CKD	Higher-than-referent	1.21 (1.14-1.29)	1.19 (1.10-1.28)
Cardiac troponin					**Pooled HR overall mortality**	**Pooled HR cardiovascular mortality**
Li, 2015[55]	23/27	17 (5,605)	CKD	Higher-than-referent	2.93 (1.97-4.33)	3.27 (1.67-6.41)
Michos, 2015[56]	25/27	2 (357)	CKD	Higher-than-referent	3.41 (1.06-10.99)	
Michos, 2015[56]	25/27	2 (2,594)	CKD	Higher-than-referent	1.73 (1.17-2.65)	
NT-proBNP					**Pooled RR overall mortality**	**Pooled HR cardiovascular mortality**
Schaub, 2015[57]	26/27	9 (10,777)	CKD	Higher-than-referent	1.59 (1.41-1.80)	
4-variable risk equation[a]					**C-statistic for the 2-year predicted probability of kidney failure**	**C-statistic for the 5-year predicted probability of kidney failure**
Tangri, 2016[58]	22/27	31 (721,357)	CKD		0.90 (0.89-0.92)	0.88 (0.86-0.90)

HR, hazard ratio; ESRD, endstage renal disease.
[a]Variables used in risk equation: age, sex, sCR-based eGFR, ACR.

Table 7 Clinical value of biomarkers in CKD based on systematic reviews and meta-analyses, authority approval, and estimated assay costs

	Clinical value (color indicates performance, +/- indicates level of evidence)					
Biomarker	Diagnosis	Monitoring	Prognosis	Actionability	Approved for clinical use	Estimated assay costs ($/sample)
eGFR (sCR)	++	++	+	++	EMA/FDA	$5.20
eGFR (sCysC)	-	-	+	-	EMA/FDA	$18.94
eGFR (β2-microglobulin)	-	-	+	-	-	$16.75
eGFR (sβ-trace protein)	-	-	+	-	-	$12.50
Albuminuria	++	+++	+	++	EMA/FDA	$18.04
uNGAL	-	-	++	-	EMA	$19.62
uKIM-1	-	-	++	-	-	$19.72
CRP	-	-	+	-	EMA/FDA	$4.40
Cardiac troponin	-	-	++	-	EMA/FDA	$8.35
NT-proBNP	-	-	++	-	EMA/FDA	$20.04

The green or red colors are indicative of good (green) or poor (red) performance of biomarkers in different settings of AKI based on systematic reviews and meta-analyses and whether there are mixed results (purple) or no data was found (gray). We use + or – to indicate the level of evidence found in literature. – indicates no meta-analyses have been found for this biomarker on their diagnostic, monitoring or prognostic value, + indicates one meta-analysis with a PRISMA-score <25, ++ indicates one meta-analysis with a PRISMA-score ≥25, and +++ indicates multiple meta-analyses with PRISMA-scores ≥25. The "approved" column indicates whether a biomarker is approved by EMA and/or FDA for clinical use. Prices are indicative and expressed in US dollars, based on the cheapest human ELISA kit, per triplicate measurement. Prices are a gross underestimation of the true costs, because personnel expenses and costs for GLP-setup (good laboratory practice) etc. are not included in this estimation.

Biomarkers used in nephrology are often classified as functional biomarkers, damage biomarkers, risk factors, or tools for risk prediction. Functional biomarkers refer almost exclusively to biomarkers of glomerular filtration rate, neglecting the homeostatic and regulatory functions of the kidney, such as excretion of sodium, free water, potassium, phosphate, and uric acid, control of blood pressure, acid-base balance, and the humoral aspects of red blood cell production and bone and mineral metabolism. By neglecting these renal functions and their corresponding biomarkers, a whole dimension of actionable biomarkers is overlooked. An important reason to consider the role of such markers, which we propose to name "metabolic biomarkers" (**Figure 1**), is their dynamic character, and hence their potential for actionability by lifestyle-related interventions, pharmacological interventions, or their combination. Metabolic biomarkers closely reflect a known (patho)physiological process for which (non)pharmacological interventions are already available. In contrast, damage biomarkers do not evidently lead to direct therapeutic targets, except for albuminuria and urinary sediment abnormalities.

Some metabolic biomarkers, such as urinary sodium and urea excretion, and serum potassium, bicarbonate, calcium, phosphate, and PTH, are already incorporated in clinical practice guidelines. Daily salt intake is adequately reflected by 24-h urinary sodium excretion.[63] Salt reduction reduces blood pressure and proteinuria considerably and consistently.[64,65] Hence, in daily practice, 24-h sodium excretion is an actionable metabolic biomarker and reflects the efficacy of adherence to a sodium-restricted diet. Moreover, lower sodium intake is associated with an enhanced renoprotective effect of renin-angiotensin system blockade.[66,67]

Daily 24-h urea excretion can be used to estimate daily protein intake[68] and to counsel patients on adequate intake, weighing the potential benefits and dangers of varying dietary protein intake.

Existing data support prevention of hyperphosphatemia and associated secondary hyperparathyroidism in CKD. Calcium/phosphate dysbalance, vitamin D deficiency, or enhanced parathyroid hormone levels may be improved by reducing phosphate intake or using phosphate binders, vitamin D supplementation, or calcium receptor sensitizer agents. Emerging data suggest that vitamin D deficiency, particularly when combined with high sodium intake, may contribute to the development of albuminuria,[69] and that treatment with vitamin D analogs (along with limiting sodium intake) may reduce albuminuria in established CKD.[70,71] However, in general, the evidence for recommended targets to restore mineral metabolism in CKD, and the strategies to achieve these targets, is merely observational.[35] Hence, more clinical trials are needed, especially in patients with nondialysis CKD, to substantiate the actionability of serum calcium, phosphate, and PTH.

A serum bicarbonate level <22 mmol/l has been associated with an increased risk of CKD progression and increased risk of death and serum bicarbonate levels guide treatment with oral bicarbonate supplementation.[35]

From these perspectives on metabolic biomarkers, there are several other candidate metabolic biomarkers, for which therapeutic interventions are already available. However, in these cases, future clinical trials are needed before routine measurements can be implemented in daily practice. For example, functional vitamin K deficiency is common in patients with CKD and is independently associated with an increased risk of all-cause

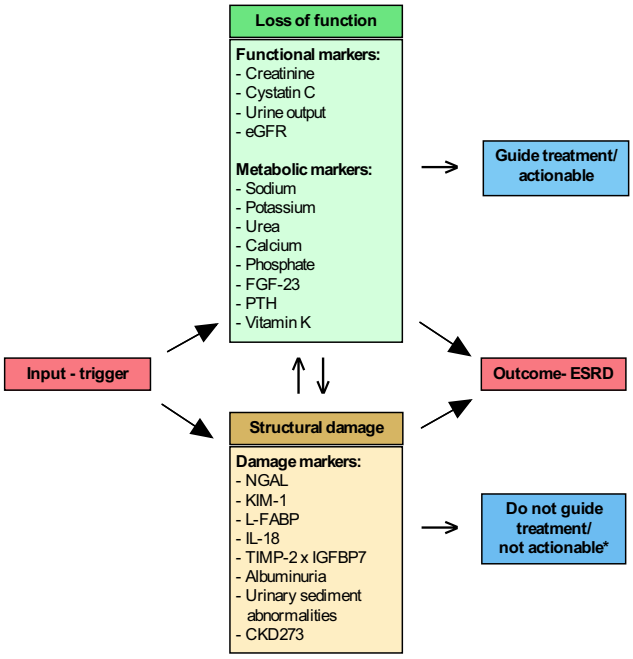

Figure 1 The availability of specific biomarkers permits recognition of kidney damage separately from changes in kidney function. Kidney damage and changes in function may precede each other or occur concurrently in response to a trigger. We propose a new subgroup of functional biomarkers, namely, metabolic biomarkers. In contrast to damage biomarkers, metabolic biomarkers are markers for which interventions are already available and most of them are highly actionable.

mortality.[72–74] Given the potential to modulate vitamin K intake by dietary interventions and the availability of vitamin K supplements, vitamin K insufficiency seems an attractive target for therapeutic intervention. Furthermore, the KDIGO CKD guideline lists hyperuricaemia as a potential contributor to progression of CKD,[35] based on evidence describing the association of hyperuricaemia with CKD and adverse cardiovascular outcomes. Hyperuricaemia can be reduced with xanthine oxidase inhibitors such as allopurinol. Furthermore, a recent meta-analyses showed that allopurinol therapy is associated with significantly improved endothelial function in subjects at risk of CVD risks, and the beneficial effects of allopurinol seemed not to be related to its uric acid lowering action.[75]

However, further large trials are required to better understand the potential benefit of uric acid-lowering agents for the specific purpose of delaying CKD progression or lowering the risk of cardiovascular events.[35]

Numerous reports have linked elevated fibroblast growth factor 23 (FGF23) to progression to ESRD, cardiovascular disease, and death in patients with CKD. FGF23 levels may be improved by reducing phosphate intake or using phosphate binders, vitamin D supplementation, or calcium receptor sensitizer agents. However, large randomized controlled trials are needed to evaluate if these therapeutic strategies lead to improved survival.[76]

In the less advanced CKD-stages, dietary interventions to increase potassium intake, while reducing salt intake, by stimulating the intake of fruit and vegetables, has the potential to improve cardiovascular risk management.[77] Furthermore, dietary changes to reduce salt intake are accompanied by reduced phosphate intake. Therefore, it might be speculated that the blood pressure-lowering effect observed with salt restriction is partly explained by this concomitant phosphate reduction.[78] Both observations need to be further explored in randomized controlled trials.

All the above-mentioned examples illustrate the added value of clinical studies with actionable metabolic biomarkers, complementary to all the research done on damage biomarkers, risk stratificators, and tools for risk prediction.

OVERALL CONCLUSION

Despite an increasing body of literature assessing the accuracy of biomarkers to detect kidney damage or to predict outcome, the question of how patients whose risk is stratified by a biomarker level should be treated remains mostly unanswered. In other words, knowledge on the actionability of novel biomarkers is mostly lacking. In this regard, most advances are expected in the near future from TIMP-2 × IGFBP7 in AKI and CKD273 in CKD. Furthermore, in CKD, we propose a different perspective on functional biomarkers by broadening this group with metabolic biomarkers, such as markers of bone mineral disorders, uric acid, urinary sodium excretion, and vitamin K status, because these biomarkers are highly actionable and in contrast to damage biomarkers, therapeutic interventions are already available in most cases. Future studies should address whether interventions targeting these actionable biomarkers results in improved outcomes in AKI and chronic kidney disease.

Additional Supporting Information may be found in the online version of this article.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

H.B. and R.H. performed the quality assessment of selected meta-analyses and systematic reviews. H.B., R.H., G.N., and M.d.B. wrote manuscript.

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