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URINALYSIS WHITE PAPER | June 2021

Chronic kidney disease (CKD)

Fighting the burden of chronic kidney disease by frequent albuminuria screening in risk group patients

Introduction

Chronic kidney disease (CKD) is a severe complication in context of various civilisation diseases, such as diabetes, hypertension, and obesity. With increasing treatment cost along the progression of CKD towards end-stage renal failure (ESRF) and the need for renal replacement therapies, CKD is not only a driver of premature mortality and diminished quality of life of affected individuals, but also puts significant burden on the society and healthcare expenditures.

The early detection of kidney damage through a frequent screening of risk patients could help to fight the burden of CKD. Cost-efficient routine urinalysis data could play a vital role in providing valuable information for screening, diagnosing, and monitoring of renal disorders.

Chronic kidney disease

CKD is a systemic condition and a result of various diseases, defined as persistent abnormalities of the kidney structure and/or function, present for more than three months. The classification of CKD is based on cause by the estimated glomerular filtration rate (eGFR) and albuminuria [1].

Risk factors of chronic kidney disease

Risk factors for CKD can be differentiated in initiating risk factors that initially cause the onset of CKD, and progression factors that promote the progression of CKD towards end-stage renal disease [2].

The main drivers for the onset of CKD are diabetes and hypertension, causing approximately two-third of all cases of chronic kidney disease [2]. Besides these factors, obesity, persistent obstructions of the urinary tract, chronic infections of the lower urinary tract, interstitial nephritis, glomerulonephritis, polycystic kidney disease, certain tumours, but also age, ethnicity and a family history of CKD are initiating risk factors [3].

Traditional progression factors include African American ethnicity, proteinuria, hypertension, high protein intake, obesity, anaemia, dyslipidaemia, smoking, nephrotoxins and cardiovascular disease.

In addition, recent studies revealed additional progression factors and markers, such as adiponectin, adrenomodulin, neutrophil gelatinase-associated lipocalin (NGAL), genetic polymorphisms and others [2].

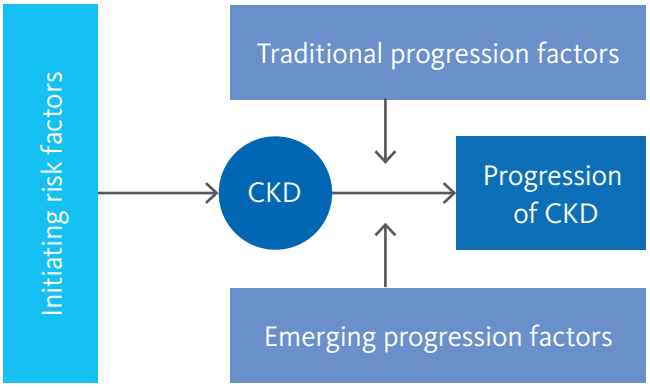

Fig. 1 *Risk and progression factors for chronic kidney disease [2].*

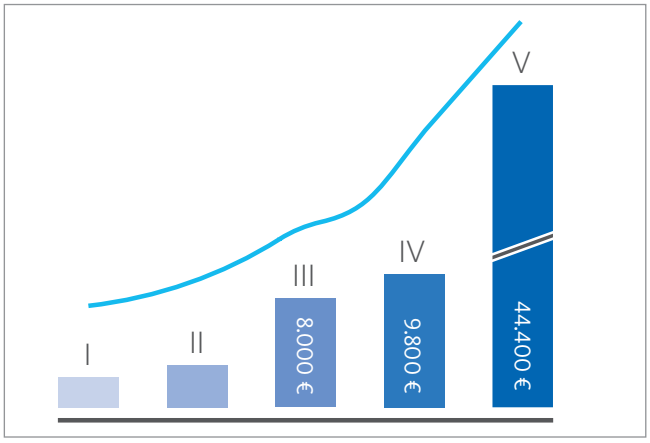

Fig. 2 *Annual treatment cost per CKD patient and stage of CKD progression in Germany [following 6].*

Prevalence of CKD

CKD puts significant burden on global health via direct effects on mortality and morbidity and indirect effects by increasing the risk for cardiovascular diseases.

In 2017, 697.5 million cases of all-stage chronic kidney disease have been reported, reflecting a global prevalence of 9.1 % and 1.2 million deaths were directly attributable to CKD. Compared to 1990, the global all-age prevalence has increased by 29.3 % and all-age mortality rate increased by 41.5 %.

Interestingly, a higher burden of CKD correlates with low and medium socio-demographic indices (SDI), causing further complications, since these areas are often limited in resources for CKD diagnostics and treatment.

In addition, CKD has a strong impact on the quality of life. In 2017, CKD caused in 35.8 million disability-adjusted life years (DALY) with diabetic nephropathy accounting for almost a third of DALYs [4].

Medicare and social costs of CKD

Besides the diminished quality of life of CKD patients, the progression of CKD has considerable effects on medical treatment costs, healthcare expenditures and the community (Evans and Taal 2011). This is fostered by increased numbers of hospital admissions, treatment of CKD-related symptoms, secondary diseases and the requirement for lifelong renal replacement therapies (RRT), such as haemodialysis and/or renal transplantation with ongoing CKD progression.

Individual costs increase exponentially with ongoing CKD progression and stage [5]. In Germany, annual individual healthcare expenditures attributable to CKD were 8,030 € at CKD stage 3, 9,760 € at CKD stage 4 and 44,374 € at stage 5 on dialysis [6].

Future development

The increasing prevalence for CKD over the last decades also correlates with the development of the prevalence for hypertension and diabetes, the main risk factors for CKD. Since 1990, the cases for hypertension (systolic blood pressure of 140 mm Hg or higher) increased by 18.5 % in 2015 [7] and for diabetes by 41 % [8].

An increase of the prevalence of CKD is thus projected in various studies [9, 10], further highlighting the need for a frequent screening of risk group patients.

Urinalysis and CKD diagnostics

Urine represents an important specimen to investigate abnormalities related to CKD, including the presence of protein, albumin, and creatine, as well as the normalised albumin-to-creatinine (ACR) and protein-to-creatinine ratios (PCR).

Table 2 *Reference ranges in spot urine*

Parameter	Reference range
PRO Protein	< 30 mg/dL
CRE Creatinine	1.0 – 1.5 mg/24 h
PCR Protein-to-creatinine ratio	<150 mg/g Cre
ACR Albumin-to-creatinine ratio	< 30 mg/g Cre

Proteinuria
Abnormally high concentrations of protein in the urine, is of pathological origin, if being persistent. Depending on the cause and affected nephrological structures, pathological proteinuria can be differentiated in pre-renal, renal and post-renal proteinuria [11].

Albumin
Is one of the most abundant proteins and crucial for homeostasis. The molecular weight of albumin of 66 kDa theoretically allows its transition trough the blood-urine barrier, followed by a re-absorption from the glomerular filtrate by proximal renal tubular epithelial cells was shown [12]. Upon glomerular damages, e.g., due to CKD, albuminuria increases.

Even low albuminuria concentration might therefore be an early sign for glomerular damage and chronic kidney disease with over 50 % of CKD cases to be missed if albuminuria is ignored [13]. Moreover, persistent albuminuria is the principal marker of kidney damage, and essential for monitoring changes in the degree of proteinuria.

Since urine shows a large physiological variability in both, biochemical composition, and quantity, protein and albumin levels must be normalised to avoid falsely low or falsely high results. This can be remedied by analysis of collected 24-hour urine or by correlation with urinary creatinine.

Creatinine
Is a catabolite of the protein metabolism that is secreted by the kidney with a constant rate of 1.0 to 1.5 mg per 24 hours, depending on age and muscle mass, but independent of the total urine volume. Thus, the albumin-to-creatinine (ACR) and protein-to-creatine ratios (PCR) allows a more reliable estimation of increased protein excretion.

Urinary sediment diagnostics

Besides the detection of molecular components of the urine, cellular and acellular particles of the urinary sediment can be detected by urinary flow cytometry to support the diagnosis of renal impairments.

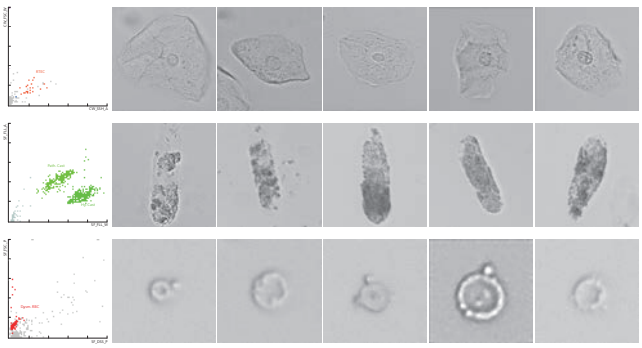

Fig. 3 *Urinary particle findings related to chronic kidney disease, including renal tubular epithelial cells (RTEC, upper row), hyaline and pathological casts (upper row) and dysmorphic RBC (lower row). Scattergrams obtained from UF-5000 analysis; cellular images obtained from UD-10 analysis*

Renal Tubular Epithelial Cells (RTEC)
Cover the renal tubules from the proximal tubule via the Henle Loop to the distal tubule. These epithelial cells play a vital role in renal regeneration, but also release proinflammatory molecules that promote the progression of chronic kidney disease [14]. Although a few RTECs may be present in the urine of healthy individuals due to normal exfoliation, the presence of ≥ 15 RTECs (per ten HPFs) indicates an active renal disease or tubular injury [15].

Urinary casts
Are a result of precipitation and aggregation of the glycoprotein uromodulin, also known as Tamm-Horsefall protein [16]. This protein is exclusively synthesized by renal tubular epithelial cells in the distal loop of Henle [17]. Various pathological casts indicate kidney damage, including RBC casts in proliferative glomerulonephritis, WBC casts in pyelonephritis or interstitial nephritis, oval fat bodies or fatty casts in diseases with proteinuria and granular casts and renal tubular epithelial cells in many parenchymal diseases [1]

Dysmorphic RBC
Represent a major manifestation of haematuria. In contrast to isomorphic RBC that are of uniform morphology resulting from renal pelvis, ureter or bladder bleedings, dysmorphic RBC show various blebs and projections originating from glomerular damage [18]. The UF-Series accurately detects RBCs and highlights the presence of isomorphic and dysmorphic RBCs [19] allowing the judgment of haematuria according to glomerular and non-glomerular origin.

Enabling a frequent CKD screening through routine urinalysis

Various technologies and analytical systems are available to assess both, proteinuria, and albuminuria. Whereas urine dipstick testing is generally considered as being of low sensitivity and specificity, immunological-based assays are commonly used for quantification of albuminuria.

Radioimmunoassays, immunonephelometry and immunoturbidimitry can detect urinary albumin from as little as 16 µg/L, 2 mg/L or 6 mg/L, respectively. High performance liquid chromatography (HPLC) with a detection limit of 2 mg/L is gaining importance, as in contrast to other assays, HPLC detects different albumin species, including intact albumin, albumin fragments, albumin aggregates and immune-unreactive albumin [20].

Improved test strip reading

The urinary test strip, though often used for screening purposes, demonstrated low sensitivities and specificities for many urinary abnormalities. In context of albuminuria, poor detection limits of > 30 mg/L have been reported [21], questioning the potential of test strip assays to accurately differentiate between physiological and pathological albuminuria.

Significant improvements of the reflectometry measurement of albumin using dye-biding test strips have been achieved due to the replacement of classical LED cameras by complementary metal oxide semiconductor (CMOS) sensor technology. CMOS sensors are well-known in industrial imaging applications but are meanwhile widely applied within molecular diagnostic instruments [22]. CMOS sensors convert detected photons, reflected from a test strip via released electrons into electrical signals, thereby recognising three different wave lengths according to the RGB colour coding.

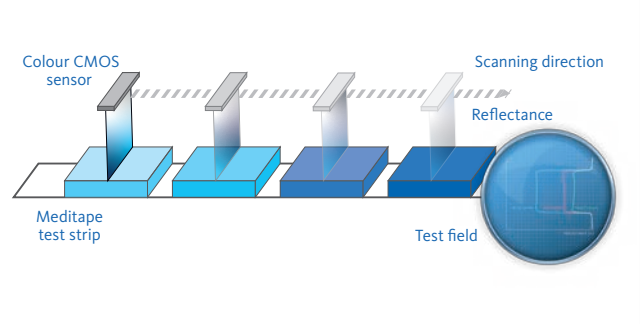

Fig. 4 *Complementary metal oxide semiconductor (CMOS) sensor technology for improved reflectometry reading of dye-binding urinary test strips.*

Quantitative quality using a semi-quantitative technology

The use of Meditape 11A test strips on the UC-3500 and its CMOS sensor technology demonstrated the potential for high-sensitive detection and quantification of albuminuria with a dye-binding based albumin test field. Using raw reflectance data, albumin concentrations cannot only be graded, but quantified with a limit of detection (LoD) as low as 5.5 mg/L [23].

A perfect correlation of albumin values obtained from immunonephelometry and test strip, supports the reliability of test strip-mediated albumin values (Fig. 5). The correlation of albumin levels with test strip obtained creatinine allows the reporting of the ACR (Fig. 6) and a further reduction of falsely high albumin values. Thus, for the first time a dye-binding test strip allows quantitative testing in the mildly increased albuminuria range and below with a limit of detection (LoD) comparable to those of HPLC and immune-based albuminuria assays [24].

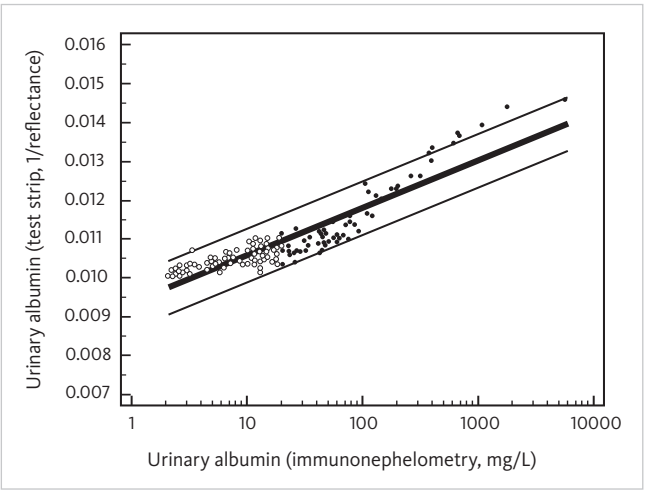

Fig. 5 *Regression analysis of urinary albumin using Meditape 11A test strips and immunonephelometry. Hollow circles represent specimens within reference range, filled circles represent samples, exceeding the upper reference limit [23].*

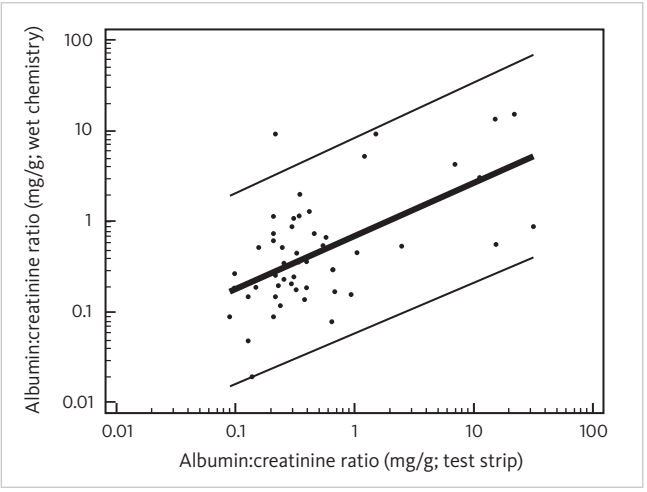

Fig. 6 *Regression analysis of urinary albumin using Meditape 11A test strips and immunonephelometry. Hollow circles represent specimens within reference range, filled circles represent samples, exceeding the upper reference limit. Regression analysis of the urinary albumin/creatinine ratio using Meditape 11A test strips and clinical wet chemistry [23].*

Urinary albumin strip assay to replace quantitative technologies

The potential of dye-binding test strip to serve as a front-line semiquantitative tool to decide upon the quantitative estimation of urinary albumin and ACR has been investigated, recently.

The semi-quantitative detection of the ACR demonstrated to be a reliable test to identify patients without pathological albuminuria values to avoid quantitative testing. In the respective laboratory setting the albumin screening workflow has been optimised by including the semi-quantitative measurement of the ACR using Meditape 11A test strips in combination with the UC-3500 automated biochemistry [Fig. 8], allowing a reduction of quantitative albumin measurements of around 40 % [25].

The implementation of the test strip-based albuminuria screening thus not only positively impacts on the laboratory workflow, but also provides significant economic savings that are re-invested in the frequent albuminuria screening of patients at risk for the development of CKD [25,26].

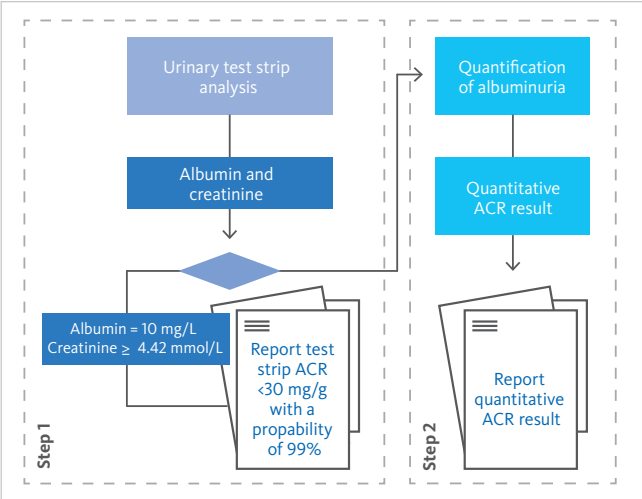

Fig. 7 *Improved albuminuria screening workflow through partial replacement of quantitative measurement technologies [25].*

Early detection in routine primary health check

In context of a primary healthcare setting, the early detection of CKD has been assessed recently, by comparison of the performance of test strip-mediated examination of proteinuria and the ACR. Both parameters have been assessed using the Meditape 11A test strip in combination with the automated urine test strip analyser UC-3500. In line with the KDIGO guideline [1], albuminuria was defined using test strip ACR ≥ 30 mg/g and test strip proteinuria as '≥ trace' or protein-to-creatinine ratio (PCR) ≥ 150 mg/g.

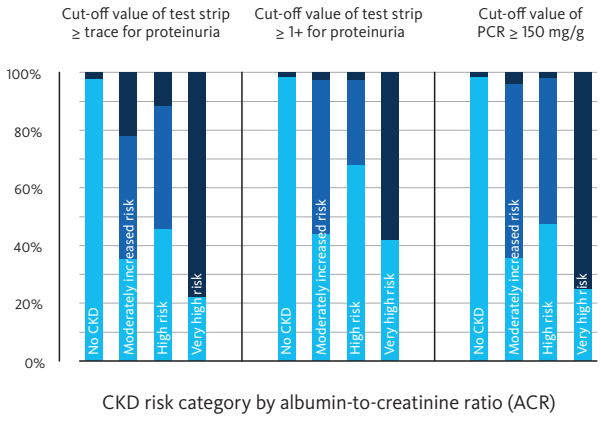

Fig. 8 *Performance of urine protein reagent strip in classification of CKD risk compared to urinary albumin-to-creatinine ratio (ACR) from dye-binding test strip [27].*

The assessment of the risk for CKD, based on proteinuria or PCR in comparison to test-strip ACR, revealed a moderate (κ = 0.567) and substantial agreement (κ = 0.683), respectively. More than 30 % of the investigated cases showed a moderately increased risk for CKD upon ACR screening, but were negative, based on proteinuria and PCR. The caused underestimation of the risk for CKD by the exclusive assessment of proteinuria and PCR therefore demands the consideration of ACR test strip examinations for screening for CKD at early and asymptomatic stages in primary care [27].

Test strip-mediated ACR screening at the point of care

Since CKD is especially a rising burden in developing countries with lower social-development indices [4], cost-efficient screening solutions are of utmost importance. With the Meditape 12S and the UC-1000 semiautomated test strip reader, the CMOS technology is also available as a point of care solution.

A recent evaluation of the diagnostic performance of the UC-1000 demonstrated high diagnostic accuracy for the ACR in context of screening for the onset of CKD and in comparison, to immuno-turbidimetric assays. A sensitivity of 0.79, a specificity of 0.84, a positive predictive value of 0.39 and a negative predictive value of 0.97 allow a reliable rule-out of albuminuria, whereas suspected cases of albuminuria require confirmation via immune-based assays. The sensitivity improved up to 0.89, in samples from individuals suffering from diabetes, hypertension, HIV infections or of an age of 65+ [28].

The high diagnostic performance of this point of care solution thereby not only allows to exclude albuminuria, but also to reduce the number of unnecessary albuminuria screening and to setup a frequent CKD screening at the point of care.

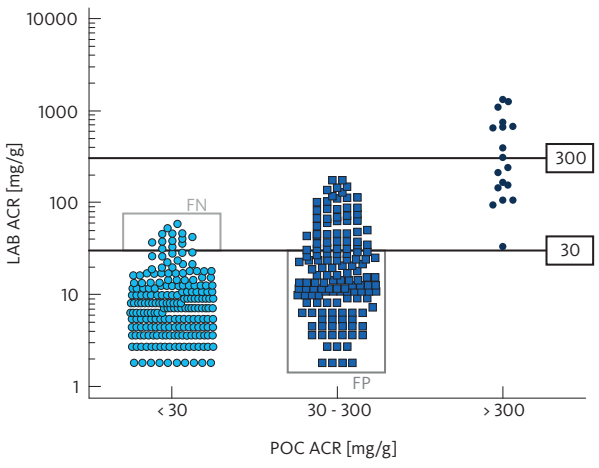

Fig. 9 *Agreement between ACR measurement on the UC-1000 point of care solution, compared to immune-based diagnostics. LAB ACR: ACR determined by quantitative immune—based assay. POC ACR: ACR obtained from UC-1000. FN: False-negative. FP: False-positive. [28].*

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