

Current updates on protein as biomarkers for diabetic kidney disease: a systematic review

Rani Sauriasari^{ID}, Dhonna Dwi Safitri and Nuriza Ulul Azmi

Ther Adv Endocrinol Metab

2021, Vol. 12: 1–13

DOI: 10.1177/

20420188211049612

© The Author(s), 2021.
Article reuse guidelines:
sagepub.com/journals-permissions

Abstract

Background: In the past decade, researchers have been focused on discovering protein biomarkers for diabetic kidney disease. This paper aims to search for, analyze, and synthesize current updates regarding the development of these efforts.

Methods: We systematically searched the ScienceDirect, SpringerLink, and PubMed databases for observational studies of protein biomarkers in patients with diabetes mellitus. We included studies published between January 2018 and April 2020, that were based on a population of patients with type-1 or type-2 diabetes mellitus aged ≥ 18 years, with an observational design such as cross-sectional, case-control, or cohort studies. The dependent variable of the research results was in the form of protein biomarkers from urine, plasma, or serum.

Results: Following the screening process, 20 research articles with available full text met the inclusion criteria. These could be categorized as glomerular biomarkers (ANGPTL4, beta-2 microglobulin, Smad1, and glypican-5); inflammatory biomarkers (MCP-1 and adiponectin); and tubular biomarkers (NGAL, VDBP, megalin, sKlotho, and KIM-1). The development of a panel of biomarkers showed more promising results than those for a single biomarker in diagnosing diabetic kidney disease.

Conclusion: All the biomarkers discussed in this review showed promising results for predicting diabetic kidney disease because they correlate with albuminuria, eGFR, or both. However, of the 11 protein biomarkers, none have prognostic value beyond albuminuria and eGFR.

Keywords: albuminuria, biomarker, diabetic kidney disease, estimated glomerular filtration rate, proteomic

Received: 11 December 2020; revised manuscript accepted: 12 September 2021.

Introduction

Diabetic kidney disease is one of the main causes of increased morbidity and mortality in patients with diabetes mellitus (DM).¹ About 20%–40% of patients with DM, both types 1 and 2, will develop diabetic kidney disease. If not treated properly, this will reach an advanced stage, known as end-stage kidney disease (ESRD).² Currently, the urinary albumin–creatinine ratio (UACR) and estimated glomerular filtration rate (eGFR) are two indicators that are commonly used in the diagnosis of diabetic kidney disease.³ Several

studies that have been conducted on the UACR value showed that not all diabetic kidney disease patients experience an increased value in the early stages of the disease, which indicates that the UACR value is not sensitive enough as a marker in the early phase of diabetic kidney disease.⁴ On the contrary, calculation of the eGFR value using serum creatinine is only accurate when the eGFR value is < 60 mL/min/1.73 m², in which case half of the kidney function may have already been lost.⁵ Therefore, a more sensitive and specific biomarker than the two biomarkers currently used is

Correspondence to:
Rani Sauriasari
Faculty of Pharmacy,
Universitas Indonesia,
Depok, 16424, Indonesia.
rani@farmasi.ui.ac.id
Dhonna Dwi Safitri
Nuriza Ulul Azmi
Faculty of Pharmacy,
Universitas Indonesia,
Depok, 16424, Indonesia

highly needed, to accurately predict diabetic kidney disease in the early phase.

In the past decade, many new biomarkers associated with diabetic kidney disease have been discovered; these include proteins, metabolite products and genes. Most of the biomarkers found were protein,⁶ a macromolecule that functions in various biological processes in the body. Given the important role of protein in the body, a method that can provide information on protein dysregulation would be useful in understanding the pathogenesis of a disease.

The proteomic method is currently one of the most promising in discovering new biomarkers.⁶ The method comprises a process of analyzing proteomes and proteins, which are expressed in various biological fluids such as urine, plasma, and serum. In recent years, several biomarkers for diabetic kidney disease have been identified. Protein in the urine can reflect damage occurring in the kidneys, such as kidney injury molecule-1 (KIM-1), which plays a role in renal tubular damage.⁶

The development of diabetic kidney disease involves various mechanisms. Therefore, a single biomarker is not sufficient to describe the entire process taking place. Instead, a biomarker panel consisting of several proteins and peptides is considered more representative of the various disease development mechanisms and a more accurate biomarker.⁶ We conducted a systematic review to explore, examine, and synthesize some of the latest findings regarding protein biomarkers, either single biomarkers or biomarker panels, which can potentially diagnose diabetic kidney disease in the early phase. In addition, the latest situation regarding the application of these biomarkers in the clinical field is also presented.

Methods

Study search

The systematic review followed recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The research articles used in this systematic review were obtained from Internet searches of databases from ScienceDirect, SpringerLink, and PubMed, and limited to ones

published from January 2018 to April 2020. The search was carried out using keywords: ‘diabetic kidney disease’, ‘biomarker for diabetic nephropathy’, and ‘biomarker for diabetic kidney disease’.

Eligibility criteria

The inclusion criteria set were that (1) the study was published in January 2018 to April 2020; (2) the research was based on a population of patients with type-1 or type-2 DM aged ≥ 18 years; (3) the research study design was observational, such as cross-sectional, case-control, or cohort; and (4) the dependent variable of the research results was in the form of protein biomarkers from urine, plasma, or serum.

In addition, a study was not included if: (1) the article was not published in English; (2) the full-text article was not available; and (3) it was not related to diabetic kidney disease.

Study selection

The search process conducted is briefly described in Figure 1. Based on the search results from several databases using predefined keywords, 17,054 research articles were obtained. After the screening process, 20 of these were judged to meet the criteria set and were subsequently reviewed.

Results and discussion

Diabetic kidney disease is one of the main causes of mortality and morbidity in DM patients. Currently, albuminuria and eGFR are the gold standard markers used to diagnose and monitor diabetic kidney disease. However, these two markers have several limitations in detecting the early phase of diabetic kidney disease.^{4,5} Therefore, a new marker or biomarker that has a more sensitive and specific prognosis ability is needed. We conducted a systematic review to explore, examine, and synthesize some of the latest findings regarding protein biomarkers, either single biomarkers or biomarker panels, which can potentially diagnose diabetic kidney disease. The biomarker measure was the mean difference comparing biomarker in patients to the control group. Table 1 summarizes the key points for each review article included in this systematic review and Table 2 summarizes the characteristics of each biomarker.

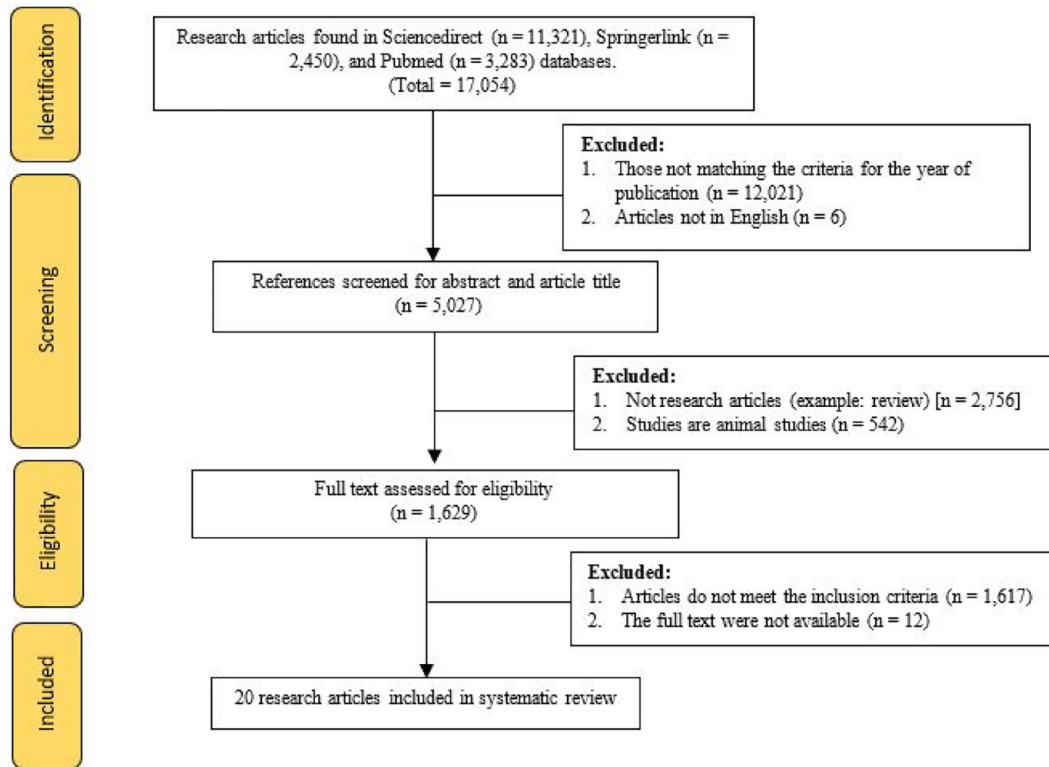


Figure 1. Flowchart of information search strategy according to a four-phase flow diagram of PRISMA schematic guidelines.

Biomarkers related to tubular damage

Vitamin D-binding protein (VDBP) is a plasma protein that plays a role in various physiological functions of the body, including as a carrier for vitamin D3 metabolites in the blood circulation; the binding and absorption of actin; and inflammation and the immune system.³⁹ Tian *et al.*⁴⁰ revealed that increased excretion of VDBP in urine was associated with tubular dysfunction. Therefore, it is thought that an increase in VDBP excretion can also occur in patients with diabetic kidney disease. In their study, it was shown that the concentration of VDBP in urine significantly increased in type-2 DM patients with various levels of albumin secretion when compared with the healthy control group. These results were similar to those of previous studies. Apart from an increase in urine, VDBP concentrations were also significantly increased in the microalbuminuria group. VDBP in urine and serum shows a relationship with the UACR.¹¹

KIM-1 is a transmembrane protein that includes an immunoglobulin-like domain and a mucin domain expressed on proximal tubular epithelial cells. It is thought to have the potential to be used

as a marker to determine renal tubular damage in diabetic kidney patients.⁴¹ Gohda *et al.*¹² found that the KIM-1 concentration in serum was significant in patients with renal insufficiency, showing an association with better eGFR value than KIM-1 in urine. In addition, KIM-1 in serum also has a relationship with the duration of suffering from diabetes; it was found to be elevated in patients with diabetes duration of <5 years. The results indicate that KIM-1 has the potential to be used as a biomarker in the early phase of diabetic kidney disease.¹³

In this review, three articles discuss the potential of neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for diabetic kidney disease. Kaul *et al.*⁹ and Li *et al.*⁸ conducted studies on its potential as a biomarker for diabetic kidney disease. The results of both studies indicated that the concentration of NGAL in urine increased as diabetic kidney disease progressed. Correlation analysis shows that NGAL associates with albuminuria and eGFR values. In addition, NGAL in serum and plasma was also found to be elevated in diabetic kidney disease patients.^{9,10}

Table 1. Summary of all biomarker studies in the systematic review.

Biomarker	Authors	Population and sample	Research design	eGFR (mL/min/1.73 m ²)	ACR (mg/g)/UAER (mg/24 h)/24 h albumin excretion (mg)/AER (µg/min)	Biomarker scores	Research results
ANGPTL4 in plasma	Al Shawaf <i>et al.</i> ⁷	N = 122 (n = 37 DM type 2, n = 49 diabetic kidney disease, n = 36 controls)	Cross-sectional	1. Control group: 80.22 ± 2.27 2. DM type-2 group: 86.64 ± 3.03 3. DKD group: 64.08 ± 3.48	ACR 1. Control group: 9.99 ± 1.34 2. DM type-2 group: 10.24 ± 1.12 3. DKD group: 707.07 ± 217.78	1. Control group: 178.43 ± 2409 µg/mL 2. DM type-2 group: 176.88 ± 14.11 µg/mL; 3. DKD group: 241.56 ± 14.2 µg/mL	ANGPTL4 has a relationship with the albumin-creatinine ratio, serum creatinine, and eGFR
NGAL in urine, plasma, and serum	Li <i>et al.</i> ⁸	N = 209 DM type 2	Cross-sectional	Data not displayed	Data not displayed	Data not displayed	NGAL (AUC: 0.674) in urine is a potential biomarker for type-2 DM patients with normoalbuminuria and renal insufficiency
	Kaul <i>et al.</i> ⁹	N = 198 (n = 144 DM type 2, n = 54 control group)	Cross-sectional	1. Control group: 104.23 (100.82–107.33) 2. Normoalbuminuria group: 95.39 (89.58–103.54) 3. Microalbuminuria group: 88.26 (80.55–98.27) 4. Macroalbuminuria group: 80.90 (75.35–91.97)	ACR 1. Control group: 13.5 (9–17.25) 2. Normoalbuminuria group: 21 (12.75–25) 3. Microalbuminuria group: 149 (107–194) 4. Macroalbuminuria group: 553 (486–632)	NGAL in serum 1. Control: 42.56 ng/mL (25.94–56.6) 2. Normoalbuminuria: 113.62 ng/mL (88.51–135.04) 3. Microalbuminuria: 263.19 ng/mL (217.26–329.06) 4. Macroalbuminuria: 474.88 ng/mL (434.03–494.89) NGAL in urine 1. Control: 7.9 ng/mg (5.5–13.18) 2. Normoalbuminuria: 31.25 ng/mg (22.59–55.47) 3. Microalbuminuria: 110.33 ng/mg (63.17–148.54) 4. Macroalbuminuria: 352.93 ng/mg (166.18–410.25)	There were significant differences in serum and urine NGAL concentrations between the group of patients with type-2 DM and the control group
	Kim <i>et al.</i> ¹⁰	N = 400 (n = 376 DM types 1 and 2, n = 24 healthy control groups)	Cross-sectional	1. Control group: 118 ± 25.1 2. Normoalbuminuria group: 117.7 ± 26.2 3. Microalbuminuria group: 94.5 ± 12.3 4. Macroalbuminuria group: 51.6 ± 19.6	No data available	NGAL in plasma 1. Control group: 61.9 ± 5.3 ng/mL 2. DM type-1 and -2 groups: 93.4 ± 71.8 ng/mL	Measurement of the concentration of NGAL in plasma can play a role in the diagnosis of diabetic kidney disease. Combination of NGAL in plasma and urinary albumin secretion is thought to detect glomerular and renal tubular damage

(continued)

Table 1. (continued)

Biomarker	Authors	Population and sample	Research design	eGFR (mL/min/1.73 m ²)	ACR (mg/g)/UAER (mg/24 h)/24 h albumin excretion (mg)/AER (µg/min)	Biomarker scores	Research results
VDBP in urine and serum	Fawzy and Abu ASe ¹¹	N = 160 (n = 120 DM type 2, n = 40 healthy control group)	Cross-sectional	<ol style="list-style-type: none"> Control group: 102.4 ± 17.6 Normoalbuminuria group: 111.2 ± 36.6 Microalbuminuria group: 107.9 ± 17.2 Macroalbuminuria group: 113.3 ± 22.9 	<ol style="list-style-type: none"> Control group: 16.7 ± 8.7 Normoalbuminuria group: 10.5 ± 7.8 Microalbuminuria group: 77.5 ± 65.5 Macroalbuminuria group: 803.5 ± 355 	<ol style="list-style-type: none"> Control: 127.7 ± 21.9 ng/mg Normoalbuminuria: 193.1 ± 141.0 ng/mg Microalbuminuria: 820.4 ± 402.8 ng/mg Macroalbuminuria: 1,458.1 ± 210 ng/mg <p>VDBP in serum</p> <ol style="list-style-type: none"> Control: 210.3 ± 33.8 µg/mL Normoalbuminuria: 202.4 ± 43.9 µg/mL Microalbuminuria: 248.4 ± 36.5 µg/mL Macroalbuminuria: 299.2 ± 50.6 µg/mL 	VDBP concentrations in serum and urine experienced a significant increase in type-2 DM patients compared to healthy controls
KIM-1 in urine and serum	Gohda <i>et al.</i> ¹²	N = 602 DM type 2	Cross-sectional	<ol style="list-style-type: none"> eGFR group ≥ 60 mL/min/1.73 m²: 80 (69–91) eGFR group = 45–59 mL/min/1.73 m²: 53 (48–57) eGFR < 45 mL/min/1.73 m² group: 40 (36–43) 	<ol style="list-style-type: none"> eGFR group ≥ 60 mL/min/1.73 m²: 19 (8–55) eGFR group = 45–59 mL/min/1.73 m²: 33 (12–217) eGFR < 45 mL/min/1.73 m² group: 179 (21–781) 	<ol style="list-style-type: none"> eGFR group ≥ 60 mL/min/1.73 m²: 1.28 ng/g (0.77–1.96) eGFR 45–59 mL/min/1.73 m²: 1.26 ng/g (0.81–2.1) eGFR group < 45 mL/min/1.73 m²: 1.56 ng/g (1.01–2.14) <p>KIM-1 in serum:</p> <ol style="list-style-type: none"> eGFR group ≥ 60 mL/min/1.73 m²: 96 pg/mL (62–151) eGFR = 45–59 mL/min/1.73 m²: 145 pg/mL (92–216) eGFR < 45 mL/min/1.73 m² group: 207 pg/mL (124–314) 	<p>Increased KIM-1 concentration has a correlation with the albumin-creatinine ratio and decreased eGFR value. However, KIM-1 in serum showed a better correlation than KIM-1 in urine</p>
MCP-1 in urine	Khan <i>et al.</i> ¹³	N = 85 (n = 60 DM; n = 25 healthy controls)	Cohort	No data available	Data not displayed	<ol style="list-style-type: none"> Control group: 7.52 ± 0.77 ng/mL Normoalbuminuria group: 20.91 ± 14.9 ng/mL 	<p>Increased KIM-1 concentration in serum was found to be good in type-2 DM patients</p>
MCP-1 in urine	Satirapoj <i>et al.</i> ¹⁴	N = 83 DM type 2	Cohort	<ol style="list-style-type: none"> Group with decreased LFG < 25% per year: 51.9 ± 27.6 Group with LFG reduction ≥ 25% per year: 36.4 ± 27.4 	<ol style="list-style-type: none"> eGFR decrease < 25% per year: 49.7 (19.9–261.3) eGFR decrease ≥ 25% per year: 673.4 (412.6–2627.6) 	<ol style="list-style-type: none"> Group with decreased LFG < 25% per year: 1.66 ng/mg (1–2.38) Group with decreased LFG ≥ 25% per year: 3.12 ng/mg (2.17–7.97) 	<p>MCP-1 in urine and MCP-1/EGF ratio provided promising results as markers of renal progression in type-2 DM patients</p>

(continued)

Table 1. (continued)

Biomarker	Authors	Population and sample	Research design	eGFR (mL/min/1.73 m ²)	ACR (mg/g)/UAER (mg/24 h)/24 h albumin excretion (mg)/AER (µg/min)	Biomarker scores	Research results
Soluble klotho in plasma	Fountoulakis et al. ¹⁵	N = 92 DM type 2	Cohort	90.7 ± 20.0	UAER 24.5 (9.0–90.2)	204.4 pg/mL (156.8–281.6)	Low concentrations of klotho are associated with albuminuria and decreased kidney function
Soluble klotho in serum	Bob et al. ¹⁶	N = 63 DM	Cohort	65.15 ± 32.45	ACR 381 ± 986	326.36 ± 246.78 pg/mL	There was an increase in the concentration of klotho in patients with eGFR values < 60 mL/min/1.73 m ²
Smad1 in urine	Doi et al. ¹⁷	N = 554 DM type 2	Cohort	1. Groups with IgG4 < 39.2 µg/g: 76.53 ± 18.13 2. Groups with IgG4 > 39.2 µg/g: 69.71 ± 22.76	No data available	1. Groups with IgG4 < 39.2 µg/g: 0.319 ± 0.398 µg/g 2. Groups with IgG4 > 39.2 µg/g: 0.398 ± 0.570 µg/g	Smad1 has potential as a biomarker in the development of diabetic kidney disease
Megalyn in urine	Akour et al. ¹⁸	N = 209 DM type 2	Cross-sectional	106.51 ± 55.03	ACR 34.80 ± 12.93	62.8 pg/g kreatinin	Megalyn in urine has a positive correlation with risk factors for diabetic kidney disease
Adiponectin in urine and serum	Yamakado et al. ¹⁹	N = 83 (n = 59 DM, n = 24 kontrol sehat)	Cross-sectional	Data not displayed	Data not displayed	Adiponectin in urine 1. DM group: 14.88 ± 3.16 ng/mg kreatinin 2. Control group: 3.06 ± 0.33 ng/mg kreatinin Adiponectin in serum 1. DM group: 1.29–22.30 µg/mL 2. Control group: 4.91–10.04 µg/mL	Adiponectin concentration in urine experienced an increase in diabetes patients. Adiponectin in urine is a potential biomarker for the diagnosis of diabetic kidney disease
	Bjornstad et al. ²⁰	N = 1407 (n = 646 DM type 1, n = 761 healthy control)	Cohort	1. Male type 1 DM group: 100 ± 27 2. Female type 1 DM group: 105 ± 28 3. DKD group: 55 ± 33	ACR 1. Type-1 male DM group: 12 (10–15) 2. Female type-1 DM group: 10 (9–12) 3. DKD group: 156 (36–332)	Adiponectin in urine 1. Type-1 DM male: 12.5 ± 7.2 µg/mL 2. Female type-1 DM: 18.6 ± 9.4 µg/mL 3. Control: 10.5 ± 6.4 µg/mL 4. DKD: 19.5 ± 10.8 µg/mL	Adiponectin has a higher concentration in type-1 DM patients, and women with type-1 DM have a higher adiponectin concentration than men with type-1 DM
Glypican-5 in urine	Li et al. ²¹	N = 77 (n = 57 DM type 2, n = 20 healthy controls)	Cohort	1. Control group: 94.09 ± 14.13 2. DM type-2 group: 91.31 ± 21.41 3. DKD group: 55.52 ± 27.94	24-h albumin excretion 1. Control group: 8.5 (4.55–19.13) 2. DM type-2 group: 8.45 (4.2–16.13) 3. DKD group: 1.818 (1.102–3.411,5)	1. Control group: 1.29 ± 0.39 ng/g 2. DM type-2 group: 1.55 ± 0.46 ng/g 3. DKD group: 2.37 ± 1.46 ng/g	Glypican-5 experienced a significant increase in concentration in DKD patients when compared with DM patients and the healthy control group

(continued)

Table 1. (continued)

Biomarker	Authors	Population and sample	Research design	eGFR (mL/min/1.73 m ²)	ACR (mg/g)/UAER (mg/24 h)/24 h albumin excretion (mg)/AER (µg/min)	Biomarker scores	Research results
β ₂ -MG in urine	Jiang <i>et al.</i> ²²	N = 302 (n = 252 DM type 2, n = 50 healthy controls)	Cross-sectional	<ol style="list-style-type: none"> Control group: 110.9 ± 11.6 mL/min/1.73 m² eGFR group ≥ 90 mL/min/1.73 m²: 105.6 ± 13.8 eGFR group = 60–89 mL/min/1.73 m²: 73.9 ± 9.1 eGFR group = 30–59 mL/min/1.73 m²: 47.8 ± 7.9 	<ol style="list-style-type: none"> Control group: 15.4 (12.8–24.9) eGFR group ≥ 90 mL/min/1.73 m²: 22.7 (13.8–41.2) eGFR group = 60–89 mL/min/1.73 m²: 45.7 (16.1–143.3) eGFR group = 30–59 mL/min/1.73 m²: 71.4 (23.6–442.3) 	<ol style="list-style-type: none"> Control group: 0.3 mg/g (0.2–0.5) eGFR group ≥ 90 mL/min/1.73 m²: 0.5 mg/g (0.3–0.9) eGFR group = 60–89 mL/min/1.73 m²: 0.5 mg/g (0.3–1.8) eGFR group = 30–59 mL/min/1.73 m²: 1.1 mg/g (0.4–5.8) 	The four biomarkers studied had increased concentrations in patients with type-2 DM. However, only α1-MG and β2-MG had a correlation with the eGFR value
Biomarker panels	Currie <i>et al.</i> ²³	N = 155 DM type 2	Cohort	<ol style="list-style-type: none"> CKD group > 0.343: 86 ± 18 CKD group < 0.343: 90 ± 15 	<ol style="list-style-type: none"> CKD group > 0.343: 148 (70–385) CKD group < 0.343: 55 (29–99) 	<ol style="list-style-type: none"> CKD group > 0.343: 0.546 (0.369–1.231) CKD group < 0.343: 0.041 (–1.078 to 0.347) 	The CKD273 classification was associated with mortality in type-2 DM patients with microalbuminuria, even after adjustment for cardiovascular and renal biomarkers
Biomarker panels (17 biomarkers in plasma)	Heinzel <i>et al.</i> ²⁴	N = 481 DM type 2	Cohort	<ol style="list-style-type: none"> Groups with decreased stable kidney function: 85 (65–96) Groups with rapid decline in kidney function: 82 (63–94) 	<ol style="list-style-type: none"> Groups with decreased kidney function stable: 8.2 (4.6–21) Groups with rapid decline in kidney function: 9.2 (5–36.5) 	No data available	Twelve of the 17 biomarker candidates had a correlation with eGFR values, but the prognostic ability of these biomarkers was low
Biomarker panels (nine biomarkers in plasma and urine and conventional biomarkers)	Nowak <i>et al.</i> ²⁵	N = 1032 DM type 2	Cohort	<ol style="list-style-type: none"> Normoalbuminuria group: 95 (84–105) Albuminuria group: 97 (83–105) 	<ol style="list-style-type: none"> Normoalbuminuria group: 4 (2–7) Albuminuria group: 44 (20–141) 	No data available	The biomarker panel tested in this study did not have a good prognostic value for predicting decreased renal function in type-2 DM patients with normoalbuminuria
Biomarker panels (46 biomarkers)	Colombo <i>et al.</i> ²⁶	N = 403 DM type 2	Cohort	<ol style="list-style-type: none"> SDR study group: 52.6 (42.2–58.5) GoDARTS study group: 53.4 (43.3–63.8) CARDS study group: 62.1 (54.5–68.7) 	No data available	No data available	The combination of KIM-1 and β2-MG biomarkers in serum significantly increases the predictive ability of decreased renal function in type-2 DM patients

ACR, albumin-creatinine ratio; ANGPTL4, angiotensin-like protein 4; AUC, area under the curve; β2-MG, beta-2-microglobulin; CARDS, collaborative atorvastatin in diabetes study; CKD273, chronic kidney disease 273; DKD, diabetic kidney disease; DM, diabetes mellitus; EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; GoDARTS, Genetics of Diabetes Audit and Research in Tayside; KIM-1, kidney injury molecule-1; MCP-1, monocyte chemoattractant protein-1; n, number of subjects in each group; N, number of all subjects in one study; NGAL, neutrophil gelatinase-associated lipocalin; SDR, Scania Diabetes Registry (Sweden); Smad1, suppressor of mothers against decapentaplegic transcription factor 1; UAER, urine albumin-creatinine ratio; UAER, urinary albumin excretion rate; VDBP, vitamin D-binding protein. Data are shown as mean ± SD or median (minimum–maximum).

Table 2. Characteristics of all protein biomarkers in the systematic review.

Biomarker	Molecular weight	Physiological source	Physiological matrix	Physiological role
Glomerular biomarkers				
ANGPTL4 ²⁷	50 kDa	Synthesized and secreted from several metabolically active tissues	Plasma	Modulating triacylglycerol homeostasis, by inhibiting lipoprotein lipase and stimulates intracellular adipocyte lipolysis. Angptl4 can directly stimulate cAMP-dependent PKA signaling and lipolysis
β ₂ -MG ²⁸	11.8 kDa	Found on the surface of all nucleated cells	Urine	Associated with class-I major histocompatibility complex proteins. Produced in response to systemic inflammation, some acute viral infections, and a number of malignancies
Smad1 ²⁹	52 kDa	Translocated from cytoplasm to the nucleus after phosphorylated and further forms the complex with Smad4 upon the activation of BMP type 1 receptors	Urine	Mediating the bone morphogenetics proteins (BMPs) signaling by forming the heteromeric complex with Smad 4 to act as DNA-binding transcriptional modulator that is activated by BMP type 1 receptors
Glypican-5 ³⁰	64 kDa	Expressed in fetal tissues such as brain, lung, and liver, while in the adult mainly in the brain tissue. It can be secreted from cell surface	Urine	Its function essentially in cell growth and development, play a role as co-receptor for several heparin-binding growth factors to modulate their activity, and able to regulate a variety of pathways such as Wnt, hedgehog, fibroblast growth factor, and bone morphogenetic protein
Inflammatory biomarkers				
MCP-1 ³¹	11–13 kDa	Produced by a variety of cell types, including endothelial, fibroblasts, epithelial, smooth muscle, mesangial, astrocytic, monocytic, and microglial cells, with the major source is monocyte/macrophages	Urine	Plays a role in the recruitment of macrophages and monocytes
HMW-adiponectin ³²	300 kDa	Synthesized in adipocytes	Urine or serum or plasma	Functions as insulin sensitizer, involved in energy homeostasis, and also shows the effect of anti-diabetic, anti-inflammatory, and anti-atherogenic
Tubular biomarkers				
NGAL ³³	25 kDa	Produced by neutrophils and various epithelial cells including kidney tubular cell	Urine or serum or plasma	Contributed to several roles such as in iron metabolism, innate immunity to bacterial, and mycobacterial infection, kidney development, and as a growth factor
VDBP ³⁴	52–59 kDa	Expressed in liver	Urine or serum	A carrier (binding and transporting) of all vitamin D3 metabolites, actin monomers, fatty acids, and membranes proteoglycans of leukocytes and activation of complement C5 system
Megalin ³⁵	38–50 kDa 600 kDa	Expressed in kidney, brain, and central nervous system. In the kidney, the expression is in clathrin-coated pits and proximal tubule epithelial cell microvilli Megalin is also found in intestinal brush border cells, gallbladder epithelial cells, thyroid follicular cells, ocular ciliary bodies, fallopian tubes, and uterus	Urine	Plays a role mainly in receptor-mediated endocytosis and particularly in the proximal tubular uptake of glomerular-filtered albumin and other low-molecular-weight proteins

(continued)

Table 2. (continued)

Biomarker	Molecular weight	Physiological source	Physiological matrix	Physiological role
Soluble klotho ³⁶	135 kDa 130 kDa	Synthesized in kidney as the major source and in brain. Soluble klotho is released from the cell membrane	Plasma or serum	Implicated in increase the transient receptor potential cation channel subfamily V member 5 (TRPV5) and renal outer medullary potassium channel (ROMK) 1 that important in calcium and potassium re-absorption, participate in phosphate homeostasis, suppress the oxidative stress, block the TGF- β signaling, and may be involved in several processes such as apoptosis, cell cycle, and immune system
KIM-1 ³⁷	104 kDa	Produced by the human kidney after injury, specifically in the proximal tubule	Urine and serum	Recognizing and phagocytizing the apoptosis cells in the kidney after injury and thus limiting the proinflammatory response. KIM-1 may also be involved in the interstitial fibrosis development and regeneration process

ANGPTL, angiopoietin-like protein 4; β_2 -MG, beta-2-microglobulin; BMP, bone morphogenetics proteins; HMW, high molecular weight; KIM-1, kidney injury molecule-1; MCP-1, monocyte chemoattractant protein-1; NGAL, neutrophil gelatinase-associated lipocalin; PKA, protein kinase A; ROMK, renal outer medullary potassium channel; Smad1, suppressor of mothers against decapentaplegic transcription factor 1; VDBP, vitamin D-binding protein.

Apart from KIM-1, NGAL is also thought to play a role in renal tubular damage. In healthy individuals, NGAL is secreted by various organs. It is then filtered by the glomerulus and reabsorbed in the proximal tubule. If there are abnormalities in the kidneys, NGAL will be synthesized and quickly regulated in the renal tubules, increasing the excretion of NGAL in the urine.³⁸

In diabetics, endocytosis of advanced glycation end products (AGEs) by megalin in proximal tubular epithelial cells can cause cellular toxicity.³⁵ Studies of megalin as a tubular biomarker showed that increased concentrations of megalin in urine correlated with the severity of diabetic kidney disease.⁴²

Inflammation-related biomarkers

Biomarkers of the inflammatory process also show promising results in predicting the development of diabetic kidney disease.⁴³ MCP-1, which plays a role in the recruitment of macrophages and monocytes, was found to be increased in people with DM without albuminuria. A significant increase occurred in the levels of MCP-1 in the urine of type-2 DM patients with macroalbuminuria compared with other groups of type-2 DM patients and healthy controls.⁴⁴

Adiponectin, which functions as an anti-inflammatory agent, decreases in concentration as diabetic kidney disease develops. Adults with type-1 DM experienced a significant increase in adiponectin than healthy adults. The difference in concentration between the two groups also remained significant during the follow-up period of 6 years after adjustments for eGFR and albumin excretion ratio. In addition, diabetic kidney disease patients

with a rapid decrease in eGFR values also had higher adiponectin concentrations than type-1 DM patients without diabetic kidney disease.²⁰

Biomarkers related to glomerular damage

Beta-2 microglobulin (B2M) has shown a promising ability to detect glomerular damage in diabetic kidney disease. B2M concentrations increased in diabetic patients with normal kidney function (eGFR ≥ 90 mL/min/1.73 m²).²² Glypican-5 and Smad1 showed involvement in the occurrence of glomerular morphological changes, especially in mesangial cell dysfunction.⁴⁵

An *in vivo* study found that GPC5 levels were significantly elevated in mice with induced diabetes, especially in the mesangial cells and kidney podocytes.⁴⁵ Diabetic patients experienced a significant increase in GCP5 concentrations compared to the healthy control group. After a 52-week follow-up period, in the diabetic kidney disease patients, GCP5 was estimated to have a strong correlation with decreased eGFR values ($r = -0.786$) and albumin secretion ($r = 0.346$). Therefore, GCP5 has the potential to be used as a biomarker for diabetic kidney disease. However, further studies are needed regarding the mechanism of the association of GCP5 with other clinical parameters.²¹

In the development of diabetic kidney disease, Smad1 plays a role in the overproduction of type-IV collagen in mesangial cells in animals, which is induced by diabetes acting on the TGF- β receptor. Type-IV collagen is a component that plays a major role in the expansion of the mesangial matrix in diabetic kidney disease.⁴⁶ A study showed that high Smad1 concentrations correlated with the rate of mesangial cell expansion in diabetic kidney disease.¹⁷

ANGPTL4 is also thought to play a role in the breakdown of glomerular podocytes. Physiologically, it plays a role as a regulator in lipid metabolism by inhibiting lipoprotein lipase (LPL) activity and also plays a role in the pathophysiological mechanisms of cardiovascular disease and metabolic syndrome.²⁷ Clement *et al.*⁴⁷ explained that ANGPTL4 plays a part in the proteinuria process in nephropathic syndrome, in which the high concentration of ANGPTL4 produced by podocytes can cause changes in the glomerular basement membrane and reduce the ability of the podocyte diaphragm slit in experimental animals.

Increased secretion of ANGPTL4 in podocytes causes a decrease in the function of the podocyte diaphragm slit. In a study of type-2 DM patients by Al Shawaf *et al.*,⁷ the plasma ANGPTL4 concentration was significantly higher in diabetic kidney disease patients compared to type-2 DM patients and the control group. In addition, ANGPTL4 was found to have a correlation with the eGFR value and albumin-creatinine ratio.

The kidneys play an important role in klotho homeostasis by maintaining its circulation in the body. In a cross-sectional study of patients with chronic renal failure, the soluble Klotho (sKlotho) concentration was found to decrease in the early stages of the disease, but decreased as the disease progressed.⁴⁸ In a study conducted on diabetic kidney disease patients, patients with low sKlotho concentrations showed a faster decrease in eGFR values from baseline than patients with higher concentrations.¹⁵

However, research conducted by Bob *et al.*¹⁶ obtained contradictory results. sKlotho showed an increase in concentration in patients with eGFR values <60 mL/min/1.73 m². The difference in the results of this study are thought to be due to technical differences when measuring biomarkers, as there is no standardization in

commercially available kits. In addition, it is important to remember that the concentration of biomarkers does not always decrease as the disease progresses.⁷ Therefore, further studies are needed to determine whether sKlotho can predict the longitudinal progression of diabetic kidney disease.⁴⁸

An increase or decrease in the concentration of biomarkers in urine, serum, and plasma indicates that biomarkers play a role in various disease pathogenesis mechanisms, such as glomerular and tubular morphological changes, and inflammatory events. In addition, the single biomarkers discussed in this review are associated with albumin excretion in the urine, decreased eGFR values, or both.

Biomarker panels

Diabetic kidney disease involves various pathogenetic processes in its development. Therefore, the use of one single biomarker is considered insufficient to describe the overall disease progression process.^{49,50} Several studies on biomarker panels have been conducted to improve disease diagnostics, prognostics, and therapeutic responses.⁴⁹⁻⁵³ A multimarker score increased prognostic accuracy and reclassification compared with traditional clinical variables alone.⁵² One of the most researched biomarker panels is the CKD273 classification.⁵⁰ This value was used to classify patients based on the level of risk of decreased kidney function. This can be useful for providing interventions according to the patient's needs to reduce medical costs and prevent unwanted side effects.⁵¹ However, other biomarker panel studies showed that the biomarker panel they analyzed did not have a good prognostic ability to predict decreased kidney function in diabetic kidney disease patients.^{52,53}

Limitations of the research on protein and peptide biomarkers

Apart from the emergence of various new biomarkers that provide promising results, some studies have limitations, such as too few samples and too short follow-up periods.²³ In addition, the results of one study to another are not always similar and consistent, which is due to the use of different analysis methods and conditions.²³ Other factors, such as lifestyle and population ethnicity, must also be considered when presenting research results.

Researchers are still using albuminuria and eGFR values as final parameters in research related to diabetic kidney disease. To date, no new biomarkers have been found that have a prognostic ability beyond albuminuria and eGFR values. However, some experts claim that new biomarkers can better describe disease progression than albuminuria and eGFR value.³ Therefore, further studies are needed on developing this biomarker, especially biomarker panels, to predict decreased kidney function and therapeutic responses in DM patients.

Conclusion

All the biomarkers discussed in this systematic review showed promising results for predicting diabetic kidney disease because they correlate with albuminuria, eGFR, or both. These could be categorized as glomerular biomarkers (ANGPTL4, B2M, Smad1, and glypican-5); inflammatory biomarkers (MCP-1 and adiponectin); and tubular biomarkers (NGAL, VDBP, megalin, sKlotho, and KIM-1). However, of the 11 protein biomarkers, none showed a prognostic value beyond albuminuria and eGFR.

The use of single biomarkers or biomarker panels in clinical practice is still very limited. Apart from the various limitations that arise in the process of discovering new biomarkers, the development of proteomic technology in the effort to find new biomarkers for diabetic kidney disease must still be implemented.

Acknowledgements

RS and DDS contributed equally to this work.

Author contributions

RS: conceptualization; funding acquisition; methodology; supervision; writing—original draft; and writing—review and editing. DDS: conceptualization; formal analysis; methodology; writing—original draft; and writing—review and editing. NUA: methodology; supervision; writing—original draft; and writing—review and editing.

Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship,

and/or publication of this article: The study was supported by PUTI KI Grant from Directorate of Research University Indonesia (grant no. NKB-752/UN2.RST/HKP.05.00/2020).

ORCID iD

Rani Sauriasari  <https://orcid.org/0000-0001-7861-4369>

References

1. Stanton R. Clinical challenges in diagnosis and management of diabetic kidney disease. *Am J Kidney Dis* 2014; 63(2, Suppl. 2): S3–S21.
2. Persson F and Rossing P. Diagnosis of diabetic kidney disease: state of the art and future perspective. *Kidney Int Suppl* 2018; 8: 2–7.
3. Colhoun HM and Marcovecchio ML. Biomarkers of diabetic kidney disease. *Diabetologia* 2018; 61: 996–1011.
4. Said S and Nasr S. Silent diabetic nephropathy. *Kidney Int* 2016; 90: 24–26.
5. Bjornstad P, Cherney D and Maahs D. Update on estimation of kidney function in diabetic kidney disease. *Curr Diab Rep* 2015; 15: 57.
6. Pena M, Mischak H and Heerspink H. Proteomics for prediction of disease progression and response to therapy in diabetic kidney disease. *Diabetologia* 2016; 59: 1819–1831.
7. Al Shawaf E, Abu-Farha M, Devarajan S, *et al.* ANGPTL4: a predictive marker for diabetic nephropathy. *J Diabetes Res* 2019; 2019: 4943191.
8. Li A, Yi B, Liu Y, *et al.* Urinary NGAL and RBP are biomarkers of normoalbuminuric renal insufficiency in type 2 diabetes mellitus. *J Immunol Res* 2019; 2019: 5063089.
9. Kaul A, Behera M, Rai M, *et al.* Neutrophil gelatinase-associated lipocalin: as a predictor of early diabetic nephropathy in type 2 diabetes mellitus. *Indian J Nephrol* 2018; 28: 53–60.
10. Kim S, Jeong T, Lee W, *et al.* Plasma neutrophil gelatinase-associated lipocalin as a marker of tubular damage in diabetic nephropathy. *Ann Lab Med* 2018; 38: 524–529.
11. Fawzy M and Abu AlSel BT. Assessment of vitamin D-binding protein and early prediction of nephropathy in type 2 Saudi diabetic patients. *J Diabetes Res* 2018; 2018: 8517929.
12. Gohda T, Kamei N, Koshida T, *et al.* Circulating kidney injury molecule-1 as a biomarker of renal

- parameters in diabetic kidney disease. *J Diabetes Investig* 2019; 11: 435–440.
13. Khan FA, Fatima SS, Khan GM, *et al.* Evaluation of kidney injury molecule-1 as a disease progression biomarker in diabetic nephropathy. *Pak J Med Sci* 2019; 35: 992–996.
 14. Satirapoj B, Dispan R, Radinahamed P, *et al.* Urinary epidermal growth factor, monocyte chemoattractant protein-1 or their ratio as predictors for rapid loss of renal function in type 2 diabetic patients with diabetic kidney disease. *BMC Nephrol* 2018; 19: 246.
 15. Fountoulakis N, Maltese G, Gnudi L, *et al.* Reduced levels of anti-ageing hormone Klotho predict renal function decline in type 2 diabetes. *J Clin Endocrinol Metab* 2018; 103: 2026–2032.
 16. Bob F, Schiller A, Timar R, *et al.* Rapid decline of kidney function in diabetic kidney disease is associated with high soluble Klotho levels. *Nefrologia* 2019; 39: 250–257.
 17. Doi T, Moriya T, Fujita Y, *et al.* Urinary IgG4 and Smad1 are specific biomarkers for renal structural and functional changes in early stages of diabetic nephropathy. *Diabetes* 2018; 67: 986–993.
 18. Akour A, Kasabri V, Bulatova N, *et al.* Urinary megalin in association with progression factors of diabetic nephropathy. *Bratisl Lek Listy* 2019; 120: 532–535.
 19. Yamakado S, Cho H, Inada M, *et al.* Urinary adiponectin as a new diagnostic index for chronic kidney disease due to diabetic nephropathy. *BMJ Open Diabetes Res Care* 2019; 7: e000661.
 20. Bjornstad P, Pyle L, Kinney G, *et al.* Adiponectin is associated with early diabetic kidney disease in adults with type 1 diabetes: a Coronary Artery Calcification in Type 1 Diabetes (CACTI) study. *J Diabetes Complications* 2017; 31: 369–374.
 21. Li R, Zhang L, Zhang S, *et al.* Levels and clinical significances of glypican-5 in urine of type 2 diabetic nephropathy cases. *Iran J Kidney Dis* 2019; 13: 173–181.
 22. Jiang X, Zhang Q, Wang H, *et al.* Associations of urinary, glomerular, and tubular markers with the development of diabetic kidney disease in type 2 diabetes patients. *J Clin Lab Anal* 2017; 32: e22191.
 23. Currie G, von Scholten B, Mary S, *et al.* Urinary proteomics for prediction of mortality in patients with type 2 diabetes and microalbuminuria. *Cardiovasc Diabetol* 2018; 17: 50.
 24. Heinzl A, Kammer M, Mayer G, *et al.* Validation of plasma biomarker candidates for the prediction of eGFR decline in patients with type 2 diabetes. *Diabetes Care* 2018; 41: 1947–1954.
 25. Nowak N, Skupien J, Smiles A, *et al.* Markers of early progressive renal decline in type 2 diabetes suggest different implications for etiological studies and prognostic tests development. *Kidney Int* 2018; 93: 1198–1206.
 26. Colombo M, Looker H, Farran B, *et al.* Serum kidney injury molecule 1 and β 2-microglobulin perform as well as larger biomarker panels for prediction of rapid decline in renal function in type 2 diabetes. *Diabetologia* 2018; 62: 156–168.
 27. Koliwad S, Gray N and Wang JC. Angiopoietin-like 4 (Angptl4): a glucocorticoid-dependent gatekeeper of fatty acid flux during fasting. *Adipocyte* 2012; 1: 182–187.
 28. Li L, Dong M and Wang XG. The implication and significance of beta 2 microglobulin: a conservative multifunctional regulator. *Chin Med J* 2016; 129: 448–455.
 29. Lin Y, Martin J, Gruendler C, *et al.* A novel link between the proteasome pathway and the signal transduction pathway of the bone morphogenetic proteins (BMPs). *BMC Cell Biol* 2002; 3: 15.
 30. Thway K, Selfe J and Shipley J. GPC5 (glypican 5). *Atlas Genet Cytogenet Oncol Haematol* 2011; 15: 557–559.
 31. Deshmane SL, Kremlev S, Amini S, *et al.* Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res* 2009; 29: 313–326.
 32. Achari AE and Jain SK. Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. *Int J Mol Sci* 2017; 18: 1321.
 33. Soni SS, Cruz D, Bobek I, *et al.* NGAL: a biomarker of acute kidney injury and other systemic conditions. *Int Urol Nephrol* 2010; 42: 141–150.
 34. Rozmus D, Ciesielska A, Płomiński J, *et al.* Vitamin D binding protein (VDBP) and its gene polymorphisms—the risk of malignant tumors and other diseases. *Int J Mol Sci* 2020; 21: 7822.
 35. De S, Kuwahara S and Saito A. The endocytic receptor megalin and its associated proteins in proximal tubule epithelial cells. *Membranes* 2014; 4: 333–355.
 36. Xu Y and Sun Z. Molecular basis of Klotho: from gene to function in aging. *Endocr Rev* 2015; 36: 174–193.

37. Mussap M, Noto A, Fanos V, *et al.* Emerging biomarkers and metabolomics for assessing toxic nephropathy and acute kidney injury (AKI) in neonatology. *Biomed Res Int* 2014; 2014: 602526.
38. Phan V, Brophy P and Fleming G. Chapter 39. Acute renal failure: prevention, causes, and investigation. In: Geary DF and Schaefer F (eds) *Comprehensive pediatric nephrology*. Philadelphia, PA: Elsevier, 2008, pp. 607–627.
39. Bouillon R. The vitamin D binding protein DBP. In: Feldman D, Pike JW and Adams JS (eds) *Vitamin D*. San Diego, CA: Academic Press, 2011, pp. 57–72.
40. Tian XQ, Zhao LM, Ge JP, *et al.* Elevated urinary level of vitamin D-binding protein as a novel biomarker for diabetic nephropathy. *Exp Ther Med* 2014; 7: 411–416.
41. Anadón A, Castellano V and Martínez-Larrañaga M. Biomarkers in drug safety evaluation. In: Gupta RC (ed.) *Biomarkers in toxicology*. Philadelphia, PA: Elsevier, 2014, pp. 923–945.
42. Ogasawara S, Hosojima M, Kaseda R, *et al.* Significance of urinary full-length and ectodomain forms of megalin in patients with type 2 diabetes. *Diabetes Care* 2012; 35: 1112–1118.
43. Xiang A, Ekinci E and MacIsaac R. Inflammatory proteins in diabetic kidney disease—potential as biomarkers and therapeutic targets. *Ann Transl Med* 2019; 7(Suppl. 6): S243.
44. Kim M and Tam F. Urinary monocyte chemoattractant protein-1 in renal disease. *Clin Chim Acta* 2011; 412: 2022–2030.
45. Okamoto K, Honda K, Doi K, *et al.* Glypican-5 increases susceptibility to nephrotic damage in diabetic kidney. *Am J Pathol* 2015; 185: 1889–1898.
46. Abe H, Matsubara T, Iehara N, *et al.* Type IV collagen is transcriptionally regulated by smad1 under advanced glycation end product (AGE) stimulation. *J Biol Chem* 2004; 279: 14201–14206.
47. Clement L, Avila-Casado C, Macé C, *et al.* Podocyte-secreted angiotensin-like-4 mediates proteinuria in glucocorticoid-sensitive nephrotic syndrome. *Nat Med* 2010; 17: 117–122.
48. Pavik I, Jaeger P, Ebner L, *et al.* Secreted Klotho and FGF23 in chronic kidney disease stage 1 to 5: a sequence suggested from a cross-sectional study. *Nephrol Dial Transplant* 2013; 28: 352–359.
49. Schrauben SJ, Shou H, Zhang X, *et al.* Association of multiple plasma biomarker concentrations with progression of prevalent diabetic kidney disease: findings from the Chronic Renal Insufficiency Cohort (CRIC) study. *J Am Soc Nephrol* 2021; 32: 115–126.
50. Pontillo C, Zhang ZY, Schanstra JP, *et al.* Prediction of chronic kidney disease stage 3 by CKD273, a urinary proteomic biomarker. *Kidney Int Rep* 2017; 2: 1066–1075.
51. Puthumana J, Thiessen-Philbrook H, Xu L, *et al.* Biomarkers of inflammation and repair in kidney disease progression. *J Clin Invest* 2021; 131: e139927.
52. Perco P, Pena M, Heerspink H, *et al.* Multimarker panels in diabetic kidney disease: the way to improved clinical trial design and clinical practice. *Kidney Int Rep* 2019; 4: 212–221.
53. Lindhardt M, Persson F, Zürlbig P, *et al.* Urinary proteomics predict onset of microalbuminuria in normoalbuminuric type 2 diabetic patients, a sub-study of the DIRECT-Protect 2 study. *Nephrol Dial Transplant* 2016; 32: 1866–1873.

Visit SAGE journals online
journals.sagepub.com/
home/tae

 SAGE journals