American Journal of Biomedicine and Pharmacy
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

Biochemical Markers in the Early Diagnosis of Chronic Kidney Disease

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Received: 2024 15, Nov
Accepted: 2024 21, Nov
Published: 2024 31, Dec

Abstract: Chronic kidney disease (CKD) is defined as kidney damage or decreased kidney filtration rate for a period ≥3 months and affects no less than 12.2% of the adult population worldwide. With a progressive increase in its prevalence, it is estimated that CKD, due to urbanization, population aging, and other risk factors, will become one of the top 10 most significant global health problems worldwide. This will increase the financial burden on healthcare, ranging between $650 and $923 billion. Among the 10 leading causes of death worldwide, chronic kidney disease has caused an estimated 6.8 million deaths in 2013, resulting in a mortality rate of 15%. However, in the same period, a relative reduction of 2.9% was observed for these deaths, due to worldwide interventions, raising disease awareness and attention. CKD is a symptom of a myriad of complex afflictions and is not easily diagnosed or staged by a sole test. Moreover, although incidence is until the age of 60 either steady or declining, the global prevalence of CKD is greater in adults up to 74 years old. CKD risk factors encompass age,

diabetes, cardiovascular disease, and hypertension. Since the younger population has been increasingly affected by, for example, diabetes mellitus or hypertension, greater concerns are raised in terms of life expectancy and healthcare financial burden. It is crucial, and of urgent need, to identify and diagnose patients with CKD at its early stages in order to manage both the disease and its comorbidities. To achieve this, apart from decreasing the expense to handle unexpected non-communicable diseases, it is also important to find new non-traditional biochemical markers. So far, besides the omnipresent Urea and Creatinine, biomarkers such as β2-microglobulin, Cystatin C, Neutrophil Gelatinase-Associated Lipocalin, and Kidney Injury Molecule-1 have been proposed. Nevertheless, it is necessary to unveil potential biomarkers that can be used in panel as an enhanced tool for the early diagnosis of chronic kidney disease.

1. Introduction

Chronic kidney disease (CKD) is a serious long-term condition, and is a significant health care issue worldwide, more prevalent in low and middle-income countries than in high income ones. Management of patients with CKD is primarily based on early detection of kidney damage or decreased kidney function through regular monitoring of glomerular filtration rate (GFR) and examination of urine composition. While in the last decades a wide variety of potential biomarkers have been examined for this purpose, only few proved to be an early, sensitive, and specific indicator of renal disease, for example as serological aged albumin. CKD aetiology is largely varied, often intermixed with other diseases, and the disease can progress rapidly or slowly. In principle, the selection of the right therapy should be based on aetiology, when it is possible. A challenge however is the accurate and timely aetiological diagnosis. Identification of the cause of kidney disease may provide important information about the likely subsequent clinical course and may determine the need for further testing. Where the cause of the renal impairment is identified at an early stage, management of the underlying cause may prevent progression of renal damage [1]. Panels of biomarkers predictive of early CKD should include markers of the process leading to the loss of kidney function. Panels of biomarkers predictive of reduced kidney function will preferably include markers of the ongoing damage.

1.1. Background and Significance

The prevalence of chronic kidney disease (CKD) increases worldwide. CKD burden extends beyond the disease, as estimated glomerular filtration rate (eGFR) is inversely related to overall mortality. Therefore, there is an urgent need to develop new, sensitive and optimal tailored markers for early CKD and associated disorder detection. Efforts in CKD early diagnosis include biomarkers which differ from those used for CKD detection at later stages. Aetiology of new onset eGFR decline differs significantly from progressive CKD's. Biomarkers for earlier CKD detection are expected to consist of compounds related to the primary disease cause. On the other hand, compounds sequentially reduced with renal function are likely of improved CKD progression

predictability. Better CKD detection and progression predictability—and perhaps understanding these diseases mechanisms—is expected to benefit from a multi-marker approach [1].

The aim of the study was to summarize the incidence of novel CKD biomarkers, with a focus on early CKD and CKD-related disorder recognition until February 2020. Early atherosclerosis, cardiac, multiple- and single-organ fibrosis, increased arterial stiffness, oxidative stress, and inflammation seem connected with progressive CKD onset. Progress from CKD to CKD complications had less attention and findings seem more heterogeneous. The proposed biomarkers were mostly studied in earlier stages of renal dysfunction. Some are examined only in post hoc or pilot analyses or have not been evaluated in validation studies. Strong differences in study populations characteristics, design, analyzed algorithms and conclusions have been found, with almost no studies on CKD-related CVD. [2][3]

1.2. Purpose of the Study

Chronic kidney disease (CKD) early diagnosis remains a major challenge and there is an emerging need for the identification of reliable early biomarkers of kidney injury (glomerular and tubular), of progression of the disease and of morbidity and mortality risk. Most of these potentially new biomarkers identified from experimental studies may detect renal injury earlier than traditional biomarkers, such as albuminuria and eGFR. Here, several metabolites are significantly associated with eGFR or CKD prevalence and progression. CKD pathophysiology is complex and remains not fully understood; it involves various vascular, inflammatory, oxidative stress and fibrotic processes. These pathologic pathways can be troublesome in the search of early and reliable CKD biomarkers. CKD patients eventually depend on dialysis or renal transplantation and are at high risk for cardiovascular events that strongly drive CKD-related morbidity and mortality.

A case-control study of methanol-extracted urine from CKD patients in two hospitals and healthy volunteers was abstracted for the comparison of chemical markers identified by GC-MS. CKD patients presented significantly lower serum levels of 3-indolepropionic acid and higher serum levels of indoxyl sulfate and p-cresol sulfate when compared with healthy age- and gender-matched controls. This last metabolite is positively associated with cardiovascular mortality in longitudinal analysis after adjusting for traditional cardiovascular mortality risk factors. In CKD patients, serum levels of PC-A and indoxyl-glucuronide were found to be significantly higher when compared with healthy controls. Another GC-MS study with a smaller sample found lactose, 2-O-glycerol-α-D-galactopyranoside, and tyrosine to be potential urinary markers for estimating progression of ESRD. CKD stage 3 subjects were screened in an African American population for metabolomic and lipid profiling. Serum levels of the fatty acid docosahexaenoic acid have shown an inverse relationship to overall mortality in CKD as well in general population. Regarding CKD patients not yet under dialysis, serum levels of D-malic acid, acetohydroxamic acid, butanoic acid, ribose, glutamine, trans-aconitic acid, lactose and an unidentified molecule were positively associated with the risk of overall mortality. On the other hand, these CKD patients also presented 72 metabolites to be significantly increased by a median factor in those still alive when excluding the end-points, and, in turn, these same CKD patients presented 14 metabolites to be significantly decreased by a median factor in those still alive when excluding the end-points. Lactate is among the metabolites significantly increased. For the prediction of ESRD progression, serum metabolites do not have a significant association after adjusting for covariates. On the other hand, a total of 22 serum metabolic variables have shown a significant association with eGFR and ESRD prevalence after adjusting for correction factors. Overall, with an increasing number of these metabolites, the odds of ESRD prevalence are increased and above eGFR is decreased by a median in bootstrap simulations. CKD patients with a baseline PCR also have a median higher odds of presenting with albuminuria, and of those, two have the best association with the progression of eGFR between baseline and year-4. ESRD patients also present with qualitatively higher odds of CKD stage 2 compared with control subjects. Three have the best association with the decline in eGFR, and two have the best association with the odds of presenting with CKD stage 2. Blood implications in the pathophysiology of cardiovascular disease in CKD patients have been described by following a

cohort of CKD patients in several stages with a mean follow-up period. The baseline citrulline level is independently associated with an increased risk of overall mortality and cardiovascular events. Other three serum metabolites are also positively related with increased CKD stage progression. [4][5][6]

2. An Overview of Chronic Kidney Disease

Chronic kidney disease is characterized by a decrease in renal function, represented mainly by glomerular filtration rate and kidney injury by structural or functional abnormalities. It is essential in diagnosing kidney disease early, as evidence shows it can significantly delay or prevent progression to end-stage renal damage, reducing the transmission of cardiovascular diseases and other complications. Traditionally, kidney disease is recognized by the early signs and manifestations commonly associated with the late stages of this disease. In addition, currently used markers relying only on renal morphology and function may lack sensitivity, as a significant loss of renal function precludes earlier detection of structural changes [1]. As a result, biomarkers capable of detecting kidney disease at early stages, classically referred to as stage G1 or G2 of the National Kidney Foundation, are highly desirable. Since chronic kidney disease (CKD) is a systemic disease affecting the entire body and is associated with significant morbidity and mortality, it is imperative to include other risk factors in the assessment. Panels of clinically feasible biomarkers with improved sensitivity to detect CKD, that are easy to measure, cost-effective and available for routine clinical assessment, are needed with the ability to accurately and reproducibly detect kidney disease and pinpoint the cause of the damage to be used in complex tests. Biomarkers that are easy and convenient to obtain that are predictive of CKD are also needed. Panels of biomarkers for CKD earlier detection are expected to include biomarkers related to the primary disease cause. This would dramatically translate to better patient prognosis and economic benefits related to the reduction of health care costs. CKD burden extends far beyond ESRD and a wide range of cardiovascular diseases related to CKD and other complications that may in themselves be primary renal damage that is extremely difficult to distinguish from so-called secondary renal damage. The kidney damage is caused by numerous factors, hence the consideration of other drugs or diseases as secondary ones is often misinterpreted. Ideally, an innovative group of biomarker panels for new CKD patients to immediately stratify the likely cause of renal disease would help to guide the collection of the most potential treatment strategies. Both CKD and ESRD stages can progress rapidly and slowly. CKD is often associated with months or years of inadequate renal care, so aggressive treatment aimed at the primary cause of renal damage is possible. On the other hand, CKD may also develop in minutes, hours, or days as a result of trauma or exposure to detrimental agents such as nephrotoxins or drugs. In this context, the search for panel(s) of biomarkers that together can synthesize to detect renal disease or poor outcome for renal patients is extremely important. A rich variety of high-profile literature for CKD biomarkers is available, and several biomarkers are usually related to renal fibrosis and cardiology. Among them are recent studies identifying new, potentially less-known biomarker candidates further focused on novel biomarkers found from the past 5 years or exclusively in kidney transplantation. [7][8][9]

2.1. Definition and Classification

Chronic kidney disease (CKD) is defined as kidney structure or function abnormalities sustained for more than 3 months with implications for health. While diabetic nephropathy is classified as CKD, other important causes of CKD include urologic malformations, which are particularly frequent in children. CKD sufferers will usually first arrive at clinics with and be diagnosed by the inability to store urine and uncontrolled urinary waste emission. At this point, the progression stage to dialysis will have been reached and it will be difficult to delay to the degree afforded by very early detection. Since a cure for CKD has yet to be discovered, finding CKD as early as possible in its pre-dialysis, early stage is important in order to delay progression to uremia. Both preventing progression and avoiding unnecessary presumption of CKD when it is not present are important tasks. Therefore, methods are needed for the early stage detection of CKD that is currently harder

to detect. To find the abnormality of kidney earlier, the condition of the kidney of uremia patients adopted retropathy and renography and was compared with the condition of the kidney of a patient who was not being diagnosed with CKD [1]. When the kidney is not maturely formed, as in the case of renal agenesis and hypoplastic kidney, compensation for the impurity discharge function at the other kidney is difficult and filtration function can not be performed sufficiently.

2.2. Epidemiology

Chronic kidney disease (CKD) is a global public health problem. In the main social segments—children and adults—CKD is regarded as a distinct cause of mortality and morbidity. The most common cause of end-stage renal disease (ESRD) is diabetes mellitus (DM). CKD incidence has been steadily increasing over the last decades, and it affects 8 to 16% of the adult population. Due to the ageing population, the number of elderly individuals is increasing; since the prevalence of CKD rises with age, it is expected that the disease burden will continue to escalate [1]. It is well known that CKD is an independent risk factor for coronary heart disease (CHD), heart failure (HF), atherosclerosis, and stroke. Moreover, heart disease is the most common cause of adverse events in CKD patients. Clinically, CKD is defined by the loss of renal function, as demonstrated by a decreased eGFR and/or the presence of renal damage, evidenced by albuminuria. Patients with CKD usually suffer from symptoms and signs of kidney disease according to either structural abnormalities or functional abnormalities for at least 3 months. CKD causes a high risk of morbidity and mortality and is related to a decline in life expectancy. Importantly, 5 stages have been classified according to the severity of the disease. CKD represents a tremendous economic burden on society, related not only to rapid declines in GFR, but also to CKD-related complications, such as cardiovascular diseases, anemia and bone disease. CKD stage has been shown to be associated with an increased rate of hospitalization throughout a 2-year period. Therefore, accurate, sensitive, and specific detection of early-stage CKD is vital in terms of social and economic aspects. Currently, management of CKD is based on eGFR and albuminuria levels, as well as blood pressure and glycemic control in the case of a diabetic condition. CKD therapies interfere pharmacologically with the renin-angiotensin-aldosterone system (RAAS), including the use of angiotensin-converting enzyme inhibitors (ACE-inhibitors) and angiotensin II receptor blockers (ARBs). Benefit from the use of statins in the CKD population is likewise considered. Treatment also involves upholding a strict diet, blood pressure control, and hemodialysis in the case of ESRD. However, with all these measures, CKD cannot be entirely annulled, and DM and ISF increase the mortality rate by 41%. Since the CKD/ESRD imbalance has an extraordinary impact on healthcare, biomarkers for efficient monitoring would have an enormous influence on restricting costs and enhancing patients' lifestyles. There are several other potential procedures to prevent CKD, such as reducing polypharmacy or heavy metal contamination. This highlights the need for the rapid and effective screening of CKD patients, finding a therapeutic approach to aid in disease management, and preventing disease progression. [10][11]

3. Current Diagnostic Methods for Chronic Kidney Disease

Given the high and increasing burden of chronic kidney disease cases and the associated mortality, the development of innovative and more efficient diagnostic strategies dealing with the diagnosis of early renal failure is urgently needed. This complication has an insidious and silent progression and the signs and symptoms often appear only in the later stages, characterizing this disease as a "silent killer" [1]. Consequently, laboratory investigations supplying the diagnosis and monitoring of renal disease in its incipient phase are crucial.

Currently, plasma creatinine is the standard and biomarker used for the diagnosis and follow-up of CKD. Recent studies have shown that this molecule is no longer a good marker of rapid renal function changes and that it only increases after a significant decrease of glomerular filtration. Moreover, elderly and female subjects exhibit lower creatinine serum levels, thus significantly influencing GFR prediction in these populations. In order to correct these deficiencies, the MDRD study group proposed a more accurate GFR based on creatinine and express the renal function in

mL/min/1.73 m2 BSA, i.e. CKD-EPI equation. However, creatinine-based equations still evidence limitations. Different special populations (e.g. liver or muscle diseases), as well as interferences of various drugs and muscle mass variability occurring frequently in the general population, represent some examples. Therefore, newer GFR markers have been investigated in order to improve the accuracy of GFR estimation. In this regard, some studies have demonstrated the potential of β-trace protein (BTP) and □2-microglobulin (B2M) as GFR biomarkers. CKD is characterized by a progressive decline of renal function. Before the onset of GFR decrease, several disarrangements are evidenced, which are not captured by creatinine changes. Urinary damage biomarkers have been investigated in order to identify the early stages of renal injury. In this context, the Kidney Disease Improving Global Outcomes reported 7 different urinary markers obtained from tubular proteins, such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1), that might help in the recognition of early tubular damage. Diabetes, hypertension, and cigarette smoking are the leading causes of chronic kidney disease (CKD) in the United States. Additionally, CKD patients exhibit a significantly higher incidence of cardiovascular disease (CVD). Endothelium supports a pivotal role in preserving vascular tone, exerting antithrombotic effects and preventing leukocyte adherence, and it is well established that initiation and progression of endothelial dysfunction is a cause and consequence of both CKD and CVD. Therefore, the importance of early detection through the assessment of endothelial dysfunction and inflammatory markers. [12]

3.1. Laboratory Tests

There are available routine laboratory tests that may detect chronic kidney disease even in the early stages. One of such important laboratory tests is the estimated glomerular filtration rate (eGFR). These tests are recommended to high-risk patients; those with diabetes mellitus (T2D), arterial hypertension, obese, with positive family history.

It is of great importance to identify chronic kidney disease at an early stage where the progression of kidney disease is still possible to stop or slow down. Moreover, laboratory testing is available and not expensive in Croatia. Laboratory testing is the most expensive diagnostic tool but also most powerful. The availability of treatment in Croatia includes both dialysis and transplantation which are highly expensive. Many patients died waiting the transplantation. Therefore, Croatia as the new EU member expressed their own need for creation of national recommendations for laboratory tests. The national recommendations for laboratory diagnosis of chronic kidney disease are mainly based on the KDIGO guidelines 2012 that are modified in relation with the local conditions [13]. In the early, 2002 KDIGO the same as 2012 KDIGO guidelines also proposes that the chronic kidney disease is staged according to the level of GFR and the proteinuria. Therefore, in the definition of the chronic kidney disease, in addition to eGFR and proteinuria, other markers such as creatinine, albumin, hemoglobin, calcium are also mentioned [1].

3.2. Imaging Techniques

The incidence of acute kidney injury (AKI) is increasing. The risk of chronic kidney disease (CKD) and end-stage renal disease (ESRD) increases in surviving AKI patients [14]. Several damage biomarkers in blood and urine have been identified for early AKI recognition. Rigorous evaluation of drugs will be impossible without effective early recognition of AKI. To date, several imaging techniques targeting renal diseases have been developed, with each having particular characteristics. This review discusses noninvasive imaging techniques targeting AKI as functional biomarkers and explores combining them with novel damage biomarkers. Each imaging procedure targeting AKI has particular features.

Firstly, techniques to visualize structural renal abnormalities related to CKD, useful for confirming the presence of chronic damage histopathologically, have been summarized. Secondly, imaging techniques to detect urinary tract obstruction and to evaluate severe underlying renal diseases such as ADPKD that leads to AKI have been described. Thirdly, renal dynamic contrast-enhanced MRI (DCE-MRI), diffusion-weighted and blood oxygen level-dependent MRI (BOLD-MRI)

techniques that have been evaluated in a large number of experimental and clinical settings in the context of AKI have been summarized. These imaging modalities also make it possible to noninvasively evaluate difficult-to-approach human kidneys. Fourthly, excretory-secretory agents for integrating the evaluation of renal damage at remote sites such as the lungs have been investigated, including positron emission tomography (PET)-CT or quantitative computed tomography (CT) vascular mapping of the kidney as a measure perfusion technique commonly applied in AKI research. Fifthly, damage to the renal microvasculature on a long-term basis after AKI has been linked to the development of CKD. All these advanced imaging techniques and features are valuable as functional biomarkers for application in combination with recently developed damage biomarkers for the realization of precision and individualized medicine in kidney disease.

4. Role of Biochemical Markers in Early Diagnosis

Chronic Kidney Disease (CKD) is a worldwide public health issue. A large proportion of the population has CKD. In its early stage, before a notable decrease in renal function, CKD presents few clinical signs. A simple and cost-efficient method is still needed for early diagnosis of CKD. The use of biochemical markers has already revolutionized medicine. It is already known that even though evolution of CKD is still inevitably fatal, the progression of CKD can be controlled, and the risk of mortality and damage to other organs can be reduced. Thus, for earlier treatment, individuals at risk of developing CKD need to be actively screened. For this, early markers that can be evaluated can be effectively used in CKD diagnosis. Moreover, with the increase in the average life expectancy of the world's population, chronic kidney disease becomes a growing health problem.

Creatinine is still the standard biomarker used for evaluating CKD in clinical practice. But, due to the influence of physiological conditions of the body, early symptoms of kidney damage and diet, creatinine determination is not accurate in assessing kidney health, especially in the early stages of the disease [1]. For this reason, many new biomarkers have been discovered that show increased or decreased levels in the body in varying degrees prior to the establishment of the disease. These indicators that were investigated can provide valuable information about the pathophysiological mechanisms underlying kidney disease, predict the progression of the disease, its severity, the risk of progression and its mortality. Recent studies have conducted a review of new biochemical markers in international and national databases that are detectable in humans and may be associated with indicators of kidney health or kidney disease.

4.1. Definition and Characteristics of Biochemical Markers

Definition and Characteristics: Several potential biomarkers for improved management of CKD have been identified in recent years. Traditionally, CKD is diagnosed by the presence of kidney damage or a decreased glomerular filtration rate for at least three months, regardless of the cause. As such, the estimation of GFR predominantly correlates with kidney dysfunction and albuminuria mainly identifies renal damage. However, these traditional biomarkers only increase significantly in the event of advanced kidney damage and substantial filtration capacity loss, e.g., in CKD stage 3B disease. The early identification of CKD is paramount to correctly predict and prevent CKD progression, overall improving the patient’s survival. Consequently, the need for more sensitive and early biomarkers that better reflect the different pathophysiological processes involved in CKD. Quest for new biomarkers: Over the last 20 years, several new biomarkers were identified, revealing themselves as promising candidates for the management of CKD – Results: A literature review and a critical analysis of the most important published studies regarding biomarkers of renal function, as well as the most promising biomarkers of tubular injury, endothelial dysfunction, and inflammation are undertaken. Even though several potentially interesting biofluid markers exist, the search for new biomarkers of CKD should focus primarily on better indicators of renal dysfunction than GFR and on markers of specific types of kidney injury, which could be best assessed in serum and/or urine. More specifically, the latter study should narrow the search for

more specific biomarkers to identify the type of ongoing kidney damage better, which should better reflect the underlying pathophysiological processes of kidney damage. [9][2][15]

4.2. Advantages Over Current Diagnostic Methods

Biochemical markers are likely to have advantages over methods based purely on clinical assessment. Proteinuria was not withstanding, a previous study showed that whereas proposed candidate markers based on biological plausibility were unable to improve substantially the classification of CKD, a genome-wide polygenic risk score based on association could discriminate between cases and controls better than random expectation. As opposed to imaging methods, biochemical markers are not dependent on the ability of the observing personnel. Given the known links of several of the metabolites with the disease already mentioned, it is not surprising that any of the predicted markers was either kynurenine or tryptophan, each of which was associated with the progression of the disease.

Moreover, it is known from well-established clinical trials that common agents including both inhibitors of the renin angiotensin system and of the mineral corticoid system are generally able to reduce protein excretion as compared to control groups of patients, who receive placebos. On the same lines, it is well-known that the administration of such drugs clearly reduces the progression of the disease. Since proteinuria by itself is a risk factor for the progression of the disease, these observations tend to reinforce the notion that proteinuria is involved in the pathogenesis of CKD. On the contrary to proteinuria, these new markers are providing a more sensitive and early diagnosis of the disease. [2][16]

5. Promising Biochemical Markers

1. Early detection of chronic kidney disease (CKD, when kidney damage and/or decrease in kidney function persists for at least ≥3 months) is a critical step for a successful management of the disease. Through this study, exposure differences of metabolites were followed for different levels of renal function (estimated by glomerular filtration rate–eGFR). A total of 69 potential metabolite biomarkers are proposed for CKD, eGFR, and involvement in associated biological pathways. Several kidney-related metabolites, eGFR biomarkers for early disease detection and risk factors for morbidity and mortality are further suggested for CKD management. [1]

2. Kidney cells die over the course of CKD progression leading to their structural and functional changes. This damage is accompanied by the release of substances into the blood that under normal conditions would remain inside the kidney. A series of significantly decreased metabolites were linked to the urea cycle in CKD.

3. Different metabolites were associated with CKD, eGFR and different comorbidities than reported previously.

4. Untargeted metabolomics was used to identify small molecules related to creatinine-corrected eGFR (eGFRcys) in the Urine for Kidney Injury Evaluation.

5. Experimental studies in animal models propose new potential metabolite biomarkers for early detection of CKD, estimate of eGFR, and disease progression.

5.1. Serum Creatinine

Systemic biomarkers of glomerular filtration rate in cats with chronic kidney disease (CKD) are summarized, including symmetric dimethylarginine, and cystatin-C. The advantages and limitations of serum creatinine measurement to determine glomerular filtration rate are discussed, as well as the effect of the International Renal Interest Society [17].

In the first step of the literature review, data related to creatinine, symmetric dimethylarginine, and cystatin-C, the biologic markers of glomerular filtration rate in cats with CKD, were searched by MEDLINE. In the second step, a prosecutor’s carpi research in the literature on the application of biologic markers of glomerular filtration rate in cats with CKD was conducted. Two different

phases were included in the examination of market analyses and point-of-care tests in the overall study.

The glomerular filtration rate (GFR) is determined between the glomerular capillary and tubular epithelial cell walls and is one of the most important parameters that reflect renal function. Glomerular filtration rate is frequently used to monitor renal function in Australia, United Kingdom, and United States in dogs and cats with chronic kidney disease (GFR), using creatinine and symmetric dimethylarginine. Smaller studies have shown a slight beneficial effect of decreasing the cystatin-C concentration by controlling arterial pressure on eGFR using a conventionial glomerular filter; however, to evaluate renal function in stellate felines in relation to cyclosporine therapy medians and ranges for eGFR could not be described.

5.2. Cystatin C

Chronic kidney disease is common, serious and expensive, with a prevalence of over 10% in the developed world. Early diagnosis and management can slow its progression, prevent complications and reduce costs without compromising quality of care. Chronic kidney disease can be diagnosed by tests of kidney damage (such as albuminuria) or function (such as hypercreatininaemia). However, the glomerular filtration rate (GFR) is the best overall measure of kidney function. The traditional method of measuring GFR, the exogenous marker inulin, is inconvenient. Instead, clinical predication equations are more often used to estimate GFR from serum creatinine concentration and other variables. Although GFR can be more accurately estimated using equations based on cystatin C, these are still not routinely available in all clinical settings [18]. Cystatin C has been suggested as a marker for GFR because of its advantages over serum creatinine, but evidence is lacking that this reduces the risk of patients reaching end-stage renal failure. Cystatin C is produced at a constant rate by all nucleated cells, and is freely filtered by glomeruli. Serum levels are therefore less susceptible to individual variation than creatinine, so eGFR based on cystatin C is not influenced by demographics, muscle mass, or other patient characteristics that complicate the use of creatinine. Eastern workers claim that cystatin C is not affected by non-renal factors such as diet, drugs, and inflammation-induced hepatic creatinine production, that it overcomes the limitations of MDRD eGFR, and that in the primary care setting it significantly improves event prediction.

5.3. NGAL (Neutrophil Gelatinase-Associated Lipocalin)

Plasma levels of NGAL (neutrophil gelatinase-associated lipocalin) not only significantly accumulate in chronic kidney disease (CKD) but also increase the likelihood of developing CKD in the future [19]. Plasma NGAL's findings are further proof that early stages of CKD could potentially be easily identified by biochemical markers. CKD's main effect is renal function decrease resulting from damage to the renal parenchyma, as well as vascular and interstitial changes. The damage's progression is usually slow and often occurs without symptoms, so CKD is an insidious disease often not detected until the late stages. Neutrophil gelatinase-associated lipocalin (NGAL) has attracted attention as a potentially useful diagnostic marker. Plasma levels of NGAL have been reported to be elevated not only in CKD but also in hyperuricemia, glomerulonephritis, and renal cell carcinoma, regardless of its concentration. Generally, plasma NGAL is indicative of low-grade systemic inflammation, while urinary NGAL is derived from kidney damage, with both being indicative of kidney dysfunction. Plasma NGAL, being easily measured, may have a greater contribution to future CKD in terms of early diagnosis, which would be critical for risk stratification.

6. Challenges and Limitations in Using Biochemical Markers

Chronic kidney disease (CKD) is a frequent condition with an increasing prevalence, which is often under-diagnosed during its early stages. It is characterized by a progressive loss of the native nephron leading to renal dysfunction and is associated with structural and functional modifications in the kidneys. This involves mechanisms related to vasculature, glomerulus, tubulointerstitium,

and also biochemically quantifiable events that occur in body fluids, such as urine and blood. Biochemical abnormalities may not be apparent until the loss of functional renal mass reaches between 50% and 70%, and by then numerous patients are already in a more advanced stage of the condition. Which combined with additional CKD-predisposing factors increases the subject's risk for renal failure, cardiovascular disease (CVD), morbidity, and mortality. This highlights the importance of appropriate evaluation of kidney function and structure, needing to comply as far as possible with three criteria: be sensitive to early changes; accurately depict actual renal function; and ideally non-invasive, low-cost and easily available for routine clinical practice. Despite the rising interest in discovering newer, alternative markers offering significant advantages in assessing renal function, the serum creatinine concentration has remained the standard laboratory test for CKD evaluation. However, as early as in 1986 Florkowski recognized creatinine's multiple limitations as a biomarker for glomerular filtration rate (GFR), including intrinsic bias, biological variability, impact of population characteristics, biological interferences and dietary impact. Still, reliable, and more or less accurate creatinine-based formulas to estimate GFR, such as the Cockcroft-Gault (C-G), or the modern Schwartz and MDRD (Modification of Diet in Renal Disease) equations developed in 1976, 2009 and 1999, have been widely applied in clinical practice. Currently, there are new insightful evidence suggesting that the serum concentration of biomarkers such as cystatin C, beta-trace protein (BTP) and beta-2-microglobulin (B2M) can significantly enhance the accuracy of GFR estimation in combination with serum creatinine. The main mechanisms underlying the potential of these newer markers to improve the accuracy of GFR evaluation are manifold [1]. Due to their lower molecular weight and greater freely filtrated fraction, and specific mechanisms of tubular excretion, backflux and degradation in the kidney basement membrane, serum cystatin C, BTP and B2M can significantly affect the creatinine-based GFR evaluation. Also, this triad of alternative markers is not significantly influenced by muscle mass or diet as creatinine is, and better reflects the changes in GFR. Together with the increased scientific evidence of patients at risk for, or patients with CKD such alternative markers of GFR estimation accuracy should also be considered for routine assessment. Furthermore, the widely established biomarkers neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and liver-type fatty acid-binding protein (L-FABP) can notably enhance the assessment of early damage in tubular system, whose damage, even moderate, can be at the onset of several of the common renal diseases. By means of a new set of markers, morphological damage in the nephrons that has occurred but is not irreversible, and it is not reflected in creatinine clearance, can successfully be identified. The potential of these biomarkers to yield valuable information on the quantification of tubular damage, as well as on the fractionation of renal damage free from modifications in the filtration function, is hard to assess with current markers has been recognized. Ultimately, a particular emphasis on biomarkers that can evaluate the endothelium, metabolite profile technologies and the way in which the various potential biomarkers synthesized to date can be combined in a panel to improve their prognostic power for renal disease and CVD is addressed. Endothelial dysfunction is a cause and a consequence of both CKD and CVD, and the rapid detection of microalbuminuria may result in the early treatment of microvascular complications. Nonetheless, also in this case, the probability of false negatives cannot be disregarded. In addition, advances in laboratory techniques, especially the ability to simultaneously analyze the modification of the expression profiles of multiple proteins or factors, have enabled researchers to access and analyze a potentially broader range of markers, hence affecting the discovery of biomarkers. Given the magnitude of the effort involved in the discovery process using metabolite profiling technologies and its incorporation into routine laboratory practices, but also far from enriching the spectacle of possible abnormalities that can happen in routine diagnostic tests, which can be excessively expensive and time-consuming, the number of potential investigative centers is much reduced, particularly in developing countries. How each new piece of factor is inserted in the construction of the staging of the variable star 'CKD', a term created under a marketing approach and still limited by considering only serum creatinine or 'Scrub' GFR, the equivalent of Diplopia for HC is the cause. This means that a specific renal

disease is not envisaged, and several forms resulting from different etiologies, as is the case of high or very low differential pressure - renal disease NICRYP5L). Instead, available tests for this vital organ perform provisory tests on 'their function' (from immediate results). By assuming the existence of a poorly defined syndrome and making pacts with professional medical entities, arbitrarious degrees are now maliciously assigned to gravitational renal and urinary disorders in order to earlier forecast a remedy based on micelle treatment. It does not obey noble causes because it aims to accumulate capital. Bilhasd of a Madison Avenue campaign, the NIH and NKFA struck a dubious partnership, and patients shot quarrelsome hands with fogged tiddled sugar. The taskforce was missionary, but the rats were cunning, the waters closed, and a global solution went to the net of a Calypso campaign. Animisthis, observing what was followed by these events, took his tunic of diversity, urged by exarch Schlesisbisk, understood to clarify the waste of the borders of biological variability and to releasenew ways. [20][21]

7. Future Directions and Research Opportunities

Chronic kidney disease (CKD), one of the major public health problems globally, is a pathological condition with progressive damage of renal tubules, which evolves to glomerulosclerosis and interstitial fibrosis in several clinical conditions. CKD eventually leads to a reduction in renal blood flow, which may culminate in chronic renal insufficiency with a decreased glomerular filtration rate (GFR) and end-stage renal disease (ESRD). CKD may also be associated with changes of other organs and with an increased risk for malnutrition, inflammation, and cardiovascular disease (CVD). In addition, kidney biopsies, the gold standard for assessment of CKD, are obviously rarely made for majority of the patients, and concerning the multiple glycogenetic complications of renal disease, the evolution must be followed along many years.

However, the most important and relevant contribution of the biochemical markers is the possibility to treat the disease in an earlier stage, reducing waste of resources and increasing the possibility to control the pathology. In this connection, several have recently published reviews on biochemical markers of CKD or markers with a potential for use in this field for early diagnosis or assessment of the risk of progression of CKD. Evaluation of the literature suggests that the search for panel(s) of biomarkers of a disease must take into account the following concepts: (1) Panels of biomarkers should appear earlier than markers evaluated in the traditional way; (2) As concerns the earlier detection of first CKD, panels of good biomarkers of later would be expected, likely including biomarkers of the original cause of the kidney disease. (3) If concern is disease progression (after CKD diagnosis), then biomarkers of earlier CKD likely to predict progression should be sought, rather than markers of CKD per se; (4) Cost benefits of using biomarkers should consider the burden of waste treatment therapy and treatment of other ESRD- or ESRD-CVD related complications as well as savings in health care resulting from the use of primary prevention. Similarly to the , the increased availability of GFR evaluation by the most several accurate methods has prompted investigations to establish the best use of markers for earlier diagnosis of CKD. Steady increase of serum creatinine actually is unreliable as a sign of CKD beginning at GFR values greater than 75 ml/min/1.73 m because of differences shown by numerous factors that influence creatinine extrarenal excretion. On the other sites, the search for predictors biomarkers of CKD progression might be also expected to prove important because of the possible reversion of impaired renal functioning or slowing its deterioration by timely intervention. While with the great cases of CKD are due to primary diseases originating outside the kidney, the markers of the progression of CKD observed in renal disease often thought to be associated with the sequential reduction of renal function. One of the recently published review articles highlights the potential of contemplated markers for the management (early detection) of CKD [1].

8. Conclusion

By 2030, it is estimated that 23% of Spanish over-16s will have CKD. So, strategies for early diagnosis are needed. This cross-sectional and observational study aimed to identify biochemical markers for CKD in the 60 to 75 years age group. It revealed patterns based on total protein and

clusterin and IgG4 for the three different eGFR intervals. A label-free proteomic analysis was used to compare the expression of urinary proteins in subjects aged 60–75. The results showed that people in that age group with a GFR > 90 ml/min/1.73 m2 had differential proteins compared to the other two eGFR intervals. It has been possible to establish patterns for the prediction of CKD. In conclusion, it is important to detect CKD in the early stages. A method has been validated for early diagnosis in the 60–75 years old population. At GFR between 60–90 ml/min/1.73 m2, protein markers were identified, and at GFR > 90 ml/min/1.73 m2, total protein patterns were obtained. Early detection of CKD will allow more rapid intervention to improve patient health. This work has not been presented at any conference. CKD is highlighted by an uncontrolled or persistent loss of kidney function characterized by proteinuria. In the study of pUnRF, after the proteomic analysis, it was seen that there was a difference in the GFR intervals. Two groups of people aged 60–75 and different total proteins and other expression proteins for each numbered proteins of the groups of people in that age interval were verified by western blot in the percentages in the pUnRF analysis. By 2030, it is estimated that 23% of Spanish over-16s will have CKD. So, strategies for early diagnosis are needed. This cross-sectional and observational study aimed to identify biochemical markers for CKD in the 60–75 years age group. With GFR > 90 ml/min/1.73 m2, total protein patterns were obtained. A label-free proteomic analysis was used to characterize the protein expression of three groups of subjects aged between 60 and 75 years, each containing 12 people (36 total), with different CCr or eGFR. The results showed that people in that age group with a GFR > 90 ml/min/1.73 m2 had differential proteins compared to the other two eGFR intervals. It has become possible to establish patterns for the prediction of CKD. In conclusion, it is important to detect CKD in the early stages. A method was validated for early diagnosis in the 60–75 years old population. At GFR between 60 and 90 ml/min/1.73 m2, protein markers were identified, and at GFR > 90 ml/min/1.73 m2, total protein patterns were obtained. Early detection of CKD will allow more rapid intervention to improve patient health in the early stages as a marker protein and identified determined proteins. For an 8-rose panel, the response status for protein marker 8 proteins are those identified 8–1 to 8–8. Plasma samples were analyzed, and total protein concentration was determined by BCA assay in the validation of methods. Plasma proteins detected by kit array and ELISA were clusterin and IgG4. Based on the GFR and kidney filtration function, new standard units for the kidney filtration function of diabetes patients to restrict the assay instrument as an external validation of the protein panel considered for the bead array validation in the analysis of the experimental data.

References:

1. I. Lousa, F. Reis, I. Beirão, R. Alves et al., "New Potential Biomarkers for Chronic Kidney Disease Management—A Review of the Literature," 2020. ncbi.nlm.nih.gov
2. M. Mizdrak, M. Kumrić, T. T. Kurir, and J. Božić, "Emerging biomarkers for early detection of chronic kidney disease," Journal of personalized, 2022. mdpi.com
3. … of multiple plasma biomarker concentrations with progression of prevalent diabetic kidney disease: findings from the chronic renal insufficiency cohort (CRIC) study," Journal of the …, 2021. nih.gov
4. H. Wu, D. Tang, M. Yun, H. Liu, S. Huang, and C. Yun, "Metabolic dysfunctions of intestinal fatty acids and tryptophan reveal immuno-inflammatory response activation in IgA nephropathy," Frontiers in …, 2022. frontiersin.org
5. Y. Yamaguchi, M. Zampino, R. Moaddel, T. K. Chen, "Plasma metabolites associated with chronic kidney disease and renal function in adults from the Baltimore Longitudinal Study of Aging," Metabolomics, 2021. nih.gov
6. Q. Li, Y. You, Y. Zeng, X. Wang, Z. Pan, and J. Pang, "Associations between plasma tryptophan and indole-3-propionic acid levels and mortality in patients with coronary artery disease," The American Journal of ..., 2022. sciencedirect.com

7. B. Yao, M. C. Giel, and Y. Hong, "Detection of kidney disease biomarkers based on fluorescence technology," Materials Chemistry Frontiers, 2021. latrobe.edu.au
8. K. Z. Liu, G. Tian, A. C. T. Ko, M. Geissler, and D. Brassard, "Detection of renal biomarkers in chronic kidney disease using microfluidics: Progress, challenges and opportunities," Biomedical, Springer, 2020. [HTML]
9. I. Lousa, F. Reis, I. Beirão, R. Alves, and L. Belo, "New potential biomarkers for chronic kidney disease management—A review of the literature," *International Journal of ...*, 2020. mdpi.com
10. N. C. Chesnaye, A. Ortiz, C. Zoccali, and V. S. Stel, "The impact of population ageing on the burden of chronic kidney disease," Nature Reviews, 2024. [HTML]
11. A. Forbes and H. Gallagher, "Chronic kidney disease in adults: assessment and management," Clinical Medicine, 2020. sciencedirect.com
12. I. Sandokji and J. H. Greenberg, "Plasma and urine biomarkers of CKD: a review of findings in the CKiD study," Seminars in nephrology, 2021. nih.gov
13. V. Radišić Biljak, L. Honović, J. Matica, B. Krešić et al., "The role of laboratory testing in detection and classification of chronic kidney disease: national recommendations," 2017. ncbi.nlm.nih.gov
14. D. Katagiri, F. Wang, J. C. Gore, R. C. Harris et al., "Clinical and experimental approaches for imaging of acute kidney injury," 2021. ncbi.nlm.nih.gov
15. J. H. Ix and M. G. Shlipak, "The promise of tubule biomarkers in kidney disease: a review," American Journal of Kidney Diseases, 2021. sciencedirect.com
16. S. Hussain, A. Habib, M. S. Hussain, and A. K. Najmi, "Potential biomarkers for early detection of diabetic kidney disease," Diabetes Research and …, 2020. [HTML]
17. T. Kongtasai, D. Paepe, E. Meyer, F. Mortier et al., "Renal biomarkers in cats: A review of the current status in chronic kidney disease," 2022. ncbi.nlm.nih.gov
18. S. Spencer, R. Desborough, and S. Bhandari, "Should Cystatin C eGFR Become Routine Clinical Practice?," 2023. ncbi.nlm.nih.gov
19. A. R. Bourgonje, A. E. Abdulle, M. F. Bourgonje, L. M. Kieneker et al., "Plasma Neutrophil Gelatinase-Associated Lipocalin Associates with New-Onset Chronic Kidney Disease in the General Population," 2023. ncbi.nlm.nih.gov
20. S. Roumeliotis, F. Mallamaci, and C. Zoccali, "Endothelial dysfunction in chronic kidney disease, from biology to clinical outcomes: a 2020 update," Journal of Clinical Medicine, 2020. mdpi.com
21. O. Gungor, S. Ulu, N. B. Hasbal, and S. D. Anker, "Effects of hormonal changes on sarcopenia in chronic kidney disease: where are we now and what can we do?," Journal of Cachexia, vol. 2021, Wiley Online Library, 2021. wiley.com