Review Article

Albuminuria in diabetic patients: how to measure it?—a narrative review

Guillaume Résimont[1], Etienne Cavalier[2], Régis P. Radermecker[3], Pierre Delanaye[1,4]

[1]Nephrology-Dialysis-Transplantation, University of Liège, CHU Sart Tilman (CHU ULg), Liège, Belgium; [2]Department of Clinical Chemistry, University of Liège, CHU Sart Tilman, Liège, Belgium; [3]Diabetes, Nutrition and Metabolic Diseases Department, Clinical Pharmacology, University of Liège, CHU Sart Tilman (CHU ULg), Liège, Belgium; [4]Department of Nephrology-Dialysis-Apheresis, Hôpital Universitaire Carémeau, Nîmes, France

Contributions: (I) Conception and design: G Résimont, P Delanaye; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: None; (V) Data analysis and interpretation: None; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Guillaume Résimont. Nephrology-Dialysis-Transplantation, CHU Sart Tilman, 4000 Liège, Belgium.
Email: guillaume.resimont08@gmail.com.

Abstract: Diabetes is the leading cause of chronic kidney disease (CKD) and end stage renal disease (ESRD) worldwide. Glomerular filtration rate (GFR) and urine albumin to creatinine ratio (ACR) define the severity of CKD and predict cardiovascular outcomes of patients with CKD. ACR is preferred over protein to creatinine ratio (PCR) because of a greater sensitivity for low level of proteinuria, higher reproducibility in between laboratories and better cardiovascular risk stratification. Interventions aiming at decreasing ACR [such as use of renin-angiotensin-aldosterone system (RAAS) or more recently, sodium glucose cotransporter type 2 inhibitors (SGLT2is)] slow down the rate of progression of CKD. Therefore, global recommendations state that ACR needs to be assessed at least once a year, ideally on an early morning void, in patients with diabetes or CKD. However, quantification of urinary albumin is not standardized yet and heterogeneity in the laboratory methods available or expression of albuminuria may lead to misinterpretation of ACR. Pre-analytical considerations such as characterisation of the urinary sediment is also important as abnormalities (infection or haematuria) may interfere with the ACR. Moreover, atypical features such as an active sediment, fast progression of albuminuria or decline in GFR, short duration of diabetes, absence of diabetic retinopathy, nephrotic syndrome, must lead to further investigation since non-diabetic renal disease is not rare in patients with diabetes and might benefit from targeted therapeutics. Nephrologists and diabetologists should be aware of these limitations when interpreting the results in order to offer incisive and adequate therapeutic management.

Keywords: Chronic kidney disease (CKD); urinary protein; urinary albumin; albuminuria; proteinuria

Received: 16 September 2021; Accepted: 19 November 2021; Published: 30 January 2022.
doi: 10.21037/jlpm-21-58
View this article at: https://dx.doi.org/10.21037/jlpm-21-58

Introduction

Chronic kidney disease (CKD) demonstrates few symptoms until late stages. Early identification and management of CKD with detection of albuminuria is cost effective and reduces risk of progression of kidney diseases and cardiovascular outcomes in diabetic patients (1-5).

International guidelines recommend assessment of urine albumin to creatinine ratio (ACR), ideally on an early morning void, as the first method for initial testing of proteinuria since protein to creatinine ratio (PCR) has a lower level of sensitivity for low range proteinuria (6-10). Since creatinine excretion is quite constant through the day, ACR or PCR are preferred to quantify albuminuria or

proteinuria alone in order to reduce the dilution variable of the spot sample. Spot urine samples are preferred since 24 hours collection is more cumbersome and prone to errors. Moreover, diabetic nephropathy (DN) is mainly characterized by glomerular injury, and ACR is thus again preferred, as it is more sensitive and specific for detection of changes in glomerular permeability. ACR is also predictive of cardiovascular events (11).

Several different methods exist for detecting albuminuria. However, quantification of urinary albumin is not standardized because of the absence of a reference system including a reference measurement procedure and certified reference materials (12,13). Hence the National Institute of Standards and Technology (NIST), the National Kidney Disease Education Program (NKDEP) and the Working Group for the Standardization of Albumin Assays in Urine (WG-SAU) of the International Federation of Clinical Chemistry (IFCC) are currently working on such a standardization of albuminuria measurement (12). Regarding urine protein measurement, it seems quite difficult to standardize because of the heterogeneity of urine protein composition and reactants.

In the current article, we will define diabetic kidney disease (DKD) and DN, then briefly discuss the analytical challenges of albuminuria and proteinuria measurement and eventually underline the limitations of the current methods used to quantify albuminuria in routine laboratories.

We present the following article in accordance with the Narrative Review reporting checklist (available at https://jlpm.amegroups.com/article/view/10.21037/jlpm-21-58/rc).

Definitions/natural history of DKD

CKD affects 5% to 10% of the general population and is a risk marker for cardiovascular events, especially when albuminuria is high (2-5,10,14). Diabetes is the leading cause of CKD and end stage kidney disease (ESKD) in western countries (15,16). Optimal blood pressure and glycaemic control are keystones to slow down the progression of DKD to ESKD. The American Diabetes Association (ADA) recommends to assess albuminuria and estimation of the glomerular filtration rate (eGFR) annually in patients with type 1 diabetes with duration of at least 5 years and in all patients with type 2 diabetes (17). The European Society of Cardiology (ESC)/European Association for the Study of Diabetes (EASD) also recommend yearly assessment of eGFR and ACR in diabetes (18). When ACR is >30 mg/g and eGFR <60 mL/min/1.73 m², ADA recommends that patients should be monitored twice a year. Consistently, Kidney Disease: Improving Global Outcomes (KDIGO) guidelines state that eGFR and albuminuria should be assessed at least annually in people with CKD and more often in individuals at higher risk of progression or if measurement will impact therapeutic decisions (6). In the United Kingdom, the National Institute for Health and Care Excellence (NICE) adds further recommendations on the cut off for a confirmatory repeat test: the NICE recommend to repeat an ACR between 3 and 70 mg/mmol (30 and 700 mg/g) on another early morning void to confirm the result. When the ACR is >70 mg/mmol (>700 mg/g), a repeat sample is not needed (10). The NICE also recommends to appreciate proteinuria with urine ACR in adults, children and young people with diabetes; adults with an eGFR <60 mL/min/1.73 m²; and adults with an eGFR >60 mL/min/1.73 m² if there is a strong suspicion of CKD. As for non-diabetic children and young people, the <60 mL/min/1.73 m² threshold is not appropriate since any reduction in GFR in this population should lead to quantify albuminuria. The threshold with children and young people without diabetes is a value of serum creatinine above the upper limit of the age-appropriate reference range (10).

GFR (divided in 6 stages) and albuminuria categories (A1-A2-A3 see below) define together the prognosis of CKD. Despite these recommendations, measuring the ACR in clinical practice is too often overlooked: an American study gathering half a million type 2 diabetes patients showed that only half of them underwent ACR testing in the previous year, and 74% in the previous 3 years, contrasting with the higher rate of eGFR testing (89.5% in the previous year and 97% in the 3 previous years) and HbA1c testing (91% in the previous year and 96% in the 3 previous years) (19). Inhibitors of the renin-angiotensin-aldosterone system (RAAS) are first line medications that slow down the loss of GFR and reduce albuminuria (20-22). Recently, sodium-glucose co-transporter 2 inhibitors (SGLT2is), Glucagon like peptide 1 receptor agonists (GLP-1 RA) and Finerenone also showed huge benefits with reduction of albuminuria and decrease of the slope of eGFR (23-27).

DKD

DKD is defined by the presence of albuminuria or decreased eGFR in patients with diabetes. DKD does not describe a specific pathological phenotype and many other aetiologies such as hypertensive nephrosclerosis, unresolved acute kidney failure, infections or other nephropathies might be

intricated. The pathophysiology of DKD is multifactorial and includes metabolic and hemodynamic factors (15). Therefore, atypical features such as short duration of diabetes, absence of diabetic retinopathy, haematuria, nephrotic syndrome, fast progression of albuminuria or decline in GFR or other sign or symptoms associated with other causes of kidney damage should lead to kidney biopsy to exclude more specific renal diseases (15,17). Fiorentino *et al.* showed in a meta-analysis that non-diabetic renal disease is not rare in patients with diabetes which may lead to specific treatment for delaying ESKD (16).

DN

DN is a histological diagnosis based on structural and functional changes seen in the kidneys of patients with type 1 or type 2 diabetes directly caused by the effects of diabetes on the kidney. Clinical presentation includes albuminuria, hypertension and progressive decline in GFR. Typical histological findings include thickening of glomerular basement membrane, arteriolar hyalinosis, mesangial expansion with diffuse or nodular glomerulosclerosis (Kimmelstiel-Wilson lesion), interstitial fibrosis and progressive tubular atrophy (28,29).

Incipient nephropathy/"Microalbuminuria"/KDIGO A2

Microalbuminuria is the word generally used to define the presence of low quantity of albumin in urine (30–300 mg/g on a spot sample or 30–300 mg/24 h on a timed collection) (30). In 2012, the KDIGO Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease suggested that this term should no longer be used because it is confusing. Indeed, the term microalbuminuria could suggest a different, smaller form of albumin in urine, which is not the case (6). Microalbuminuria is now referred as A2 albuminuria (30–300 mg/g on a spot sample or 30–300 mg/24 h on a timed collection). The classification with cut-off ranges is more clinically relevant for the patient follow up and more representative of a continuous variable that affects morbidity and mortality.

Indeed, patients presenting low but detectable albumin concentrations in urine are prone to develop CKD, i.e., progression to higher ACR and/or towards more severe GFR stages. Early recognition of this A2 stage is crucial, since intervention in controlling hypertension and diabetes may slow down the progression to ESKD (31). Beyond the control of these risk factors, blocking the RAAS and SGLT2i administration '*per se*' also slow down the progression of CKD and reduce the risk of cardiovascular disease. Of note, GFR is often preserved or even supranormal (hyperfiltration) in these patients (30).

Overt nephropathy/"Macroalbuminuria"/KDIGO A3

Overt DN is defined by a decline in GFR and/or marked albuminuria (A3 which is >300 mg/g or >300 mg/24 h). Blood pressure usually rises, leading to a faster decline of kidney function.

Measurement of urine albumin

Pre-analytical considerations

Exercise, posture, pregnancy and fever are well known factors that may increase urine albumin excretion (12,32). Therefore, sampling should be performed on a first morning sample to attenuate this effect.

A positive urine sediment (haematuria, pyuria, leukocyturia) overestimates albuminuria or proteinuria (33). Thus, a positive ACR in presence of haematuria and/or leukocyturia result should always be confirmed on another sample without haematuria and/or leukocyturia.

Albumin adhesion to the wall of the plastic containers is neglectable (<1%) and does not lead to falsely low concentrations (34).

A fresh midstream sample is always preferable (35). Albumin is stable in urine for at least one week and up to 8 weeks when stored at 4 ℃. For long term storage, urine should be stored at −70 ℃ or lower since significant degradation is reported when stored at −20 ℃ (12,36). However, the stability of the analyte could be method dependent and immunonephelometry seems less affected than high performance liquid chromatography (HPLC) by long-term storage at −20 ℃ and even −80 ℃ (37). Urine creatinine appears to be stable for a week at 4 or 20 ℃ and is not affected by freezing at either −20 or −80 ℃ (13). Bacterial growth can affect creatinine stability when samples are stored at +4 ℃ (36).

Urine pH has a wider range than plasma and albuminuria is therefore subject to a wider ionic exposure. Other factors may also modify albumin such as high concentration of urea, ascorbate, or glucose. Other modification may also take place with glycation or cleavage as described below (13).

Spot or timed collection: limitations of the ratio

Twenty-four-hour timed collection remains the method of reference for albumin quantification, but numerous

international guidelines recommend measuring ACR on a spot sample because creatinine excretion is fairly constant through the day (38). Indeed, 24-hour timed collection is cumbersome and prone to errors (spills, errors in timing, incomplete bladder emptying) and may lead to mis-estimation of albuminuria (1,6-9,38,40). There is a good concordance between ACR on a random urine sample and 24 hour collection sample (41,42).

The day-to-day biological variation of ACR must carefully be taken into consideration when interpreting longitudinal follow-up of patients. Indeed, day-to-day variability is important and influenced by the basal concentration of urine albumin. Naresh *et al.* showed that, in lower concentrations, like in the A1 stage, an increase of 467% in ACR values is required to observe a clinically significant change with a 95% probability (43). In later stages, this relative increase will be less important, i.e., 170% for A2 and 83% for A3, respectively—even if the absolute values are more important in these later stages. In other words, a patient presenting a basal ACR at 15 mg/g whose ACR increases to 60 mg/g in the next sample (i.e., 300% relative increase but 45 mg/g absolute increase) will not present a significant variation of the ACR, but another patient presenting a basal ACR at 500 mg/g whose value would increase to 750 mg/g (i.e., 200% relative increase but 250 mg/g absolute increase) will present a clinically significant variation of the ACR. Reversely, this approach should also be considered when evaluating the impact of a therapeutic treatment on the ACR. The large intra-individual variability, especially in A1 stage, means that a single observation should always be confirmed by a second one (42).

From an analytical perspective, analytical performances specifications (APS) have been set for albuminuria and albumin excretion ratio (AER) based on a study involving 87 diabetic patients with persistent A2 albuminuria (36). In this study, the intra-individual biological variation of the AER on a 24-hour timed collection was 25.7% whereas biological variation of ACR was 11.8%. Accordingly, these data helped defining analytical objectives for imprecision for albuminuria [desirable coefficient of variations (CV) of 12.9% and 5.9% for AER and ACR, respectively].

Another limitation is that creatinine excretion may vary because of non-kidney related factors such as age, muscular mass, race or obesity. For instance, an old lady with a small urine creatinine excretion might have a raised ACR ratio without a pathologic proteinuria, just because urine creatinine excretion is very low (1). On the other hand, high creatininuria may lead to underestimation of the ACR ratio in individuals with high muscular mass. In those individuals with extreme muscle mass or diet, a 24-hour timed collection should be preferred with a result expressed as an AER. Some equations, based on an ACR and an estimated creatinine excretion ratio (eCER), have also been developed to approach the AER, but they are few used in daily practice (44,45). Finally, acute kidney injury provides a non-steady state for creatinine excretion which interferes with the ratio (7).

Multiple forms of urine albumin and work towards standardization

Albumin is a 583 amino-acid protein of 66 kDa with three homologous domains forming a heart shaped molecule (13,46). With 17 disulfide bonds, 4 globular domains, it binds to numerous ligands (fatty acids, bilirubin, calcium and magnesium). Multiple molecular forms of albumin can be found in urine due to proteolysis while passing through the urinary tract (filtration, tubular uptake), or truncation at both ends (N and C terminal) or chemical modifications (proteolysis, glycation, oxidants, free radicals and other ligands) during storage (46). Hence, fragmented, partially degraded, glycated forms of proteins are found in urine (47). Interference may also occur with the measurement methods due to the urine matrix. All these factors contribute to the complexity for measurement of urinary albumin since the composition of albumin molecules can vary significantly, even within healthy people (13).

The complexity is enhanced since there are several methods for quantifying albuminuria. Standardization of measurement is in progress with a reference measurement procedure based on liquid chromatography-isotope dilution tandem mass spectrometry (LC-IDMS/MS) using human serum albumin as calibration and urine albumin certified reference materials (12,36,48,49). This should allow calibration for routine methods (essentially immunoassays), and eventually lead to a standardization of the measurement in the future.

Laboratory methods for urine albumin quantification

Several methods are available to measure albuminuria: immunoassays, enzyme-linked immunosorbent assay (ELISA), electrophoresis, western blot, size exclusion HPLC, liquid chromatography with mass tandem spectometry (LCMS/MS) and point of care (POC) methods. In this article, we will focus on the most popular ones (POC

and immunoassays) on one side and the reference method (LCMS/MS) on the other side.

LCMS/MS: the higher order method and Candidate Reference Method Procedure

Two candidate reference method procedures by LCMS/MS have been described so far for the quantification of urine albumin after trypsin digestion (50,51). These methods allow clinical urine albumin measurements of certified reference and high-order calibration materials. The limit of quantification (LOQ) of such methods is compatible with the detection of albumin in urine at the lower stage of A2 level. Accordingly, this is a great step toward standardization of albumin in urine (52).

Immunoassays: the routine measurements procedures

Numerous forms of albumin can be found in urine (fragments, glycated, partial degradation etc). Albuminuria fragmentation may be due to kidney degradation or chemical modification during sample conservation (53). Thus, albumin found in urine may differ in structure from plasma albumin and have a compromised immunoreactivity (49). In the last decade, it was assumed that some form of modified albumin (immune-unreactive albumin) was not detected by immunochemical assays, because size exclusion HPLC yielded higher values than immunoassays (46,54,55). The difference was mostly noted at lower levels of albuminuria. It was first suggested that assays that could quantify immunoreactive and immuno-unreactive albumin might better foresee the development of DN, cardiovascular events and mortality. However, the PREVEND (56) and HOPE (57) studies, as well as another study including 741 Aboriginal high risk patients by Wang *et al.* (58) did not show any difference in predicting a composite cardiovascular endpoint between HPLC and immunoassays.

Studies have shown that immunoassays are also able to recognize modified forms of albumin (47,49), probably because albumin is very antigenic. Even if proteolysis or chemical changes in albumin destroy some epitopes, there is a broad distribution of reactive sites on albumin for antibodies to react to and polyclonal antibodies will still bind to another epitope and lead to detection (49). Sviridov *et al.* showed that use of a monoclonal antibody leads to a very different sensitivity to structural modification of albumin (49). The NKDEP-IFCC and Laboratory Medicine Working Group on Standardization of Albumin recommend quantifying urine albumin with polyclonal immunoassays since they have higher sensitivity compared with monoclonal antibodies, because as explained above, they are able to detect multiple epitopes of albumin (35) since urinary albumin has multiple antigenic sites that may be recognized by polyclonal antibodies. However, difference in composition of said antibodies may also lead to significant difference between commercially available kits (59): in 2014, Bachmann *et al.* compared 17 (including 16 quantitative and one semiquantitative) immunoassays with an isotope dilution mass spectrometry (60). Bias was the main source of discordance among the routine kits with median biases between –35% and 34% at 15 mg/L. At the clinically relevant threshold of 30 mg/L, 9 measurement procedures had biases over ±10% in comparison to the LC-MS/MS measurement. The difference of results may lead to misclassification of patients regarding their risk of kidney disease. Another comparison of albumin measuring methods with LC-MS/MS drew the same conclusion and showed that mean biases are greater at lower albumin concentration (–20% at 16 mg/L; –12% at 36 mg/L; –11% at 184 mg/L) (12). The Laboratory Working Group of the NKDEP and the IFCC and Laboratory Medicine Working Group for Standardization of Albumin in Urine recommended that the higher order method used as reference system for calibration of clinical laboratory measurement should aim at a desirable bias goal of 13% and optimal bias goal of ≤7% (61). Shaikh *et al.* showed that comparability between a LC-MS and an immunoturbidimetric method substantially improved when both methods used the same calibrators with the same calibrator value assignments with a mean bias upgrading from –37.8% to 2.2% (62).

POC methods: quick, but maybe not so cheap (and not that easy)

POC methods can be divided into semi-quantitative or quantitative methods for screening of proteinuria or albuminuria. Semi quantitative tests such as Clinitek, reports ACR as <30, 30–300 or >300 mg/g.

Theoretically, POC testing are quick, easy-to-do, and cheap. They do not need transportation or other laboratory management. Also, results may be discussed during the visit with immediate treatment management. However, ACR and its related clinical decision are all about accuracy. Moreover, regarding high intra-individual variability and lack of standardization, current recommendations state that a positive POC result should be confirmed on a laboratory ACR (6,7,9), which questions the real cost/effectiveness of POC methods. In the context of screening, a negative result (i.e., <30 mg/g of creatinine) should be particularly

reliable to rule out A2 albuminuria. The ADA and the American Association for Clinical Chemistry suggested that semiquantitative or qualitative screening tests ought to have a clinical sensitivity >95% (63). A meta-analysis by McTaggart *et al.* showed that semiquantitative POC test (essentially Clinitek, Siemens HealthCare Diagnosis, Tarrytown, USA) is not sensitive enough (76%) for ruling out albuminuria in patients at risk for kidney disease (64) especially when read by a clinical operator (sensitivity 67%) instead of a laboratory professional (sensitivity 83%), which corresponds to real life practice. The purposes of immediately discussing the results with the patients is thus questionable, especially if the result would have to be confirmed by a laboratory test. On the other hand, the quantitative POC (DCA, Siemens HealthCare Diagnostics, Tarrytown, USA) (sensitivity 96%) met the evidence based required cut off with a sensitivity >95%. However, sensitivity dropped to 91% when the POC was read by a clinical operator. Despite the fact that these methods perform better than conventional urinary dipsticks for total urinary proteins, these POC testing do not meet the clinical need and laboratory methods should be preferred when screening diabetic, CKD or at risk patients for renal disease (65).

Total urinary protein measurement

Despite international recommendations, PCR is more frequently performed than ACR in the everyday clinical setting, because urine protein quantification is less expensive than urine albumin determination and because some countries do not allow refunding of albuminuria in all patients. Another reason to, theoretically, prefer PCR over ACR is that the presence of light or heavy chains of immunoglobulins or tubular proteinuria may not be detected when performing ACR alone (especially at the beginning of the disease, when damage from the immune deposit still does not lead to glomerular injury). It should be however noted that most methods aiming at proteinuria detection tend to react more strongly with albumin than with globulin or other proteins and lack precision at low concentration of these proteins (6). Hence, measuring PCR when non-albumin proteinuria is suspected may not meet the pathological threshold for proteinuria and therefeore the KDIGO guidelines recommend that if non-albumin proteinuria is suspected (when testing for tubular proteinuria of myeloma for instance), one should perform a specific assay (alpha 1 microglobulin, monoclonal heavy or light chains or immuno-electrophoresis). As previously mentioned, assessment of ACR is more sensitive and specific, compared with PCR (1,6). The heterogeneity of urinary protein composition makes it impossible for standardization since numerous available methods may react differently to the panel of proteins present in urine. Moreover, urinary total proteins measurement may not be sensitive enough for detecting clinically relevant urinary albumin concentration: indeed there is no global accepted definition of proteinuria (normal ranges between <150 mg/L to <300 mg/L) whereas normal albuminuria range has been defined <30 mg/L (1,13,66). Also, variability of non-protein molecules or important content of inorganic ionised compounds can interfere with the measurement. Finally, there are several methods of measurement for total urinary protein (colorimetry or turbidimetry for instance) that have different sensitivity and specificity for total proteins which may contribute to variability of the results (13).

Conclusions

Albuminuria is an important biomarker regarding not only kidney function and prognosis of CKD, but also cardiovascular events, morbidity and mortality. It is an early marker of renal involvement in diabetes and other kidney diseases. Accurate measurement of albuminuria is therefore of critical clinical interest for early therapeutic management (with initiation of effective treatments that slow down the loss of kidney function and progression toward ESRD) and follow up. Despite this recognized clinical importance, measuring albuminuria is not an easy task: urine is a very heterogeneous fluid in terms of pH and chemical composition, numerous methods are available with their own limitations and work towards standardization is in progress. LC-MS/MS measurement of albumin specific peptides after trypsin digestion in a controlled environment is foreseen as a reference method for measurement of urinary albumin. Development of a reference material based on pure human albumin is also a crucial step. This will allow standardized calibration for polyclonal immunoassays that are easier to perform in everyday clinical practice and able to recognise several modified form of albumin coming from its journey through the nephron.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editor (Rafael Noal Moresco) for the series "Laboratory Medicine in Diabetic Kidney Disease" published in *Journal of Laboratory and Precision Medicine*. The article has undergone external peer review.

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://jlpm.amegroups.com/article/view/10.21037/jlpm-21-58/rc

Peer Review File: Available at https://jlpm.amegroups.com/article/view/10.21037/jlpm-21-58/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jlpm.amegroups.com/article/view/10.21037/jlpm-21-58/coif). The series "Laboratory Medicine in Diabetic Kidney Disease" was commissioned by the editorial office without any funding or sponsorship. Pierre Delanaye serves as an unpaid editorial board member of *Journal of Laboratory and Precision Medicine*. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Johnson DW. Global proteinuria guidelines: are we nearly there yet? Clin Biochem Rev 2011;32:89-95.
2. van der Velde M, Matsushita K, Coresh J, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. Kidney Int 2011;79:1341-52.
3. Astor BC, Matsushita K, Gansevoort RT, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with mortality and end-stage renal disease. A collaborative meta-analysis of kidney disease population cohorts. Kidney Int 2011;79:1331-40.
4. Chronic Kidney Disease Prognosis Consortium; Matsushita K, van der Velde M, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. Lancet 2010;375:2073-81.
5. Gansevoort RT, Matsushita K, van der Velde M, et al. Lower estimated GFR and higher albuminuria are associated with adverse kidney outcomes. A collaborative meta-analysis of general and high-risk population cohorts. Kidney Int 2011;80:93-104.
6. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Int Suppl 2013;3:1-150.
7. Inker LA, Astor BC, Fox CH, et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for the evaluation and management of CKD. Am J Kidney Dis 2014;63:713-35.
8. National Clinical Guideline Centre (UK). Chronic Kidney Disease (Partial Update): Early Identification and Management of Chronic Kidney Disease in Adults in Primary and Secondary Care. London: National Institute for Health and Care Excellence (UK); July 2014.
9. Haute Autorité de Santé (France). Evaluation du rapport albuminurie/créatininurie dans le diagnostic de la maladie rénale chronique chez l ' adulte. 2011.
10. NICE. Chronic kidney disease: assessment and management (NG203). Accessed 4 November 2021. Available online: https://www.nice.org.uk/guidance/ng203
11. Márquez DF, Ruiz-Hurtado G, Segura J, Ruilope L. Microalbuminuria and cardiorenal risk: old and new evidence in different populations. F1000Res 2019;8:F1000 Faculty Rev-1659.
12. Seegmiller JC, Miller WG, Bachmann LM. Moving Toward Standardization of Urine Albumin Measurements. EJIFCC 2017;28:258-67.
13. Martin H. Laboratory measurement of urine albumin and urine total protein in screening for proteinuria in chronic kidney disease. Clin Biochem Rev 2011;32:97-102.
14. Jonsson AJ, Lund SH, Eriksen BO, et al. The prevalence of chronic kidney disease in Iceland according to KDIGO

criteria and age-adapted estimated glomerular filtration rate thresholds. Kidney Int 2020;98:1286-95.
15. Sugahara M, Pak WLW, Tanaka T, et al. Update on diagnosis, pathophysiology, and management of diabetic kidney disease. Nephrology (Carlton) 2021;26:491-500.
16. Fiorentino M, Bolignano D, Tesar V, et al. Renal biopsy in patients with diabetes: a pooled meta-analysis of 48 studies. Nephrol Dial Transplant 2017;32:97-110.
17. American Diabetes Association. 11. Microvascular Complications and Foot Care: Standards of Medical Care in Diabetes-2020. Diabetes Care 2020;43:S135-51.
18. Cosentino F, Grant PJ, Aboyans V, et al. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Eur Heart J 2020;41:255-323.
19. Stempniewicz N, Vassalotti JA, Cuddeback JK, et al. Chronic Kidney Disease Testing Among Primary Care Patients With Type 2 Diabetes Across 24 U.S. Health Care Organizations. Diabetes Care 2021;44:2000-9.
20. Anderson S, Rennke HG, Brenner BM. Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. J Clin Invest 1986;77:1993-2000.
21. Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). Lancet 1997;349:1857-63.
22. Xie X, Liu Y, Perkovic V, et al. Renin-Angiotensin System Inhibitors and Kidney and Cardiovascular Outcomes in Patients With CKD: A Bayesian Network Meta-analysis of Randomized Clinical Trials. Am J Kidney Dis 2016;67:728-41.
23. Scheen AJ. Effects of glucose-lowering agents on surrogate endpoints and hard clinical renal outcomes in patients with type 2 diabetes. Diabetes Metab 2019;45:110-21.
24. Heerspink HJL, Kosiborod M, Inzucchi SE, et al. Renoprotective effects of sodium-glucose cotransporter-2 inhibitors. Kidney Int 2018;94:26-39.
25. Delanaye P, Wissing KM, Scheen AJ. SGLT2 inhibitors: renal outcomes according to baseline albuminuria. Clin Kidney J 2021;14:2463-71.
26. American Diabetes Association. 9. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes-2021. Diabetes Care 2021;44:S111-24.
27. Pitt B, Filippatos G, Agarwal R, et al. Cardiovascular Events with Finerenone in Kidney Disease and Type 2 Diabetes. N Engl J Med 2021;385:2252-63.
28. Tervaert TW, Mooyaart AL, Amann K, et al. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol 2010;21:556-63.
29. Amann K, Daniel C. Histology of human diabetic nephropathy. In: Wolf G. editor. Diabetes and Kidney Disease. Oxford, UK: Wiley-Blackwell, 2012:62-9.
30. Mogensen CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease. With emphasis on the stage of incipient diabetic nephropathy. Diabetes 1983;32 Suppl 2:64-78.
31. Giampietro O, Penno G, Clerico A, et al. Which method for quantifying "microalbuminuria" in diabetics? Comparison of several immunological methods (immunoturbidimetric assay, immunonephelometric assay, radioimmunoassay and two semiquantitative tests) for measurement of albumin in urine. Acta Diabetol 1992;28:239-45.
32. Levey AS, Becker C, Inker LA. Glomerular filtration rate and albuminuria for detection and staging of acute and chronic kidney disease in adults: a systematic review. JAMA 2015;313:837-46.
33. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 2002;39:S1-266.
34. Robinson MK, Caudill SP, Koch DD, et al. Albumin adsorption onto surfaces of urine collection and analysis containers. Clin Chim Acta 2014;431:40-5.
35. Miller WG, Bruns DE, Hortin GL, et al. Current issues in measurement and reporting of urinary albumin excretion. Clin Chem 2009;55:24-38.
36. Mosca A, Paleari R, Ceriotti F, et al. Biological variability of albumin excretion rate and albumin-to-creatinine ratio in hypertensive type 2 diabetic patients. Clin Chem Lab Med 2003;41:1229-33.
37. Brinkman JW, de Zeeuw D, Lambers Heerspink HJ, et al. Apparent loss of urinary albumin during long-term frozen storage: HPLC vs immunonephelometry. Clin Chem 2007;53:1520-6.
38. Zuo Y, Wang C, Zhou J, et al. Simultaneous determination of creatinine and uric acid in human urine by high-performance liquid chromatography. Anal Sci 2008;24:1589-92.
39. Xin G, Wang M, Jiao LL, et al. Protein-to-creatinine ratio in spot urine samples as a predictor of quantitation of proteinuria. Clin Chim Acta 2004;350:35-9.
40. Newman DJ, Pugia MJ, Lott JA, et al. Urinary protein and albumin excretion corrected by creatinine and specific

gravity. Clin Chim Acta 2000;294:139-55.
41. Price CP, Newall RG, Boyd JC. Use of protein:creatinine ratio measurements on random urine samples for prediction of significant proteinuria: a systematic review. Clin Chem 2005;51:1577-86.
42. Mariat C, Delanaye P, Bastard JP, et al. Urinary protein or albumin/creatinin ratio for reporting measurements results. Expression des résultats d'une protéinurie: le ratio protéines ou albumine sur créatinine urinaire. Ann Biol Clin (Paris) 2018;76:643-50.
43. Naresh CN, Hayen A, Weening A, et al. Day-to-day variability in spot urine albumin-creatinine ratio. Am J Kidney Dis 2013;62:1095-101.
44. Fotheringham J, Campbell MJ, Fogarty DG, et al. Estimated albumin excretion rate versus urine albumin-creatinine ratio for the estimation of measured albumin excretion rate: derivation and validation of an estimated albumin excretion rate equation. Am J Kidney Dis 2014;63:405-14.
45. Abdelmalek JA, Gansevoort RT, Lambers Heerspink HJ, et al. Estimated albumin excretion rate versus urine albumin-creatinine ratio for the assessment of albuminuria: a diagnostic test study from the Prevention of Renal and Vascular Endstage Disease (PREVEND) Study. Am J Kidney Dis 2014;63:415-21.
46. Speeckaert MM, Speeckaert R, Van De Voorde L, et al. Immunochemically unreactive albumin in urine: fiction or reality? Crit Rev Clin Lab Sci 2011;48:87-96.
47. Molinario R, Pocino K, Daloiso PD, et al. Urinary Albumin Detection: Comparison of Two Different Methods. J Clin Lab Anal 2016;30:888-91.
48. Chen Y, Liu H, Loh TP, et al. Measurement of urine albumin by liquid chromatography-isotope dilution tandem mass spectrometry and its application to value assignment of external quality assessment samples and certification of reference materials. Clin Chem Lab Med 2020;59:711-20.
49. Sviridov D, Drake SK, Hortin GL. Reactivity of urinary albumin (microalbumin) assays with fragmented or modified albumin. Clin Chem 2008;54:61-8.
50. Seegmiller JC, Barnidge DR, Burns BE, et al. Quantification of urinary albumin by using protein cleavage and LC-MS/MS. Clin Chem 2009;55:1100-7.
51. Beasley-Green A, Burris NM, Bunk DM, et al. Multiplexed LC-MS/MS assay for urine albumin. J Proteome Res 2014;13:3930-9.
52. Lieske JC, Bondar O, Miller WG, et al. A reference system for urinary albumin: Current status. Clin Chem Lab Med 2013;51:981-9.
53. Benz-de Bretagne I, Roger C, Carlier MC, et al. Urinary albumin measurement methods. Ann Biol Clin (Paris) 2018;76:633-7.
54. Osicka TM, Comper WD. Characterization of immunochemically nonreactive urinary albumin. Clin Chem 2004;50:2286-91.
55. Klapkova E, Fortova M, Prusa R, et al. Determination of Urine Albumin by New Simple High-Performance Liquid Chromatography Method. J Clin Lab Anal 2016;30:1226-31.
56. Brinkman JW, Bakker SJ, Gansevoort RT, et al. Which method for quantifying urinary albumin excretion gives what outcome? A comparison of immunonephelometry with HPLC. Kidney Int Suppl 2004;(92):S69-75.
57. McQueen MJ, Gerstein HC, Pogue J, et al. Reevaluation by High-Performance Liquid Chromatography: Clinical Significance of Microalbuminuria in Individuals at High Risk of Cardiovascular Disease in the Heart Outcomes Prevention Evaluation (HOPE) Study. Am J Kidney Dis 2006;48:889-96.
58. Wang Z, Hoy WE, Nicol JL, et al. Predictive Value of Nephelometric and High-Performance Liquid Chromatography Assays of Urine Albumin for Mortality in a High-Risk Aboriginal Population. Am J Kidney Dis 2008;52:672-82.
59. Liu R, Li G, Cui XF, et al. Methodological evaluation and comparison of five urinary albumin measurements. J Clin Lab Anal 2011;25:324-9.
60. Bachmann LM, Nilsson G, Bruns DE, et al. State of the art for measurement of urine albumin: Comparison of routine measurement procedures to isotope dilution tandem mass spectrometry. Clin Chem 2014;60:471-80.
61. Miller WG, Seegmiller JC, Lieske JC, et al. Standardization of Urine Albumin Measurements: Status and Performance Goals. J Appl Lab Med 2017;2:423-9.
62. Shaikh A, Seegmiller JC, Borland TM, et al. Comparison between immunoturbidimetry, size-exclusion chromatography, and LC-MS to quantify urinary albumin. Clin Chem 2008;54:1504-10.
63. Sacks DB, Arnold M, Bakris GL, et al. Executive summary: Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem 2011;57:793-8.
64. McTaggart MP, Newall RG, Hirst JA, et al. Diagnostic accuracy of point-of-care tests for detecting albuminuria:

a systematic review and meta-analysis. Ann Intern Med 2014;160:550-7.
65. Résimont G, Piéroni L, Bigot-Corbel E, et al. Urinary strips for protein assays: easy to do but difficult to interpret! J Nephrol 2021;34:411-32.
66. Lamb EJ, MacKenzie F, Stevens PE. How should proteinuria be detected and measured? Ann Clin Biochem 2009;46:205-17.

doi: 10.21037/jlpm-21-58
Cite this article as: Résimont G, Cavalier E, Radermecker RP, Delanaye P. Albuminuria in diabetic patients: how to measure it?—a narrative review. J Lab Precis Med 2022;7:4.