MANAGEMENT OF PATIENTS WITH NON-DIALYSIS DEPENDENT CHRONIC KIDNEY DISEASE (ND-CKD)

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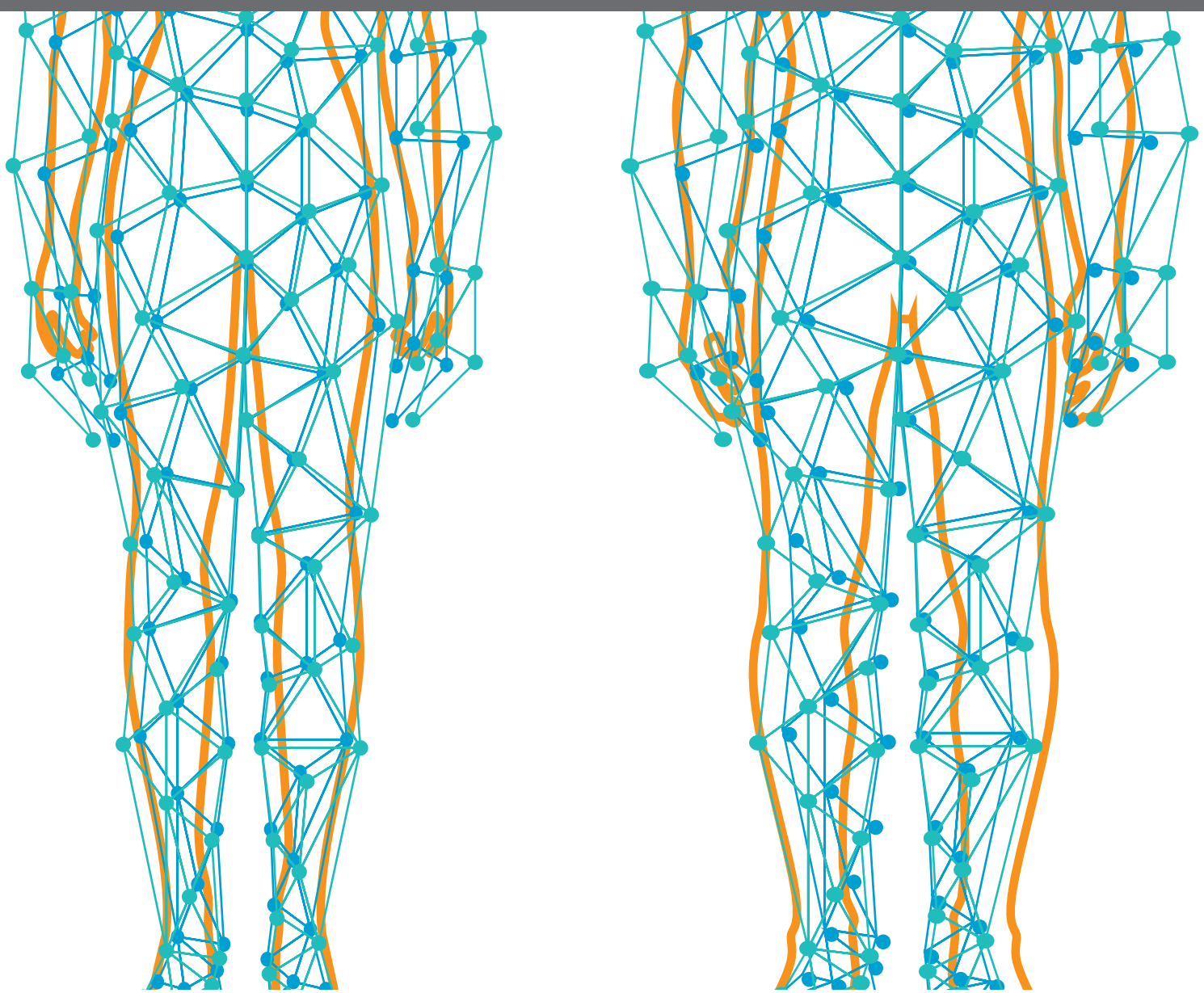

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MANAGEMENT OF PATIENTS WITH NON-DIALYSIS DEPENDENT CHRONIC KIDNEY DISEASE (ND-CKD)

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EDITORIAL
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Editorial: Management of Patients With Non-dialysis Dependent Chronic Kidney Disease (ND-CKD)

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Keywords: chronic renal failure, prognosis, albuminuria, ESKD (end stage kidney disease), cardiovascular risk

Editorial on the Research Topic

Management of Patients With Non-dialysis Dependent Chronic Kidney Disease (ND-CKD)

Chronic Kidney Disease (CKD) is a clinical condition defined by a reduction in renal function (estimated glomerular filtration rate, eGFR < 60 mL/min/1.73 m^2) and/or the presence of structural abnormalities in the kidney and/or increased (>30 mg/24 h) urine albumin excretion, which persist for at least 3 months (1). Patients with CKD and without appropriate treatment are likely to develop relevant clinical outcomes, such as cardiovascular events (especially heart failure), End-Stage-Kidney-Disease (ESKD), and death (2). The onset of ESKD is often a prelude to the need for renal replacement therapies, such as dialysis or kidney transplant, and, in the patient's perspective, it is certainly an event that marks a substantial change in the quality of life. Owing to this background, great motivation from physicians to improve the diagnosis, treatment, and prognosis of CKD patients is more than expected. With the present Research Topic published in *Frontiers in Medicine* we can definitely confirm our impression. In fact, many authors have examined and provided interesting details around risk factors for the onset of cardiorenal outcomes, pathophysiologic mechanisms active in CKD, and novel therapeutic strategies.

Among the risk factors, the role of intimal and medial vascular calcification (VC) has been revised (Bover et al.). The authors highlighted that prevalence of VC in moderate to severe CKD (G3a-G5 stage) exceeds 50% of patients and that VC is not a unique expression of the mineral bone diseased disorder, but rather a complex clinical entity associated with different endpoints, mainly represented by left ventricular hypertrophy and heart failure for medial VC and endothelial-ischemic damage for intimal VC. Moreover, a combination of calcium and phosphate deposition, in the form of calciprotein particles, and differentiation of vascular smooth muscle cells toward secretory phenotype plays a role in VC development. The implementation of omics techniques in detecting and differentiating VC in CKD patients is a promising and very interesting tool for future clinical application.

Still, in the context of cardiovascular risk in CKD, D'Marco et al. discussed the association between perirenal and epicardial factors with kidney measures (eGFR and urine albumin excretion) and cardiorenal outcomes, such as cardiovascular events, mortality, and eGFR decline. Thus, the authors pointed out the role of these two markers specifically in CKD patients and, interestingly, report the link between adipose tissues, fibroblast growth factor 23 (FGF23), inflammatory cytokines (IL-6), and cardiovascular risk.

Inflammatory cytokines also play a role in the context of diabetic kidney disease (Donate-Correa et al.). Urinary and serum levels of cytokines, like IL-6, IL-8, IL-18, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ which exert pro-inflammatory actions, are increased in these patients

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and play a significant role in triggering pathophysiological mechanisms, such as inflammation, extracellular matrix accumulation, and fibrosis. More importantly, it has been demonstrated that novel antidiabetic drug agents, namely sodium-glucose cotransporter type 2 inhibitors (SGLT2is) and glucagon-like peptide-1 receptor agonist (GLP-1RA), have a cardioprotective effect, which is partially mediated by anti-inflammatory effect, in addition to other unexpected effects on renal hemodynamics, a decrease of renal and cardiac workload and hypoxia and improving myocardial and renal energetics.

There are few studies demonstrating that treatment with these drugs is followed by a change in inflammatory parameters. However, the development of drugs that specifically target inflammatory pathways and that encourage the use of recent technological innovations, such as antibodies or microRNAs (miRNAs), can really represent a very interesting opportunity for future research. In the context of cardiac markers, in their original article Liu et al. reported that performing coronary angiography within 7 days before cardiac surgery procedures did not modify the risk for the onset of acute kidney injury; that observation should be considered in the preoperative management of high-risk patients.

Obesity and anemia are two important prognostic factors in CKD patients and have been also examined in the Research Topic (García-Carro et al.; Portolès et al.). In both Research Topics, novel interesting future perspectives have been discussed. Regarding obesity, Perticone et al. performed original research by stratifying obese patients into those with Obstructive sleep apnea syndrome (OSAS) and those without OSAS, reporting that the presence of OSAS was significantly associated with a lower eGFR and higher albuminuria levels. Furthermore, a holistic approach has been proposed to manage CKD patients and the results of novel randomized studies testing the efficacy of SGLT2is, GLP-1RA, mineralocorticoids receptor agonists, alone or in combination with classical treatments for obesity are eagerly expected to be obtained. With respect to anemia, a great milestone is represented by the novel Hypoxia-inducible factor prolyl hydroxylase (HIF) inhibitors, which are valid alternatives to the traditional Erythropoiesis-stimulating agents (ESAs) (3). However, Portolés et al. in their article also mentioned on the basis of previous intervention studies that hemoglobin targets in CKD patients should be personalized, this being a valid example of personalized medicine.

Rare diseases are recognized as an important cause of kidney disease and need to be detected as soon as possible in order to plan a specific treatment for each patient. Especially after the demonstration that the cause of CKD (and not only the presence/absence of CKD) *per se* influences prognosis (4). Zhang et al. presented a case series including 19 patients with fibronectin glomerulopathy, an inherited autosomal dominant disease characterized by the presence of proteinuria, microscopic hematuria, and arterial hypertension. All patients enrolled in this study underwent renal biopsy. Interestingly, albeit the sample size was very low, it was still possible to detect subgroups of patients (nephrotic range proteinuria and focal glomerular sclerosis on histology) with a faster renal function decline.

Another inherited disease, the Fabry Disease, has been associated with a very poor prognosis since it causes fast CKD progression, cerebrovascular, and cardiac events. In this volume, Battaglia et al. highlighted the importance of early detection of the disease, which is often misdiagnosed, especially in the pre-dialysis stage of CKD.

Siligato et al. revised the pathophysiology, outcomes, and treatment of nephrotic syndrome due to primary glomerulonephritis (GN) during pregnancy, stratified by type of GN. Based on the accurate literature review summarizing previous studies, those authors found that membranoproliferative glomerulonephritis and focal segmental glomerulosclerosis are associated with a worse prognosis as compared to minimal change disease and membranous nephropathy.

However, this Research Topic seems of great interest with respect to the attitude of nephrologists toward young female patients. Among the category of tubule-interstitial diseases, kidney stones reached a non-negligible prevalence, especially in developed countries. In this Research Topic, Xun et al. developed a predictive model, based on radiomic features and clinical features, for stone-free rate in patients undergoing flexible ureteroscopy. Radiomic refers to computer-assisted techniques of image extraction that optimize the selection of useful features from digital medical images. Other characteristics, which predicted outcome, were stone volume, hydronephrosis level, and operator experience. It is important to note that the authors used an excellent methodology, including least absolute shrinkage and selection operator (LASSO) regression for selecting radiomic features, but also model calibration, model validation, and built a nomogram. These tools are important to apply the clinical finding at the individual level and have been largely evoked for CKD patients (5, 6).

The management of Kidney Transplant recipients (KTR) has made important progress in the past decades, in particular for the knowledge and control of acute rejection (7). Nevertheless, KTRs are at increased risk for long-term graft failure and further effort should be directed toward the comprehension of the underlying mechanisms. Codina et al. have described in detail the causes of damage in KTR focusing on the mechanisms of intrinsic kidney repair that should be improved to reduce the burden of chronic graft failure. Moreover, the role of traditional risk factors of CKD progression, which have been widely investigated in pre-dialysis CKD patients, is less investigated in KTR. The authors have analyzed the effect of the recurrence of glomerulonephritis in kidney transplantation, as well as other factors that may influence the progression of CKD in kidney transplantation, such as drug nephrotoxicity, the post-transplant diabetic nephropathy, and the role of infection and cold ischemia time and ischemia-reperfusion injury. The authors also describe reparation mechanisms of renal lesions in those patients. In a cohort study of 286 KTR, Bielopolski et al. found that microalbuminuria, measured after kidney transplant, was associated with an increased risk for cardiovascular events and death over time, with the association becoming significant after 2 years from albuminuria measurement. This is an important confirmation of what

has already been found in CKD patients, where proteinuria is recognized as an independent and strong risk factor for cardiovascular outcomes (8). Thrombotic microangiopathy has been also found as one of the possible complications in KTR (Ávila et al.).

Other than detecting CKD patients at increased cardiorenal risk, there is presently a growing interest in improving treatment and reducing the risk for future events in these patients (9). Novel drugs have been introduced with the aim of reducing the residual risk in CKD patients, on top of the standard-of-care which consists, following the current guidelines, of drugs intervening in the Renin-Angiotensin-Aldosterone system (RAAS) (Provenzano et al.; Shabaka et al.). For instance, SGLT2is have been shown to reduce cardiovascular risk and risk for CKD progression in several recent studies, enrolling both diabetic and non-diabetic CKD patients. Moreover, these drugs share mechanisms of action with a low-protein diet, namely the reduction in hyperfiltration and a correct diet may compensate for the energy-wasting determined by SGL2is, thus improving nephroprotection (Cupisti et al.).

In general, diet has always aroused interest in the nephrology community with the aim of delaying CKD progression in renal patients. Correct incomes in protein, sodium, potassium, calcium, phosphorus, and vitamins should be carefully evaluated both in patients with pre-dialysis CKD and in KTR (Molina et al.). With respect to potassium, a novel interesting therapeutic approach derives from the novel agents reducing serum potassium (sK) levels, namely Patiromer Sorbitex Calcium, and Sodium Zirconium Cyclosilicate, which are able to maintain optimal sK levels without determining hypokalemia and adverse events that were more frequent with old treatments (Morales et al.). The importance of such a finding is not only related to the risk of cardiovascular death due to hyperkalemia but also depends on the evidence that those agents allow to maintain and optimize treatment with RAAS inhibitors and thus can lead to a better prognosis in CKD patients.

Steps forwards have also been made for optimizing the treatment of CKD patients with a concomitant cardiovascular disease. In patients with CKD and concomitant atrial fibrillation, which is a common arrhythmia in CKD, treatment with vitamin K antagonists provided a clinical benefit. However, these drugs have been associated with an increased risk for renal function deterioration as compared with direct oral anticoagulants, which should be accurately tested in patients with advanced CKD (stage 4 and 5) (Cases et al.). The advent of angiotensin receptor–neprilysin inhibitor for the treatment of Heart Failure (HF) has prompted its use in CKD patients given the efficacy in reducing parameters, such as N-terminal pro–B-type natriuretic peptide (NT-proBNP), left atrial size, and the overall risk of death. Fu et al. have shown that sacubitril/valsartan may be effective in patients in CKD stage 5 and HF with a preserved ejection fraction where it reduced NT-proBNP and heart rate while alleviating symptoms of HF, even if further and larger studies should confirm this hypothesis. This Research Topic presents many interesting and stimulating concepts and sets the stage for the future work that is still needed to optimize the management of this complex, yet very interesting disease, CKD.

AUTHOR CONTRIBUTIONS

MP drafted the initial manuscript. All authors revised, approved the final version, and agree to be accountable for the content of the work.

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BRIEF RESEARCH REPORT
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Clinicopathologic Features and Outcomes in Fibronectin Glomerulopathy: A Case Series of 19 Patients

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Aims: To describe the characteristics and prognosis of 19 patients with fibronectin glomerulopathy (FNG) and evaluate prognostic factors associated with poor renal outcomes.

Methods: Included in this retrospective study was 19 FNG patients in Nanjing Glomerulonephritis Registry system. Associations between the clinical parameters, pathological features, and renal outcomes were evaluated by Kaplan-Meier survival analysis.

Results: Of the 19 FNG patients included in this study, 8 (42.1%) were women. The median age of the 19 FNG patients was 31 (17–71) years, and the median disease duration 48 (1–175) months at diagnosis. At the time of renal biopsy, the mean serum creatinine (Scr) was 1.22 ± 0.16 mg/dl and urinary protein was 6.24 ± 0.97 mg/24 h. Renal biopsy showed a lobular appearance with cellular mesangial nodules expanded by matrix in 14 cases. After a median follow-up period of 87 months (interquartile range 34–114.5 months), 8 FNG patients developed renal function decline, including 7 progressing into end-stage renal disease (ESRD) and 1 presenting with by a 2-fold-increase in Scr. Scr and proteinuria remained stable in the remaining 11 patients. Kaplan-Meier survival analysis showed that nephrotic range proteinuria ($P = 0.022$) and focal glomerular sclerosis ($P = 0.028$) were associated with renal function decline.

Conclusions: Nephrotic range proteinuria and focal glomerular sclerosis were associated with renal function decline during the follow-up period of the FNG patients in our series. FNG Patients at risk of renal function decline should be identified preferentially and given more progressive and effective therapies to prevent further disease progression.

Keywords: fibronectin glomerulopathy, outcome, prognosis, renal pathology, proteinuria

INTRODUCTION

Fibronectin glomerulopathy (FNG) is a rare autosomal dominant inherited renal disease with typical clinical features of proteinuria, microscopic hematuria, and hypertension. The diagnosis of FNG mainly depends on renal biopsy. Pathologically, FNG is featured by massive deposition of fibronectin in the mesangium and along capillary walls. Electron microscopically, fibronectin deposition is shown as finely granular or fibrillary substructures with randomly arranged 12–16-nm fibrils (1–3). Fibronectin 1 (FN1) gene mutation was detected in about 40% of patients and is believed to be responsible for the occurrence of the disease (4–6). However, the exact pathogenic mechanism of FNG is not fully understood and no specific treatment is currently available.

So far only about 75 cases have been reported in the literature, mainly in case reports and case series with small sample sizes (7). The disease course and outcome of these cases need to be fully described. Moreover, it is believed that FNG patients usually show slow progression to ESRDover 15–20 years (8). In the series reported by Strøm et al. (1) some patients progressed into dialysis in 2 years, while renal function of some patients remained stable in over 10 years, suggesting that the clinical symptoms and renal function decline were variable over time, even among family members of the same pedigree. We report herein a largest series of patients with biopsy-proven FNG with long-term follow-up periods at a single center. The aim of this retrospective study was to describe the clinico-biological characteristics, renal pathology, and renal outcome of such patients and screen prognostic factors associated with poor renal outcomes.

MATERIALS AND METHODS

Patients Selection

Nineteen biopsy-proven FNG registered in Nanjing Glomerulonephritis Registry System from 2007 to 2018 were enrolled and reviewed. The baseline and follow-up data of these patients were obtained from the database of the said registry system. All follow-up data were updated to September 2019. This study was conducted in compliance with the Good Clinical Practice protocol and the Declaration of Helsinki principles, and approved by the institutional review board.

Follow-Up Observations and Outcome Measures

Clinical, biological, and histological data were collected retrospectively from the medical records of the patients at the time of kidney biopsy and at last follow-up. Hypertension was defined by systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg and/or use of antihypertensive medications. Renal parameters included serum creatinine(SCr), urine protein and the estimated glomerular filtration rate (eGFR) estimated using the four-variable Modification of Diet in Renal Disease (MDRD) formula. Proteinuria and nephrotic range proteinuria were defined by urine protein >0.3 and >3 g/d, respectively. Nephrotic syndrome was defined by proteinuria >3.5 g/d with albuminemia <30 g/l. End-stage renal disease (ESRD) was defined as eGFR <15 mL/min/1.73 m^2, initiation of dialysis or transplantation. Renal function decline was classified as a 2-fold increase in Scr after biopsy, initiation of dialysis, transplantation, or death based on the last clinical visit or report available.

Biopsy Processing

Kidney biopsy samples were processed for light and immunofluorescence microscopy according to standard techniques. Sections were systematically stained with Congo red and examined under polarized light. At light microscopy, presence of glomerular sclerosis, endocapillary and mesangial cell proliferation, extracapillary proliferation, tubular atrophy and interstitial fibrosis, and vascular lesions were evaluated. The presence of immune deposits was determined on the basis of the immunostaining for IgG, IgM, IgA, C3, C1q, kappa, lamda light chains performed at the time of renal biopsy. Poly-clonal rabbit antibody (DAKO, A0245) was used in staining of fibronectin. The renal biopsy samples were reviewed blindly and independently by two experts in renal pathology.

Statistical Methods

Descriptive statistics included the mean ± standard deviation (SD) or median (and range) as appropriate for continuous variables, and frequency (percentage) for categorical variables. Group comparisons were made using Student's *t*-test, Chi–square test, or Fisher's exact test, as appropriate. Renal survival was analyzed by using Kaplan–Meier curves and the log-rank test. A $p < 0.05$ was considered to indicate statistical significance. Data analyses were performed using SPSS software version 18.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Patient Baseline Characteristics

The 19 patients (8 female and 11 male) with biopsy-proven FNG, including previously published ones, were identified in this study (9). The patient baseline characteristics are detailed in **Table 1**. The median age at diagnosis was 31 (range 17–71) years. The intervals between initial onset of renal disease and the date of renal biopsy varied (median 48 months, range 1–175). The family history of renal disease in 9 patients was listed in **Table 1**. The baseline median SCr was 1.07 mg/dl (range 0.6–3.13). Distribution of the patients by 2012 KDIGO CKD guideline (CKD evaluation and management) at baseline using the MDRD formula is as follows: 11 (57.9%) within G1A3, 5 (26.3%) within G2A3, 2 (10.5%) within G3aA3, and 1 (5.3%) within G3bA3. All patients had proteinuria with median 5.73 g/d (range 1.3–17.8) and no patient demonstrated gross hematuria. Nephrotic range proteinuria was detected in 14 patients (73.7%). Median serum albumin was 31.6 g/L (range 21.6–47.2). Full-blown nephrotic syndrome was observed in 6 patients (31.6%). Hypertension occurred in 13 patients (68.4%). Genetic tests were performed in 2 patients, including one with heterozygous

TABLE 1 | Patient demographics and renal characteristics.

Patients	Age	Gender	Follow-up time	Hypertension	Nephrotic syndrome	Creatinine at renal biopsy (mg/dl)	Proteinuria (g/24 h)	Serum albumin	Family history of renal disease
1	65	Female	25	Yes	No	1.07	5.56	31.6	No
2	71	Male	71	Yes	Yes	1.13	6.99	27.5	Two sisters on dialysis
3	22	Male	40	Yes	No	1.55	2.49	39.1	One cousin diagnosed with MCD
4	31	Male	40	Yes	Yes	1,27	7.9	28.4	Mother with proteinuria
5	43	Female	34	No	No	0.6	1.5	36.5	Father on dialysis
6	29	Female	25	No	No	0.72	3.71	30.3	Father, one brother and one sister on dialysis
7	30	Male	113	Yes	No	0.78	5,73	35.9	Mother on dialysis, one sister diagnosed with FNG
8	34	Female	95	Yes	Yes	0.74	5.36	18.9	Mother on dialysis, one brother diagnosed with FNG
9	35	Male	87	No	No	1.83	6.02	32.1	Father, aunt and a cousin on dialysis
10	45	Female	140	No	No	0.72	1.44	39.7	No
11	24	Male	92	Yes	No	2.81	3.91	31.2	No
12	45	Male	140	Yes	No	0.9	6.85	39	A sister diagnosed with polycystic kidney disease
13	32	Male	147	No	No	0.83	1.33	47.2	No
14	27	Female	118	No	No	0.71	1.89	36.9	No
15	26	Male	109	Yes	Yes	1.37	8.2	22.4	No
16	19	Male	14	Yes	Yes	1.07	11.4	25.5	No
17	32	Female	116	Yes	Yes	3.31	9.13	21.6	No
18	29	Male	53	Yes	No	1.22	11.47	30.7	No
19	19	Female	24	No	No	0.59	17.78	36.3	No

MCD, minimal change disease; FNG, fibronectin glomerulopathy.

missense mutation L1974P. No specific comorbidities including neoplasms or congenital alterations was detected in our series.

Renal Biopsy Findings

The renal biopsy samples of all patients were available for review (**Table 2**). Lobular lesions (73.6%) were the predominant histologic pattern, characterized by mesangial expansion, lobular accentuation, positive periodic acid-Schiff staining, and fuchsinophilic deposits. Lobular accentuation of the glomerular tuft was observed in all cases. There was a range of 0–78.6% global glomerulosclerosis (mean 14.2%). Focal glomerular sclerosis was present in 5 cases (26.3%) with a mean of 8.1%. Interstitial fibrosis and tubular atrophy were reported in 15 cases (78.9%), including 5 cases of the moderate form. The acute form tubular necrosis was seen in 2 cases (10.5%) and moderate-to-severe form in one case (5.26%). Arteriolar hyalinosis and arterial intimal thickening were observed in 12 (63.1%) and 5 (26.1%) cases, respectively. Besides strong positive staining of fibronectin, immunofluorescence microscopy also showed slight and irregular deposition of IgM in 5 cases, IgG in 6, IgA in 7, C3 in 7 cases on the mesangial and capillary wall. Congo-red staining was negative in all cases. Electron microscopy showed massive granular deposits in the mesangial and subepithelial area.

Treatment and Renal Outcomes

Renal outcomes, treatment and follow-up findings of the patients are presented in **Table 3**. All the 19 patients were treated with renin-angiotensin system blockade, including 11 patients who were treated with Tripterygium Wilfordii Hook (TWHF), and 4 patients with corticosteroid therapy in combination with immunosuppressive therapies, including 2 with mycophenolate mofetil (MMF) and 2 with tacrolimus.

Last follow-up data were available in 15 of the 19 patients. The mean follow-up duration was 78 months (rang 14–147 months, median 87). At last follow-up, 7 patients progressed to ESRD despite supportive therapy and required initiation of dialysis, 2 of whom received renal transplantation. Scr increased by 2-fold in one patient. Of the 8 patients, the mean SCr and mean

TABLE 2 | Renal biopsy findings.

Patients	Lesion pattern	% Global glomerusclerosis	% Focal glomerular sclerosis	Interstitial fibrosis	Immunofluorescence microscopy
1	Lobular	35.7	0	Mild	IgA (1+), C3 (1+)
2	Lobular	17.6	11.8	Mild	IgA (1+), IgG (1+), C3 (1+)
3	MPGN	25	0	Moderate	IgM (1+), C3 (1+)
4	Lobular	0	0	Mild	C3 (1+)
5	Lobular	2.7	0	Negative	Negative
6	Lobular	0	0	Mild	Negative
7	Lobular	10.2	5.13	Negative	IgG (1+), IgA (1+)
8	Lobular	7.7	3.8	Mild	IgM (1+), C3 (1+)
9	MPGN	20	0	Moderate	IgG (1+), IgA (1+), IgM (1+)
10	Lobular	3	0	Mild	Negative
11	Lobular	78.6	0	Moderate	IgG (1+), IgA (1+), IgM (1+)
12	MPGN	0	0	Mild	IgA (1+), IgG (1+)
13	Lobular	0	0	Negative	IgM (1+)
14	Lobular	0	0	Negative	Negative
15	Lobular	9.3	0	Mild	IgG (1+), IgA (1+), C3 (1+)
16	Lobular	20	5	Moderate	C3 (1+)
17	MPGN	25.7	0	Moderate	Negative
18	Lobular	12.5	15	Mild	Negative
19	MPGN	3.3	0	Mild	Negative

MPGN, membranoproliferative glomerulonephritis.

proteinuria at time of renal biopsy was 1.62 ± 0.31 mg/dl and 7.10 ± 0.85 mg/24 h, respectively. The time from renal biopsy to the initiation of dialysis ranged from 21 months to over 109 months (median 71). The mean SCr in patients not on dialysis remained stable from 0.91 ± 0.09 mg/dl at the time of renal biopsy to 1.01 ± 0.11 mg/dl at the last follow-up and the mean proteinuria improved from 5.62 ± 1.56 mg/24 h to 3.14 ± 0.97 mg/24 h, respectively.

Factors Associated With Renal Function Decline

The general characteristics, laboratory results, and histological characteristics of these patients were included for statistical analysis. The results showed that the median follow-up duration did not differ between patients with stable renal function and those with renal function decline [40 months [IQR 25–129 months] vs. 93.5 months [IQR 83–110 months]; $P = 0.26$]. Patients with nephrotic range proteinuria had a significantly higher probability of renal function decline than non-nephrotic range proteinuria patients [8 of 14 patients [57.1%] compared to 0 of 5 patients [0.0%]; $P = 0.022$]. Pathologically, patients with focal glomerular sclerosis also had a trend toward higher risk of renal function decline than non-focal glomerular sclerosis patients [4 of 5 patients [80.0%] compared to 4 of 14 patients [28.5%]; $P = 0.028$] (**Figure 1**).

DISCUSSION

In this study, we described the clinicopathologic features of FNG patients in a single center. To the best of our knowledge, this is the first study to have demonstrated the factors associated with poor renal outcomes in FNG patients.

Most FNG patients clinically present with mild proteinuria, microscopic haematuria and hypertension. In the 76 cases reported by Takii et al. (8), proteinuria was the most common presenting symptom in all cases, of which 35 out of 69 (50.7%) had nephrotic-range proteinuria. In our series, the proportion of nephrotic range proteinuria was 73.6%, suggesting that high-grade proteinuria is a common occurrence in Chinese FNG patients. It is generally accepted that high-grade proteinuria is positively correlated with increased risk of ESRD (10, 11). Indeed, nephrotic range proteinuria is also indicated as a poor renal prognostic factor in FNG patients in our series.

Also, 6 (31.5%) patients in our study presented with abnormal serum creatinine (> 1.24 mg/dl) and 4 (21.0%) with decreased renal function (GFR < 60 ml/min) in our study. Among the 76 cases reported by Takii et al. (8) Scr or renal function was

TABLE 3 | Renal outcomes, treatment, and follow-up findings.

Patients	F/U serum creatinine (mg/dl)	Dialysis at last FU	F/U proteinuria (g/24 h)	Duration of F/U in months	Duration of renal biopsy to dialysis in months	Treatment
1	1.06	No	1.26	25		Prednisone/MMF/ARB
2	ND	Yes	ND	71	71	TWHF/ARB
3	1.53	No	0.68	40		TWHF/ARB
4	1.01	No	10.9	40		ARB
5	0.61	No	0.1	34		ARB
6	0.59	No	4.11	25		Prednisone/Tacrolimus/ARB
7	ND	Yes	ND	113	28	TWHF/ARB
8	ND	Yes	ND	95	90	TWHF/ARB
9	ND	Yes	ND	87	39	ARB
10	0.7	No	0.8	140		TWHF/ARB
11	ND	Yes	ND	92	92	Prednisone/TWHF/ARB
12	1.58	No	1.19	140		ARB
13	1.19	No	6.81	147		TWHF/ARB
14	0.85	No	2.31	118		Prednisone/Tacrolimus
15	ND	Yes	ND	109	109	TWHF/ARB
16	1.49	No	3.68	14		TWHF/ARB
17	ND	Yes	ND	116	20	TWHF/ARB
18	2.48	No	10.9	53		TWHF/ARB
19	0.57	No	2.77	24		Prednisone/MMF

F/U, follow-up; MMF, mycophenolate mofetil; TW, Tripterygium wilfordii Hook F; ARB, angiotensin receptor blockers; ND, not done.

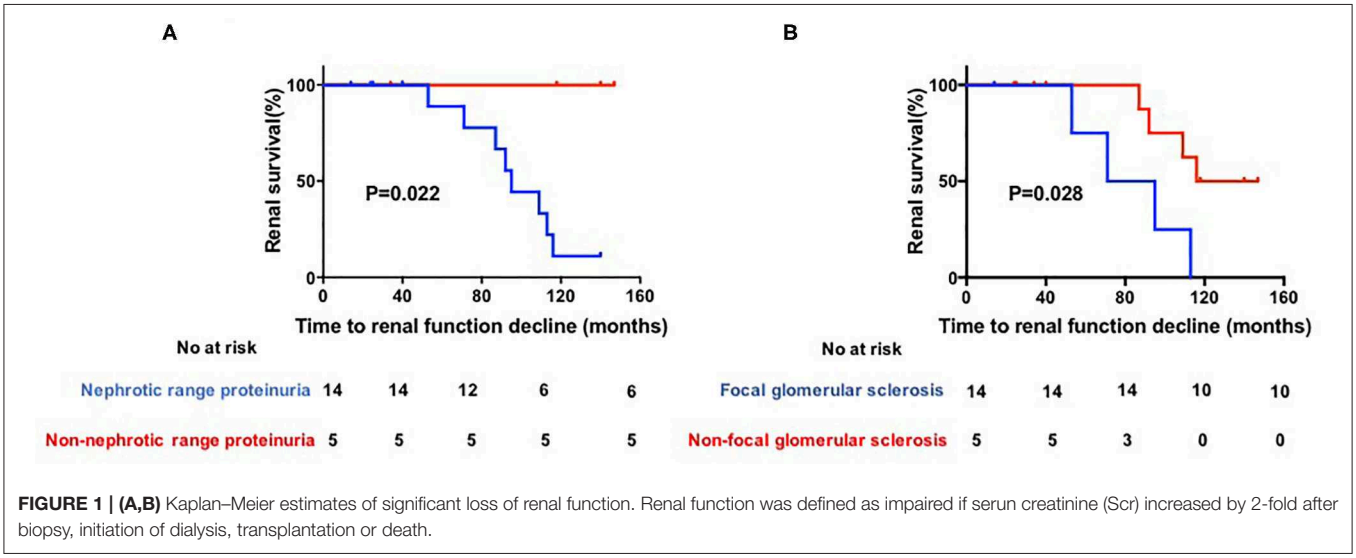

FIGURE 1 | (A,B) Kaplan–Meier estimates of significant loss of renal function. Renal function was defined as impaired if serun creatinine (Scr) increased by 2-fold after biopsy, initiation of dialysis, transplantation or death.

recorded in 58 patients. Abnormal Scr (> 1.24 mg/dl) or renal function (GFR < 60 ml/min) was detected in 20 patients (34.4%), two of whom presented with ESRD at the time of diagnosis. The rate of renal function decline is similar to that exhibited in our study.

Typically, FNG is regarded as a slowly progressive glomerular disease into ESRD over the period of 15–20 years (2, 10). Most FNG patients progressed into ESRD in the 2nd to 6th decade of life. However, the period of progressing into ESRD varies based on previous studies. Strom et al. (1) reported that the mean time of 4 FNG patients progressing into dialysis was 93 months, while Gemperle et al. (12) reported 5 patients who needed dialysis with 160-month follow-up (1). In our series, the mean time of progression into ESRD or dialysis is 71 months, which may

mean that the rate of disease progression in FNG used to be underestimated previously (13).

A genetic test should be performed when FNG is suspected pathologically (6, 14). In 2008, Castelletti et al. (5) firstly confirmed mutations at the FN1 gene locus at 2q32. They identified three heterozygous missense mutations Y973C, W1925R, and L1974R. Subsequently, Ohtsubo et al. (4) identified six FN1 mutations including five novel FN1 mutations. In our study, genetic test results were only available in 2 patients, including heterozygous missense mutation L1974P in one patient, and the mutation site in this patient is consistent with that reported by Castelletti et al. (5). However, none of these previously identified FN1 mutations was found in the other patient.

Deposition of immunoglobulins or complement components in the glomeruli is a commonly observed phenomenon in immunofluorescent study. Yoshino et al. (15) reported detection of immunoglobulin, fibrinogen, or complement components in 15 of their 43 cases (35%) (16). The link between immunoglobulin or complement component deposition and prognosis has not been clearly defined. In our study, we observed co-deposition of fibronectins with immunoglobulin or C3 in the mesangium in 12 (63.1%) of the 19 cases. Whether immunoglobulin or C3 deposition is non-specific or represents immune complex-mediated glomerulonephritis needs further investigation. Focal glomerular sclerosis as a typical histological change was rarely mentioned in FNG in previous studies. Indeed, focal glomerular sclerosis was only detected in five patients in our series and found to be associated with poor renal survival. Surprisingly, tubulointerstitial fibrosis or vascular changes was not found to be significantly correlated with the renal outcome.

Our study has several limitations. First, the population of enrolled FNG patients is relatively small and therefore the implications and outcomes should be interpreted with caution. Second, although our study provided a mean 10-years follow-up observation, loss to follow-up still occurred in some cases. Finally, as a result of the retrospective nature study, we were unable to evaluate the effect of treatment on outcomes. Because treatment of FNG is always individualized, it is therefore difficult to accurately evaluate the influence of treatment on renal outcomes.

In conclusion, the disease progression of FNG may be underestimated, which should not be ignored in some progressive renal diseases. In our series, nephrotic range proteinuria and focal glomerular sclerosis were found to be associated with renal function decline in FNG patients during follow-up. It will help clinicians to identify patients at risk of renal progression in clinical practice. Further studies regarding to underlying mechanism of FNG and finding effective treatments are necessary.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

TZ and ZC: research conception and study design and research and data analysis. TZ and WZ: manuscript preparation and clinical data collection. TZ and KZ: research sample preparation. All authors have read the journal's authorship agreement and agree with the statements. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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A Novel Clinical-Radiomics Model Pre-operatively Predicted the Stone-Free Rate of Flexible Ureteroscopy Strategy in Kidney Stone Patients

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Purpose: The purpose of the study is to develop and validate a novel clinical–radiomics nomogram model for pre-operatively predicting the stone-free rate of flexible ureteroscopy (fURS) in kidney stone patients.

Patients and Methods: Altogether, 2,129 fURS cases with kidney stones were retrospectively analyzed, and 264 patients with a solitary kidney stone were included in a further study. For lower calyx calculi, a radiomics model was generated in a primary cohort of 99 patients who underwent non-contrast-enhanced computed tomography (NCCT). Radiomics feature selection and signature building were conducted by using the least absolute shrinkage and selection operator (LASSO) method. Multivariate logistic regression analysis was employed to build a model incorporating radiomics and potential clinical factors. Model performance was evaluated by its discrimination, calibration, and clinical utility. The model was internally validated in 43 patients.

Results: The overall success rate of fURS was 72%, while the stone-free rate (SFR) for lower calyx calculi and non-lower calyx calculi was 56.3 and 90.16%, respectively. On multivariate logistic regression analysis of the primary cohort, independent predictors for SFR were radiomics signature, stone volume, operator experience, and hydronephrosis level, which were all selected into the nomogram. The area under the curve (AUC) of clinical–radiomics model was 0.949 and 0.947 in the primary and validation cohorts, respectively. Moreover, the calibration curve showed a satisfactory predictive accuracy, and the decision curve analysis indicated that the nomogram has superior clinical application value.

Conclusion: In this novel clinical–radiomics model, the radiomics scores, stone volume, hydronephrosis level, and operator experience were crucial for the flexible ureteroscopy strategy.

Keywords: clinical-radiomics model, flexible ureteroscopy, kidney stone, computed tomography, lithotripsy

INTRODUCTION

As a common urological disease, the prevalence rates for kidney stones vary from 1 to 20%. Especially in western developed countries such as the USA, kidney stone prevalence is notably high (>10%) (1). Related treatment costs are estimated to be several billion dollars per year in such countries (2). With the continuous development of the flexible ureteroscope and auxiliary equipment, flexible ureteroscopy (fURS) is considered to be one of the first-line treatments for the active removal of renal stones smaller than 2 cm (3). Furthermore, recent studies have shown fURS to be less dangerous for severe complications than percutaneous nephrolithotomy (PCNL) and also has low possibility for retreatment compared with shock wave lithotripsy (SWL) (4, 5).

However, the complete stone-free rate (SFR) for fURS is relatively low compared with PCNL (6). Numerous factors such as stone characteristics, stone location, renal anatomy, and hydronephrosis level may affect the success rate of fURS. Among these factors, the characteristics of the stone are very crucial. Several articles documented that the composition or Hounsfield units (HU) of the stone have a great impact on the efficacy of fURS (7, 8). However, HU based on computed tomography (CT) only represents the average value of the stone and thus cannot reflect the intracalculi structure, which is eminently related to the SFR. In addition, our previous research discovered that CT texture analysis (CTTA) of urinary tract stones may better predict the SFR on ESWL patients (9). Apparently, the limitations of this previous study included solitary enrollment of simple first-order parameters, and failure to combine clinical factors and lack multivariate analysis.

Recent advances in computer-assisted imaging techniques have enabled the high-throughput extraction of quantitative features from digital medical images. Actually, this new methodology, named radiomics, has been proven to be capable of influencing and altering the diagnosis and treatment strategies in the field of tumors (10, 11). Moreover, several investigations showed that predictive model based on radiomics or machine learning can better predict the post-operative outcome of certain surgical treatments (PCNL or SWL) (12, 13). It is important to develop a novel predictive model for fURS that combines radiomics features and clinical indicators, particularly for the lower renal stones.

Accordingly, the objective of this study was to develop and validate a novel clinical–radiomics nomogram model for pre-operative assessment and prediction SFR of fURS.

MATERIALS AND METHODS

Patients

Ethical approval was obtained for this retrospective analysis, and the requirement for informed consent was waived. In the present study, we retrospectively enrolled 2,129 fURS for renal stone removal performed between December 2014 and March 2019 (**Supplementary Figure 1** shows flowchart of patient selection). In total, 264 patients with solitary kidney stone met the inclusion criterion, including 122 cases of non-lower calyx calculi and 142 cases of lower calyx calculi. Lower calyx calculi treated with fURS were included in further research and randomly divided into two independent cohorts: primary cohort and validation cohort with a ratio of 7:3 based on the 10-fold cross-validation principle.

Meanwhile, patients' pertinent clinical data and stone characteristics were extracted pre-operatively, including age, sex, hydronephrosis level, the burden of calculi, pre-operative catheterization, the experience of the surgeon, etc. The follow-up procedure was conducted on the basis of the results of the CT scan or X-ray of kidney–ureter–bladder (KUB) review 3 months after operation. The standard of stone-free status was defined as free from stones or residual stone fragments <2 mm (14).

Surgical Techniques

Rigid ureteroscopy was routinely used for ureteral dilation before fURS. Thereafter, a 0.035-mm straight guidewire was placed through the ureteric orifice to the renal pelvis under direct rigid ureteroscope vision. Then, we placed a 14-F ureteric access sheath (Cook Medical, Bloomington, IN, USA) by a straight guidewire. A 7.5-F flexible ureteroscope (Flex-X2, Karl Storz, Germany) was passed through the ureteric access sheath to access the stone. Once the location of stones was confirmed, the Ho:YAG laser was used to fragment stones. After lithotripsy, 6-F double-J stent was routinely left in all cases for 2–4 weeks.

CT Image Acquisition, Region of Interest Segmentation, and Radiomics Feature Extraction

All included patients underwent NCCT using a 64-slice MDCT scanner (Discovery CT750 HD, GE Healthcare, USA). The related CT imaging acquisition parameters are as follows: tube voltage, 100–120 kV; automatic tube current, 200–350 mA; rotation time, 0.5 s; scan slice thickness, 5 mm; and reconstruction thickness, 1.25 mm.

Stone regions of interest (ROIs) were manually segmented on each transverse slice CT images, in the format of DICOM, using an open-source software 3D Slicer (version 4.9.0; www.slicer.org). Then, the following features were documented: (a) stone size, defined as maximum diameter on images; (b) stone volume, the ROIs would be fused and become the volume of interest (VOI); (c) stone location, subclassified as lower and non-lower calyx calculi; and (d) the degree of hydronephrosis, defined as severe and non-severe.

Radiomics feature extraction was performed using in-house texture analysis software with algorithms implemented in Matlab 2015a (MathWorks, Natick, Mass). In our research, a total of 604 radiomics features, including first-order statistics, shape- and size-based features, textural features, and wavelet features, were generated from each original CT image. Specific details of feature algorithms are shown in Supplement 1 in **Supplementary Material**.

Radiomics Feature Selection and Signature Construction

Dimension reduction and signature building process were arranged by LASSO logistic regression algorithm (15). With

penalty parameter tuning conducted by 10-fold cross-validation, LASSO was performed to select robust and non-redundant features from the primary cohort. A radiomics signature was created by a linear combination of selected features weighted by their respective coefficients, and the relevant radiomics score (Rad-score) was calculated for each patient.

Development, Performance, and Validation of a Clinical–Radiomics Nomogram Model

A model that incorporated the radiomics signature and clinical factors for predicting stone-free (SF) status was built based on multivariate logistic regression analysis in the primary set. We initially excluded some variables from the multivariate model for multicolinearity using variance inflation factor (VIF).

To provide a visual tool for clinical decision-making, a clinical–radiomics nomogram was then generated based on multivariate logistic regression analysis of corresponding pre-operative factors. Model performance was typically measured in terms of discrimination and calibration. The area under the curve (AUC), calculated by receiver operating characteristic (ROC) curve, was used to quantify the discrimination performance of established models (16). Calibration curves were portrayed to evaluate the predictive accuracy of the clinical–radiomics nomogram, followed by the Hosmer–Lemeshow goodness-of-fit test (a significant test statistic means that the model does not calibrate perfectly) (17).

To evaluate its predictive accuracy, the performance of nomogram was tested in the validation cohort. The logistic regression formula formed in the primary cohort was applied to all patients of the validation cohort, with total points for each patient calculated. Finally, the ROC and calibration plot were generated based on the regression analysis.

Clinical Utility of the Clinical–Radiomics Nomogram Model

Finally, to determine the clinical value of the radiomics model that incorporates clinical consequences, the decision curve analysis (DCA) was conducted to demonstrate a quantification of the net benefits at different threshold probabilities (18).

Statistical Analysis

Statistical analysis was conducted with Statistical Package for Social Sciences (SPSS) software version 24.0 and R (version 3.4.4) with R packages listed in Supplement 2 in **Supplementary Material**. The normality of all continuous variables was evaluated by the Kolmogorov–Smirnov test. Univariate analysis (chi-square test for categorical variables and *t*-test or rank sum test for continuous variables) and multivariate statistical analysis (logistic regression) were performed to identify significant independent predictors. A two-sided $p < 0.05$ was described as significant.

TABLE 1 | Comparison of single kidney stone patient and stone characteristics according to SF at 3 months after fURS.

Variable	SF	Non-SF	*p*
Number of patients, *n*	190	74	
Age, mean ± SD, years	49.26 ± 12.00	49.12 ± 11.45	0.933[a]
Gender, *n* (%)			0.244
Male	111 (69.4%)	49 (30.6%)	
Female	79 (76.0%)	25 (24.0%)	
BMI, mean ± SD, kg m^{-2}	23.71 ± 3.35	23.53 ± 3.49	0.778[a]
Pre-operative stenting, *n* (%)			0.312
No	168 (73.0%)	62 (27.0%)	
Yes	22 (64.7%)	12 (35.3%)	
Pre-operative ESWL, *n* (%)			0.884
No	163 (71.8%)	64 (28.2%)	
Yes	27 (73.0%)	10 (27.0%)	
History of stone surgery, *n* (%)			0.097[b]
No	160 (74.1%)	56 (25.9%)	
fURS	15 (71.4%)	6 (28.6%)	
PCNL	7 (43.8%)	9 (56.3%)	
Ureterolithotomy	4 (80%)	1 (20%)	
Pyelolithotomy	4 (66.7%)	2 (33.3%)	
Hypertension, *n* (%)			0.677
No	147 (71.4%)	59 (28.6%)	
Yes	43 (74.1%)	15 (25.9%)	
Diabetes, *n* (%)			0.795
No	179 (71.6%)	71 (28.4%)	
Yes	11 (78.6%)	3 (21.4%)	
Stone laterality, *n* (%)			0.089
Left	96 (67.6%)	46 (32.4%)	
Right	94 (77.0%)	28 (23.0%)	
Stone location, *n* (%)			<0.001
Lower calyx	80 (56.3%)	62 (43.7%)	
Non-lower calyx	110 (90.2%)	12 (9.8%)	
Hydronephrosis			<0.001
No/mild	168 (87.5%)	24 (12.5%)	
Severe	22 (30.6%)	50 (69.4%)	
Experience of operator, *n* (%)			<0.001
fURS ≥ 100	115 (87.8%)	16 (12.2%)	
fURS < 100	75 (56.4%)	58 (43.6%)	
Stone diameter (cm), *n* (%)			0.067
≤1	106 (76.8%)	32 (23.2%)	
>1	84 (66.7%)	42 (33.3%)	
Stone volume (cm^3), *n* (%)			<0.001
≤1	134 (79.8%)	34 (20.2%)	
>1	56 (58.3%)	40 (41.7%)	

SD, standard deviation; fURS, flexible ureteroscope; SF, stone free.
[a] *t-test.*
[b] *Rank sum test.*
Others are chi-square test.

RESULTS

Clinical Characteristics

Baseline characteristics of all patients are shown in **Table 1**. A total of 264 patients, 160 (60.6%) men and 104 (39.4%) women, were enrolled in this study. The overall rate of SF was about 72%. Significant difference was revealed between the SF and non-SF groups in the following indicators: stone location ($p < 0.001$),

TABLE 2 | Influencing factors on the success rate after fURS between lower calyx stone and non-lower calyx stone patients.

	Lower calyx			Non-lower calyx		
	SF	**Non-SF**	***p***	**SF**	**Non-SF**	***p***
Number of patients, *n*	80	62		110	12	
Age, mean ± SD, years	50.15 ± 11.42	48.85 ± 12.01	0.513[a]	48.61 ± 12.42	50.50 ± 8.22	0.608[a]
Gender, *n* (%)			0.682			0.004
Male	45 (54.9%)	37 (45.1%)		66 (84.6%)	12 (15.4%)	
Female	35 (58.3%)	25 (41.7%)		44 (100%)	0 (0%)	
BMI, mean ± SD, kg m^{-2}	23.97 ± 3.38	23.34 ± 3.49	0.367[a]	23.10 ± 2.99	24.84 ± 1.06	0.418[a]
Stone laterality, *n* (%)			0.151			0.952
Left	42 (51.2%)	40 (48.8%)		54 (90.0%)	6 (10.0%)	
Right	38 (63.3%)	22 (36.7%)		56 (90.3%)	6 (9.7%)	
Hydronephrosis			<0.001			0.008
No/mild	66 (79.5%)	17 (20.5%)		75 (96.2%)	3 (3.8%)	
Severe	14 (23.7%)	45 (76.3%)		35 (79.5%)	9 (20.5%)	
Experience of operator, *n* (%)			<0.001			0.003
fURS ≥ 100	57 (79.2%)	15 (20.8%)		58 (98.3%)	1 (1.7%)	
fURS < 100	23 (32.9%)	47 (67.1%)		52 (82.5%)	11 (17.5%)	
Stone diameter (cm), *n* (%)			<0.001			0.796
≤1	60 (68.2%)	28 (31.8%)		46 (92.0%)	4 (8.0%)	
>1	20 (37.0%)	34 (63.0%)		64 (88.9%)	8 (11.1%)	
Stone volume (cm^3), *n* (%)			<0.001			0.890
≤1	68 (65.4%)	36 (34.6%)		66 (89.2%)	8 (10.8%)	
>1	12 (31.6%)	26 (68.4%)		44 (91.7%)	4 (8.3%)	

SD, standard deviation; fURS, flexible ureteroscope; SF, stone free.
[a]*t-test.*
Others are chi-square test.

hydronephrosis ($p < 0.001$), operator experience ($p < 0.001$), and stone volume ($p < 0.001$).

However, in the subgroup analysis of stone location, the SFR for lower calyx calculi and non-lower calyx calculi was 56.3% (80/142) and 90.16% (110/122), respectively (**Table 2**). In the lower calyx group, stone diameter, hydronephrosis, experience of operator, and stone volume (all $p < 0.001$) were found to be the significant factors effecting fURS results, while SF status was significantly associated with hydronephrosis ($p = 0.008$) and experience of operator ($p = 0.003$) in the non-lower calyx group.

Subsequently, we further conducted studies on the lower calyx cases. Ninety-nine patients were included in primary groups to establish a predictive model, and 43 patients were enrolled in the validation groups to verify the accuracy and reliability of the generated model. Both two groups were further divided into SF and non-SF cases separately in accordance with the results of follow-up of each patient. Univariate analysis revealed the possible association in five factors (hydronephrosis level, operator experience, stone diameter, stone volume, and radiomics score) and the SFR, as presented in **Table 3**.

Feature Selection, Radiomics Signature Construction, and Validation

Using the 604 extracted radiomics features, LASSO analysis was performed, and 28 features with non-zero coefficients were screened based on the primary group (**Figure 1**).

A radiomics score calculation formula was then constructed by using corresponding coefficients of the chosen signature, presented in Supplement 3 in **Supplementary Material**. Distributions of the radiomics score and post-operative outcome for each patient in the primary and validation groups are shown in **Supplementary Figure 2**.

A significant difference in radiomics score was initially evidenced between SF and non-SF patients in the primary group ($p < 0.001$), and later confirmed in the validation group ($p < 0.001$), which can be noted in **Table 3**. The results show that the index based on radiomics analysis is significantly and positively correlated with post-operative SFR. Namely, patients with an SF outcome generally had higher Rad scores in the primary cohort.

Development, Performance, and Diagnostic Validation of Prediction Models

Results of multivariate regression analysis are shown in **Table 4**. The VIFs of three potential predictors ranged from 1.2 to 1.42, showing that there was no multicolinearity. A radiomics model that is composed of four different parameters (stone volume, operator experience, hydronephrosis level, and radiomics signature) was constructed and presented as a nomogram (**Figure 2**). Higher total point reflects corresponding case with higher probability for post-operative treatment success.

The observed AUC value for nomogram predictions was 0.949 (95% CI, 0.910–0.989). The established nomogram was then

TABLE 3 | Characteristics of lower calyx stone patients in the primary and validation cohorts.

	Primary cohort			Validation cohort		
	SF	**Non-SF**	***p***	**SF**	**Non-SF**	***p***
Number of patients, *n*	56	43		24	19	
Age, mean ± SD, years	50.34 ± 10.82	48.40 ± 12.31	0.406[a]	49.71 ± 12.94	49.89 ± 11.55	0.961[a]
Gender, *n* (%)			0.350			0.553
Male	34 (53.1%)	30 (46.9%)		11 (61.1%)	7 (38.9%)	
Female	22 (62.9%)	13 (37.1%)		13 (52.0%)	12 (48.0%)	
BMI, mean ± SD, kg m^{-2}	24.64 ± 3.75	23.15 ± 3.76	0.136[a]	23.71 ± 3.24	24.12 ± 3.20	0.754[a]
Stone laterality, *n* (%)			0.056			0.759
Left	27 (48.2%)	29 (51.8%)		15 (57.7%)	11 (42.3%)	
Right	29 (67.4%)	14 (32.6%)		9 (52.9%)	8 (47.1%)	
Hydronephrosis			<0.001			<0.001
No/mild	46 (78.0%)	13 (22.0%)		20 (83.3%)	4 (16.7%)	
Severe	10 (25.0%)	30 (75.0%)		4 (21.1%)	15 (78.9%)	
Experience of operator, *n* (%)			<0.001			0.001
fURS ≥ 100	40 (78.4%)	11 (21.6%)		17 (81.0%)	4 (19.0%)	
fURS < 100	16 (33.3%)	32 (66.7%)		7 (31.8%)	15 (68.2%)	
Stone diameter (cm), *n* (%)			0.007			0.012
≤1	42 (66.7%)	21 (33.3%)		18 (72.0%)	7 (28.0%)	
>1	14 (38.9%)	22 (61.1%)		6 (33.3%)	12 (66.7%)	
Stone volume (cm^3), *n* (%)			<0.001			0.002
≤1	49 (71.0%)	20 (29.0%)		19 (76.0%)	6 (24.0%)	
>1	7 (23.3%)	23 (76.7%)		5 (27.8%)	13 (72.2%)	
Radiomics score, median (interquartile range)	1.348 (0.467–2.243)	−0.462 (−1.429 to 0.260)	<0.001[b]	0.557 (−0.175 to 2.009)	−0.895 (−1.482 to −0.119)	<0.001[b]

SD, standard deviation; fURS, flexible ureteroscope; SF, stone free.
[a]t-test.
[b]rank sum test.
Others are chi-square test.

verified in a validation cohort, with AUC of 0.947 (95% CI, 0.883–1) supported the increased predictive efficacy (**Figure 3**). The calibration curve and the Hosmer–Lemeshow test ($p = 0.344$) demonstrated favorable calibration of the nomogram in the primary group (**Figure 4A**). In the validation cohort, the calibration curve showed that there was a good agreement between nomogram predicted probability of SF and actual SF rate (**Figure 4B**). Meanwhile, the Hosmer–Lemeshow test yielded a non-significant statistic ($p = 0.099$), which suggested that there was no departure from perfect fit. **Figure 5** showed a specific clinical case decision procedure utilizing the generated clinical–radiomics nomogram.

Clinical Use

The DCA for the clinical–radiomics nomogram is portrayed in **Figure 6**. The decision curve showed that if the threshold probability of a patient is above 0.1, using the predictive model to predict SFR provides a better net benefit than either the treat-all-patients scheme or the treat-none scheme.

DISCUSSION

In recent years, fURS has become a mainstream surgical treatment for upper urinary calculi with its advantages of non-invasiveness, safety, and having a short learning curve. The indications for fURS have gradually expanded, and some scholars have even tried to use it for the treatment of staghorn calculi. It is very likely to be an alternative to ESWL and PCNL and to become the preferred surgical procedure for upper urinary calculi (19–21). After the craze, some urologists began to reflect on whether this technique is so widely applicable. It is undeniable that some patients have unsatisfactory results after receiving fURS and even fail to touch the stones, causing patients to undergo ineffective surgery and need to be treated again, which brings huge safety risks and waste of medical resources (22). Therefore, it is particularly important to pre-operatively assess the effect of fURS surgery and accurately select patients who are suitable for fURS.

Stone characteristics have been considered as a significant factor determining the final efficacy because various stone characteristics can result in different times and extents of pulverization under the function of laser (7, 8, 23). Stones

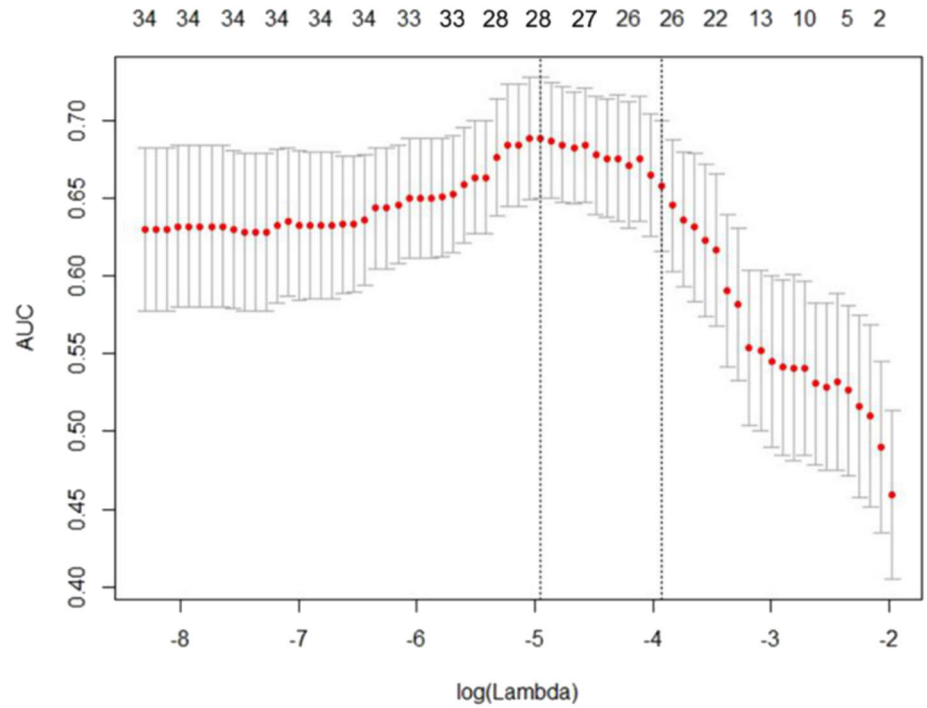

FIGURE 1 | Least absolute shrinkage and selection operator (LASSO) regression analysis uses the minimum standard and a 10-fold cross-validation method. The coefficients of the model are compressed by introducing a penalty adjustment parameter (λ) so that the coefficients of the irrelevant variables tend to be zero, and then, the automatic screening of the variables is realized.

TABLE 4 | Multivariate analysis of the influencing factors on the success rate after fURS in lower calyx patients.

Variables	B	SE	OR	95% CI	*p*
Male	−0.045	0.746	0.956	0.222–4.129	0.952
Left	−1.328	0.758	0.265	0.060–1.170	0.080
No/mild hydronephrosis	2.293	0.762	9.908	2.224–44.132	0.003
Stone volume ≤1 cm^3	2.121	0.945	8.337	1.309–53.106	0.025
fURS ≥ 100	2.413	0.854	11.169	2.095–59.550	0.005
Radiomics score	0.986	0.333	2.679	1.395–5.146	0.003

fURS, flexible ureteroscope.

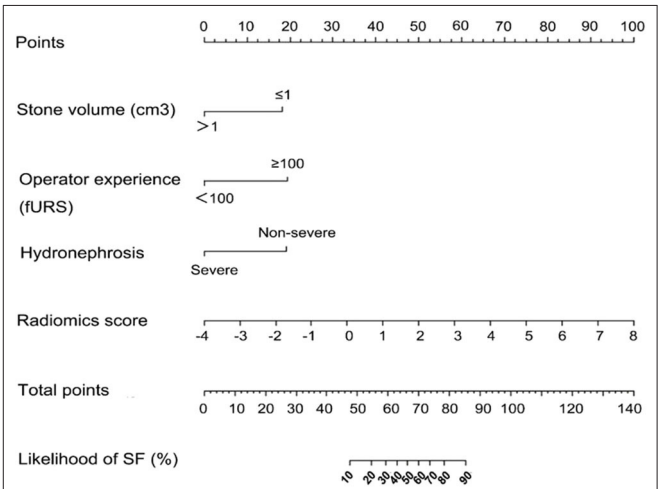

FIGURE 2 | Established clinical–radiomics nomogram model. The clinical–radiomics nomogram was generated in the primary cohort, with the radiomics signature, stone volume, operator experience, and hydronephrosis level incorporated.

with longer pulverization times are more likely to reposition during the process, which may lead to an unsuccessful outcome. However, comprehensively identifying stone characteristics prior to surgery still remains an intractable challenge. On the one hand, traditional stone composition analysis can only depend on post-operative or intraoperative *vitro* testing, which is not feasible for pre-operative assessment. On the other hand, simple measurement of Hounsfield unit or density is unilateral, since the situation of intracalculi is often uneven and complicated. This explains why many studies failed to include this crucial factor into the evaluation scoring system (24, 25).

In this study, a radiomics signature consisting of 28 robust features was identified to be an independent factor for the SFR of fURS in patients with the lower calyx calculi. This multifeature-based radiomics signature also successfully stratified patients into

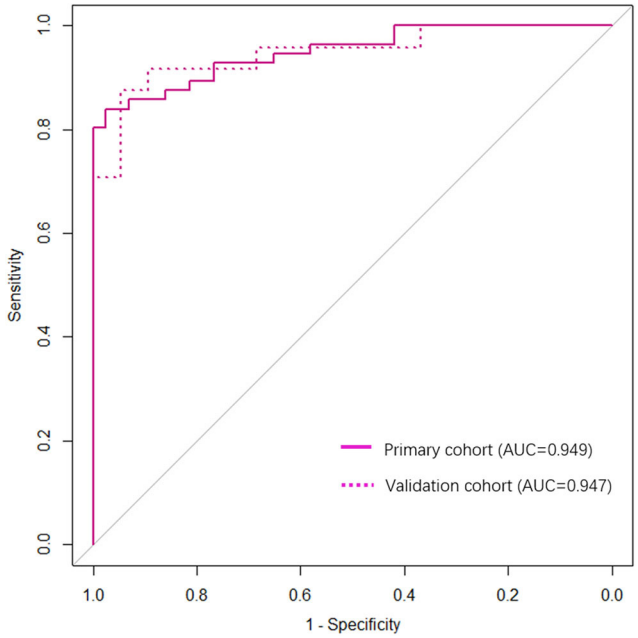

FIGURE 3 | Receiver operating characteristic (ROC) based on clinical–radiomics nomogram.

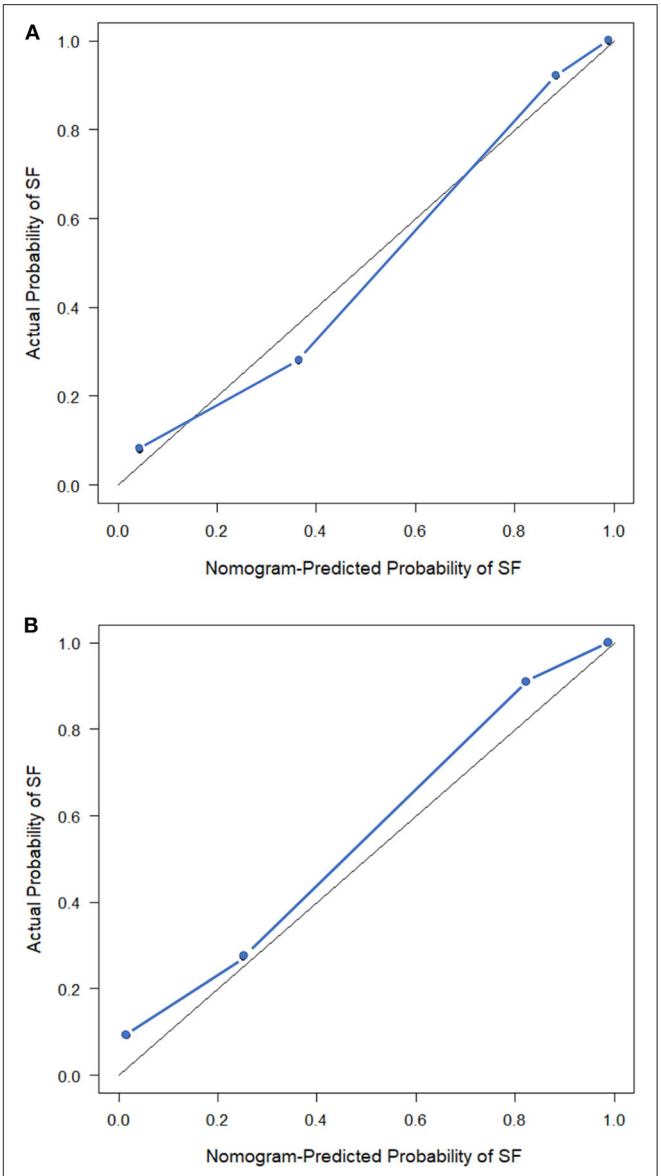

FIGURE 4 | Calibration curves of the clinical–radiomics nomogram. **(A)** Calibration curve of the clinical–radiomics nomogram in the primary cohort. **(B)** Calibration curve of the clinical–radiomics nomogram in the validation cohort. Calibration curves describe the calibration of the model with respect to the agreement between nomogram predicted probability of stone free (SF) and actual SF rate. The y-axis depicts actual SF rate. The x-axis depicts the nomogram predicted probability of SF. The diagonal solid line depicts an excellent prediction by a supreme model. The blue dotted line represents the performance of the nomogram.

successful and unsuccessful groups in the validation dataset. Our previous study on SWL also found that CTTA, a quantitative analysis method, may be useful in improving medical decision-making on ESWL patients (9). Similarly, several pieces of research showed that establishing a prediction model utilizing radiomics or machine learning may contribute to a better predictive efficacy for pre-operative estimation of PCNL or SWL outcomes (12, 13).

Obviously, the radiological features alone were not enough, and it has been considered that relying on a solitary strong risk indicator could fail to evaluate the comprehensive post-operative outcome of individual patients (26). Therefore, we generated a clinical–radiomics model, which is the combination of the radiomics signature and potential clinical indicators. The established clinical–radiomics nomogram demonstrated superior discrimination and calibration in both training and validation cohort, with an AUC of 0.949 and 0.947, respectively. Likewise, the decision curve analysis indicated that the clinical–radiomics nomogram was more beneficial than the treat-all scheme or the treat-none scheme across the majority of the scope of rational threshold probabilities.

Indeed, with respect to clinical predictors, there are some similarities between our study and Ito's original study (24). The two studies both agreed that the location, stone volume, hydronephrosis level, and surgeon's experience are significant predictors of post-operative SF status. Many articles have reported that the stone volume greatly affects the success rate of fURS (27), and our research also showed similar results. With the increase in stone burden, it would require a longer pulverization time, and the stone fragments are more likely to move. At the same time, it may increase the probability of intraoperative bleeding, which can lead to blurred vision, affecting normal surgical procedure and thus leading to stone residues after surgery. In addition, hydronephrosis causes enlargement of the renal pelvis and calyces, which makes breaking and basketing stones trickier, thus increasing the likelihood of stone residues after the procedure. Furthermore, Ito et al. reported on urologists

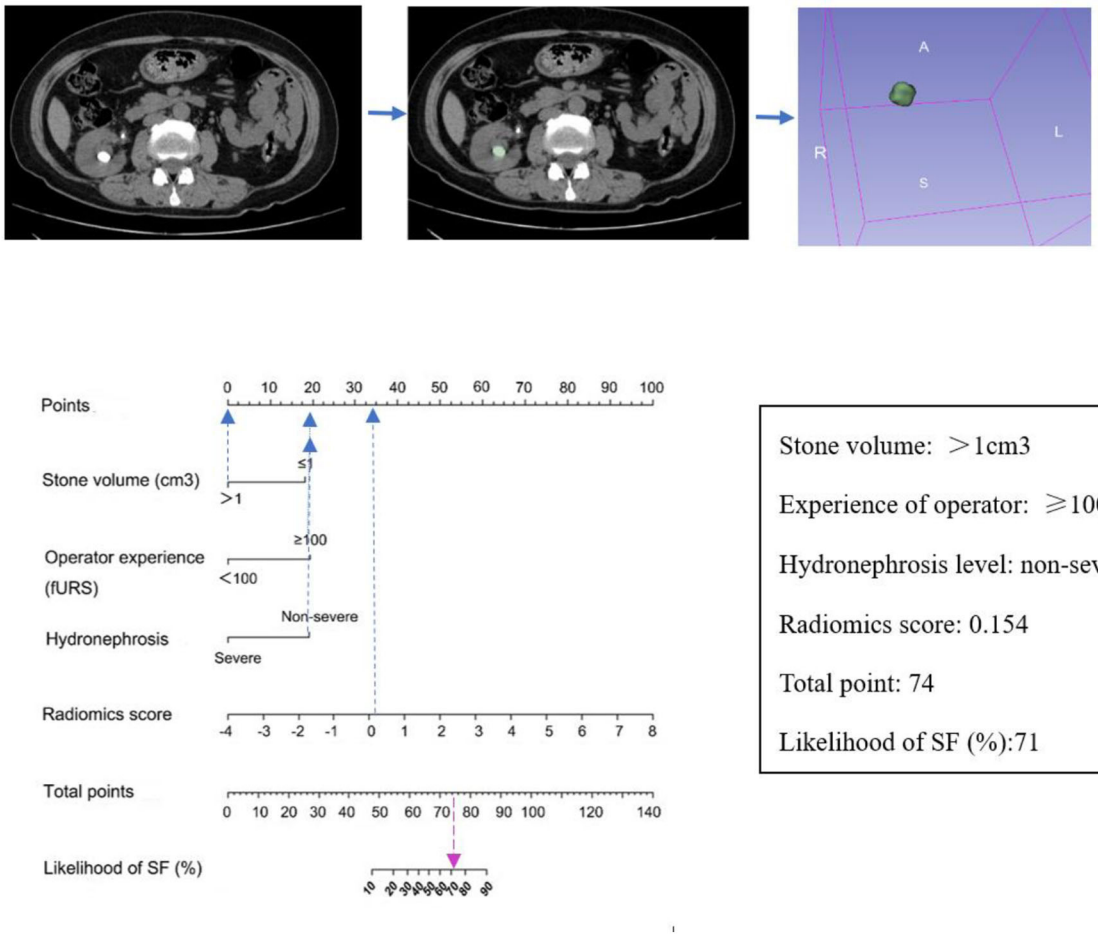

FIGURE 5 | An example of how to use the clinical–radiomics nomogram to predict SF status in a 58-year-old female patient with SF outcome after flexible ureteroscopy (fURS). Locate the patient's Rad-score on the Rad-score axis. Draw a line straight upward to the points' axis to determine how many points the patient receives for his or her Rad-score. Conduct a similar process for other indicators. Sum the points calculated for each of the risk factors and track down the added sum on the total points axis. Draw a line straight down to find the patient's likelihood of SF.

with experience of >100 fURS that were associated with a satisfactory post-operative outcome. Meanwhile, Cho et al. reported that 56 cases were required for reaching a plateau in the learning curve (28). Another study indicated that surgeon experience affects the outcomes of fURS mainly in terms of safety (29). Similarly, in our department, we found that operators with experience of more than 100 procedures can achieve more skilled surgical techniques and deal with complications more efficiently. Being proficient in using ureteroscopy and its ancillary equipment, they can reduce some dangerous complications, such as intraoperative bleeding, or even prevent them from happening.

Unlike prior prognostic investigations that mostly analyzed all kinds of patients regardless of stone location, our current study focused exclusively on patients with the lower calyx calculi. Among various treatments for single lower calyceal stone, Bozzini et al. (30) reported that fURS and PCNL were more effective than SWL to obtain a better SFR and a lower auxiliary and retreatment rate. fURS, compared with PCNL, offers the best outcome in terms of procedure length, radiation exposure, and hospital stay. De et al. (31) suggested that PCNL provides overall significantly higher stone-free rates than fURS, at the expense of higher complication rates, blood loss, and a longer length of stay. Nevertheless, fURS can provide higher stone-free rates compared with minimally invasive percutaneous procedures. In our study, the overall rates of SF of fURS process was about 72%, which is consistent with other studies (ranging between 65 and 92%) (32). However, in the subgroup analysis based on location, the SF rate for the lower calyx calculi only reached 56.3%, which was obviously lower than the figure for the non-lower calyx calculi (90.16%). It is also verified in another study that the stone treatment in the lower pole is less effective compared to elsewhere in the kidney (33). Compared to middle and upper pole stones, the fURS treatment of lower pole stones is more complicated because of the anatomical factors and the limited deflection angle of flexible ureteroscopes (34). Additionally, Tonyali et al. reported that patients with lower pole stones are 2.25 times more

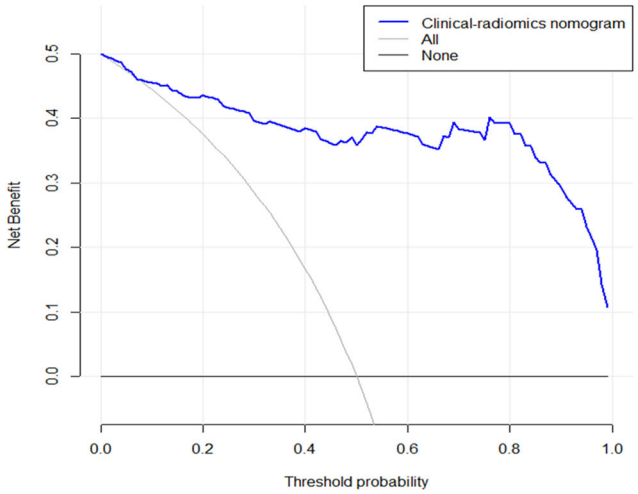

FIGURE 6 | The y-axis measures the net benefit. The blue line represents the clinical–radiomics nomogram. The gray line represents the assumption that all patients have SF outcome. The black line represents the presumption that no patients have SF status. The decision curve showed that if the threshold probability is above 0.1, then using an imaging colinearity chart to predict post-operative result is more beneficial for making clinical decisions. For example, if the personal threshold probability of a patient is 50% (i.e., the patient would opt for treatment if his probability of SF was 50%); then, the net benefit is 0.36 when using the clinical–radiomics nomogram to make the decision of whether to undergo treatment, with added benefit than the treat-all scheme or the treat-none scheme).

likely to have residual stones after fURS compared to patients having stones at other locations (35). Evidently, the spontaneous passing of stone fragments after the surgery was more difficult due to the position of the lower pole. Therefore, we mainly focused on investigating the factors affecting the success rate of the lower calyx calculi.

Nevertheless, in the studies focusing on lower calyceal stones, some researchers have looked at specific factors that can affect the SFR for fURS. Several studies used the Elbahnasy method to calculate the infundibular pelvic angle (IPA) and reported that IPA and other pelvicaliceal anatomy-correlated parameters associate with a lower SFR (36). However, Danuser et al. (37) reported that influence of the collecting system anatomy on disintegrate clearance from the lower calyx could not be demonstrated. The effect of the lower calyx anatomy on the stone-free rate of fURS is still controversial. In addition, controversies also exist on the measurement of pelvicaliceal anatomy. To measure the IPA, urologists need to rely on intravenous urography (IVU) or contrast-enhanced CT (CCT), which are dependent on the use of a contrast agent. First of all, patients with kidney stones generally do not perform these two tests. Moreover, patients with a contrast agent allergy or moderate to severe renal insufficiency are not suitable for these tests. Last but not least, the anatomy of the renal pelvis and calyx will expand with the perfusion of water during the operation, and the pre-operative measurements cannot play an accurate predictive role in the operation. Herein, the IPA was not analyzed in our present study because of the low feasibility in most of the patients.

The current study included several limitations. First, this was a retrospective study, which may cause possible selection bias. Second, the sampling included was relatively inadequate. Moreover, the model was established based on single-center data, and prospective multicenter studies are needed to further validate our results. Finally, due to practical constraints, this paper cannot provide a comprehensive review of multiple stones and lower calyx anatomy, which may be the aim of a future prospective study.

In conclusion, this study indicated that patients with higher radiomics score, smaller stone volume, non-severe hydronephrosis level, and with a more experienced operator were more likely to reach a successful outcome when choosing the flexible ureteroscopy strategy. This clinical–radiomics model may serve as an effective pre-operative prediction method for clinical decision-making for kidney stone patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SW and DH devised the experiment. CL and ZL developed and organized the paper. PL and ZZ designed the tables and figures. QX and HD performed the data analysis. IK and PT participated in the revision of the manuscript. MC and YX wrote the original draft. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.576925/full#supplementary-material

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Nephroprotection by SGLT2i in CKD Patients: May It Be Modulated by Low-Protein Plant-Based Diets?

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Sodium-glucose-transporter 2 inhibitors (SGLT2i) are a new class of anti-diabetic drugs that in large trials such as CREDENCE have shown also a reduction of glomerular hyperfiltration and albuminuria in type 2 diabetic patients. Hence, the interest toward SGLT2i is focused toward this potential nephroprotective effect, in order to reduce the progression to overt nephropathy, and it seems to be confirmed in the most recent DAPA-CKD trial. This is the reason why the indication for SGLT2i treatment has been extended to chronic kidney disease (CKD) patients with eGFR up to 30 ml/min, namely with CKD stage 1–3. In patients with CKD stage 3 to 5, the most recent KDIGO guidelines recommend low-protein diet and plant-based regimens to delay end-stage kidney disease (ESKD) and improve quality of life. Similarly to SGLT2i, low-protein diets exert renal-protective effects by reducing single nephron hyperfiltration and urinary protein excretion. Beyond the glomerular hemodynamic effects, both protein restriction and SGLT2i are able to restore autophagy and, through these mechanisms, they may exert protective effects on diabetic kidney disease. In this perspective, it is likely that diet may modulate the effect of SGLT2i in CKD patients. Unfortunately, no data are available on the outcomes of the association of SGLT2i and low-protein and/or vegan diets. It is therefore reasonable to investigate whether CKD patients receiving SGLT2i may have further advantages in terms of nephroprotection from the implementation of a low-protein and/or plant-based diet or whether this association does not result in an additive effect, especially in vascular nephropathies.

Keywords: CKD-chronic kidney disease, SGLT2 inhibitors, diabetic nephropathy, diet, low protein diet, plant based diet

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INTRODUCTION

Sodium-glucose-transporter 2 inhibitors (SGTL2i) are a new class of anti-diabetic drugs able to improve glycemic control. While this primary effect is undoubtedly interesting, the major point of interest in these drugs is related to their cardiovascular and renal protective properties. SGLT2i have been shown to reduce several cardiovascular endpoints in high-comorbidity patients with type 2 diabetes (1, 2). Of note, the CREDENCE trial was pre-maturely terminated at the ad interim analysis because of the evident benefits toward the primary composite endpoint of doubling of serum creatinine and renal or cardiovascular death, in treated patients (3).

Randomized controlled trials such as EMPAREG, CANVAS and DECLARE-TIMI, have shown that different SGLT2i (empagliflozin in EMPAREG, canagliflozin in CANVAS and dapagliflozin in DECLARE-TIMI) reduced glomerular hyperfiltration and albuminuria in type 2 diabetic patients (1, 3, 4). As a consequence, the interest toward SGLT2i shifted from their role in diabetes control to their potential to reduce the incidence of overt nephropathy (5). The randomized controlled trial CREDENCE was specifically designed to assess renal survival in a large cohort of type 2 diabetic patients with chronic kidney disease (CKD) and confirmed the favorable effect of canagliflozin on the risk of kidney failure (2). In fact, proteinuric patients with CKD stage 2 and 3 treated with canagliflozin showed a 30% decreased risk of reaching the composite end-point of end-stage kidney disease, doubling of serum creatinine levels or death for renal or cardiac causes over a media follow-up of 2.62 years. Moreover, the benefits of canagliflozin seemed to be greater in patients with worst kidney function and more severe proteinuria (2). It has also been reported that Dapagliflozin was able to improve endothelial function and arterial stiffness as well as renal resistive index, likely mediated by oxidative stress reduction in type 2 diabetics (6, 7).

The nephroprotective effect of SGLT2i has been mainly attributed to the modulation of glomerular hemodynamics, as we will discuss in detail in the next paragraphs.

The intrarenal effects of SGLT2 inhibitors aroused great interest which was initially limited to diabetic patients with increased, normal or only slightly reduced kidney function. More recently, accumulating evidences suggested testing these molecules in more advanced stages of CKD in the course of type 2 diabetes mellitus. The extraordinary results recorded in diabetic patients, independently of glycemic control, suggest today a role for SGLT2i even in non-diabetic nephropathies.

Recent data of the DAPA-CKD trial that included CKD patients with and without diabetes, suggested a significant protective effect of dapagliflozin on CKD outcomes (8). This beneficial effect seemed to be even greater in non-diabetic patients. Again, the DAPA-CKD study was terminated earlier based on the suggestion on an independent committee because of the superior efficacy compared to the placebo. A sub-analysis of the DAPA-HF study showed that, in patients with reduced ejection fraction hearth failure, dapagliflozin was able to reduce the progression of kidney disease in both diabetic and non-diabetic patients over a 2 year follow-up (9). Finally, the DIAMOND study showed no benefits on proteinuria in 53 non-diabetic CKD patients with a mean GFR of 58 ml/min, over a 6 week therapy with a cross-over design (10).

It appears that dapagliflozin, like other SGLT2i, can be useful also for non-diabetic patients, at least up to CKD stage 3. These data progressively extended canagliflozin prescription indications and it can now be prescribed to patients with a moderate reduction of renal function with an estimated glomerular filtration rate (eGFR) up to 30 ml/min/1.73 m^2; furthermore, once started at an eGFR $>$ 30 ml/min/1.73 m^2, the treatment can be continued even in the presence of lower eGFR values, namely CKD stage 4.

SGTL2i are not the only drugs able to modify glomerular hemodynamics: non-steroidal anti-inflammatory drugs increase the resistance of the afferent arteriole whereas RAAS inhibitors reduce resistance of efferent arteriole (11) (**Figure 1**). In addition, calcium channel blockers induce vasodilation of both the afferent and efferent arteriole, which is greater at the former than at the latter site (12) (**Figure 1**).

Besides drugs, significant changes of renal hemodynamics are also modulated by the amount and quality of protein intake (**Figure 1**).

Indeed, the role of diet in managing CKD has recently been sharply underlined by the new KDOQI guidelines on nutrition in kidney disease which extended the indication to prescribe a protein-restricted diet to delay ESKD in CKD patients since stage 3 (graded evidence level 1A) (13).

The combination between these two approaches is therefore appealing, but unfortunately, no data exist about the effects of SGLT2i in patients on low-protein diets vs. patients on unrestricted protein intake. Furthermore, we do not know if the effect of SGLT2i is modulated by salt intake, raising concerns about possible interferences or synergistic effects on renal outcomes.

The aim of this perspective paper is to review the similarities and differences of renal protection in the course of SGLT2i treatment or low-protein diet regimens and hypothesize their interferences, as a mean to call for studies on these emerging issue in the prevention and care of CKD.

EFFECTS OF SGLT2i ON RENAL HEMODYNAMICS AND KIDNEY FUNCTION

Tubuloglomerular Feed Back

Sodium glucose transporter 2 inhibitors act inhibiting the renal proximal tubule reabsorption of glucose and sodium (14). Murine models have shown that SGLT2 inhibitors are able to modulate renal hemodynamics, leading to reduction of glomerular hyperfiltration and urine albumin excretion. In fact, SGLT2 inhibitors reduce sodium reabsorption in the proximal tubule causing increased sodium delivery to the macula densa which, in turn, stimulates the tubuloglomerular feedback (TGF). As a consequence, vasoconstriction of the afferent glomerular arteriole occurs and causes a decrease in intraglomerular pressure and renal blood flow (15). This hypothesis was initially postulated by Cherney (16) in a cohort of young adults with Type 1 diabetes who underwent empagliflozin therapy for 8 weeks and was later confirmed in a murine model using multi-photon microscopy (17).

Other Hemodynamic Effects

Despite the prevailing view that SGLT2i are able to restore pre-glomerular arteriolar resistance, Van Bommel et al. (18) observed that dapagliflozin acted lowering efferent arteriolar resistance in type 2 diabetes. In this context, a randomized, double-blind trial compared hemodynamic effects of gliclazide vs. dapagliflozin over 3 months in type 2 diabetic patients with eGFR $>$ 60 ml/min/1.73 m^2 and overt proteinuria. Dapagliflozin reduced

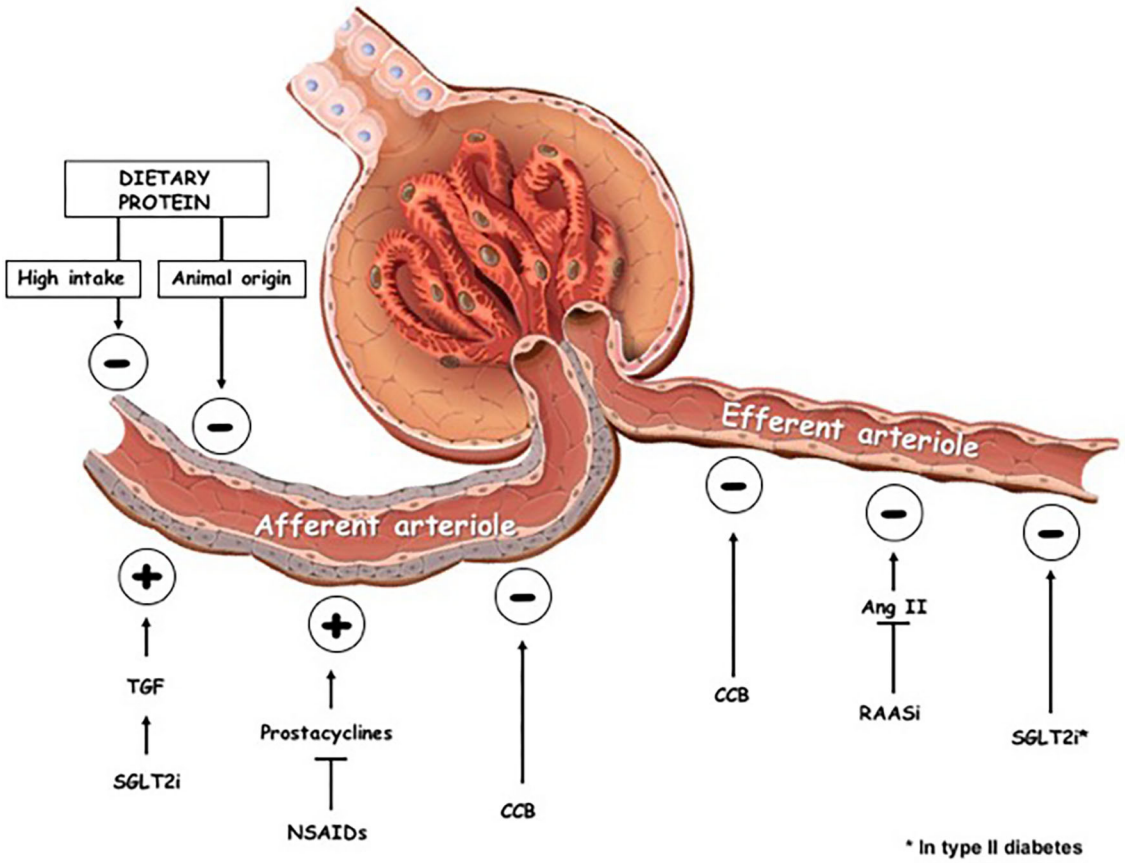

FIGURE 1 | Effects on glomerular hemodynamics of dietary changes and pharmacologic interventions. TGF, tubuloglomerular feedback; SGLT2i, sodium-glucose-transporter 2 inhibitors; NSAIDs, non-steroidal anti-inflammatory drugs; CCB, calcium channel blockers; RAASi, renin-angiotensin-aldosteron system inhibitors; ⊕, vascular tone increase; ⊖, vascular tone decrease.

GFR, filtration fraction and kidney vascular resistances through vasodilation of the efferent arteriole.

An increase in prostaglandin production has been reported in patients treated with dapagliflozin; prostaglandin release may cause post-glomerular arteriolar dilation, preventing TGF-mediated pre-glomerular vasoconstriction (19). This pattern resembles what happens reducing the protein intake (20) and, probably, sodium intake (21).

Modulation of the Effect on Renal Hemodynamics by Type of Diabetes

There is no clear explanation regarding the discrepancy about the renal effects of SGLT2i in Type 1 or Type 2 diabetic patients but the possible causes are many. In hyperfiltrating, young type 1 diabetic patients, pre-glomerular resistance is low and the tubuloglomerular feedback, activated by empagliflozin, may cause constriction of the afferent artery via adenosine release (22), thus reducing intra glomerular pressure and glomerular filtration rate. Conversely, in older type 2 diabetic patients, glomerular hyperfiltration is less prominent and the potential for pre-glomerular vasoconstriction is limited due to arterial stiffness.

Even if renin-angiotensin-aldosterone system (RAAS) blockers should be included early in the treatment of all diabetic patients, especially at the appearance of proteinuria, it is possible that patients with type 2 diabetes are more often treated by these drugs at higher doses, for their anti-hypertensive effect (18).

Effects on GFR and Albuminuria

From a clinical point of view, several studies describe an initial fall of eGFR (3, 4), followed by a raise and subsequent stabilization in the long term: this eGFR pattern has been called the "check-mark" sign (√) (23).

The drop in eGFR is probably one of the main mechanisms of nephroprotection and is rapidly reversible after discontinuation of therapy (15). Notably, this reduction of GFR following SGLT2i therapy is also observed in diabetic individuals without hyperfiltration and in patients with non-diabetic glomerular disease (24, 25), suggesting that it is not just due to an improved glycemic control.

In the long-term, treatment with SGLT2i is associated with a reduction of albuminuria and a slower decline of GFR when compared to placebo (26). These endpoints were achieved

independently of changes of blood pressure or glucose control; indeed, the reduction of albuminuria with SGLT2i was associated to the reduction of intraglomerular pressure (15, 27).

Notably, the composite endpoint of retarding progression of kidney disease, expressed as doubling of serum creatinine or development of overt albuminuria, was also reached in a study conducted in with overt diabetic kidney disease (15).

Autophagy

Recently, restoration of autophagy, a cell defense mechanism, was suggested as a contributor to the renoprotective effects of SGLT2i (28). Autophagy is a self-degradative process that occurs to save energy sources in conditions of nutrient deprivation. Autophagy allows disposing misfolded or aggregated proteins and damaged organelles, such as mitochondria, endoplasmic reticulum and peroxisomes, by pro-inflammatory or pro-oxidative stress.

An impairment of the kidney's autophagic capacity in conditions of increased oxidative stress has been shown to contribute to renal injury in type 2 diabetes (29, 30). The production of proinflammatory cytokines stimulated by cellular stress leads to cellular dysfunction and loss, and ultimately to inflammation and fibrosis (31, 32).

In the course of diabetes, autophagy dysregulation is attributed to an impaired nutrient deprivation signaling (SIRT1/AMPK) in podocytes and tubular cells (33–35). Moreover, diabetes may also impair the adaptive actions of hypoxia-inducible factors (36, 37).

SGLT2i appear to enhance autophagic capacity of kidney tissue and mitigate oxidative stress, through induction of hypoxia-like transcriptional patterns that enhance SIRT1/HIF-2a signaling (38). These molecular effects are peculiar of SGLT2i and are distinct from other glucose-regulating agents (28).

EFFECTS OF DIETARY MANIPULATION ON RENAL HEMODYNAMICS AND KIDNEY FUNCTION

Hemodynamic Effects of Protein Restriction

Glomerular filtration is influenced by dietary protein intake in health and disease. As underlined by the recent KDGO guidelines, low-protein diets are a mean to reduce the progression of chronic nephropathies, in addition to the metabolic control of urea and phosphate levels and acidosis.

The effects of a low-protein diet have indeed many similarities with those of SGLT2i.

In animal models of CKD induced by subtotal nephrectomy, a low-protein diet is able to reduce hyperfiltration and hypertrophy in the remaining nephrons (39).

The reasons of this effect are manifolds: an increased glomerular filtration of amino acids and the consequent increased proximal tubular reabsorption increases sodium reabsorption in the proximal tubule and a decreased sodium delivery to the distal nephron. This, in turn, inhibits the tubuloglomerular feedback, lowering the resistance of the afferent arteriole, thus allowing glomerular hyperfiltration.

In CKD patients, a fall of GFR is observed in the first weeks of protein restriction; afterwards, however, kidney function stabilizes and the disease tends to progress slower than in patients on an unrestricted diet. This observation, derived from the classic MDRD trial, is consistent with an initial reduction of hyperfiltration followed by a slower CKD progression and is comparable to the "check-mark" sign ($\surd$) described in the course of treatment with SGLT2i, as mentioned above (23).

The quality of dietary proteins modulates glomerular hemodynamics and the perm-selectivity of the glomerular basement membrane. An acute load of animal proteins determines an increase not only in GFR and plasma renal flow, but also in the permeability of the glomerular basement membrane to albumin, in the absence of modification of the filtration fraction (39). This suggests a decrease in pre-glomerular vascular resistances. Conversely, an acute load of vegetable proteins (soy) is not associated with the aforementioned changes (40, 41). Moreover, subjects undergoing a plant-based diet, both in experimental as well as observational studies in vegan communities, usually display a lower GFR compared to subjects on a diet containing the same amount of mixed or animal-derived proteins (42).

Effect of the Quality of Dietary Proteins

It has been hypothesized that the effect of plant-based diets is mediated by the lower serum concentrations of IGF-1 in vegans-vegetarians compared to omnivorous.

Recently, it has been reported that an omnivorous diet is associated with a higher GFR compared to a vegan diet, while a lacto-ovo-vegetarian diet was associated to an intermediate GFR value (43). In other words, the glomerular "reserve," associated with the vasodilation capacity of the afferent arteriole, is not elicited by vegetable proteins, a mechanism probably, at least in part, mediated by phytoestrogens. Indeed, serum concentrations of isoflavones increase during a soy-based diet and inversely correlate with urinary albumin to creatinine ratio

TABLE 1 | Beneficial effects of sodium-glucose-transporter 2 (SGLT2) inhibitors and low-protein/plant-based diets.

Effect	SGLT2 inhibitors	Low-protein/ plant-based diet
Decreased sodium proximal tubular reabsorption	+ (14)	+ (39)
Restoration of the tubuloglomerular feedback	+ (15–17)	+ (39)
Reduction of glomerular hyperfiltration	+ (15)	+ (39)
Restoration of autophagy	+ (28, 38)	+ (58)
Slowing of CKD progression	+ (15, 26)	+ (41)
Reduction of unfavorable cardiovascular outcomes	+ (1, 2, 4)	+ (47–49)
Reduction of proteinuria	+ (26)	+ (44, 46)
Reduction of the acid load	-	+ (62)
Reduction of phosphate load	-	+ (63)

Present: +. Absent: - (reference).

(44). In addition, soy proteins, as well as other plant derived proteins, may have anti-inflammatory properties, modulating interleukin 6, tumor necrosis factor alpha and nuclear factor-kB, displaying also an antioxidant activity at the renal level (45). In animal models, a soy-based diet may also reduce RAAS activity, decreasing resistance at the afferent arteriole and decreasing proteinuria (46). Finally, plant-based regimens have been shown to reduce cardiovascular risk (47–49). These data supported a plant-based diet for nephroprotection in healthy individuals as well as in renal patients (41), in particular with mild proteinuria and diabetic nephropathy (50, 51).

Effect of Dietary Proteins on Tubulo-Glomerular Feedback and on the Hormonal Milieu

In diets rich in animal proteins, the inhibition of the tubuloglomerular feedback, the increase in glucagon secretion and the local production of vasodilatory prostacyclin have been called as explanations of hyperfiltration and increased glomerular permeability (52, 53). Indomethacin, an old, non-specific, antiproteinuric drug, reduces proteinuria in human and animal studies, supporting a major role of prostaglandins in the pathogenesis of hyperfiltration and proteinuria (54, 55).

In murine models, an isocaloric, protein restricted diet for 6 weeks decreases mitochondrial ROS production in the kidney (56). Low-protein diets may exert an anti-fibrotic effect through down-regulation of transforming growth factor-β (TGF-β) in mesangial cells (57).

In addition, animal models have shown that low-protein diets may reduce tubular cell damage, tubulointerstitial oxidative stress and apoptosis by decreasing the accumulation of abnormal mitochondria. This finding could be explained by the restoration of autophagy; furthermore, low protein diets reduce the mammalian target of rapamycin complex 1 (mTORC1) activity, promoting autophagic capacity (58, 59).

POTENTIAL SYNERGY BETWEEN SGLT2i AND LOW-PROTEIN DIETS

So far, no study evaluated whether the type of diet, and its protein content, has a synergistic, neutral or counteracting effect on the action of SGLT2i. The main point is the site and mechanism of action.

In the case of RAAS inhibitors, the synergy between reduced intake of animal proteins has been investigated (60, 61). Conversely, if a competitive action of SGLT2i and protein

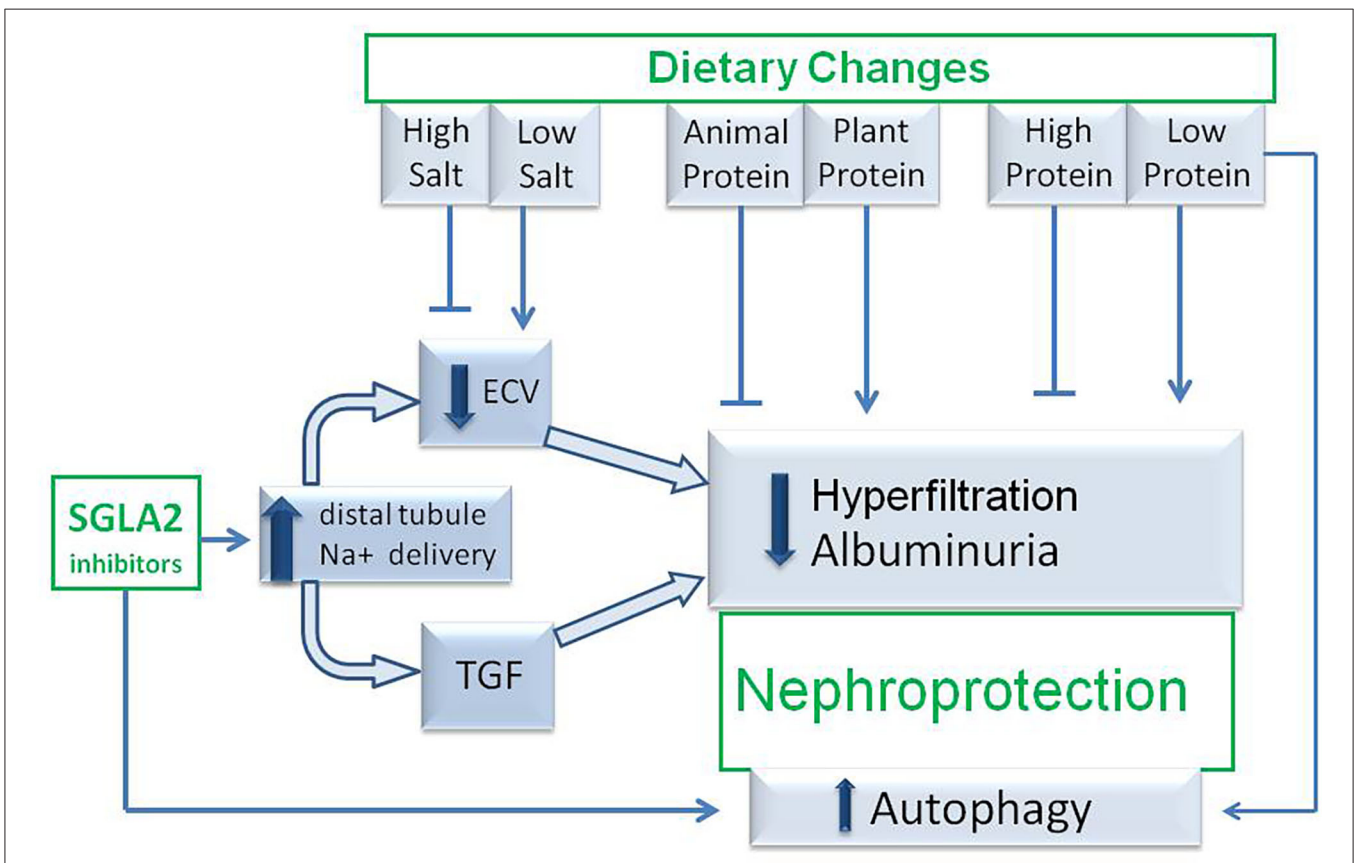

FIGURE 2 | Hypothetic mechanisms of nephroprotection occurring during association between SGLT2 inhibitor therapy and medical nutritional therapy. TGF, tubuloglomerular feed-back; SGLT2i, sodium-glucose-transporter 2 inhibitors; ECV, extracellular volume.

restriction at the same level exists, the addition of a low-protein diet to SGLT2i therapy may not result in an additive effect.

The baseline protein intake may be important: a diet relatively rich in protein and restricted in lipids and carbohydrates is often recommended in diabetic patients. However, a high protein diet, especially if of animal origin, induces glomerular hyperfiltration that could blunt the favorable effects of SGLT2i. In this context, at least restoring a normal protein intake may allow setting a favorable stage for SGLT2i activity. Indeed, the recent Nutritional Guidelines in CKD (K-DOQI 2020) recommend normalizing the protein intake (0.8 g of proteins/Kg of ideal body weight/day) in diabetic patients (13). This strategy, overall, is expected to result in a reduction of the actual protein intake.

Furthermore, beyond hemodynamic effects, plant based, moderately protein-restricted diets have several additional advantages: they decrease the acid load (62) and phosphate intake, this easing the management of CKD-MDB (63). Moreover, the partial restoration of autophagy shared by low-protein diet and SGLT2i, may amplify the nephroprotective effects (**Table 1**).

Conversely, in diabetic patients, insulin resistance or sub-optimal glycemic control could compromise the adaptation to protein restriction, increasing the risk of protein energy wasting (64). Of note SGLT2i induce a loss of up to 300 Kcal/day, which can enhance protein catabolism. Under these conditions, nutritional management should be careful and a plant-based diet, with normal protein intake (0.8-g of proteins/Kg/day), may be a nutritionally safe option, possibly enhancing nephroprotection.

CONCLUSIONS

SGLT2i and low-protein and/or vegetarian diets offer interesting opportunities of nephroprotection in both diabetic and non-diabetic CKD patients and they share some mechanisms of nephroprotection (**Figure 2**). Even if the pleiotropic effects of protein-restricted and plant-based diets may be expected to exert a favorable effect on top of a SGLT2i therapy, further studies should be targeted at assessing whether or not patients receiving a combined therapy have additional benefits in terms of nephroprotection and, if so, which dietary regiments have the safest and most efficient profile.

AUTHOR CONTRIBUTIONS

AC and GBP: conceptualization, original draft preparation, writing, review and editing. DG, DM, CD'A, and MT: writing, review and editing. All authors contributed to the article and approved the submitted version.

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Maternal and Fetal Outcomes of Pregnancy in Nephrotic Syndrome Due to Primary Glomerulonephritis

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Chronic kidney disease (CKD) affects 3% of pregnancies, impacting on maternal and fetal outcomes, and at the same time, a recurrent question in nephrology regards gestation impact on kidney function. Observational studies stated that CKD stage, pre-existent hypertension, and proteinuria are the main predictors of possible complications, such as maternal CKD progression, maternal or fetal death, prematurity, small for gestational age (SGA) newborn, or admission to the neonatal intensive care unit. In this regard, given the prominence of proteinuria among other risk factors, we focused on primary nephrotic syndrome in pregnancy, which accounts for 0.028% of cases, and its impact on materno-fetal outcomes and kidney survival. Data extracted from literature are scattered because of the small cohorts investigated in each trial. However, they showed different outcomes for each glomerular disease, with membranous nephropathy (MN) having a better maternal and fetal prognosis than focal and segmental glomerulosclerosis (FSGS), membranoproliferative glomerulonephritis (MPGN), or minimal change disease (MCD). Nephrotic syndrome does not have to discourage women to undertake a pregnancy, but the correct management may include a specific evaluation of risk factors and follow-up for adverse materno-fetal events and/or maternal kidney disease progression.

Keywords: pregnancy, nephrotic syndrome, focal and segmental glomerulosclerosis, membranous nephropathy, membranoproliferative glomerulonephritis, minimal change disease

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INTRODUCTION

In the past, fear of chronic kidney disease (CKD) progression could prevent nephrologists from sustaining their female patients' idea of conceiving or carrying on gestation, even if evidence of adverse outcomes was scattered. Today, mild CKD affects nearly 3% of women in childbearing age, whereas stage 3–5 CKD can be detected in 0.7% of same age women (1). However, the real prevalence in pregnant patients cannot be assessed, given that gestational screening guidelines usually include only urinalysis to identify urinary tract infections (UTIs) or proteinuria. On the other hand, mild CKD reports at more extended laboratory examinations performed in pregnancy are rising, likely because of increasing maternal age and higher incidence of hypertension, obesity, diabetes mellitus type 2 (DMT2), or smoking habits. Thus, first pregnancy is frequently the real touchstone to discover metabolic disorders, impaired renal function, or UTIs. Among primary kidney diseases, glomerulopathies, particularly nephrotic syndrome, are

rare conditions whose management in pregnancy must include specific follow-up and evaluation of risk factors for adverse materno-fetal events and/or maternal renal disease progression. All the trials based on miscellaneous CKD cases demonstrate that stage of renal impairment, but above all baseline proteinuria and hypertension, are significant risk factors for adverse outcomes. The aim of our review is to investigate the outcomes of women affected by nephrotic syndrome due to primary glomerulopathies, including focal and segmental glomerulosclerosis (FSGS), minimal change disease (MCD), membranous nephropathy (MN), and membranoproliferative glomerulonephritis (MPGN).

NEPHROTIC SYNDROME AND PREGNANCY, A DIFFICULT DATA ANALYSIS

Ethical concerns about pregnancy prevent starting interventional randomized controlled trials, so literature in the field is mainly based on retrospective or, to a lower extent, prospective observational studies. Moreover, statistical analysis of materno-fetal or kidney outcomes in those trials is often based on CKD stage rather than on the specific underlying causes that range from hypertension and/or proteinuria, diabetic nephropathy (DN), and glomerulonephritis to autosomal dominant polycystic kidney disease (ADPKD), kidney malformations, nephrolithiasis, or chronic UTIs. It is worth considering that some of them may expose patients to specific risks, such as elevated rate of fetal deformity in DN or hereditary transmission of ADPKD, in addition to other adverse outcomes as maternal CKD progression or maternal and fetal death, prematurity, small for gestational age (SGA) babies, or admission to the neonatal intensive care unit (NICU), which are common for all CKD causes. Finally, long-term effects of moderate–severe CKD have to be contemplated and result in a higher incidence of hypertension and ischemic heart disease in women affected by materno-fetal complications. Except for IgA nephropathy (IgAN) and systemic lupus erythematosus that result in the most common glomerular diseases in childbearing age women, investigating peculiar outcomes of other glomerulopathies appears to be quite complex because it is hard to find a pregnant cohort big enough to reach statistical significance after stratification of individual risk factors.

Superimposed Preeclampsia or Kidney Disease Progression?

Nephrotic syndrome is estimated to occur in 0.028% of gestations (2), but an increased or new-onset proteinuria has to be distinguished from preeclampsia. Usually, time and clinical presentation are diagnostic criteria when proteinuria rises twice the baseline value if it is >2 g/day or 5-fold if it is lower, after gestation week 20, associated with the onset of hypertension and severe headaches or epigastric pain, as well as with laboratory abnormalities, such as thrombocytopenia and titer of serum aspartate aminotransferase >70 U/L (3).

At the moment, podocyturia and nephrinuria have been considered as direct urinary markers of podocyte damage. Podocyturia has higher sensitivity (88%) and specificity (79%) with immunofluorescence techniques targeting podocalyxin rather than synaptopodin or podocin. Nephrinuria is defined as an increase in urine nephrin level, preceding the onset of clinical manifestation of preeclampsia by about 9 days, with the best sensitivity (100%) and specificity (97%) during the 3rd trimester of pregnancy. However, their benefit as predictors of preeclampsia may not be extremely relevant in differential diagnosis between this condition and a new-onset or progression of nephrotic syndrome. Placental growth factor (PlGF) is an angiogenic factor whose production increases until gestation weeks 26–30 in healthy women. On the contrary, in preeclampsia, reduced titer of PlGF may be an expression of a placental dysfunction. Soluble FMS-like tyrosine kinase receptor 1 (sFlt-1) counteracts PlGF and also demonstrated to downregulate the expression of slit diaphragm components responsible for proteinuria. sFlt-1/PlGF ratio appears to be a promising placental specific marker of preeclampsia, in terms of both specificity and sensitivity (4), and correlates with disease severity, anticipating its onset by about 5 weeks.

Specific Outcomes in Primary Nephrotic Syndrome

Maternal and fetal outcomes in FSGS patients were studied from 1980 to 2017 in several case series of pregnant women affected by biopsy-proven primary glomerular diseases. Except for a prospective trial and two retrospective studies each including only a case of FSGS and reporting, respectively, a case of progression of kidney disease, stillbirth, and preterm delivery (5–7), other studies included a number of pregnancies ranging from 10 to 84, with term delivery in 16–78.5% of cases (8–14). The analysis of adverse materno-fetal outcomes showed a high rate of incidence of spontaneous abortion (5.9–28.6%), preterm delivery (5.9–58.8%), and perinatal death (11.7–45.2%) (8–14). Regarding maternal renal outcomes, hypertension emerged in 20–71% of pregnancies, but persisted after 6 months after delivery in 4–66% of women (8–14). The highest rate of postpartum hypertension was reached in a Saudi cohort of 84 pregnancies in 18 women affected by FSGS, who had undergone 38 pregnancies before the onset of nephrotic syndrome. Malik et al. compared the materno-fetal outcomes of their pregnancies demonstrating no statistical significance of the increased abortion rate registered after FSGS diagnosis. Conversely, higher rate of preterm delivery (5.9%, $p = 0.0006$) and SGA infants (16.6%, $p = 0.0001$) in FSGS cohort than in control group gestations resulted to be significant (13). Interestingly, proteinuria increased from 3.8 ± 3.4 g/day in the first pregnancy to 4.8 ± 2.0 g/day in the last one, but the difference was not significant ($p = 0.275$). At last follow-up, occurring at a mean of 10.5 ± 3.3 years after FSGS diagnosis, half of their cohort had CKD progression, and four women (22.2%) reached end-stage renal disease (ESRD), requiring renal replacement therapy (13). These results are in line with the previous experience of Packham et al., who reported a transient impairment of renal function in 49% of pregnancies, persisting

in four patients and leading to ESRD in three of them (19%), respectively, after 2, 7, and 18 years, in contrast to higher renal survival rate (93% at 10 years from diagnosis) of a control group of 58 non-pregnant women affected by FSGS (11).

Six studies evaluated outcomes of pregnancy and MCD (2, 5, 6, 8, 14). Two retrospective studies, including only seven MCD patients in their report, showed 50–66% of SGA newborns. Regarding renal outcomes, they appeared limited to gestation, with increased level of proteinuria and onset of hypertension registered in only a pregnancy for each trial, both resolving postpartum (2, 5). In line with these results, Surian (8) recorded no complications in two pregnancies. In 1985, Abe et al. studied 17 pregnancies of 10 MCD patients, highlighting materno-fetal complications (SGA infants, preterm delivery, polyhydramnios, premature abruptio placentae, and severe bleeding after delivery) in 12% of gestations and fetal loss and abortion in 12 and 17% of cases, respectively. The main risk factors identified were hypertension and glomerular filtration rate <70 ml/min (6). More recently, a retrospective trial included seven pregnancies of which 71.4% hesitated in preterm delivery and 14.3% in fetal loss beyond gestation week 20. Only a patient experienced a stable increase in proteinuria postpartum (14).

Trials investigating MN and pregnancy highlighted a reduced impact of this glomerulopathy on the incidence of abortion and stillbirths in the absence of risk factors, such as nephrotic range proteinuria during pregnancy (2, 5, 6, 8, 9, 12, 14–20). A transient development of hypertension was registered in 7.7–45.4% of pregnancies, and increased proteinuria in up to 60% of cases confirmed an association of MN with worse fetal and maternal outcomes. Packham et al. reported the highest rate of materno-fetal complications with fetal loss in 24% of cases, preterm delivery in 43% of pregnancies, CKD progression in 9%, but the onset of hypertension in 46% of pregnant patients, and higher proteinuria in 54.5% (17). A Saudi cohort study comparing the maternal outcomes of nine women experiencing pregnancies before ($n = 21$) and after ($n = 30$) MN diagnosis demonstrated that the different rates of abortion (4.7 vs. 3.3%, $p = 0.843$), preterm delivery (0 vs. 6.6%, $p = 0.152$), and perinatal mortality (0 vs. 3.3%, $p = 0.317$) between the two groups were not statistically significant (18). Moreover, the rise in mean serum creatinine level experienced from the first gestation after MN diagnosis to the last one of the same cohort was also not significant (mean SCr 68 ± 17 vs. 80 ± 48 μmol/L, respectively, $p = 0.489$) (18).

MPGN defines a pathologic pattern characterized by subendothelial, subepithelial, and mesangial deposits of immune complexes (due to infections, autoimmune or monoclonal disorders) or of complement (due to alternative pathway dysregulation). Idiopathic MPGN cases were studied in five trials accounting for a total number of 36 patients (5–9). Two studies included only a patient and obtained opposite results, with a neonatal death (6) and a physiologic pregnancy (7). The other retrospective trials showed full-term deliveries in up to 50% of patients, whereas 16.7–23.8% had preterm delivery. Fetal death was more frequent in FSGS and MN patients evaluated in the same studies, with both an abortion rate and peripartum mortality in 0–11.1% of gestations (5–9). Moreover, Barcelò et al., stratified MPGN, FSGS, and MN patients' outcomes based on proteinuria and detected a significant association only of proteinuria levels >2.5 g/day with preterm delivery and low birth weight (1,930 ± 301 g) (9).

New data come from De Castro et al.'s retrospective study on 26 pregnancies in 19 patients affected by biopsy-proven primary nephrotic syndrome with a mean proteinuria of 8.33 ± 6.7 g/day (19). In 26 pregnancies described, glomerulopathies most represented were FSGS (42%), MN (16%), and IgAN (16%), followed by MCD, MPGN, C3 glomerulonephritis (C3GN), and fibrillary glomerulonephritis, each accounting for 5%. A new diagnosis was performed in 12 women during pregnancy at mean gestational week 18.6 ± 9.1, and kidney biopsy was performed in eight patients within a mean gestational age of 21 weeks. Only two minor biopsy complications were reported in this group of women (hematoma and hematuria), compared with one adverse event recorded in 11 patients who had pathologic diagnosis before or after their pregnancies (1 small arteriovenous fistula), but the procedure proved to be worth the risk because it allowed to change the management of nephrotic syndrome in six patients who started prednisone in a dose ranging from 60 to 120 mg/day. One patient affected by known C3GN was given eculizumab. The main adverse maternal outcomes described were preeclampsia (27%), acute kidney injury (AKI, 23%), and cellulitis (12%), which was not associated with steroid therapy. FSGS accounted for half of AKI episodes, one of which progressed to ESRD, whereas the remaining happened in IgAN and C3GN patients. Fetal outcomes included preterm delivery and low birth weight (under 2,500 g) in 54% of pregnancies, intrauterine growth restriction (IUGR) in 11%, and NICU admission in 31%. Two cases of premature rupture of membranes were identified in patients treated with high dose glucocorticoids. In this cohort, MN confirmed having the best maternal–fetal and kidney survival outcomes, with three patients with stable serum creatinine during pregnancies and a transient increase in proteinuria, which reverted to non-nephrotic range in a year after delivery. Statistical analysis of risk factors of the whole cohort confirmed the strong association of proteinuria with adverse outcomes, such as preeclampsia ($p = 0.002$), low birth weight ($p = 0.002$), and preterm delivery ($p = 0.02$) (19).

The most recent retrospective study performed by Liu et al. reported preserved kidney function but the occurrence of adverse materno-fetal complications including fetal loss (11%), preterm delivery (26%), and severe preeclampsia (15%) in 27 pregnancies from 2008 to 2018 in a cohort of women affected by MN (20). Consistent with De Castro' observations (19), Liu also demonstrated a correlation of proteinuria before week 20 (intended as both time-averaged urine protein, OR 67.5, $p < 0.001$; maximum proteinuria >3.5 g/day, OR 29.3, $p = 0.001$), hypoalbuminemia (time-averaged albumin, OR 67.5, $p < 0.001$; minimum serum albumin <25 g/L, OR 10.9, $p = 0.01$), and no-remission during pregnancy (OR 21.6, $p = 0.004$) with adverse materno-fetal outcomes. In particular, time-averaged proteinuria and serum albumin were associated with birth weight percentile of neonates. Moreover, anti-PLA2R antibody positivity ($p = 0.03$) appeared as a specific risk factor for pregnant MN patients (20). Interestingly, a case report of PLA2R positive MN

TABLE 1 | Retrospective trials on primary nephrotic syndrome and materno-fetal and renal outcomes.

	Pregnancies/ patients	Abortions	Preterm delivery	SGA	Neonatal death	Hypertension during gestation/follow-up*	Proteinuria during gestation/follow-up*
FSGS							
Katz et al. (5)	1/1	0	0	0	0	0	1 (100)/0
Surian et al. (8)	25/19	2 (8)	6 (24)	1 (4)	4 (16)	5 (20)/1 (4)	0/0
Abe et al. (6)	1/1	0	0	0	1 (100)	1	1 (100)/1 (100)
Barcelò et al. (9)	17/13	1 (5.9)	3 (17.6)	0	0	4 (23.5)/1 (7.7)	3 (17.6)/1 (7.7)
Kincaid-Smith and Fairley (10)	28/15	8 (28.6)	14 (50)	0	0	20 (71)/3 (18)	11 (39)/4 (14)
Packham et al. (11)	31/21	0	12 (38.7)	5 (16.1)	14 (45.2)	23 (68)/4 (12.9)	15 (49)/4 (19)
Jungers et al. (12)	10/6	1 (10%)	3 (30)	3 (30)	1 (10)	0/0	4 (40)/1 (16.6)
Alexopoulos et al. (7)	1/1	0	1 (100)	0	0	1 (100)/0	1 (100)/1 (100)
Malik et al. (13)	84/18	7 (8.3)	5 (5.9)	14 (16.6)	11 (13)	52 (61)/12 (66)	13 (72)/12 (71)
O'Shaughnessy et al. (14)	17/10	0	10 (58.8)	4 (23.5)	2 (11.7)	11 (68.8)	5 (38.5)/3 (37.5)
De Castro et al. (19)	13	0	2	2	0	0	5 (38.5)/5 (38.5)
MCD							
Studd and Blainey (2)	4/4	0	0	2 (50)	0	1 (25)	0/0
Katz et al. (5)	6/3	0	1 (16.6)	4 (66.6)	0	1 (16.6)/0	1 (16.6%)/0
Surian et al. (8)	2/2	0	0	0	0	0/0	0/0
Abe et al. (6)	17/10	3 (17.6)	2 (11.8)	2 (11.8)	2 (11.8)	1 (5.9)/0	1 (5.9)/0
O'Shaughnessy et al. (14)	7	0	5 (71.4)	0	1 (14.3)*	0/0	2 (28.6)/1 (14.3)
De Castro et al. (19)	1	0	0	0	0	0	1 (100)/1 (100)
MN							
Studd and Blainey (2)	12/7	2 (6.6)	4 (33.3)	11 (41.6)	0	8/0	0/0
Forland and Spargo (15)	5/4	2 (40)	0	0	0	0/0	3 (60)/3 (75)
Noel et al. (16)	9/7	1 (11.1)	0	0	1 (11.1)	0/0	1 (11.1)/1
Katz et al. (5)	10/7	0	1 (10)	1 (10)	0	4 (40)/5 (71.4)	6 (60)/4 (57.1)
Surian et al. (8)	8/7	0	0	0	0	1 (12.8)/0	0/0
Abe et al. (6)	13/7	0	1 (7.7)	0	1 (7.7)	1 (7.7)/0	0/0
Barcelò et al. (9)	9/7	0	2 (22.2)	0	0	3 (33.9)/0	2 (22.2)/0
Packham et al. (11)	33/24	8 (24)	14 (43)	11 (33)		15 (46)/0	2 (22.2)/0
Jungers et al. (12)	37/17	13 (35)	(30%)	0	0	(35)/0	0/0
Malik et al. (18)	30/9	1 (3.3)	2 (6.6)	3 (10)	1 (3.3)	1/1 (11.1)	0/0
O'Shaughnessy et al. (14)	6	0	2 (33.3)	0	1 (16.6)†	3 (75.0)	2 (33.3)/1 (16.6)
De Castro et al. (19)	4	0	1 (25)	1 (25)	0	0/0	4 (100)/0
Liu et al. (20)	27/25	0	7 (25.9)	7 (25.9)	1 (3.7)	0/0	0/0
MPGN							
Katz et al. (5)	4/4	0	0	1 (25)	0	3 (75)/1 (25)	4 (100)/3 (75)
Abe et al. (6)	1/1	0	0	0	1 (100)	1 (100)/0	0/1 (100)
Surian et al. (8)	18/14	2 (11.1)	3 (16.6)	2 (11.1)	2 (11.1)	4 (22.2)/3 (21.4)	3 (16.6)/2 (14.3)
Barcelò et al. (9)	21/16	2 (9.52)	5 (23.8)	0	1 (4.76)	5 (23.8)/2 (12.5)	4 (19)/2 (12.5)
Alexopoulos et al. (7)	1/1	0	0	0	0	0	0

*SGA, small for gestational age; FSGS, focal and segmental glomerulosclerosis; MCD, minimal change disease; MN, membranous nephropathy; MPGN, membranoproliferative glomerulonephritis. *Renal outcomes during follow-up have been evaluated in percentage on the number of patients in each trial and not on pregnancies total number. †Fetal death after 20 weeks of gestation. All values represent n (%).*

patient undergoing pregnancy demonstrated the transplacental passage of these autoantibodies, which were detected in the fetus' cord blood (21). Sachdeva et al. confirmed this finding in a new case report and also showed a PLA2R passage to newborn during breastfeeding, which raises questions about the opportunity of this practice and of checking PLA2R titer, serum albumin, and urinalysis in children of mother affected by primary MN (22).

Symptomatic Treatment of Nephrotic Syndrome in Pregnancy

Primary glomerulonephritis may require the administration of immunosuppressive drugs, such as mycophenolic acid and cyclophosphamide, whose teratogen risk is known and should therefore be replaced before gestation (23). Monoclonal antibodies, such as anti-CD20 rituximab, should be avoided because of their transplacental transport in the last trimesters of pregnancy, which can induce transient B cell depletion in fetuses (24). Conversely, short half-life corticosteroids, such as prednisone and methylprednisolone, are considered a valid alternative because of their inactivation by placental 11β-hydroxysteroid dehydrogenase type 2 that prevents steroid adverse effects on fetus (23). Calcineurin inhibitors (CNIs), such as cyclosporin (CsA) or tacrolimus (TAC), appear to be safe, with no evidence of increased risk of teratogenicity (25), and experimental rat models show how CsA transplacental passage is impaired by P-glycoprotein (26).

Angiotensin-converting enzyme inhibitors (ACEIs) and/or angiotensin II receptor blockers (ARBs) are widely recommended in KDIGO guidelines as the first-line therapy of nephrotic syndrome for their nephroprotective effects (27). Their teratogen effect on the cardiovascular, urinary, skeletal, and central nervous systems makes them contraindicated in pregnancy particularly during the second trimester of gestation, so general advice is to have them immediately discontinued at the beginning of pregnancy (28–30). Hoeltzenbein et al. conducted a prospective observational trial comparing 329 women accidentally exposed to ACEIs during their first trimester of gestation and no longer than week 20 and 654 control pregnant women without a history of hypertension (31). The significantly increased rate of major birth defects in the ACEIs cohort confirmed the results of previous studies on these drugs in pregnancy (32, 33), but they also investigated maternal hypertension impact on malformations, already demonstrated in other trials (34, 35). Comparing patients affected by chronic hypertension exposed to ACEIs vs. patients treated with methyldopa, they concluded that malformations rate was not significantly different, also suggesting the potential role of severe hypertension *per se* as a risk factor for adverse fetal outcomes (31).

A safe option to maintain blood pressure in pregnant women affected by CKD within values of 135/85 mmHg is represented by α2 adrenergic receptor blocker, such as methyldopa, whereas clonidine may impact fetal growth, and it is usually avoided for high risk of hypertensive rebound at discontinuation. Other antihypertensive choices are represented by calcium blockers, such as long-acting nifedipine or labetalol, a combined α- and β-blocker, with efficacy and safety comparable to methyldopa. Other β-blockers represent a second choice because of the risk of adverse events in newborns (usually transient bradycardia, hypotension, and hypoglycemia). Diuretics are usually avoided in pregnancy and may be adopted in nephrotic syndrome refractory to immunosuppressive therapy under strict control to avoid volume depletion and AKI risk.

Regarding albumin infusion in nephrotic syndrome in pregnancy, no study is conclusive about its potential benefit. On the contrary, hyperfiltration risk has to be considered, with a paradox increase in proteinuria, and this discourages the systematic use of albumin in this setting (36, 37).

Nephrotic syndrome is also characterized by a hypercoagulability state that has to be treated when the albumin level is lower than 2 g/dl. Low molecular-weight heparin is a valid choice for both prophylaxis and treatment of thromboembolic complications during pregnancy and postpartum, because it does not cross the placenta. Heparin should be discontinued before labor induction or planned cesarean section, but can be resumed after delivery (38). During pregnancy, low-dose acetylsalicylate is also a useful option preventing preeclampsia in high-risk patients, such as CKD and proteinuric patients. Despite guidelines suggesting to start therapy after the first trimester to avoid hemorrhagic risk in case of spontaneous abortions, the protective effects on placentation are higher when acetylsalicylate is started early (39, 40).

The positive effect of a low protein diet with supplementation of amino acids and ketoacids in pregnant CKD patients has to be considered as a therapeutic option, in the absence of a specific anti-proteinuric therapy (41, 42).

CONCLUSIONS

Our review focused on materno-fetal and kidney survival outcomes reported in pregnant patients with nephrotic syndrome (**Table 1**). Baseline levels of proteinuria, as well as hypertension and CKD stage, are the most important risk factors for poor outcomes, as confirmed in other trials involving pregnant women affected by CKD. Incidence of C-sections and preterm deliveries are higher than healthy controls, as reported by Piccoli et al.'s findings in a prospective trial in CKD patients (54.8 and 33.4%, respectively) (43). Among nephrotic syndromes, MN resulted to be the most favorable in terms of outcomes, whereas MPGN and FSGS expose patients to a higher degree of both materno-fetal and renal adverse events. Adequate counseling appears to be indispensable to increase women awareness of the benefit of pregnancy planning to manage potential teratogen therapies and to minimize risks of gestational complications and disease progression, especially if a complete and stable remission is not obtained before conceiving. More prospective trials are required to better evaluate specific predictors of severe adverse outcomes and to reach statistical significance after stratifying individual risk factors.

AUTHOR CONTRIBUTIONS

RS, GG, and DS: conceptualization and writing—original draft preparation. RS, VC, GG, and DS: methodology. RS and DS: investigation. RG and DS: resources. RS, RG, and VC: data curation. RS, RG, AS, FT, VC, and DS: writing—review and editing. RS, RG, AS, FT, and DS: visualization. RG, DS, and GG: supervision. All authors contributed to the article and approved the submitted version.

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Inflammatory Cytokines in Diabetic Kidney Disease: Pathophysiologic and Therapeutic Implications

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Diabetic kidney disease (DKD) is the leading cause of end-stage renal disease and a main contributing factor for cardiovascular morbidity and mortality in patients with diabetes mellitus. Strategies employed to delay the progression of this pathology focus on the control of traditional risk factors, such as hyperglycemia, and elevated blood pressure. Although the intimate mechanisms involved in the onset and progression of DKD remain incompletely understood, inflammation is currently recognized as one of the main underlying processes. Untangling the mechanisms involved in the appearing of a harmful inflammatory response in the diabetic patient is crucial for the development of new therapeutic strategies. In this review, we focus on the inflammation-related pathogenic mechanisms involved in DKD and in the therapeutic utility of new anti-inflammatory strategies.

Keywords: diabetic kidney disease, inflammation, inflammatory cytokines, SGLT2i, GLP-1RA, DPP-4i, pentoxifylline

INTRODUCTION

Diabetic kidney disease (DKD) is a frequent complication in patients with diabetes mellitus (DM) and constitutes the first cause of kidney disease and an important risk factor for cardiovascular disease in this population (1, 2). DKD is characterized by a plethora of alterations including hemodynamic and metabolic abnormalities, the activation of the renin–angiotensin system (RAS), oxidative stress, and fibrosis, which together trigger the elevation of systemic and intraglomerular pressure, and the appearing of various symptoms related to the development of kidney failure: glomerular hypertrophy, proteinuria, and decreased glomerular filtration (3). Until recently, kidney involvement was only considered as the result of alterations in hemodynamic and metabolic factors; however, recent advances show that DKD is a complex and multifactorial process.

An increasing number of clinical and experimental studies have pointed out that inflammation contributes to the development and progression of DKD (4). Therefore, inflammatory mediators may represent new targets for the development of therapeutic strategies for the treatment of this complication. In this review, we discuss the pathophysiologic and therapeutic implications of the inflammatory cytokines in DKD.

INFLAMMATORY CYTOKINES IN THE SETTING OF DKD

Diverse inflammatory parameters are predictors of the evolution of the DM (5, 6), including the initiation and progression of DKD (4). These include inflammatory cytokines which, in addition to their role as regulators of the immune response, also exert important actions as cardinal effectors of injury. The increase in the levels of these molecules in the diabetic patient leads to microvascular complications such as the development of nephropathy (7–9). Proinflammatory cytokines, both circulating and those synthesized and secreted by inflammatory cells in the renal tissue, are elevated in patients with DKD (10–13), being directly associated with urinary albumin excretion (UAE) levels and with clinical markers of glomerular and tubulointerstitial damage (14, 15) (**Figure 1**).

Urinary levels of interleukin (IL) 6 and IL8 are significantly increased in microalbuminuric diabetic patients with early progressive renal function decline when compared to microalbuminuric or normoalbuminuric subjects with stable renal function (16). IL6 levels are upregulated in patients with DM and nephropathy (17); in the same way, the expression of mRNA encoding IL6 is also increased in renal cells (glomerular, epithelial, mesangial), as well as in kidney infiltrating-cells in patients with DKD compared to diabetic patients without kidney disease (17). Importantly, the expression levels of IL6 mRNA are positively related with the severity of mesangial expansion, a characteristic histological manifestation of DKD.

IL18 is another potent pro-inflammatory cytokine that exhibits elevated serum and urinary levels in patients with DKD, showing significant and direct correlations with UAE levels and with the evolution of albuminuria (7, 18, 19). Additionally, serum levels of IL18 are associated with urinary β-2 microglobulin, a marker of tubular dysfunction (18). IL18 is capable of regulating the synthesis of pro-inflammatory molecules such as IL1 and tumor necrosis factor (TNF) α, and interferon (IFN) ɣ (20), in turn increasing chemokine receptors in mesangial cells (21). Furthermore, IL8 increases the expression of intercellular adhesion molecule 1 (ICAM-1) (22) and promotes endothelial cell apoptosis (23). In addition to infiltrating cells, kidney tubular cells of patients with DKD also express increased levels of IL18 (24), which is related to the activation of the mitogen-activated protein kinase (MAPK) pathways by transforming growth factor (TGF) β (25).

Both serum and urinary levels of the cytokine TNFα are also elevated in patients with DKD (26). Moreover, this increase parallels the progression of renal damage, which may indicate a relationship with the development and progression of renal impairment in the diabetic patient (7, 9, 27). In these subjects, the rise in TNFα has been related with renal damage mediated by cytotoxic effects (26). Similar to IL18, TNFα is also expressed in endothelial, epithelial, mesangial, and tubular renal cells (28, 29). Importantly, animal models of diabetes present increased levels of TNFα in kidney glomeruli and tubules (9, 30–33) being directly and independently related with UAE (32).

Animal models of DKD show upregulation of IL1 expression in many types of kidney cells (31, 34). This increment has been related with the expression of ICAM-1, the vascular cell adhesion molecule-1 (VCAM-1), and the endothelial-leukocyte adhesion molecule-1 (ELAM-1) (35, 36), molecules involved in chemotaxis and adhesion processes. Rat kidney mesangial cells are stimulated to produce prostaglandin E2 after being incubated with recombinant IL1 (37) in response to Ang II (38), which is related to the appearing of abnormalities in intraglomerular hemodynamics. Finally, IL1 has been also related with the production of hyaluronan in the proximal tubule (39), which is related with the development of experimental hypercellularity (40).

The cytokine IL17A is a member of the IL17 family mainly produced by activated T helper (Th) lymphocytes Th17 and, at lesser extent, by macrophages, neutrophils, natural killer, dendritic, and mast cells. Circulating IL17A levels have been related with the severity of kidney disease, showing a progressive decrease from subjects with normal glucose tolerance to subjects with DM with and without DN (41, 42). In opposite with these findings, other authors have found increased IL17A plasma values in patients with DN compared to healthy controls (43). These paradoxical findings might by the fact that the modulatory effects of IL17A on inflammation may be dependent on disease context, tissue, isoform, and receptor-ligand interactions (44).

PATHOPHYSIOLOGIC IMPLICATIONS

The pathophysiological implications of the inflammation on DKD occur at various levels. In a first level, inflammatory cytokines, acting in a paracrine or autocrine form, trigger the epithelial-to-mesenchymal transition process in the kidney (45), causing extracellular matrix accumulation. Secondly, the up-regulation of chemoattractant cytokines and adhesion molecules stimulates the attraction of circulating cells and facilitate their infiltration into the renal tissue. Finally, these cells amplify the inflammatory reaction, generating more cytokines and other mediators that contribute to the development and progression of renal injury (**Figure 1**).

The first clue suggesting the existence of a pathogenic role of these molecules in the DKD was obtained *in vitro* by incubating macrophages in a glomerular basement membrane derived from diabetic rats; these macrophages presented increased production of IL1 and TNFα when compared to macrophages incubated with membranes from normal animals (8).

The interleukins IL1, IL6, and IL8, have been clearly related to renal disorders in DKD (31, 34). IL1 has been linked with the proliferation of mesangial cells and matrix synthesis, thereby altering the renal architecture, with increased permeability of vascular endothelial cells, and with intraglomerular hemodynamic abnormalities secondary to alterations in prostaglandin production (37, 46). In DKD patients, the expression of mRNA molecules encoding *IL6* is positively related with the severity of mesangial expansion (17). The up-regulation of this cytokine have been also related to another functional and structural abnormalities related to

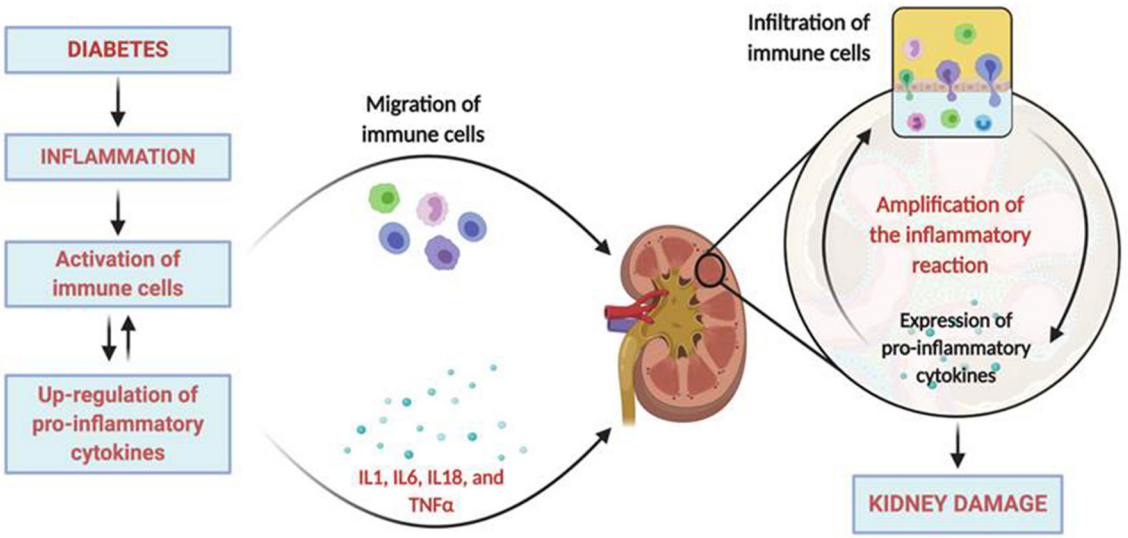

FIGURE 1 | Inflammatory pathophysiology in diabetic kidney disease. The diabetic milieu drives to the development of inflammation that includes the activation of immune cells and the upregulation of pro-inflammatory cytokines. Activated immune cells migrates and infiltrates the renal tissue locally producing more inflammatory mediators and chemokines that recruit more immune cells into the kidney. Moreover, activated resident renal cells can also produce additional proinflammatory mediators, contributing to sustained inflammation, and the induction of kidney damage. Created with BioRender.com.

TABLE 1 | Therapeutic strategies in the DKD with potential anti-inflammatory properties.

Drug	Primary target	Main outcomes in DKD	Anti-inflammatory effects	References
RAS blockers	Inhibition of ACE or blockade of angiotensin II receptor.	Reduce proteinuria and the progression of nephropathy.	Inhibition of NF-κB, MCP1 gene expression, and macrophage infiltration.	(68, 69)
SGLT2 inhibitors	Blockade of glucose reabsorption by SGLT2 at the proximal tubule.	Improved glycemic control. Slower progression of kidney disease and lower rates of clinically relevant renal events.	Reduction of inflammation by targeting the IL1ß and reduction of hsCRP, TNFα, IL6, and IFNγ.	(70–90)
DPP4 inhibitors and GLP-1 receptor agonists	Stimulation of glucose-dependent insulin release.	Improved glycemic control and body weight reductions. Renoprotective actions	Reduction in levels of inflammatory markers including CRP, TNFα, IL6, and IL18.	(91–97)
Pentoxifylline	Inhibition of phosphodiesterases.	Reduced progression of renal disease and proteinuria.	Downregulation of NF-κB signaling and reduction of inflammatory biomarkers.	(98–114)

RAAS, Renin-Angiotensin Aldosterone System; ACEI, angiotensin converting enzyme; NF-κB, nuclear factor-κB; MCP1, monocyte chemoattractant protein 1; SGLT2, type 2 sodium-glucose cotransporter; IL, interleukin; hsCRP, high sensitivity C reactive protein; TNFα, tumor necrosis factor α; IFN-γ, interferon γ; DPP4, dipeptidyl-peptidase-4; GLP-1, glucagon-like peptide-1.

DKD including increased vascular endothelium permeability (46), mesangial expansion and fibronectin expression (47), thickening of the glomerular basement membrane (48, 49), and renal hypertrophy (50). High serum and urine levels of the IL18 in type 2 diabetes have been proposed as early predictors of renal dysfunction, being related with the appearing of macroalbuminuria (18) and with the triggering of MAPK pathways by TGF-β (24). High serum TNFα levels have been clearly related with the pathophysiology of DKD including the development of renal hypertrophy and hyperfiltration (33). Levels of sodium retention, which is present in the early stages of DKD, has been related with urinary TNFα (51). Sodium retention in turn induce the expression of TGFβ and the development of renal hypertrophy (52). Moreover, the rise in both urinary and kidney-expressed TNFα levels precede the rise in albuminuria (53). These harmful effects elicited by TNFα in the kidney are mediated by the cytotoxicity on glomerular, mesangial, and epithelial cells (54–57). TNFα also change the permeability of endothelial cells, altering the equilibrium between vasoconstriction and vasodilation and the intraglomerular blood flow, which reduces glomerular filtration rate (GFR) independently from alterations in hemodynamic factors (58, 59). Moreover, TNFα also increases ROS levels in kidney cells independently of hemodynamic mechanisms,

altering the glomerular capillary wall and, consequently, increasing UAE (51, 59, 60).

IL17A exerts mainly proinflammatory responses via the activation of the NF-κB pathway and downstream regulation of proinflammatory genes (61). In podocytes *in vitro*, IL17A increased the expression of IL 6 and TNFα, particularly under conditions of high glucose concentration (62). Similarly, cultured tubular epithelial cells stimulated with this cytokine also present up-regulation of proinflammatory gene expression and monocyte chemoattractant protein 1 (MCP1) production (62, 63). Importantly, IL17A also induced epithelial-to-mesenchymal transition in cultured proximal tubular epithelial cells (64), indicating that this could be another mechanism of renal damage triggered by this cytokine. In experimental models, administration of IL17A in mice significantly upregulated kidney Mcp-1 and Rantes gene expression and the recruitment of inflammatory cells to the kidney (65). In leptin deficient BTBR ob/ob mice, the administration of a neutralizing anti-IL17A antibody resulted in an inhibition of NF-κB activation (66). Similarly, neutralization of IL17A also decreased proinflammatory genes and inflammatory cell infiltration in an experimental angiotensin II-induced renal damage model (67). Taken together, these observations suggest that local IL17A production in diabetic kidneys could activate resident renal cells to produce proinflammatory cytokines and chemokines, such as MCP-1, thereby amplifying the inflammatory response. Although many studies have addressed circulating or urinary IL17A levels in DN patients, the determination of local renal levels of IL17A has not been investigated yet.

INFLAMMATION AS A THERAPEUTIC TARGET IN DKD

Up to the present time, the main therapeutic strategy to slow the development and progression of DKD focus on the tight regulation of glucose levels and blood pressure by the utilization of RAS blockers: angiotensin converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARBs). Although this therapeutic approximation reduces the risk of nephropathy progression, they do not completely halt the evolution toward end-stage renal disease (ESRD), with a still significant residual renal risk.

The important role played by inflammation, and specifically the involvement of inflammatory cytokines in the pathophysiology of ERD, constitute a new therapeutic opportunity in DKD to improve kidney function by the pharmacological reduction of the levels of these molecules (**Table 1**). Interestingly, the reduction in proteinuria and the slowdown in the progression of diabetic and non-diabetic nephropathies derived from RAS blockade has been related not only to hemodynamic/antihypertensive but also to anti-inflammatory/antifibrotic effects. This anti-inflammatory effect is mediated by the inhibition of NF-κB dependent pathways (68), which include the production of the pro-inflammatory cytokines IL6 and TNFα (69).

In recent years, new antidiabetic drugs have emerged (**Table 1**). These drugs include SGLT2i (sodium-glucose cotransporter type 2 inhibitors), GLP-1RA (glucagon-like peptide-1 receptor agonist), and DPP-4i (dipeptidyl peptidase-4 inhibitors). These compounds improve albuminuria and other traits of DKD in diabetic patients but also have been demonstrated to exert anti-inflammatory effects with potential benefits in the delay of the progression of DKD. The group of SGLT2i (canagliflozin, dapagliflozin, and empagliflozin, among others) are very effective hypoglycemic agents that, beyond glycemic control, have shown potent cardiovascular and renal protective effects in the diabetic patient (70–72). The underlying mechanisms of these effects are not fully understood, but recent findings suggest that are related with the modulation of inflammatory cytokines at renal and systemic levels. A few small clinical pilot studies have shown reductions of inflammatory markers in type 2 diabetes patients treated with SGLT2i. In this sense, mildly reductions in serum concentrations of C-reactive protein (CRP), TNFα, IL6, IL1β, and IFN-γ were observed in diabetic patients treated with canagliflozin, dapagliflozin, and empagliflozin (74–80). While robust data on short and mainly long-term effects of SGLT2i are obtained from clinical studies with a larger number of patients, most of what is known about the anti-inflammatory actions of these drugs comes from experimental approximations, which have made possible to delve into the underlying mechanisms of this beneficial immunomodulatory effect. In HK2 cells, a line of human kidney proximal tubule cells, empagliflozin attenuated the expression of Toll-like receptor-4 (TLR4) induced by high glucose, NF-κB and activator protein1 (AP1) transcription factors binding to nuclear DNA, and secretion of collagen IV and IL6 (81). In vascular endothelial cells, canaglifozin inhibited IL1β-stimulated IL6 and MCP1 secretion by AMP-activated protein kinase dependent and independent mechanisms (82). In obese diabetic or prediabetic animals, SGLT2i reduces albuminuria and tubulointerstitial injury (83–85). Thereby, the slowing in the progression of renal complications in prediabetic rats treated with dapagliflozin was related with the suppression of renal inflammation (84). The expression of TNFα, IL1β, and IL6, as well as the infiltration of cells into atheromatous plaques, were reduced in the atherosclerosis mice model ApoE $^{-/-}$ after treatment with empagliflozin (86). Empagliflozin achieved significant reductions in albuminuria in animal models with type 1 diabetes that were related with lower levels of renal NF-κB, MCP1, and IL6 (87). The inhibition of SGLT2 in experimental type 2 diabetes models, reduced glomerular macrophage infiltration and sclerosis (88) and attenuated the overexpression of NOX4, TGFβ, osteopontin, and MCP1 in the tubular cells induced by high glucose (89).

Several clinical trials suggest that the anti-diabetic drugs DPP-4i and GLP-1RA also possess beneficial renal effects (90, 91), potentially modulating the inflammatory response. The DPP-4i vildagliptin and sitagliptin reduced the levels of markers of inflammation in diabetic patients (92). Administration of GLP-1RA, exenatide or dulaglutide, reduced the levels of CRP in diabetic patients (93). Different clinical trials are presently

evaluating the effects of these anti-diabetic therapeutic agents on systemic and renal inflammation: GLP-1RA (exenatide or liraglutide) *vs.* a DPP-4i (NCT02150707) and liraglutide (LIRALBU; NCT02545738 and NCT01847313). In the meantime, the renoprotective effects derived from the treatment with GLP-1RA and DPP-4i have been demonstrated in the experimental setting employing animal models with kidney disease. The DPP-4i linagliptin reduced albuminuria, glomerulosclerosis, and tubulointerstitial fibrosis, independently of the glycemic control, in a mouse model of DKD (94). Sitagliptin and linagliptin also suppressed the activity of NLRP3/inflammasome in an experimental model of nephropathy (95). The model of type 2 diabetes db/db mice reduced albuminuria levels and mesangial expansion after treatment with the GLP-1RA exenatide (96). This treatment also reduced the levels of inflammatory cytokines and adhesion molecules in type 1 in diabetic rats (97).

The utilization of microRNAs (miRNAs) to regulate Th17 cell differentiation by targeting transcription factors like orphan receptor γt (RORγt) and the activation of STAT-3 or key cytokines involved in this process have been tested in different models of autoimmune disease (115–118). In the context of kidney disease, a recent study described that miRNA-155 deficiency promoted nephrin acetylation and decreased renal damage in a hyperglycemia-induced nephropathy mice model; importantly these effects were associated with a reduction in IL17A production through the up-regulation of the suppressor of cytokine signaling 1 (SOCS1) expression (119). Similarly, miR-155 knockout mice presented a significant reduction of the Th17 immune response, less severe nephritis, and reduced histologic and functional injury in an experimental model of nephritis (120).

Another potential therapeutic opportunity is the administration of neutralizing antibodies against IL17A for the treatment of chronic human inflammatory diseases is being tested in several ongoing clinical trials (121, 122). Anti-IL17A biological agents like secukinumab, ixekizumab, and brodalumab were more effective than diverse anti-TNF alpha agents such as infliximab, adalimumab, and etanercept, but less so than certolizumab. Unfortunately, although it seems clear that IL17A could play an important role in the inflammatory component of kidney disease, there are no clinical trials focused on the effects of anti-IL17A antibodies on renal inflammatory diseases like DKD or even lupus nephritis (123).

Pentoxifylline (PTX) is a methyl-xanthine clinically used for the treatment of intermittent claudication with anti-inflammatory properties. Both experimental and clinical studies in diabetic patients support the renal protective action of PTX, evidenced by a decrease in proteinuria and, in some cases, an improvement in GFR (98–101, 108). It is important to note that this antiproteinuric capacity is related to an anti-inflammatory effect (102–107), being associated with significant reductions in TNFα levels (108, 109). Similarly, CKD patients report a reduction in TNFα, fibrinogen, and CRP levels and a stabilization in kidney function after PTX treatment (110). In these same patients, 1-year treatment of PTX in addition to the ARB background therapy resulted in a reduction in proteinuria and urinary levels of TNFα and MCP1 (111). In the PREDIAN trial, DKD patients under RAS blockade and treated with PTX also presented a stabilization in the progression of kidney disease after 2 years of follow-up (112). Importantly, this outcome was accompanied by reductions in proteinuria and urinary TNFα levels. Two recent meta-analysis in patients with DKD indicate, on the one hand, that the antiproteinuric effect of PTX in these patients is due to a lower production of pro-inflammatory cytokines (113) and, on the other hand, that PTX additively reduces both proteinuria and TNFα levels in patients treated with RAS inhibitors (114).

DISCUSSION

The dramatic increase in the incidence of diabetes has prompted new therapeutic strategies to treat DKD. Inflammation has revealed as a key factor in the development and progression of this complication, allowing the development of therapeutic approaches focused on the modulation of inflammatory processes. However, at the present time, clinical experience on the inhibition of inflammatory molecules and pathways in diabetic patients is scarce and more clinical trials are needed to examine their potential renoprotective efficacy.

AUTHOR CONTRIBUTIONS

JN-G, JD-C, and CF conceived the idea, prepared the final version, and finalized the manuscript. CF searched the literature, prepared the first and last part of the draft, and reviewed and prepared the revised version. FS-Q prepared the third part of the draft. AP-C, AG-L, EM-N, and CM-F reviewed the literature, critically reviewed the final version, and approved the final manuscript. All authors have substantially contributed to the preparation of the manuscript and agree with the study.

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MINI REVIEW
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Deficiency in the Screening Process of Fabry Disease: Analysis of Chronic Kidney Patients Not on Dialysis

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Fabry Disease (FD), a rare and progressive, X-linked lysosomal storage disorder, is caused by mutations in the α-galactosidase A (GLA) gene which leads to enzymatic deficiency of GLA. Misdiagnosed and undiagnosed FD cases are common for the variable FD phenotype, ranging from asymptomatic and/or impairment of single organs, which is typically seen in females and in patients with late-onset mutation, to multiple organ disease, which is frequently found in males with classic GLA mutation. Consequently, for an early diagnosis and an efficient treatment of FD, three different strategies of screening, new-born screening, high-risk screening and familiar screening, have been conducted. However, most of FD screening in the CKD population has been carried out in hemodialysis patients and kidney transplant recipients, for whom the renal damage is already irreversible, so the effectiveness of enzymatic replacement therapy is limited and delayed therapeutic intervention results in worse long-term outcomes. This review investigates the actual strategies of screening initiatives for the identification of FD, examining in detail those performed in CKD patients not on dialysis.

Keywords: Fabry disease, chronic kidney disease, screening, prevention, lysosomal disorders

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INTRODUCTION

Screening is considered one of the main processes to detect healthy patients affected by latent or early symptomatic stages of the disease (1). It allows an early diagnosis and an effective treatment of the disease, reducing the cost of medical care and improving the quality of life.

According to the classic Wilson and Jungner criteria[1], this procedure takes into account whether the disease is adequately understood and is characterized by specific findings, including a delay between the detection of risk factors and the onset of the disease.

Due to the fact that chronic kidney disease (CKD) occurs asymptomatically and is clinically silent in early stages, large-scale national screening programs (2) have been only conducted in some countries, showing more than 10% presence of markers for renal damage (3).

However, a global awareness campaign, promoted by international and national societies of nephrology to reduce the impact of CKD, confirmed that risk factors, such as high blood pressure, were found in roughly 10% of both adult and adolescent populations (4–6).

[1] Available online at: https://apps.who.int/iris/bitstream/10665/37650/17/WHO_PHP_34.pdf (accessed December 1, 2020).

Although the etiology of CKD is primarily due to diabetes and hypertension (7–9), a considerable proportion of unknown CKD cases are caused by undetected genetic diseases, including Fabry Disease (FD).

This review analyzes the recent developments on different approaches of screening FD programs with a specific focus on those which were performed on not dialysis-dependent chronic kidney disease (NDD-CKD) patients.

Pathogenesis and Clinical Manifestation of Fabry Disease

Over the last 20 years, an increasing amount of the Nephrology community's attention has been captured by FD, a hereditary, X-linked, lysosomal storage disease (10). It is caused by deficiency of α-galactosidase A (GLA) and the gene encoding GLA is located on long arm of X-chromosome (Xq21-22) (11). The enzyme deficiency leads to the accumulation of glycosphingolipids, mainly globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-GB3), in lysosomes of skin, eye, kidney, heart, brain, and the peripheral nervous system (12–14). FD is a progressive disease that worsens with the age. Clinical manifestations ensue during childhood or early adolescence with angiokeratomas, lenticular and corneal opacity, microalbuminuria or proteinuria, gastrointestinal pain, recurrent fever, alterations of thermoregulation (i.e. hypohidrosis or anhidrosis) and in the peripheral nervous system (15).

In adulthood, patients show manifestations of FD in the cardiac, cerebrovascular, and renal systems (16–18). In particular, renal impairment is one of most pervasive signs of FD (19). Firstly, a glomerular hyperfiltration, likely due to the activation of the sympathetic nervous system, increased oxidative stress and inflammatory cytokines, associated with a mesangial cell proliferation occurs (20). Later, early morphologic findings of impaired renal function, such as the development of a focal and segmental glomerulosclerosis and vascular changes, are observed (21). With the progression of the disease, proteinuria and progressive renal failure leading to dialysis (ESRD) appears in ∼50% and 20% of male and female FD patients, respectively (22).

This clinical picture, which is considered the classic type of FD, leads to death of male patients during the fourth or fifth decade of life (23). By contrast, female patients commonly show milder involvement due to random inactivation of the X-chromosome, determining the development of organs which are chimeras of normal and affected cells (24).

Besides the classic type, milder and late-onset forms of FD have been described as atypical variants. They are characterized by non-specific symptoms and/or involvement of single organ, also defined cardiac and renal variants (25). Therefore, diagnosis of FD in patients with atypical variants is often difficult and it is generally made in the more advanced stages of disease (26).

However, early diagnosis is compulsory in patients with FD since early therapeutic interventions, such as enzymatic replacement therapy and chaperone therapy, significantly stabilize the disease and prevent the progression of renal, cerebrovascular and cardiovascular complications (27–29).

Screening in CKD Patients Not on Dialysis

Unfortunately, the true prevalence of FD is not well defined. Initially, underestimated prevalence (1:117,000–1:833,00) of FD was found in general population (30–32), mainly because of the lack of clear genotype-phenotype correlation. Subsequently, large new-born and high-risk screening studies showed higher FD prevalence than previously expected. A recent re-analysis of studies for pathogenic GLA mutation, conducted on high-risk populations, showed that the prevalence of FD in 5,491 (4,054 males and 1,437 female) patients with no definitive cause of left ventricular hypertrophy was 0.94% in males and 0.90% in females and among the 5,978 (3,904 males and 2,074 females) young patients with unexplained stroke, 0.13% were males and 0.14% were females (33) (more details are described in the next paragraphs). Nevertheless, few researchers have addressed the problem of screening in NDD-CKD population to detect patients affected by FD, determining its prevalence.

Although the ERBP guidelines (34), in 2013, recommended screening for FD in NDD-CKD patients, no study had been published to support this ungraded statement at the time. However, a singular study performed by Andrade et al. (35), screened 499 renal male patients (of which 141 not on dialysis, 159 on hemodialysis, 59 on peritoneal dialysis, and 138 with a kidney transplant) by plasma GLA assay in a large Canadian province (population ∼4.25 million). No new FD cases were found despite the fact that multiple racial populations, including 71 white and 26 Asian, were exanimated.

For the first time in 2016, Turkmen et al. (36) showed in their TURKFAB Study the FD prevalence was 0.95% (3/313) in stage 1–5 NDD-CKD patients with unknown etiology of 10 Turk centers. Furthermore, 8 other patients with both FD and CKD were identified from two index patients, for a total of 11 cases of 386 screened people.

To date, only four FD screening studies reported data about CKD patients, the TURKFAB Study (T1) described above (36), one conducted in the Aegean region of Turkey (T2) (37), one in Taiwan (T3) (38) and, recently, one in Canada (C1) (39) (**Table 1**).

In a total of 3,175 (313 in T1; 1,453 in T2; 1,012 in T3; 397 in C1) follow-ups of stable CKD (3–5 stages in T2 and C1; 1–5 stages in T1 and T3) patients in 23 centers (10 in T1; 7 in T2; 2 in T3; 4 in C1), the prevalence ranged between 0% and 0.95%, of which 0.4–1.8% and 0.0–1.0% in 2,255 males and 920 females, respectively.

Surprisingly, a slightly lower average age of 16 male cases (47 years) compared with 4 female cases (49 years) was identified. This finding is in contradiction with the suggestion of performing FD screening in men under 50 years and in all ages for women, according to ERBP working groups (34). Furthermore, stratifying the cases by type of GLA mutation, 9 males with classic mutations were younger than 7 males with atypical mutations (35 and 62 years, respectively). This result seems to indicate that FD screening should be conducted in men of all ages to identify not only the classical GLA mutations, but also the late-onset GLA mutations.

All CKD patients were screened by analyzing GLA activity in dried venous blood spots (DBS) and, when enzyme activity

TABLE 1 | Characteristics of Fabry disease screening studies in chronic kidney disease patients not on dialysis.

Study	Turkmen et al. (T1)	Yeniçerioglu et al. (T2)	Lin et al. (T3)	Auray-Blais et al. (C1)
Overall screened	313	1453	1012	397
Males screened	167	797	1012	279
Females screened	146	656	0	118
Screening test	Enzymatic assay	Enzymatic assay	Enzymatic assay	Gb3 and Lsyso-Gb3 urinary assay
Confirmation test	DNA test	DNA test	DNA test	Enzymatic assay or/and DNA test
FD cases, *n*	11	3	6	0
FD prevalence, %	0.95	0.2	0.59	0
Mutation classic (*n*)	p.N34H (8) p.F229V (2) p.358delE (1)		p.T410A (1) p.G138E (1)	
Mutation atypical (*n*)		A143T variant (1) D313Y variant (2)	c.639 + 919G > A (3) p.P210S (1)	
GFR, ml/min	87 (23–126)*	33.9 ± 3.9**	50.5 ± 28.0**	
Proteinuria	0.26 (0.07–5.6)*g/g***	50 ± 6.2** mg/24 h	2.0 ± 2.4** g/24 h	
FD cases with cardiovascular complications, *n*	2	2	4	
FD cases with cerebrovascular complications, *n*	0	0	2	
FD cases with other signs or symptoms, *n*	9	2	2	

**Median (IQR); **Mean ± SD; ***Spot urine total protein/creatinine ratio; FD: Fabry Disease.*

was lower than 1.2 micromole/L/h, the GLA gene was sequenced, except for 397 patients of C1 study in which an innovative method, the dosage of Gb3 and lyso-GB3 in dried urine spots, was used. Although the enzymatic activity is normal or slightly lower in roughly 30% of FD females, only one study (T3) excluded female to avoid false-negative results. Accordingly, the prevalence of FD could be underestimated in approximately one-third (females: 146 in T1; 656 in T2) of screened CKD patients. Furthermore, in the Canadian study (C1) 43% of suspected FD patients, screened with the urine test, died in the course of study before an enzymatic or genetic test was performed.

Ancillary, Nakagawa et al. (40) screened 2,325 Japanese patients affected by typical cardiac, renal, or neurological FD complications, including 374 (16.1%) subjects with proteinuria of which 129 (34.5%) were female and 245 (65.5%) were male. Of ten patients with GLA mutations (6/10 were pathogenic), two had a nephrotic range proteinuria, namely a 37-year-old female with juvenile minor ischemic stroke and a 56-year-old male with CKD. Although data of renal function among the screened are missing, this study highlights the importance of investigations not only in CKD patients with unknown causes, but also in the presence of proteinuria, an early finding of renal damage. Furthermore, the results of the ongoing study, HISTOFAB[2] (ClinicalTrials.gov identifier: NCT03869554), might improve the understanding of the FD screening benefits also in CKD patients with undetermined etiology after a renal biopsy.

Finally, in order to help identify suspected FD patients, efficient and convenient screening protocols have been proposed (34, 41) and on the basis of available data, a comprehensive flow-chart is shown in **Figure 1**.

High-Risk Screening in Dialysis Patients and Kidney Transplant Recipients

In CKD population, most screening studies have been carried out in patients receiving hemodialysis treatment or after renal transplantation (42). Doheny et al. (33) showed an FD prevalence of 0.21% in 23,954 male dialysis patients (of these 66% had the classic phenotype and 34% had late-onset phenotype) and 0.15% in 12,866 female dialysis patients (of these 68.4 and 31.6% had classic and late-onset phenotype, respectively) among the 27 reviewed studies. Also, 5 (0.24%) pathogenic mutations among the 2,031 KTR males were reported; and, none among the 1,043 KTR females was found. By contrast, other recent studies demonstrated a higher percentage of GLA mutations in females compared to males (43). Notably, of the 265 KTR screened, Veroux et al. (44) identified six patients (one male and five female) with a pathogenic GLA mutation (2.3%) and one female patient with a benign variant of GLA mutation (0.3%). This higher incidence (2.3%) of KTRs with undetected FD could be explained by the presence of many

[2]Available online at: https://www.clinicaltrials.gov/ct2/show/study/NCT03869554?term=screening&cond=Fabry$+$Disease&draw=2&rank=5 (accessed December 1, 2020).

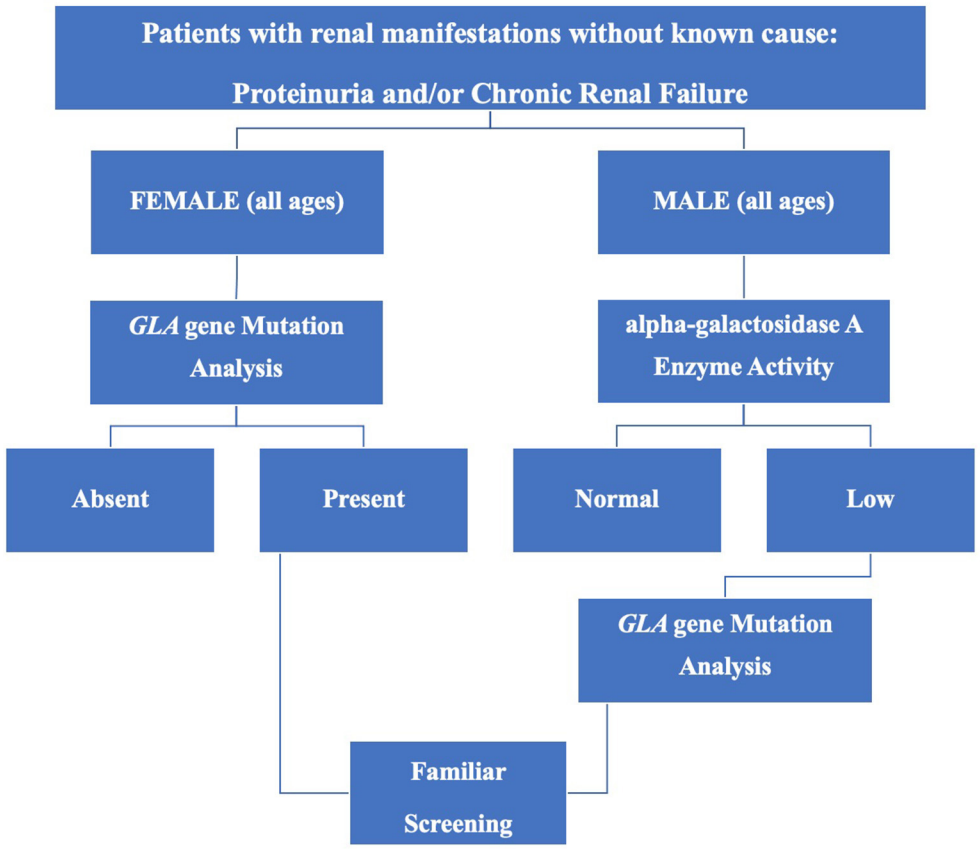

FIGURE 1 | Screening for Fabry disease in chronic kidney disease patients not on dialysis.

clusters of FD in the population and by the homogeneity of cohort.

Finally, high-risk screening in KTRs and dialysis patients is able to facilitate the detection of unrecognized FD patients even though the CKD and FD stages in these patients are remarkably advanced with a high risk of adverse outcomes and a poor efficacy of delayed treatment.

Family Screening

In order to increase the diagnostic rate of FD, currently, another different approach, the family screening, is suggested (45). It is crucial for X-linked transmission of FD. Indeed, for an index case with FD, a pedigree on average three generations is usually performed and approximately five family members are identified (46). Secondary analysis of some studies reported that the prevalence of FD among relatives of FD patients was about 2%, with higher percentage in males (47). By contrast, in a recent family screening study (48), of 1,214 relatives of 71 CKD patients with FD, it was found that the majority of the 115 (9.74%) family members with a GLA mutation were women (66.1%) and under 44 of age (74%). This conflicting data is not surprising since most of the screenings were performed with GLA activity dosage and/or with female exclusion. It may be related to lower costs of enzymatic analysis, which is adequate for screening diagnosis in males, compared to genetic sequencing, which is mandatory in females that often have normal GLA activity because of lyonization (22). However, the GLA genotyping should be performed in the patients screened with null or low GLA activity, to distinguish among the classic, the late-onset, and the benign mutations (49).

On the other hand, other studies reported only the number of cases, ranging between 8 and 24 cases (50–53). Recently, in a kidney transplant (KTR) screening study (44), seven FD probands' relatives were evaluated, finding ten females (five sisters, four daughters, and one nephew) and two males (one brother and one son) with pathogenic GLA mutation. 5/12 FD relatives received an early treatment before irreversible organ damage. This data confirms that the family screenings are able to delay the progression of FD and lead to better long-term outcomes.

New-Born Screening

In Northern Italy, Spada et al. conducted the first screening study to detect FD in the newborns, finding an incidence of GLA deficiency at 1/3,100. Most of identified GLA mutations were likely to result in atypical phenotype (54). Subsequently, several new-born screening studies confirmed higher prevalence of both FD classic males (ranging from 1:22,000 to 1:40,000) and FD atypical phenotype (1:1,000–1:3,000 in males and

1:6,000–1:40,000 in females) compared to other strategy of screening (55, 56).

However, according to the European Renal Best Practice (ERBP) (34) and the KDIGO controversies conference (45), the new-born screening to diagnose FD patients is not justified in general population for various clinical and ethical issues, including the high prevalence of atypical forms observed in the infants, as reported above. Although Hsu et al. highlighted that patients with late-onset phenotype are likely to have a silent progression of irreversible organ damage (57), the optimal time for screening late-onset variants remains debated. The best strategy to avoid irreversible damage in FD adults is an early diagnosis and effective treatment, but only a better characterization of the natural history of atypical FD forms could make the use of newborn screening advantageous.

CONCLUSION

Although expert working groups strongly recommend improvement in the screening process, few FD screening studies have been published for NDD-CKD patients and the data of FD prevalence is inconclusive. However, the importance of early detection of both CKD and FD is fundamental to start efficient treatment and to reduce the rate of morbidity and mortality. Clinicians should become conscious of the fact that FD needs to be taken into account in the differential diagnosis of CKD patients with unknown etiology even without other FD manifestations. Further multicenter systematic large-scale screening studies must be encouraged to assess the value of this screening strategy and to determine the true prevalence of FD in NDD-CKD patients.

AUTHOR CONTRIBUTIONS

YB: conceptualization. CA and AD: investigation. YB and PE: writing and editing. FF and AG: supervision. AS and RM: validation. All authors have read and agreed to the published version of the manuscript.

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Therapeutic Insights in Chronic Kidney Disease Progression

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Chronic kidney disease (CKD) has been recognized as a leading public health problem worldwide. Through its effect on cardiovascular risk and end-stage kidney disease, CKD directly affects the global burden of morbidity and mortality. Classical optimal management of CKD includes blood pressure control, treatment of albuminuria with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, avoidance of potential nephrotoxins and obesity, drug dosing adjustments, and cardiovascular risk reduction. Diabetes might account for more than half of CKD burden, and obesity is the most important prompted factor for this disease. New antihyperglycemic drugs, such as sodium-glucose-cotransporter 2 inhibitors have shown to slow the decline of GFR, bringing additional benefit in weight reduction, cardiovascular, and other kidney outcomes. On the other hand, a new generation of non-steroidal mineralocorticoid receptor antagonist has recently been developed to obtain a selective receptor inhibition reducing side effects like hyperkalemia and thereby making the drugs suitable for administration to CKD patients. Moreover, two new potassium-lowering therapies have shown to improve tolerance, allowing for higher dosage of renin-angiotensin system inhibitors and therefore enhancing their nephroprotective effect. Regardless of its cause, CKD is characterized by reduced renal regeneration capacity, microvascular damage, oxidative stress and inflammation, resulting in fibrosis and progressive, and irreversible nephron loss. Therefore, a holistic approach should be taken targeting the diverse processes and biological contexts that are associated with CKD progression. To date, therapeutic interventions when tubulointerstitial fibrosis is already established have proved to be insufficient, thus research effort should focus on unraveling early disease mechanisms. An array of novel therapeutic approaches targeting epigenetic regulators are now undergoing phase II or phase III trials and might provide a simultaneous regulatory activity that coordinately regulate different aspects of CKD progression.

Keywords: chronic kidney disease, end-stage kidney disease, drug therapy, SGLT2 inhibitors, renal fibrosis, inflammation

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INTRODUCTION

Chronic kidney disease (CKD) has been recognized as a leading public health problem worldwide. The global estimated prevalence of CKD is 13.4% (11.7–15.1%) (1) CKD has a powerful impact on global morbidity and mortality by increasing the risk of cardiovascular diseases, diabetes, and hypertension. The Global Burden of Diseases study estimated that 1.2 million deaths were due to kidney failure, and 19 million disability-adjusted life-years (DALYs) as well as 18 million years of

life lost from cardiovascular diseases were directly attributable to CKD (2). The DALYs associated with CKD have increased significantly in the last three decades (3). Even in early stages, CKD has been associated with an increased cardiovascular morbidity and mortality in both the general population as well as those patients with increased risk of CVD, therefore early detection of CKD as well as retarding the progression of kidney disease is deemed essential to reduce cardiovascular morbimortality as well as the economic burden caused by kidney disease (4).

It has been well-acknowledged that when the glomerular filtration rate (GFR) decreases below a critical level, CKD continues to progress unabatingly toward end-stage kidney disease (ESKD). The loss of a critical number of nephrons causes a vicious cycle of further nephron loss, and this damage is perpetuated even when the underlying cause of the disease is treated (5). There are several interconnected mechanisms that are involved in the progression of CKD, including hemodynamic and non-hemodynamic changes. The first occur in glomeruli, involving an increased glomerular capillary hydrostatic pressure and increased single-nephron glomerular filtration load, inducing glomerular injury and indirectly tubular injury. Hyperfiltration induces direct endothelial cell damage, increasing wall stress that may cause detachment and podocyte loss, and increased strain on the mesangial cells that can stimulate them to produce cytokines and extracellular matrix, such as transforming growth factor β (TGF-β) or isoforms of platelet-derived growth factor (6, 7). **Figure 1** summarizes the pathophysiological mechanisms of CKD progression. The mechanism by which tubulointerstitial fibrosis develops is incompletely understood. Fibrosis is part of the normal repair process that is triggered in response to injury, however, deregulation of this process leads to pathological accumulation of extracellular matrix proteins, mainly collagens. These processes result in the replacement of parenchymal tissue by extracellular matrix (8). **Supplementary Figure 1** shows how the interactions of different risk factors contribute in the progression of CKD.

In the last few decades some therapeutic agents have been identified as useful in retarding the progression of CKD. Several clinical trials have demonstrated that both renin-angiotensin-aldosterone (RAS) system inhibitors and more recently, agents that inhibit sodium-glucose cotransporter 2 (SGLT2) in the proximal convoluted tubule, reduce the loss of GFR in the long-term. The main mechanism of their action is based on the reduction of intraglomerular pressure, therefore proteinuric CKD get the most benefit from these therapies. On the other hand, and despite some positive results obtained from preclinical studies, there are no established strategies to modulate inflammation or delay progression of tubulointerstitial fibrosis. Hence, although several new therapeutic agents have been developed recently that look promising for the prevention of CKD progression, it is certainly necessary to develop agents that target different components of the fibrogenic cascade. **Figure 2** shows a proposed algorithm for prevention of CKD progression according to the current available evidence.

In this article we review the therapeutic targets for preventing CKD progression, focusing in the latest developments in treatment approaches for delaying CKD progression, we reflect on potential new biomarkers and novel therapeutic targets, as well as future lines of treatment, including regenerative therapies.

RAS BLOCKADE

Angiotensin-Converting Enzyme Inhibitor and Angiotensin Receptor Blocker Monotherapy

RAS blockade with angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs) is the cornerstone therapy to reduce proteinuria, CKD progression, and cardiovascular risk. These benefits appear to be comparable between ACEI and ARBs when they are used in equivalent doses, and carry comparable adverse effects besides cough which is exclusive to ACEi.

RAS blockade has been shown to be renoprotective in diabetic patients with microalbuminuria or overt nephropathy, as well as in non-diabetic CKD. There is an increasing body of evidence which shows that RAS blockade at doses higher than the maximum antihypertensive dose may afford additional renoprotection. In the SMART study, 269 patients who had persistent proteinuria (>1 g/day) despite seven weeks of treatment with dose of candesartan (16 mg/day) were randomized to receive candesartan at a dose of 16, 64, or 128 mg/day. After 30 weeks, the maximal dose of candesartan (128 mg/day) achieved an additional decrease in proteinuria of 33%. Reductions in BP were not different across the three treatment groups, although elevated serum potassium levels (K^+ >5.5 mEq/L) led to the early withdrawal of 11 patients (9).

Combination Angiotensin-Converting Enzyme Inhibitor and Angiotensin Receptor Blocker Therapy

Theoretically, the inhibition of RAS system at several different steps of the pathway would lead to a more complete inhibition and therefore was thought to maximize the renoprotective effects of RAS blockade. In several small clinical studies, more complete inhibition of the RAS with combination ACEI and ARB treatment was associated with additional lowering of proteinuria. A large trial of combination therapy in patients with hypertension and increased cardiovascular risk reported no additional benefit with respect to cardiovascular outcomes, and although combination therapy was associated with a greater reduction in proteinuria than monotherapy, they also induced a higher incidence of acute renal failure. It should be noted, however, that subjects were selected on the basis of cardiovascular risk profile and that the majority did not have reduced GFR or proteinuria. A similar study in patients with type 2 diabetes and albuminuria over 300 mg/g also found no benefit with respect to the primary outcome of CKD progression, ESKD, or death in participants randomized to combination ACEi and ARB therapy vs. monotherapy. Again, those patients receiving combination therapy showed a significantly higher incidence of acute kidney injury (AKI) and hyperkalemia. However, in neither of the two studies was dual therapy compared to an equipotential dose of

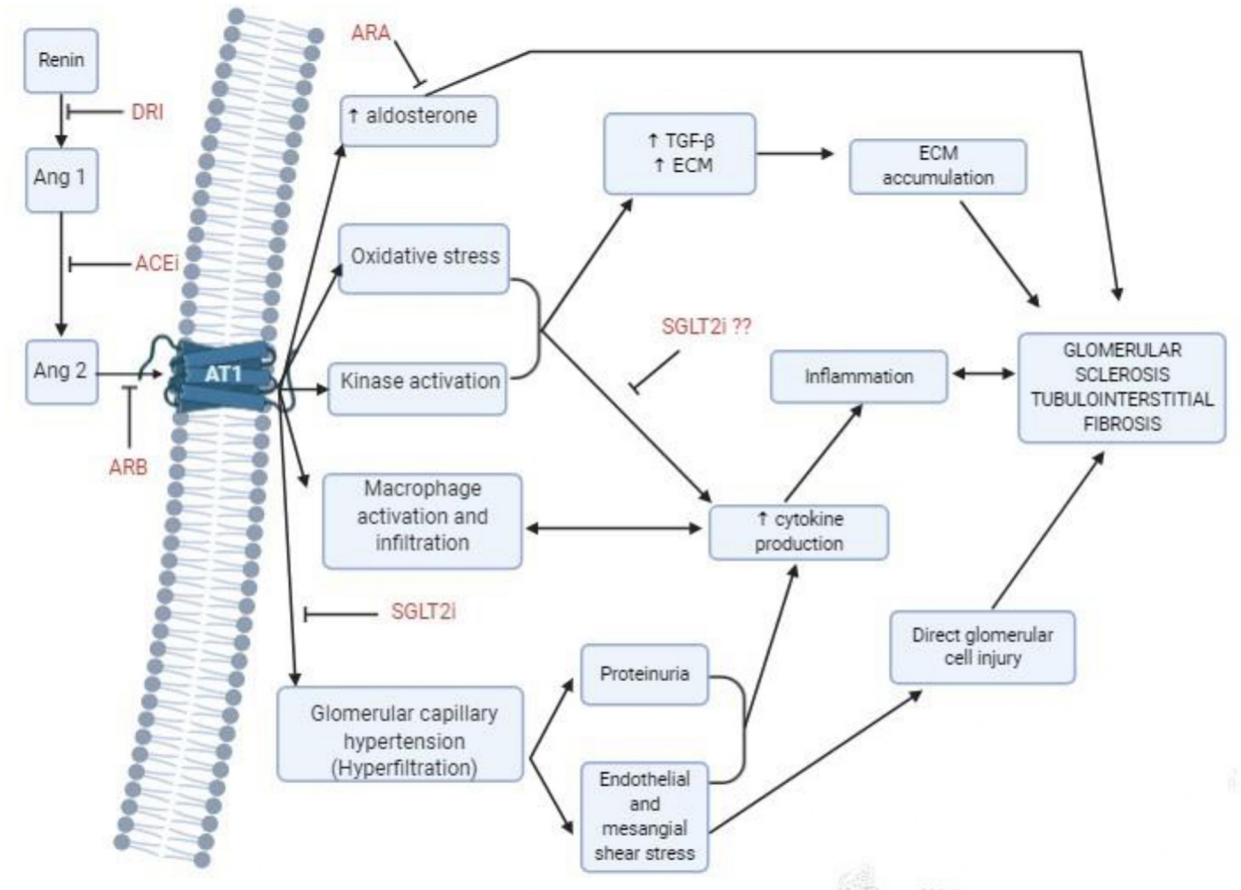

FIGURE 1 | Mechanism of progression of chronic kidney disease, and treatment options to delay progression. ACEi, Angiotensin converting enzyme inhibitors; Ang, Angiotensin; ARA, Aldosterone receptor antagonists; ARB, Angiotensin receptor blockers; AT1, Angiotensin II Type 1 receptor; DRI, Direct renin inhibitors; SGLT2i, Sodium-glucose cotransporter 2 inhibitors.

either drug in monotherapy, and therefore the dual blockade effect could not be analyzed separately from the effect carried by the use of different dosage. In the PRONEDI trial, in patients with overt diabetic nephropathy, the combination therapy consisting of lisinopril 20 mg plus irbesartan 300 mg was compared with the use of lisinopril at a dose of 40 mg and irbesartan at a dose of 600 mg in monotherapy. In this study, proteinuria decreased in the three groups similarly, with no differences in CKD progression or adverse effects (including acute kidney injury and hyperkalemia) after a median follow-up of 32 months, indicating that optimization of RAS blockade depends on dosage rather than the use of a single or combined RAS blocker (10).

Our goal should be the maximum well-tolerated RAS blockade dose, regardless of whether it is achieved with ARB, ACEI in monotherapy or their combination.

Renin Inhibitor Therapy

The development of direct renin inhibitors made it possible to inhibit the RAS at its rate-limiting step (the conversion of angiotensinogen to angiotensin I) and thereby to achieve more complete blockade. Two early randomized trials reported additional lowering of albuminuria in subjects with diabetic nephropathy receiving the combination of a direct renin inhibitor and ARB therapy vs. ARB therapy alone (11, 12). However, a large randomized trial (ALTITUDE) that included 8,561 people with type 2 diabetes and albuminuria or cardiovascular disease was stopped prematurely. Despite greater lowering of blood pressure and albuminuria with the combination of direct renin inhibitors and ARB therapy, no benefit was observed with respect to cardiovascular events, CKD progression, ESKD, or death vs. ARB monotherapy. Additionally, combination therapy was associated with a higher incidence of hyperkalemia and hypotension (13).

Therefore, Aliskiren may be a suitable alternative RAS blocker for those intolerant to either ACE inhibitors or ARBs.

Use of RAS Blockade in the Geriatric Population and in Advanced CKD

It is not known whether the benefits from RAS inhibition in proteinuric CKD can be extended to elderly patients, since they

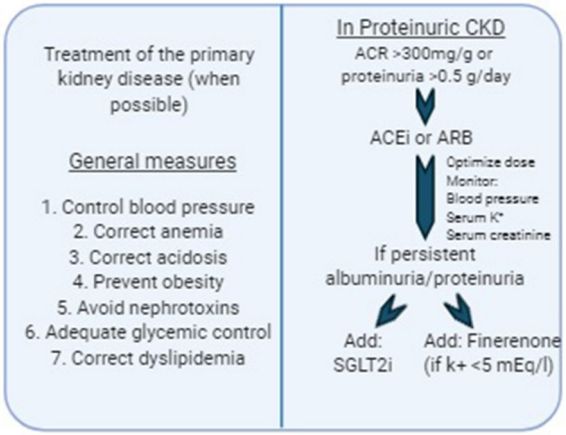

FIGURE 2 | Therapeutic approach for delaying CKD progression.

Non-hemodynamic Effects of Angiotensin II

Angiotensin II (Ang II) appears to participate in the development of tubulointerstitial fibrosis, mediated through one of the Ang II type 1 receptors that are present in the glomerulus. Ang II also contributes in cytokine and chemokine-mediated recruitment of inflammatory cells into the kidney. Overall, these effects can generate profibrotic factors such as TGF-β, connective tissue growth factor, epidermal growth factor (EGF), and other chemokines. In fact, regression of glomerulosclerosis has been observed in rodents on RAS blockade but this phenomenon has not been observed in humans.

Clinical trials have not specifically addressed the question of whether RAS blockade decreases renal fibrosis. In some *post-hoc* studies, some biomarkers of inflammation and serum fibrosis, such as interleukin 6 (IL-6) or Dickkopf 3 (DKK3), were measured sequentially; treatment with RAS blockade did not modify their levels (Sanchez-Alamo et al., submitted). Kidney tissue studies are likely needed to describe the local mechanisms that pass unnoticed in serologic test studies (**Table 1**).

are often underrepresented in clinical trials and their risk for experiencing the outcome of interest during their remaining lifetime may be different than for the younger population. In a study that included 790,342 patients over 70 years old, the number of patients necessary to treat to prevent one case of ESKD ranged from 2,500 among patients with an GFR 60 ml/min/1.73 m^2 and negative or trace proteinuria and GFR 45–59 ml/min/1.73 m^2 and no dipstick proteinuria to 16 among those with GFR 15–29 ml/min/1.73 m^2 and $\geq$2+ dipstick proteinuria. Overall, 91% of patients included in this large series should be considered as having a "low or moderate risk for kidney disease progression" and the number of patients necessary to treat to avoid an event was >100 (14).

These findings remind us that the benefit obtained from RAS inhibition for renoprotection occurs in patients with protein excretion of >1g/day, while the vast majority of patients over the age of 70 years with CKD do not belong to this category. In fact, they would be unnecessarily exposed to the adverse effects of RAS inhibition.

Another important question is whether the benefit from ACEi or ARBs extends to patients with advanced proteinuric CKD, in which the risk of hyperkalemia is significantly increased. This point was addressed in a study in which 422 patients with non-diabetic CKD were assigned to benazepril or placebo on top of standard of care. A specific group of patients ($n = 281$) included in this study had advanced CKD with serum creatinine between 3.1 and 5 mg/dL. The benefit from ACE inhibitors in terms of reaching ESKD or doubling of serum creatinine was present even in those patients with advanced CKD and especially when proteinuria was above 1 g/day. Serious hyperkalemia was similar with benazepril and placebo (15). However, the results of this study are not generalizable to all patients with proteinuric CKD, since the patients were rigorously selected and closely monitored during the study for potassium control, and also dietary intake of potassium was likely lower than that in Western patients.

ALDOSTERONE ANTAGONIST THERAPY

Spironolactone and the more selective aldosterone antagonist eplerenone have substantial antihypertensive, cardioprotective, and antiproteinuric effects even at low doses, and in the presence of combined ACEi and ARB therapy. Unlike Ang II, aldosterone is not involved in intraglomerular changes, and its mechanism of benefit may involve blockade of aldosterone effects on impaired tubuloglomerular feedback, endothelium damage and on fibrosis (34). There have been no long-term clinical trials that have studied to date the use of spironolactone or eplerenone in high-risk CKD patients, mainly due to the high risk of hyperkalemia.

Finerenone is a more selective non-steroidal mineralocorticoid receptor antagonist that reduced albuminuria in several short-term clinical trials. Preliminary studies showed that lower doses of finerenone were needed to achieve similar cardiovascular and renal effects compared to steroidal mineralocorticoid receptor antagonists (spironolactone and eplerenone), and induced less hyperkalemia. Its tissue distribution is well-balanced between cardiac and renal tissues, contrary to spironolactone and eplerenone (6 and 3 times higher concentrations in renal tissues compared to cardiac tissues, respectively) (35). This could explain the differences in mechanism and incidence of adverse events such as hyperkalemia. Recently, a large randomized clinical trial (FIDELIO) that included 5,734 patients with type 2 diabetes and CKD with albuminuria between 300 and 5,000 mg/g showed that patients with finerenone had a decreased risk of kidney disease progression or death compared to placebo, after a median follow-up of 2.6 years. Although the incidence of hyperkalemia was higher in the finerenone group, the rate of discontinuation due to hyperkalemia was relatively low (2.3%) (29).

Hyperkalemia is the main limiting factor for the use of this therapeutic group and ARB blockade. This effect could

TABLE 1 | Summary of the main clinical trials studying RAS blockade, aldosterone antagonism, endothelin antagonism, and bicarbonate therapy in delaying CKD progression.

Clinical Trials	Studied agents	Year	*n*	Baseline GFR (ml/min/1.73 m^2)	Baseline proteinuria	Patients	Follow-up period	Primary outcomes	Results
RAS blockade									
Lewis et al. (16)	Captopril vs. placebo	1993	409	84 ± 46	2,500 ± 2,500 g/day	DM nephropathy with ACR ≥ 500 mg/g and SCr <2.5 mg/dl	3 years	SCr doubling ESKD or death	Risk reduction 48% (16–69%, $p = 0.006$) Risk reduction 50% (18–70%, $p = 0.007$)
IDNT (17)	Irbesartan vs. amlodipine vs. placebo	2001	1,715	SCr 1.7 ± 0.5 mg/dl	2.9 (1.6–5.4)	DM nephropathy	2.6 years	SCr doubling, ESKD or Death	Risk reduction 20% vs. placebo ($p = 0.02$), 23% vs. amlodipine ($p = 0.006$)
RENAAL (18)	Losartan vs. placebo	2001	1,513	SCr 1.9 ± 0.5 mg/dl	ACR 1237 mg/g	DM nephropathy	3.4 years	SCr doubling, ESKD, or Death	Risk reduction 16% (2–28%), $p = 0.02$
ABCD (19)	Enalapril vs. nisoldipine	2000	470	81.8 ± 7.1	6.3 ± 0.2 mg/g	Normotensive Type 2 DM	5.3 years	Change in SCr	NS
ONTARGET (20)	Ramipril + Telmisartan vs. monotherapy	2008	25,620	73.6 ± 19.6	ACR 7.2 (6.9–7.4) mg/g	Type 2 DM with end-organ damage or atherosclerotic vascular disease	56 months	SCr doubling, ESKD, or death	HR = 1.09 [1.01–1.18]
VA-NEPHRON (21)	Lisinopril + Losartan vs. monotherapy	2013	1,448	53.6 ± 15.5	ACR 842 (495–1,698) mg/g	DM nephropathy	2.2 years*	GFR decline ≥30 ml/min if baseline >60 ml/min, or >30% if baseline <60 ml/min, ESKD, or death	HR = 0.88 [0.70–1.12]
ALTITUDE (13, 22)	Aliskiren + ACEi or ARB vs. monotherapy	2012	8,561	57 ± 22	ACR 206 (57–866) mg/g	Type 2 DM with CKD or CVD	32.9 months*	SCr doubling, ESKD, or death	HR = 1.03 [0.87–1.23]
PRONEDI (10)	Lisinopril + Irbesartan vs. monotherapy	2013	133	49 ± 21	1.32 g/24 h (1.1–1.62)	DM nephropathy	32 months	>50% increase in baseline SCr, ESKD, or death	HR = 0.96 [0.44–2.05] vs. lisinopril HR = 0.90 [0.39–2.02] vs. irbesartan
AASK (23)	Ramipril vs. Metoprolol vs. Amlodipine	2002	1,094	45.6 ± 13	0.61 ± 1.05	Hypertensive kidney disease	4.1 years	50% GFR reduction, ESKD, or death	R v M= Risk reduction 22% (1–38%, $p = 0.04$)
REIN (24)	Ramipril vs. placebo	1997	352	40.2 ± 19	5.6 ± 2.8	Non-diabetic CKD	31 months	Change in GFR, Time to ESKD, Time to overt proteinuria	52% decreased risk of overt proteinuria ($p = 0.005$). 56% decreased risk of ESKD ($p = 0.01$). No difference in rate of GFR decline
IRMA-2 (25)	Irbesartan vs. placebo	2001	590	108 ± 2	53.4 ± 2.2	Type 2 DM and Hypertension	2 years	Onset of overt albuminuria	Irbesartan 300 mg HR = 0.30 (0.14-0.61)
DETAIL (26)	Telmisartan vs. Enalapril	2004	250	91.4 ± 21.5	UAE 46.2 (4–1,011) μg/min	DM nephropathy	5 years	GFR change	

(Continued)

TABLE 1 | Continued

Clinical Trials	Studied agents	Year	*n*	Baseline GFR (ml/min/1.73 m^2)	Baseline proteinuria	Patients	Follow-up period	Primary outcomes	Results
ALLHAT (27)	Lisinopril vs. Amlodipine vs. Chlorthalidone	2012	5,545	50.2 ± 8.6	n/a	Hypertensive kidney disease	8.8 years	ESKD	HR = 0.91 [0.73–1.14] vs. Chlorthalidone
Aldosterone antagonists									
Rossing et al. (28)	Spironolactone vs. placebo	2005	21	74 ± 6	UAE 1,566 (655–4,208) mg/day	DM nephropathy	16 weeks	Albuminuria Change in GFR	−33% reduction (−41 to −25), $p < 0.001$ −3 (−6 to 0.3) ml/min, $p = 0.08$
FIDELIO (29)	Finerenone vs. placebo	2020	5,734	44.3 ± 12.6	ACR 852 (446–1,634) mg/g	DM nephropathy	2.6 years	40% decline in eGFR, ESKD, or death	HR = 0.82 [0.73–0.93]
Endothelin antagonists									
ASCEND (30)	Avosentan vs. placebo	2010	1392	33.5 ± 11	ACR 163 (83-280) mg/g	DM nephropathy	4 months*	SCr doubling, ESKD or Death	NS
SONAR (31)	Atrasentan vs. placebo	2019	2648	44 ± 13.7	797 (462–1480) mg/g	DM nephropathy	2.2 years	SCr doubling or ESKD	HR=0.65 [0.49-0.88]
Bicarbonate									
de Brito-Ashurst et al. (32)	Oral sodium bicarb vs. SOC	2009	134	20.1 ± 6.5	1.7 ± 0.8	CKD stage 4	2 years	Rate of CrCl decline Rapid CrCl decline (>3 ml/min/yr ESKD	5.93 ml/min/yr vs. 1.88 ml/min/yr, $p < 0.001$ RR: 0.15 [0.06–0.40] RR: 0.13 [0.04–0.40]
UBI (33)	Oral sodium bicarb vs. SOC	2019	740	30 ± 12	0.2 (0.07–0.4)	CKD Stage 3–5	36 months	SCr doubling ESKD Death	0.36 [0.22–0.58] 0.50 [0.31–0.81] 0.43 [0.22–0.87]

DM, Diabetes mellitus; ACR, Urine Albumin-to-creatinine ratiol; UAE, Urine albumin excretion; SCr, Serum Creatinine; CrCl, Creatinine clearance; GFR, Glomerular filtration rate; ESKD, End-stage kidney disease; CVD, Cardiovascular disease; ACEi, Angiotensin-converting enzyme inhibitor; ARB, Angiotensin receptor blocker; SOC, Standard of care; HR, Hazard ratio (95% confidence interval); RR, Relative risk (95% confidence interval).

** Terminated early due to increased adverse events.*

TABLE 2 | Summary of kidney outcomes in clinical studies with SGLT2 inhibitors.

Clinical Trial	CANVAS	DECLARE-TIMI	EMPA-REG	CREDENCE	CVD-REAL 3	DAPA-CKD
SGLT2i	Canagliflozin	Dapagliflozin	Empagliflozin	Canagliflozin	Different kinds of gliflozins*	Dapagliflozin
n	10,142	17,160	7,020	4,401	35,561	4,304
GFR (ml/min/1.73 m^2)						
Mean	76	85	74	56	91	43
Range	>30	>60	20–90	30–90	>60	25–75
ACR (mg/g)						
<30	70%	69%	60%	1%	N/A	200-1000: 51.3%
30–300	22%	24%	29%	11%		>1,000: 48.7%
>300	8%	7%	11%	88%		
Patients	Type 2 Diabetes	Type 2 Diabetes	Type 2 Diabetes	Diabetic Nephropathy	Type 2 Diabetes	Proteinuric nephropathy (diabetic and non-diabetic nephropathy)
Renal outcomes HR [95% CI]	Albuminuria progression 0.73 [0.67–0.79]	≥40% GFR decrease, ESKD, or RR- death 0.53 [0.43–0.66]	Scr doubling, GFR <45 ml/min, dialysis, RR- death 0.54 [0.40–0.75]	Scr doubling, ESKD, or RR- death 0.66 [0.53–0.81]	>50% GFR decrease or ESKD 0.49 [0.35–0.67]	>50% GFR decrease, ESKD, RR- death 0.56 [0.45–0.68]
	≥40% GFR decrease, ESKD, or RR-death 0.60 [0.47–0.77]	ESKD 0.31 [0.13–0.79]				

**Several kinds of glifozins (Dapagliflozin 58%; Empagliflozin 34%; Canagliflozin 6%; Pragliflozin; Tofogliflozin 2%; Luseogliflozin).*
Scr, serum creatinine; GFR, glomerular filtration rate; ESKD, end-stage kidney disease; RR-death, renal related death.

probably be mitigated with the use of the novel anti-hyperkalemia agents patiromer and sodium zirconium cyclosilicate, that have demonstrated a good tolerability and safety profile that remain consistent in the long term, in contrast with previous antihyperkalemic agents including resins that were generally poorly tolerated (36, 37) (**Table 1**).

SGLT2 INHIBITORS

SGLT2 inhibitors have shown remarkable additional benefits in delaying CKD progression on top of the standard RAS blockade. SGTL2i were originally developed to lower plasma glucose in type 2 diabetic patients, but large randomized controlled trials have demonstrated both renal and cardiovascular protection in both proteinuric diabetic and non-diabetic CKD patients and this effect cannot directly be explained by improved glucose control. **Table 2** summarizes the clinical trials that have studied renal outcomes with SGLT2 inhibitors.

The SGLT2i Cardiovascular outcome trials primarily demonstrated the efficacy of this treatment class in reducing CV risk among those with type 2 diabetes. However, secondary and exploratory analyses of these data highlighted a potential role for SGLT2i therapies in reducing adverse renal outcomes, even though the study populations were not generally considered to be at significant risk of progressive DKD (38).

The EMPA-REG OUTCOME and the CANVAS trials showed a reduced risk of new onset diabetic nephropathy and a large regression of albuminuria (return to ACR $\leq$ 3 mg/mmol), respectively (39, 40). Later, a large, international real-world study of patients with type 2 diabetes (CVD-REAL 3) demonstrated that initiation of SGLT2i therapy was associated with a slower rate of kidney function decline and reduced risk of major kidney events compared with other glucose-lowering drugs, with a mean follow-up was 14.9 months (41).

The CREDENCE trial was a double-blind, randomized study to assess renal treatment outcomes with canagliflozin (100 mg) among adults with type 2 diabetes and CKD. The relative risk of the composite of ESKD, doubling of the serum creatinine level or renal death was 34% lower in the canagliflozin group vs. the placebo. The components of ESKD were reduced by 32% and the risk of dialysis or kidney transplantation was reduced by 26% (42).

DAPA-CKD trial was halted early because of overwhelming efficacy, demonstrating that in patients with proteinuric CKD with or without diabetes, dapagliflozin significantly reduced the risk of 50% decline in GFR, ESKD, or death by 39% compared to placebo. The effects of dapagliflozin were similar in participants with type 2 diabetes and in those without type 2 diabetes (43).

Conclusive evidence concerning the mechanisms responsible for the renoprotective effects observed with SGLT2i is lacking, but several hypotheses have been proposed to explain the apparent slowing of DKD progression over time associated with these drugs. One of the proposed mechanisms is an improved glomerular hemodynamics due to constriction of the afferent arteriole induced by elevated adenosine levels secondary to increased membrane Na^+/K^+ ATPase activity, thus lowering glomerular hyperfiltration. Another mechanism is reduced tubular workload by reducing SGLT2 cotransporter activity and therefore reducing both the energy and aerobic

requirements of the system. An additional mechanistic proposal is that SGLT2i agents reduce inflammation and hypoxic injury in the kidney over time. Prolonged albuminuria and high intracellular glucose levels within proximal tubular cells trigger the expression of inflammatory cytokines, growth factors, and fibrotic mediators as well as the production of reactive oxygen species. Therefore, it is possible that the renoprotective effect of SGLTi may be partly due to a reduced local inflammatory response and fibrosis.

ENDOTHELIN ANTAGONISTS

Endothelins (ETs) are a family of vasoconstricting peptides. ET-1, the main isoform in human kidneys, is an important regulator of kidney function in health and disease, and its abnormal activation promotes kidney disease progression. It has been found to be increased in CKD patients and correlates well with kidney function and albuminuria. Two receptor subtypes are activated by ET-1; receptors type A and B. ET-A receptor provokes podocyte and mesangial lesions, oxidative stress, and inflammation leading to proteinuria and glomerulosclerosis. ET-B receptor inhibition induces fluid overload and precipitates congestive heart failure (44, 45).

Several studies in diabetic nephropathy, hypertensive kidney disease, and focal segmental glomerulosclerosis demonstrated that ET-1 inhibition can reduce proteinuria and improve kidney function (46). In a randomized clinical trial that tested avosentan, an ET-1A inhibitor, against placebo was ended prematurely after 4 months due to an increase in cardiovascular events in the avosentan arm, mainly due to fluid overload and congestive heart failure (30).

More recently, the SONAR study that included over 2,600 patients with type 2 diabetes, CKD with GFR between 25 and 75 ml/min and albuminuria between 300 and 5,000 mg/g and receiving optimum doses of RAS blockade, were randomized into receiving 0.75 mg atrasentan orally (another ET-1 receptor inhibitor that is more selective for ET-A receptor) or placebo after an enrichment period in which albuminuria decreased by more than 30% with no fluid overload. After median follow up of 2.2 years, Atrasentan reduced the risk of doubling serum creatinine or reaching end-stage kidney disease, with no significant differences with placebo in hospitalizations due to heart failure (3.5 vs. 2.6%) however there was a significant increase in fluid overload. Moreover, fluid retention was still more frequently observed in those treated with atrasentan (31).

Sparsentan, a dual endothelin-angiotensin II antagonist, showed promising results in reducing proteinuria in patients with FSGS after 8 weeks, but 16.4% of patients with sparsentan suffered from orthostatic hypotension and 12.3% had fluid retention, although none were considered serious and no patients were withdrawn from the study (47). The DUPLEX study is ongoing, which will evaluate the safety and long-term nephroprotective effects of sparsentan in FSGS patients (48) (**Table 1**).

BICARBONATE

Recent data support that a component of CKD progression is mediated by mechanisms used by the kidney to increase acidification in response to an acid challenge to systemic acid-base status. An acid challenge to systemic acid-base status increases nephron acidification through increased production of endothelin, aldosterone, and angiotensin II, each of which can contribute to kidney inflammation and fibrosis that characterizes CKD (49). Several small clinical trials had highlighted a potential benefit of oral bicarbonate in delaying CKD progression. A recent multicenter, randomized, controlled trial that included 740 patients with stage 3–5 CKD examined the effect of treating metabolic acidosis with oral sodium bicarbonate compared to standard of care to delay CKD progression. Patients that received sodium bicarbonate had a 64% reduced risk of creatinine doubling, a 50% lower risk of dialysis initiation, and showed a reduction of 57% in all-cause mortality, while there was no significant risk of blood pressure elevation, heart failure, or hospitalizations (33). These results confirmed those of smaller previous studies, and show that correction of metabolic acidosis with sodium bicarbonate is a beneficial tool to delay CKD progression and improve patient survival (**Table 1**).

FIBROSIS, INFLAMMATION, OTHER POSSIBLE THERAPEUTIC TARGETS

Thus far, multiple clinical trials have been designed with the aim of delaying the progression of CKD. Most of these clinical trials have aimed to limit glomerular hyperfiltration, but there are other factors such as parenchymal cell loss, chronic inflammation, and fibrosis that are known to contribute to CKD progression and need to be addressed. Therefore, only a more holistic approach targeting all of these factors will likely achieve a more complete response and better kidney outcomes, aiming not only to delay kidney progression but also to reverse CKD.

However, despite promising results in preclinical studies, therapeutic interventions targeting "other mechanisms" in humans such as cytokines, transcription factors, developmental and signaling pathways, and epigenetic modulators, particularly microRNAs, have been disappointing, and no additional treatments are available to date.

Bardoxolone, a drug that activates nrf2, a transcription factor that controls various cytoprotective proteins, was studied to target inflammation and oxidative stress. Bardoxolone improved GFR in type 2 diabetics with CKD, an effect that persisted throughout the 52 weeks of study (50). Nevertheless, a follow-up phase III study of bardoxolone that targeted renal events was stopped for safety concerns because of excessive serious adverse events and mortality in the bardoxolone arm (51). No further studies targeting this pathway are currently underway.

Novel therapies that are currently under evaluation target renal fibrosis, including the anti-fibrotic agent pirfenidone. Although its mechanism of action is not fully understood, it interrupts the TGF-β pathway. The main clinical trial was done in patients with diabetic nephropathy, with significant improvement in GFR compared to placebo, however it failed to reduce proteinuria (52). Another study conducted in FSGS patients also observed a failure to reduce proteinuria with pirfenidone (53), suggesting that this drug can ameliorate progression of kidney disease but without reducing proteinuria, due to either a structural improvement in interstitial injury or due to a hemodynamic effect on GFR.

Pentoxifylline is being studied for its inflammatory modulation and anti-oxidative stress, and has shown in several studies a significant reduction in proteinuria when it was added to RAS blockade in CKD patients (54).

BIOMARKERS

It is still challenging to predict progression in CKD. This is mainly due to a scarcity in sensitive and specific biomarkers for predicting CKD progression early. The development of end-stage kidney disease may take several years, therefore surrogate endpoints such as albuminuria and serum creatinine have been increasingly used in trials over hard endpoints to predict CKD progression (55–57). Although albuminuria may allow for an early management of CKD, the reduction of albuminuria does not always translate into slowing CKD progression, and also not all causes of CKD develop albuminuria (58). Several studies have shown that using GFR decline of 30 or 40% as alternative surrogate endpoints of CKD progression may allow a more prompt detection, allowing for trials with shorter follow-up periods (59, 60). Nevertheless both biomarkers are established kidney injury markers and we are still in search for more biomarkers that identify patients at high risk of progression at an earlier phase, allowing for earlier therapeutic intervention, as well as more specific biomarkers that could provide better tools for individual adjustment of treatments.

Dickkopf-3 (DKK3) is a stress-induced, renal tubular epithelial-derived glycoprotein that induces tubulointerstitial fibrosis through its action on the canonical Wnt/β-catenin signaling pathway. Elevated urinary DKK3 levels identify patients at high risk of rapid decline in GFR during the following 12 months regardless of the etiology of CKD. Moreover, uDKK3 levels are directly associated to the degree of tubulointerstitial fibrosis in kidney biopsies (61). In recent studies, it has been observed that high pre-surgery uDKK3 levels were an independent predictive factor for the development of postoperative AKI and for the subsequent loss of kidney function (62).

Moreover, several biomarkers of cardiovascular risk in CKD have been assessed, and could be classified into prognostic biomarkers, such as cardiac troponins and NT-ProBNP, Cystatin C, β2-Microglobulin, Galectin-3 and markers of inflammation or tissue remodeling such as matrix metalloproteinases (MMPs), and predictive biomarkers that predict response to treatments, such as proteinuria, insertion/deletion polymorphisms of the ACE gene, MMP levels, and renal resistive index in kidney ultrasound (63).

Some biomarkers such as kidney injury molecule (KIM-1) (64), neutrophil gelatinase-associated protein (NGAL) (65), apolipoprotein A-IV (apoA-IV) (66), soluble TNFα receptor 1 (TNFR1) (67), and soluble urokinase receptor (suPAR) (68) have been evaluated as potential biomarkers of kidney disease progression. Unfortunately, none of them have demonstrated to add an additional benefit to serum creatinine and albuminuria. These tubular biomarkers were described in the acute ischemia-reperfusion damage models and do not reflect tubulointerstitial fibrosis. There is a constantly increasing number of studies that are revealing novel biomarkers and potential therapeutic targets by using micro-RNA analysis, proteomics, peptidomics, and urinary transcriptomics.

FUTURE LINES OF TREATMENT: RENAL REGENERATION

There is strong evidence from animal studies that suggests that interstitial fibrosis could be reversed and therefore could be a therapeutic target in the prevention of CKD progression (69–71). The concept of kidney regeneration is emerging, and it could be achieved by using growth factors, or multipotent cells could be directed to regenerate kidneys with chronic lesions.

Stem cell-based regenerative therapy is an alternative future treatment modality. There have been studies performed with hematopoietic stem cells, mesenchymal stem cells, and endothelial progenitor cells. Patients with CKD have a decreased capacity for kidney regeneration with an altered function of endothelial progenitor cells, and several studies in animal models with CKD suggest a regenerative beneficial effect of these cell-based therapies (72, 73).

A meta-analysis of several experimental models found that stem cell-based therapy prevented progression of CKD with decreased proteinuria (74). Mesenchymal stem cells are being used in kidney transplant patients to increase immunosuppression and improve regeneration (75). On the other hand, studies involving hematopoietic stem cell-based regenerative therapies are mainly in lupus nephritis and are uncontrolled (76).

CONCLUSIONS

Progression of CKD to ESKD carries a high burden on cardiovascular morbidity and mortality. We need to improve our understanding of early mechanisms of CKD which will consequently enable early therapeutic intervention and

delay CKD progression or even reverse it. In addition to RAS blockade, SGLT2 inhibitors and bicarbonate therapy have proved to retard CKD progression, and new drugs targeting fibrosis and inflammation, as well as regenerative therapy may enhance these effects in our goal to mitigate CKD progression and consequently cardiovascular and global mortality.

AUTHOR CONTRIBUTIONS

AS, CC-C, and GF-J have designed, written, and reviewed this paper. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.645187/full#supplementary-material

Supplementary Figure 1 | Contributors to progression of Chronic Kidney Disease.

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Continuous Positive Airway Pressure Improves Renal Function in Obese Patients With Obstructive Sleep Apnea Syndrome

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Background: Obstructive sleep apnea syndrome (OSAS) is an independent risk factor for cardiovascular morbidity and mortality, and it has a detrimental effect on renal function. Obesity is the major risk factor for OSAS, and represents a risk factor for chronic kidney disease. Continuous positive airway pressure (CPAP) is the suggested therapy for moderate-to-severe OSAS. We designed this study to evaluate the effect of CPAP on estimated glomerular filtration rate (e-GFR) in a cohort of obese patients with moderate-to-severe OSAS and normal renal function.

Methods: We enrolled 198 obese subjects, divided into two groups (OSAS+ and OSAS–), on the basis of cardiorespiratory monitoring; mild OSAS patients ($n = 33$) were excluded from the study, thus the analyses were conducted on 165 patients. Comparisons between groups were made by Student t-test or χ^2 test as appropriate. Linear regression analyses were used to assess the relationship between baseline e-GFR and different covariates and, in the OSAS+ group, between Δe-GFR and different covariates. A multivariate regression analysis was performed to determinate the independent predictor of the Δe-GFR.

Results: OSAS+ subjects showed significantly increased values of systolic blood pressure, HOMA, pulse wave velocity, high-sensitivity C reactive protein and uric acid compared with OSAS– group. OSAS+ group showed significantly lower values of e-GFR and increased values of microalbuminuria. At linear regression analysis e-GFR resulted significantly and inversely related to AHI in the whole study population and in the two groups. After 6 months of CPAP therapy, OSAS+ subjects showed an improvement in respiratory parameters, as well as a significant increase in e-GFR values (104.2 + 19.0 vs. 84.0 + 13.1 ml/min/1.73 m^2, $P < 0.0001$). At multiple regression analysis, Δ apnea/hypopnea index (AHIa) resulted the main independent predictor of Δe-GFR explaining 22% of its variation.

Conclusions: Obese OSAS patients show significantly lower values of e-GFR, even if in the normal range, compared with obese non-OSAS subjects. After 6 months of CPAP, e-GFR significantly improved (+20 ml/min/1.73 m^2) and ΔAHIa resulted the most important independent predictor of Δe-GFR.

Keywords: chronic kidney disease, renal function, OSAS, CPAP, cardiovascular risk factors

INTRODUCTION

In the last years obstructive sleep apnea syndrome (OSAS) has been considered as an independent risk factor for cardiovascular (CV) morbidity and mortality, similarly to other classical risk factors such as obesity, diabetes mellitus (DM), and hypertension (1). Even if the pathogenetic mechanisms underlying the association between OSAS and CV diseases have been not completely elucidated, hypoxia-induced endothelial dysfunction (2, 3), insulin resistance (4), and sympathetic-related CV hemodynamics (5, 6) seem to play a pivotal role.

Renal function decline, evaluated by estimated glomerular filtration rate (e-GFR), is associated with increased CV morbidity and mortality in the general population as well as in various settings of patients (7–9), and mild-to-moderate renal insufficiency has emerged as a major public and clinical health problem. It has been hypothesized that chronic kidney disease (CKD) shares common pathogenetic mechanisms with OSAS, such as increased oxidative stress, metabolic alterations and sympathetic activation, which could explain the link between OSAS and renal function decline (10). Moreover, secondary analyses of intervention studies conducted in both hypertensive patients (11) and selected patients at high CV risk (12) have shown that classical CV risk factors such as hypertension, DM, hyperlipidemia, smoking, and overweight/obesity represent major correlates of renal dysfunction.

However, the relationship between OSAS and renal function decline needs to be further elucidated; it is well-known that OSAS is highly prevalent among individuals with end-stage renal disease (ESRD) (13) and that patients with early stages of CKD show an increased risk of OSAS compared to subjects with normal renal function (14), but the cause-effect link between these conditions has not yet been clearly explained. In fact, there is also evidence that ESRD may induce a progression of CKD, thus indicating a bidirectional relationship between the conditions (15).

On the other hand, several data showed that obesity, the main risk factor for OSAS, is also associated with dysregulation of renal function, such as glomerular hyperfiltration and increased albuminuria, thus representing a risk factor for CKD and its progression to kidney failure (16–18).

Currently, the gold-standard treatment of moderate-severe OSAS is continuous positive airway pressure (CPAP), regardless of body weight (19). This therapy has shown a reduction/disappearance of nocturnal hypoxic episodes which, in turn, determinates the improvement of several hemodynamic and cardiometabolic parameters.

Previous studies investigating the effect of CPAP treatment on renal function were conducted in patients with CKD (20) or in non-obese OSAS patients with normal renal function (21) but, to our knowledge, no data exist in obese OSAS subjects with normal renal function. Thus, we designed this study to evaluate the effect of CPAP therapy on renal function in a cohort of well-characterized obese patients with moderate-to-severe OSAS and preserved renal function (e-GFR > 60 ml/min/1.73 m^2).

MATERIALS AND METHODS

Study Population

We recruited 198 obese Caucasian outpatients (body mass index, BMI >30 Kg/m^2; 110 males and 55 females; mean age 55.3 ± 11.0 years) referred to the Sleep Breathing Disorders Clinic of the University Hospital of Catanzaro, who underwent cardiorespiratory monitoring (CRM) during sleep for reported daytime sleepiness and persistent (>6 months) snoring. None of the patients had history or clinical evidence of angina, myocardial infarction, valvular heart disease, arrhythmias, peripheral vascular disease, arterial hypertension, DM, coagulopathy or any disease predisposing to vasculitis or Raynaud's phenomenon, liver and respiratory disease, lung failure, CKD (defined for e-GFR values <60 ml/min/1.7 m^2) or urinary tract infections. At the time of the first evaluation, we collected medical history and performed physical examination evaluating body weight, height and BMI, waist and neck circumference (NC), determination of arterial stiffness, routine blood tests, and 75-g oral glucose tolerance test (OGTT) after 12 h fasting, 0 and 120 min of sampling for plasma glucose and insulin determination. According with clinical guidelines for OSAS management (22), all subjects participating to the study received general education about the importance of weight loss, sleep position, alcohol avoidance and risk factors modifications. Subjects who were diagnosed with moderate-to-severe OSAS were prescribed with CPAP therapy. In accordance with the aim of the study, OSAS patients treated with CPAP were specifically re-evaluated for respiratory parameters, renal function and CV risk factors 6 months after the beginning of CPAP therapy; after the observation period, appropriate anti-hypertensive and/or insulin-sensitizers medications were prescribed, when needed. At the end of the follow-up period, all subjects whose BMI remained in the obesity range were prescribed with a hypocaloric diet with a caloric deficit of 500 kcal/die, in order to achieve a BMI of 25 kg/m^2 or less. The local Ethics Committee (Calabria Centro) approved the protocol (approval number 2,012.63) and informed written consent was obtained from all participants.

All the investigations were performed in accordance with the principles of the Declaration of Helsinki.

Laboratory Determinations

All laboratory measurements were performed after a fast of at least 12 h. Plasma glucose was determined immediately by the glucose oxidase method (Glucose Analyzer, Beckman Coulter S.p.A., Milan, Italy). Triglyceride and total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol concentrations were measured by enzymatic methods (Roche Diagnostics GmbH, Mannheim, Germany). Plasma concentrations of insulin were determined by chemiluminescence test (Roche Diagnostics). Insulin resistance was determined by homeostasis model assessment (HOMA) index, calculated according with the following equation:

$$\text{HOMA} = \text{fasting insulin } (\mu\text{U/mL}) \times \text{fasting glucose (mmol/L)} /22.5 \text{ (23)}$$

that is strongly related to the euglycemic clamp, which represents the gold standard test for measuring insulin sensitivity. Creatinine measurements and uric acid (UA) were performed at baseline by use of the Jaffe methodology and the uricase peroxidase (uricase/POD; Boehringer Mannheim, Mannheim, Germany) method implemented in an autoanalyzer. Values of e-GFR (mL/min/1.73 m^2) were calculated with the equation proposed by investigators in the Chronic Kidney Disease Epidemiology (CKD-EPI) Collaboration (24). This equation was developed from a cohort of patients that included both healthy individuals and individuals with chronic kidney disease and that was much larger than the Modification of Diet in Renal Disease study. We preferred this equation because it is more accurate in subjects with GFR > 60 mL/min/1.73 m^2, which our patients were expected to have given the inclusion criteria. Total serum insulin-like growth factor-1 (IGF-1) concentrations were determined by chemiluminescent immunoassay (Nichols Advantage, Nichols Institute Diagnostic, San Juan Capistrano, CA). High sensitivity C-reactive protein (hs-CRP) levels were measured by an automated instrument (CardioPhaseH hsCRP, Siemens, Italy).

Cardio-Respiratory Monitoring

To confirm the diagnosis of OSAS, patients underwent a CRM during sleep (Compumedics, Somtè), with continuous non-invasive recordings of respiratory pressure and flow, thoracic-abdominal motion, oxygen saturation, ECG and body position. Results from the sleep studies were analyzed by a trained technician using the recommendations of the American Academy of Sleep Medicine (AASM) (25). The severity classification of OSAS was based on the apnea/hypopnea index (AHI): mild (5 < AHI < 15), moderate (15 < AHI < 30), and severe (AHI > 30). According with current clinical recommendations (22), patients with moderate or severe OSAS were prescribed with nocturnal CPAP with an automated device (ResMed AirSenseTM10 AutoSet/Elite- AutoSet TM CS-A). Moreover, all patients received counseling on sleep hygiene. CPAP has been titrated for three nights. The definitive value of CPAP is the amount of pressure that eliminates events in ∼95% of the total sleep time (95th centile). Patients have been assessed monthly for compliance and effectiveness of the intervention. Compliance to the CPAP was measured using the data from the CPAP card; a patient was considered to be compliant when using CPAP at least 4 h/night (average) in at least 70% of nights. CPAP was considered effective if the AHI falled below 5/h.

Blood Pressure Measurement

Blood pressure (BP) measurements readings of clinic BP were obtained in the non-dominant arm of the seated patient, after 5 min of quiet rest, with a mercury sphygmomanometer. Systolic and diastolic BP was recorded at the first appearance (phase I) and disappearance (phase V) of Korotkoff sounds. Baseline BP values were the average of the last two of the three consecutive measurements obtained at intervals of 3 min (26).

TABLE 1 | Baseline anthropometric, clinical, and humoral characteristics of OSAS– and OSAS+ groups.

	OSAS – $n = 100$	OSAS + $n = 65$	*P*
Age, years	54.3 ± 10.9	56.2 ± 11.0	0.251
Gender, M/F	62/38	48/17	0.159
Current smokers, *n*	25	42	0.000
BMI, kg/m^2	36.6 ± 3.3	36.9 ± 6.3	0.711
Waist, cm	114.8 ± 11.6	118.6 ± 14.1	0.059
NC, cm	44.1 ± 3.4	47.4 ± 3.2	0.0001
AHI	2.3 ± 1.4	42.2 ± 25.1	0.0001
ODI	7.5 ± 3.4	24.0 ± 11.6	0.0001
TC90, %	4.9 ± 2.8	21.1 ± 5.0	0.0001
SBP, mmHg	131.1 ± 15.2	140.0 ± 12.6	0.0001
DBP, mmHg	82.1 ± 10.4	81.7 ± 9.8	0.827
hs-CRP, mg/L	3.5 ± 2.4	6.5 ± 6.4	0.0001
Microalbuminuria, mg/dL	13.1 ± 3.6	22.7 ± 11.3	0.0001
UA, mg/dL	5.7 ± 1.4	6.5 ± 1.4	0.001
Total Chol, mg/dL	198.3 ± 23.5	200.0 ± 12.8	0.603
LDL-Chol, mg/dL	126.1 ± 23.3	129.4 ± 16.2	0.311
HDL-Chol, mg/dL	47.1 ± 10.5	42.3 ± 9.9	0.004
Triglycerides, mg/dL	125.8 ± 25.0	141.1 ± 25.0	0.0001
Fasting plasma glucose, mg/dL	98.1 ± 15.9	107.6 ± 12.7	0.0001
2 h-postload plasma glucose, mg/dL	152.6 ± 23.2	172.4 ± 25.8	0.023
Insulin, mU/L	15.7 ± 7.3	21.0 ± 3.0	0.0001
HOMA	3.8 ± 2.1	5.6 ± 1.0	0.0001
IGF-1, ng/mL	156.5 ± 28.1	141.6 ± 32.8	0.002
PWV, m/s	7.1 ± 1.0	7.4 ± 1.9	0.104

AHI, apnea hypopnea index; BMI, body mass index; DBP, diastolic blood pressure; e-GFR, estimated glomerular filtration rate; HDL-Chol, high-density lipoprotein cholesterol; HOMA, homeostatic model assessment; hs-CRP, high-sensitivity C-reactive protein; IGF-1, insulin-like growth factor; LDL Chol, low-density lipoprotein cholesterol; NC, neck circumference; ODI, oxygen desaturation index; PWV, pulse wave velocity; SBP, systolic blood pressure; TC90, sleep time percentage with oxyhemoglobin saturation (SpO_2) < 90%; Total Chol, total cholesterol; UA, uric acid.

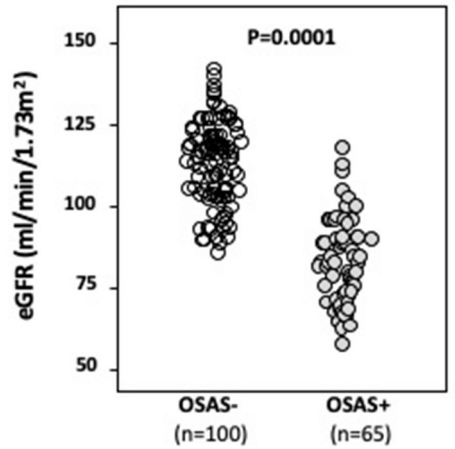

FIGURE 1 | Mean e-GFR baseline values in OSAS– and OSAS+ groups. In this figure, we graphically reported mean baseline e-GFR values of both OSAS– and OSAS+ groups. As indicated, OSAS+ subjects showed statistically significant lower baseline e-GFR values when compared to OSAS– subjects.

Arterial Stiffness

These measurements were obtained by a validated system (Sphygmocor^{TM}; AtCor Medical, Sydney, Australia) that employs high-fidelity applanation tonometry (Millar) and appropriate computer software for the analysis of pressure wave (Sphygmocor^{TM}). Aortic pulse wave velocity (PWV) was determined from carotid and femoral pressure wave forms. Carotid to femoral transit time (DT) was computed from the foot-to-foot time difference between carotid and femoral waveforms. The distance between the surface markings of the sternal notch and femoral artery was used to estimate the path length between the carotid and femoral arteries (L), and PWV computed as L/DT.

Statistical Analysis

The analysis was performed on 165 subjects, and results were expressed as mean ± SD or as percent frequency. Comparisons between groups were made by paired or unpaired Student *t*-test or χ^2 test as appropriate. Patients were divided in two groups: OSAS + (AHI>15) and OSAS– (AHI < 5). Linear regression analysis was used to assess the relationship between baseline e-GFR and different covariates in all population and in the groups separately. Thus, the same analysis was performed in the OSAS+ to evaluate the major determinant of the e-GFR variation (Δe-GFR), before and after 6 months of CPAP, considering the variation (Δ) of the examined covariates. After this, a multivariate regression analysis was performed to determinate the independent predictor of the Δe-GFR. A value of $P < 0.05$ was considered statistically significant. All calculations were made with a standard statistical package (SPSS for Windows version 16.0).

TABLE 2 | Univariate relationship between e-GFR and different covariates in the whole study population and in the two groups separately.

	All		OSAS -		OSAS +	
	r	*P*	*r*	*P*	*R*	*P*
AHI	−0.667	0.0001	−0.297	0.001	−0.319	0.005
ODI	−0.510	0.0001	0.069	0.249	0.113	0.185
TC90, %	−0.639	0.0001	0.122	0.113	0.198	0.057
HOMA	−0.332	0.0001	0.056	0.291	−0.030	0.406
Microalbuminuria, mg/dL	−0.457	0.0001	0.084	0.204	−0.217	0.041
SBP, mmHg	−0.225	0.002	0.096	0.172	−0.174	0.083
UA, mg/dL	−0.208	0.004	0.040	0.346	−0.076	0.273
IGF-1, ng/mL	0.200	0.005	0.001	0.496	0.075	0.275
hs-CRP, mg/L	−0.197	0.006	0.292	0.002	−0.046	0.357
Triglycerides, mg/dL	−0.150	0.027	0.095	0.172	0.129	0.153
HDL-Chol, mg/dL	0.147	0.029	−0.045	0.327	−0.013	0.458
DBP, mmHg	−0.088	0.129	0.189	0.030	−0.002	0.495
PWV, m/s	−0.081	0.149	0.013	0.450	0.033	0.396
BMI, kg/m^2	−0.055	0.243	−0.023	0.411	−0.076	0.274
LDL-Chol, mg/dL	0.017	0.416	0.179	0.038	−0.008	0.474

AHI, apnea hypopnea index; BMI, body mass index; DBP, diastolic blood pressure; HDL-Chol, high-density lipoprotein cholesterol; HOMA, homeostatic model assessment; hs-CRP, high-sensitivity C-reactive protein; IGF-1, insulin-like growth factor; LDL Chol, low-density lipoprotein cholesterol; ODI, oxygen desaturation index; PWV, pulse wave velocity; SBP, systolic blood pressure; TC90, sleep time percentage with oxyhemoglobin saturation (SpO_2) < 90%; UA, uric acid.

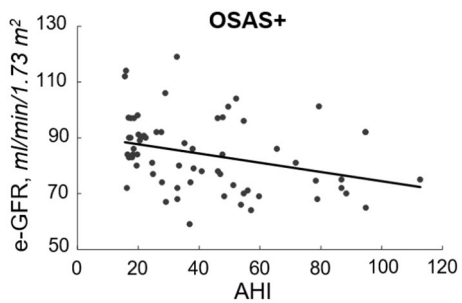

FIGURE 2 | Inverse relationship between e-GFR and AHI in OSAS+ group. In this figure, we reported the inverse relationship between e-GFR and AHI in subjects with OSAS.

RESULTS

From an initial cohort of 198 obese subjects, 33 were excluded because they were diagnosed with mild OSAS (5 < AHI < 15); baseline anthropometric, clinical and humoral characteristics of these patients are described in the **Supplementary Material**. The remaining 165 patients were divided into two groups (OSAS– $n = 100$, and OSAS+ $n = 65$) on the basis of CRM results. Baseline anthropometric, clinical, and humoral characteristics of the whole study population and of the two groups separately are listed in **Table 1**. There were no significant differences

TABLE 3 | Anthropometric, hemodynamic and humoral characteristics of OSAS+ patients at baseline and after 6 months of CPAP therapy.

	Baseline ($n = 65$)	T6 ($n = 65$)	*P*
BMI, kg/m^2	36.9 ±6.3	36.5 ±6.6	0.111
Waist, cm	118.8 ±14.1	118.0 ±14.7	0.018
AHI	42.2 ±25.1	4.9 ±2.9	0.0001
ODI	24.0 ±11.6	6.5 ±3.3	0.0001
TC 90, %	21.1 ±5.0	16.6 ±5.9	0.0001
SBP, mmHg	140.0 ±12.6	135.7 ±15.2	0.053
DBP, mmHg	81.7 ±9.8	80.9 ±9.7	0.612
hs-CRP, mg/L	6.5 ±6.4	3.1 ±2.0	0.0001
Microalbuminuria, mg/dL	22.7 ±11.3	14.1 ±6.3	0.0001
UA, mg/dL	6.5 ±1.4	5.9 ±1.4	0.014
HOMA	5.6 ±1.0	3.8 ±1.4	0.0001
IGF-1, ng/mL	141.6 ±32.8	158.3 ±27.4	0.003
PWV, m/s	7.4 ±1.9	6.7 ±1.4	0.034

AHI, apnea hypopnea index; BMI, body mass index; DBP, diastolic blood pressure; e-GFR, estimated glomerular filtration rate; HOMA, homeostatic model assessment; hs-CRP, high-sensitivity C-reactive protein; IGF-1, insulin-like growth factor; ODI, oxygen desaturation index; PWV, pulse wave velocity; SBP, systolic blood pressure; TC90, sleep time percentage with oxyhemoglobin saturation (SpO_2) < 90%; UA, uric acid.

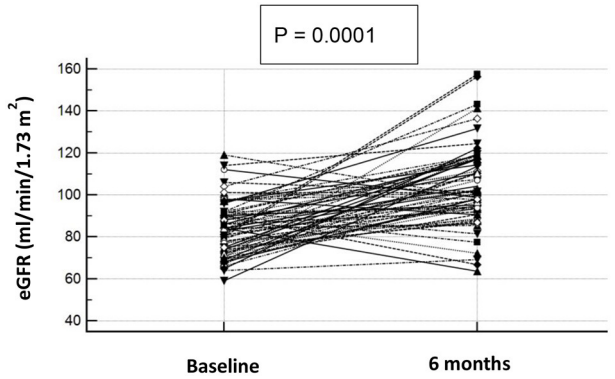

FIGURE 3 | e-GFR baseline and after 6 months of CPAP therapy in OSAS+ subjects. Graphical representation of e-GFR variation after 6 months of CPAP therapy in OSAS+ subjects. Each dot represents a single subject.

between the two groups with respect to mean age, gender, BMI, waist, DBP, total and LDL-cholesterol. As expected, patients with OSAS showed significantly increased values of AHI, oxygen desaturation index (ODI), cumulative sleep time percentage with oxyhemoglobin saturation <90% (TC90), and NC; furthermore, OSAS+ patients also showed higher values of SBP, hs-CRP, UA, triglycerides, fasting plasma glucose, 2 h-postload plasma glucose, HOMA and PWV, while IGF-1 and HDL-cholesterol were significantly lower in this group. Of interest, OSAS+ patients showed significantly higher values of microalbuminuria (**Table 1**) and significantly lower e-GFR values (84.0 ± 13.1 vs. 114.5 ± 12.8 mg/dl/1.73 m^2, $P < 0.0001$) compared with OSAS– group (**Figure 1**).

At linear regression analysis (**Table 2**) e-GFR was significantly and inversely associated with AHI ($r = -0.667$, $P < 0.0001$), ODI (-0.510, $P < 0.0001$), TC90 (-0.639, $P < 0.0001$), microalbuminuria (-0.457, $P < 0.0001$), SBP (-0.225, $P = 0.002$), UA ($r = -0.208$, $P = 0.004$), hs-CRP ($r = -0.197$, $P = 0.006$), triglycerides ($r = -0.150$, $P = 0.027$), while it was directly associated with HDL-cholesterol ($r = 0.147$, $P = 0.029$) and IGF-1 ($r = 0.200$, $P = 0.005$) in the whole study population. In OSAS– group e-GFR was significantly related to hs-CRP ($r = 0.292$, $P = 0.002$) and DBP ($r = 0.189$, $P = 0.030$), while it was inversely related to AHI ($r = -0.297$, $P < 0.001$). In OSAS+ patients, e-GFR was significantly and inversely related only with AHI ($r = -0.319$, $P = 0.005$) (**Figure 2**) and microalbuminuria ($r = -0.217$, $P = 0.041$).

Table 3 shows the clinical and biochemical characteristics of OSAS+ patients at baseline and after CPAP therapy. After 6 months, these subjects presented significantly lower values of BMI, waist, AHI, ODI, TC90, hs-CRP, UA, microalbuminuria, HOMA and PWV. Of interest, we observed a significant increase of e-GFR values (104.2 ± 19.0 vs. 84.0 ± 13.1 ml/min/1.73 m^2, $P < 0.0001$) after 6 months of CPAP therapy (**Figure 3**); in two subjects in the OSAS+ group we detected an almost doubled value of e-GFR (from 80 to 160 ml/min/1.73 m^2) at the end of the follow-up period, to be intended as a hyperfiltration due to

TABLE 4 | Univariate relationship between Δe-GFR and different covariates in OSAS+ patients.

	r	*P*
ΔAHI	−0.482	0.0001
Baseline e-GFR, ml/min/1.73 m^2	−0.543	0.0001
ΔTC 90, %	0.195	0.060
ΔMicroalbuminuria, mg/dL	−0.168	0.090
ΔTriglycerides, mg/dL	0.139	0.135
ΔODI	0.127	0.157
Δhs-CRP, mg/L	−0.104	0.205
ΔIGF-1, ng/mL	−0.067	0.298
ΔLDL-Chol, mg/dL	−0.048	0.352
ΔBMI, kg/m^2	−0.024	0.425
ΔHOMA	0.006	0.480

AHI, apnea hypopnea index; BMI, body mass index; e-GFR, estimated glomerular filtration rate; HOMA, homeostatic model assessment; hs-CRP, high-sensitivity C-reactive protein; IGF-1, insulin-like growth factor; LDL Chol, low-density lipoprotein cholesterol; ODI, oxygen desaturation index; TC90, sleep time percentage with oxyhemoglobin saturation (SpO_2) < 90%.

TABLE 5 | Multiple linear regression models for Δe-GFR with (model A) and without (model B) baseline e-GFR.

	Partial r^2 (%)	Total r^2 (%)	*P*
Model A			
Baseline e-GFR, ml/min/1.73 m^2		28.4	0.0001
ΔAHI	9.5	37.9	0.002
Model B			
ΔAHI		22.0	0.0001
ΔTC90, %	5.1	27.1	0.023

AHI, apnea hypopnea index; e-GFR, estimated glomerular filtration rate.

a weight gain >5% from the basal value. Subsequently, in the same group, a linear regression analysis was performed between Δe-GFR, as dependent variable, Δ of different covariates and baseline e-GFR. As shown in **Table 4**, Δe-GFR was significantly and inversely correlated with ΔAHI ($r = -0.482$, $P < 0.0001$) and e-GFR at baseline ($r = -0.543$, $P = 0.0001$). Then, we performed a multiple regression analysis (**Table 5**) including (Model A) and excluding (Model B) baseline e-GFR from the model. We observed that Δe-GFR was influenced by e-GFR at baseline, that explained 28.4% of its variation, and by ΔAHI that determined another 9.5% of its variation. In the second model, ΔAHI resulted the main independent predictor of Δe-GFR explaining 22% of its variation, followed by ΔTC90 that explained another 5.1% of Δe-GFR variation.

DISCUSSION

The most important finding obtained in this study, conducted in a population of obese patients with normal renal function, is that those with moderate-to-severe OSAS showed lower—even if in the normal range—baseline e-GFR values compared to subjects without OSAS; moreover, 6 months treatment with CPAP was effective in improve e-GFR by 20 ml/min/1.73 m^2. Clinically relevant, ΔAHI resulted the most important independent predictor of Δe-GFR, allowing to affirm that the improvement of nocturnal respiratory function is the main determinant of e-GFR increase in this particular setting of patients. In fact, ΔAHI -or the resolution of apneas during CPAP-, and ΔTC90 -or the improvement of oxyhemoglobin saturation during sleep-, represent the main predictors of Δe-GFR, independently from metabolic, hormonal, and inflammatory status. These findings are consistent with other published literature, which identifies the resolution of chronic intermittent hypoxic episodes during sleep as the major pathophysiological mechanism of the improvement of endothelial glomerular function (10, 27, 28). Our data are in agreement with previous literature but it appears evident that there are other pathophysiological OSAS-related mechanisms responsible of renal function impairment in this setting of patients. Our hypothesis is that arterial stiffness, in addition to the inflammatory status characterizing obese patients, may negatively influence intraglomerular hemodynamic. In fact, arterial stiffness represents an independent predictor of cardiovascular, cerebral and renal morbidity (29); mineral metabolism disturbances, vascular calcifications, formation of advanced glycation end-products, and acute and chronic volume overload, occurring in ESRD and CKD patients, have been proposed as the main pathogenetic mechanisms linking arterial stiffness to renal disease (30). In our study population, there were not significant differences in PWV (a surrogate marker of arterial stiffness) between OSAS– and OSAS+ patients, despite significantly higher SBP values in the last group. Of clinical importance, PWV was significantly lower after 6 months of CPAP therapy, leading to hypothesize a positive impact of reduced arterial stiffness on renal hemodynamic. Moreover, it is well-established that CPAP therapy reduces CV risk in OSAS patients (19, 31) through an improvement in the insulin-resistant status. In addition, in a group of subjects affected by OSAS and CKD stage III-IV, CPAP therapy was effective in reducing proteinuria and increasing e-GFR after a year of treatment (32). Other studies have demonstrated that CPAP therapy reduces glomerular hyperfiltration with a consequent increase of renal plasma flow in obese OSAS patients (33). With this regard, it is important to remark that also cigarette smoking negatively impacts on renal function (34, 35); in our study population, in the OSAS+ group we observed a greater prevalence of current smokers than in the OSAS– subjects, contributing to the impairment of e-GFR in these subjects.

The novelty of our study is that, differently from other Authors, we considered a group of obese subjects with preserved renal function. Many studies describe a major prevalence of breathing sleep disorders in subjects affected by ESRD in comparison with those with normal renal function (36, 37). On the other hand, in the last few years, some Authors focused their attention on the prevalence of OSAS in the first stages of renal dysfunction, even if the majority of the studies is based on patients' history collection and not on sleep instrumental study (36). It is well-known that obesity, a risk factor of OSAS, is associated with glomerular hyperfiltration but, on the contrary, very little is known about the impact of breathing sleep disorders on renal hemodynamic in these subjects (20, 21, 37–39).

In conclusion, even if this study has several limitations such as the single-center non-blinded design and the small sample size, our data confirm previously published literature about the detrimental effect of OSAS on renal function also in subjects free from kidney disease; this detrimental effect may be reverted, at least in part, by CPAP therapy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comitato Etico Calabria Centro. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MP, FP, and RM: Conceptualization; PS and AS: methodology; RM and PS: software; MP, GS, and FP: validation; RM: formal analysis; PS, BC, LM, MV, and ES: investigation; MP, RM, and PS: writing—original draft preparation; MP and FP: writing—review and editing; MP, AS, and FP: supervision. All authors have read and agreed to the published version of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.642086/full#supplementary-material

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REVIEW
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Anemia in Chronic Kidney Disease: From Pathophysiology and Current Treatments, to Future Agents

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Anemia is a common complication in chronic kidney disease (CKD), and is associated with a reduced quality of life, and an increased morbidity and mortality. The mechanisms involved in anemia associated to CKD are diverse and complex. They include a decrease in endogenous erythropoietin (EPO) production, absolute and/or functional iron deficiency, and inflammation with increased hepcidin levels, among others. Patients are most commonly managed with oral or intravenous iron supplements and with erythropoiesis stimulating agents (ESA). However, these treatments have associated risks, and sometimes are insufficiently effective. Nonetheless, in the last years, there have been some remarkable advances in the treatment of CKD-related anemia, which have raised great expectations. On the one hand, a novel family of drugs has been developed: the hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs). These agents induce, among other effects, an increase in the production of endogenous EPO, improve iron availability and reduce hepcidin levels. Some of them have already received marketing authorization. On the other hand, recent clinical trials have elucidated important aspects of iron supplementation, which may change the treatment targets in the future. This article reviews the current knowledge of the pathophysiology CKD-related anemia, current and future therapies, the trends in patient management and the unmet goals.

Keywords: anemia, chronic kidney disease, erythropoiesis-stimulating agents, iron, HIF stabilizer, HIF prolyl-hydroxylase inhibitor, hepcidin, COVID 19

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EPIDEMIOLOGY OF ANEMIA OF CKD

Anemia is a common complication in chronic kidney disease (CKD), and is associated with a reduced quality of life (1, 2), a worse renal survival (3), an increase in morbidity and mortality (4, 5), and higher costs (6). Several studies focused on prevalence of anemia on CKD non-dialysis dependent (NDD) report variable anemia rates up to 60%.

Anemia is more prevalent and severe as the estimated glomerular filtration rate (eGFR) declines. An analysis of the cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) in 2007–2008 and 2009–2010 (7) revealed that anemia was twice as prevalent in patients with CKD as in the general population (15.4% vs. 7.6). The prevalence of anemia raised with the progression of CKD: 8.4% at stage 1 to 53.4% at stage 5. Similar data was observed in a more recent paper by the CKD Prognosis Consortium (8). In addition, they observed an increased prevalence of anemia among diabetic patients, independent of eGFR and albuminuria.

Regarding new onset of anemia, the observational study NADIR-3 followed CKD stage 3 patients without anemia during 3 years. The authors estimated an annual rate of onset of anemia of 11% in the first year, 20% in the second year and 26% in the third year. In addition, the study revealed that those that had developed anemia significantly progressed more rapidly to CKD stages 4–5, had higher rates of hospitalizations (31.4 vs. 16.1%), major cardiovascular events (16.4 vs. 7.2%) and mortality (10.3 vs. 6.6%) (9).

With regards to the *"real-world"* management of anemia in CKD, considerable controversies and variability exist in the context of guideline recommendations. However, few studies have evaluated this issue. An Italian observational study evaluated anemia management in two visits, among 755 prevalent NDD-CKD patients. Mean eGFR was 27.5 ± 10 mL/min/ 1.73 m^2. The prevalence of severe and mild anemia was 18 and 44%, and remained unchanged at month 6 (19.3 and 43.2%). Clinical inertia to ESA was similar at baseline and at month 6 (39.6 and 34.2%, respectively, $P = 0.487$) and it was less frequent than clinical inertia to iron therapy (75.7 and 72.0%, respectively) (10). A recent observational analysis from the Swedish Renal Registry evaluated the epidemiology and treatment patterns of all nephrology-referred adult CKD patients during 2015. Among 14 415 patients [Non-Dialysis Dependent (NDD), 11,370; Dialysis-Dependent (DD), 3,045] anemia occurred in 60% of NDD and 93% of DD patients. DD patients used more erythropoiesis-stimulating agents (ESAs; 82 vs. 24%) and iron (62 vs. 21%) than NDD patients. The prescribed ESA doses were low to moderate [median 48.2 IU/kg/week (NDD), 78.6 IU/kg/week (DD)]. Among ESA-treated patients, 6–21% had hemoglobin (Hb) >13 g/dL and 2–6% had Hb <9 mg/L. Inflammation (C-reactive protein >5 mg/L) was highly prevalent and associated with ESA resistance and higher ESA doses, but not with iron use. Further, higher ESA doses (>88 IU/kg/week) were associated with an increased risk of major adverse cardiovascular events. Despite the recommendations of guidelines, the use of iron was unexpectedly low, particularly in ESA-treated NDD patients, while a fifth of the dialysis patients receiving ESA had a hemoglobin above the recommended targets (11). A multicenter cross-sectional study conducted at specialist nephrology clinics in Ireland also showed an evident variability in the implementation of different guidelines, the high rates of anemia (from 21 to 63%; $p < 0.001$, depending on the CKD Stage), the low testing rates for iron deficiency (only 45% of anemic patients), and the low use of treatment (86 % of patients with confirmed iron deficiency were not on treatment) (12).

PHYSIOPATHOLOGY OF ANEMIA IN CKD

The mechanisms of anemia in CKD are multifactorial. The progressive reduction of endogenous erythropoietin (EPO) levels has classically been considered to play a preeminent role. However, other factors have also been described to contribute to anemia in CKD patients, such as an absolute iron deficiency due to blood losses or an impaired iron absorption, an ineffective use of iron stores due to increased hepcidin levels, systemic inflammation due to CKD and associated comorbidities, a reduced bone marrow response to EPO due to uremic toxins, a reduced red cell life span, or vitamin B12 or folic acid deficiencies (13).

Hypoxia Inducible Factor System

EPO is a glycoprotein (30.4 kDa) that binds to its receptor on the surface of erythroid progenitor cells mainly in the bone marrow, and serves as a key stimulus for red cell survival, proliferation and differentiation. EPO is produced predominantly by the fibroblast-like interstitial peritubular cells of the kidneys, and in a much lesser proportion, by the perisinusoidal cells in the liver, in response to changes in tissue oxygen tension (14). The production of EPO is controlled at the level of the EPO gene transcription. One of the most important factors that regulate its expression is the hypoxia-inducible factor (HIF) system, whose activity depends on the tissue oxygen levels.

In more detail, under hypoxia or anemic stress, the HIF1 binds to the EPO gene, and activates its expression. HIF1 is composed of two subunits, HIF1α and HIF1β. HIF1β is constitutively expressed whereas HIF1α is virtually absent under normoxic conditions. However, in low oxygen tension settings, HIF1α accumulates and translocates to the nucleus, where it binds to HIF1 β. The HIF1 α-β heterodimer binds to DNA sequences called hypoxia response elements (HRE), regulating the expression of various hypoxia-sensitive genes, either downregulating or upregulating them. The purpose of this rapid adaptive response is to protect against cellular damage, by improving oxygen delivery and by decreasing oxygen consumption. Among these hypoxia-sensitive genes is the EPO gene, which is activated, leading to an increased EPO production. Other genes that are transcriptionally upregulated by the HIF complex are those encoding EPO receptor, transferrin and transferrin receptor, vascular endothelial growth factor (VEGF) or endothelin-1 (15). Recent work has shown that the HIF transcription factors are key elements in the control of cell metabolism and function (16–18). An effect of HIF on total and LDL-cholesterol levels has also been described (19), probably in part by the effects of HIF on degradation of the rate-limiting enzyme, 3-hydroxy-3-methylglutaryl-CoA reductase (20), similar to what has been observed in high altitude settings (21).

Under normoxic conditions, HIF1α is degraded. For this purpose, HIF1α is hydroxylated at two proline residues. This hydroxylation is performed by specific HIF prolyl-hydroxylase enzymes called prolyl hydroxylase domain (PHD) enzymes that need the presence of oxygen, iron, and 2-oxoglutarate as co-factors. Three forms have been described: PHD1, PHD2, PHD3. PHD2 is the main isoform regulating HIF activity (22). Once HIF1α is hydroxylated, the E3 ubiquitin ligase von Hippel-Lindau (pVHL) binds HIF1α, and is targeted for proteasomal degradation. In contrast, under low oxygen tension the action of PHDs is prevented, allowing for HIF1α stabilization and translocation to the nucleus (23, 24). This pathway is the target of the new so-called hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs) (**Figure 1**).

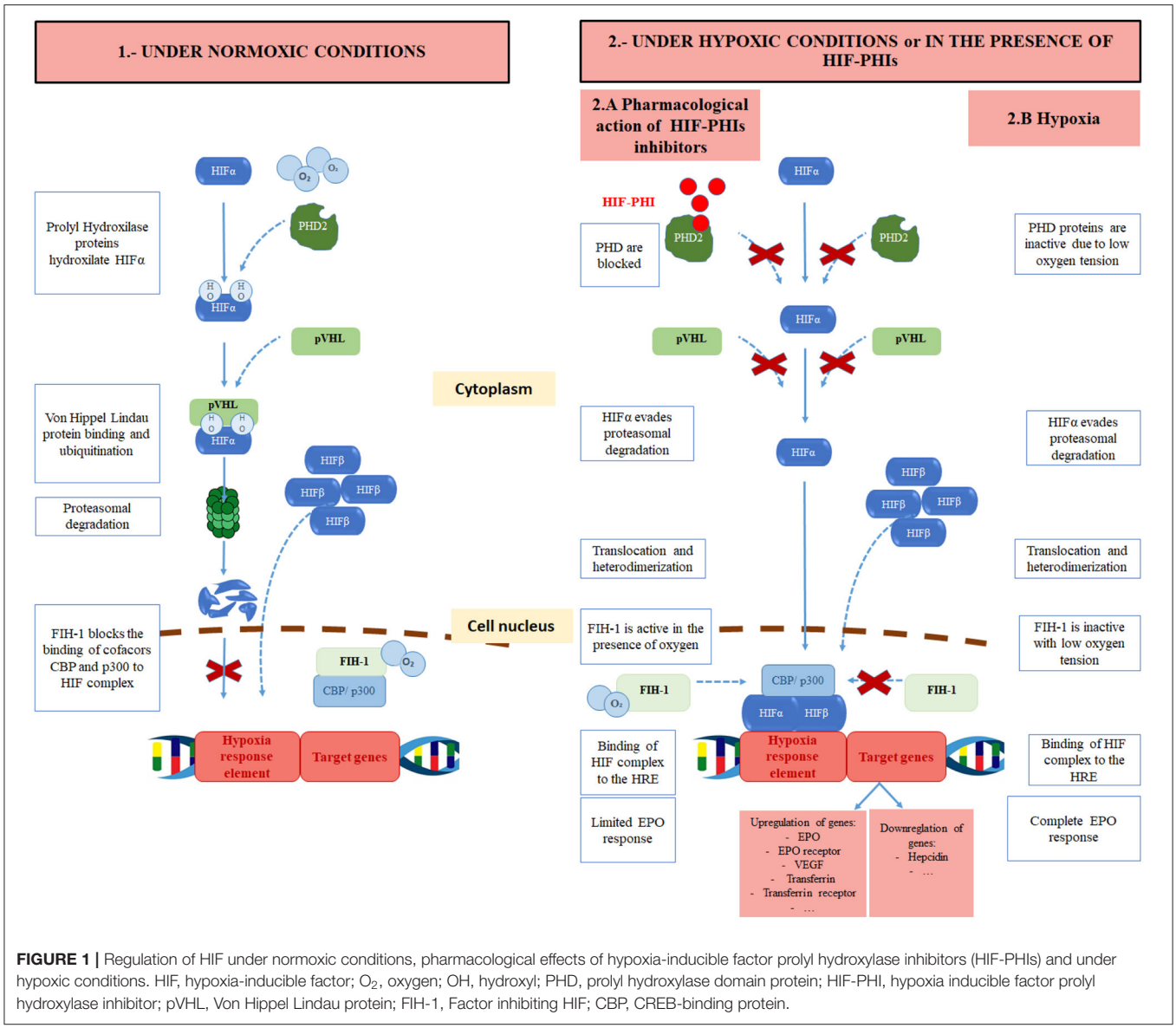

FIGURE 1 | Regulation of HIF under normoxic conditions, pharmacological effects of hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs) and under hypoxic conditions. HIF, hypoxia-inducible factor; O_2, oxygen; OH, hydroxyl; PHD, prolyl hydroxylase domain protein; HIF-PHI, hypoxia inducible factor prolyl hydroxylase inhibitor; pVHL, Von Hippel Lindau protein; FIH-1, Factor inhibiting HIF; CBP, CREB-binding protein.

HIF activity is also regulated through the hydroxylation at a carboxy-terminal asparagine residue of HIF1α by factor-inhibiting HIF (FIH) (25). This hydroxylation of HIF1α occurs with normal oxygen levels and reduces its transcriptional activity. Indeed, it prevents HIF from recruiting transcriptional coactivators such as p300 or CBP, that are needed for the transactivation of hypoxia-responsive genes (26, 27). Furthermore, angiotensin II, which is often found to be increased in CKD patients, raises the production of reactive oxygen species, leading to an inhibition of PHD enzymes, and therefore, a rise in EPO levels (28–30).

Nonetheless, recent animal studies have described important roles to other components of the HIF system. Three isoforms of HIFα have been described, that share similarities regarding oxygen-dependent hydroxylation: HIF1α, HIF2α, and HIF3α. All of them can bind to the HIFβ subunit. However, there may be some differences amongst them: while HIF1α and HIF2α activate gene transcription, HIF3α downregulates HIF1α and HIF2α activity. Moreover, their effect on the expression of some genes may also vary. HIF2α may play a more important role than HIF1α in the regulation of EPO production, as it is specifically required for renal and hepatic production of EPO. The direct role of HIF3α on erythropoiesis has not been fully described. Finally, the expression of HIF2α and HIF3α is limited to several tissues, while HIF1α is ubiquitous (31).

EPO Production in CKD

In CKD patients, EPO levels are inadequately low with respect to the degree of anemia. EPO deficiency starts early in the course of CKD, but it appears that when eGFR falls below 30 ml/min per 1.73 m^2 this deficiency becomes more severe (32). This absolute EPO deficiency can be caused by a decrease in

the EPO production and/or by errors in EPO-sensing. CKD associates an alteration in oxygen delivery to the kidneys due to a reduced blood flow. This results in an adaptation of renal tissue to consume less oxygen and the subsequent maintenance of a normal tissue oxygen gradient. As a consequence, PHD enzymes remain active, the HIF heterodimer is not formed and the EPO gene is not activated (33).

Furthermore, it has been demonstrated experimentally that hypoxia-induced EPO production is inhibited by some inflammatory cytokines such as interleukin-la (IL-la), IL-l beta, transforming growth factor-beta (TGF-beta), and tumor necrosis factor-a (TNF-a) (34, 35). It is well-known that CKD itself leads to an increase of inflammation and immune activation molecules, which would inhibit hypoxia-induced EPO production (36, 37). However, this mechanism of EPO production seems to be blunted rather than abolished in some CKD patients, as they are able to produce additional endogenous EPO in their kidneys and liver under certain circumstances. For instance, when exposed to high altitude or bleeding. Apparently, augmentation of HIF signaling can revert quiescent EPO-producing and oxygen-sensing (REPOS) cells back to EPO production (38). This has been confirmed in observational studies, where hemodialysis patients living in higher altitude require lower doses of recombinant human EPO (rhuEPO) (39).

Some CKD patients may also present with a functional EPO deficiency or EPO resistance, where normal range EPO levels coexist with low hemoglobin (Hb) levels (40), indicating that the bone marrow response to endogenous and exogenous EPO is blunted in patients with CKD. The mechanisms that have been hypothesized for the EPO resistance are various: Proinflammatory cytokines are thought to induce apoptosis as well as a to have a direct toxic effect *via* the induction of labile free radical nitric oxide on erythroid progenitor cells (41, 42). Proinflammatory cytokines are thought to downregulate the expression of EPO receptor on their surface too. It has also been shown that cytokines can induce the production of antagonistic peptides that bind to the EPO receptor, and inhibit the EPO-dependent proliferation (43–46). Moreover, hepcidin (whose production is enhanced by inflammation) might also contribute to EPO resistance, by directly inhibiting erythroid progenitor proliferation and survival (47). Lastly, neocytolysis is a homeostatic physiological process that leads to selective hemolysis of young circulating red blood cells, that has been found to contribute to resistance in CKD patients receiving exogenous EPO (48).

The search for new therapeutic options for these patients is essential. In this sense, some molecules of the HIF system have already been studied as new targets for anemia treatment with a view to increase endogenous EPO production and presumably improve the utilization of iron stores (**Figure 2**).

Iron Metabolism

Iron is required for an adequate erythropoietic response to EPO, and in anemic conditions having iron deficiency corrected allows lower exogenous EPO supplies (49).

Furthermore, iron is required in other essential non-erythropoietic effects that may help explain symptoms such as impaired exercise performance, cognitive impairment or decreased quality of life, and an increased risk in hospitalization or death in patients with heart failure and reduced ejection fraction, independent of anemia. Thus, these advocate for the need to correct iron deficiencies independent of Hb status (50). Iron is an essential component of myoglobin, which transports oxygen in the muscle cells. Iron also plays a substantive role in several oxidative reactions affecting intracellular metabolism, such as the electron transport chain or oxidative phosphorylation. Iron is involved in different mechanisms of DNA synthesis, degradation and repair. Finally, iron is an important component of cytochrome P450 family (51). In fact, there is an increasing evidence from observational studies that iron deficiency is associated with worse outcomes in CKD patients (52–54).

Most of the iron requirements are provided by recycling the iron present in senescent erythrocytes and the release of iron from storage sites (**Figure 2**). The proportion of iron that comes from the dietary uptake is much smaller. In addition, there is no physiological mechanism to regulate iron excretion. It is lost from the desquamation of intestinal epithelial cells, skin cells and blood loses and dietary iron absorption, which is regulated by hepcidin, compensate these loses.

The iron content of macrophages from the phagocytosis of senescent red blood cells, hepatocytes or enterocytes (dietary iron absorbed in the duodenum) is released into the circulation by ferroportin, the only iron exporter known. Iron is then transported through the circulation by transferrin, and delivered into target cells by transferrin binding to transferrin receptor (55). Transferrin receptors are regulated by intracellular iron quantity and cell growth. A circulating acute-phase protein produced in the liver, hepcidin, is the key regulator of iron metabolism. Its purpose is to maintain adequate systemic iron levels. Hepcidin is thought to decrease the absorption of iron in the doudenum by downregulating the expression of apical divalent metal transporter 1 (DMT1) in the enterocyte. Besides absorption, hepcidin also plays a role in iron storage. Indeed, hepcidin promotes the internalization of ferroportin into the cell for its degradation, and thus preventing iron form exiting into the circulation from enterocytes, macrophages or other iron stores. Iron overload increases hepcidin levels, whereas iron deficiency reduces its concentrations (55).

Absolute and relative iron deficiency are frequent conditions in CKD patients. Blood losses, for instance, due to blood left in the hemodialysis circuit are common. In addition, the "uremic" state and other comorbidities causing inflammation prevent from an adequate intestinal iron absorption and from the release of iron from the body stores. Proinflammatory cytokines contribute to a functional iron deficiency in several ways: They stimulate the hepatic synthesis of hepcidin, they induce the expression of DMT1 in macrophages, and induce the expression of ferritin, and inhibit that of ferroportin. They also promote the uptake of iron bound to transferrin into macrophages, *via* the transferrin receptor (56). Moreover, hepcidin is eliminated by kidney and its clearance is reduced as eGFR declines. All these mechanisms favor intracellular iron storage, limiting the availability of iron in CKD.

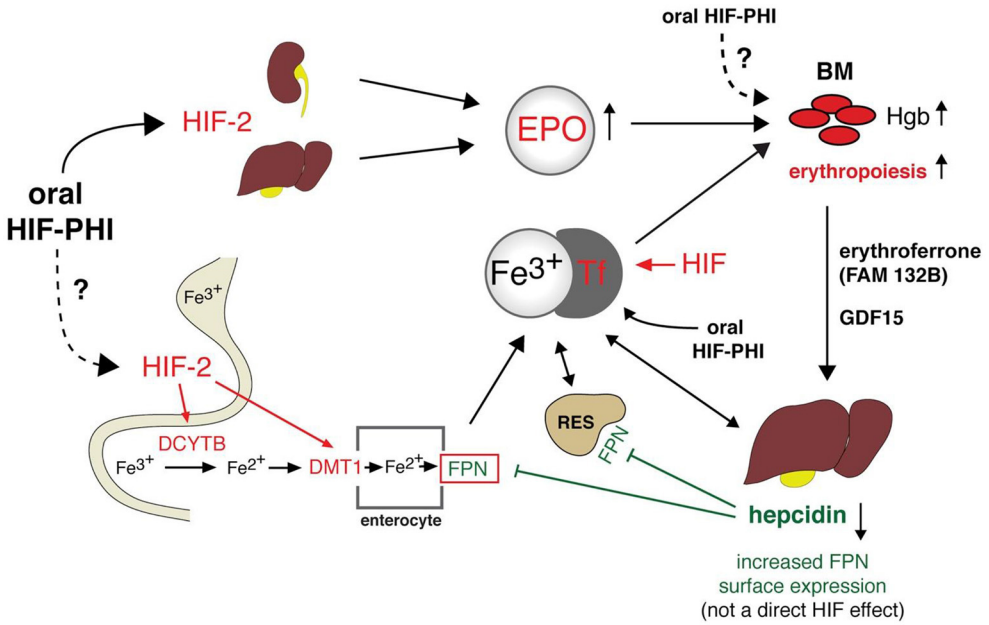

FIGURE 2 | Integrated model for CKD-anemia physiology and actual and potential treatments. HIF, hypoxia-inducible factor; Fe, iron; HIF-PHI, hypoxia inducible factor prolyl hydroxylase inhibitor; EPO, Erythropoietin; Tf, transferrin; DCYTB, duodenal cytochromeb; DMT1, divalent metal transporter 1; BM, Bone marrow; FPN, ferroportin; GDF15, Growth differentiation factor 15; Hgb, hemoglobin; RES, Reticuloendothelial system; FAM132B, Gene that codes for erythroferrone.

Under stress erythropoiesis, EPO suppresses hepcidin synthesis *via* erythroferrone (ERFE). ERFE is a hormone produced by erythroblasts in response to EPO (57). Also, HIF 1α and probably HIF2α regulates hepcidin production by directly binding to and repressing its promoter (58), while HIF-2α enhances iron availability through the activation of genes encoding DMT1 and duodenal cytochrome b (DCYTB) required for transport of dietary iron from the intestinal lumen and for the import of lysosomal iron arising from the circulation (59).

CURRENT CLINICAL PRACTICE GUIDELINES OF ANEMIA IN CKD

Anemia management in CKD has evolved dramatically: from the first oral iron supplements introduced in 1830s (ferrous sulfate), the use of red blood cell transfusions along the XX century, the appearance of the first rhuEPO use in late 1980s followed by long-acting ESAs, to finally, the widespread use of intravenous iron supplements in recent years. However, the actual management of anemia in patients with CKD varies among different countries and medical units (60, 61). Indeed, current guidelines KDIGO (13)—ERBP (62)—NICE (63) do not fully coincide with each other. Some controversies exist about the optimal Hb and iron targets. **Table 1** summarizes the main differences.

Further, these guidelines do not include more recent studies assessing the efficacy and safety of IV iron, as well as different strategies of iron repletion, which will probably change the clinical practice in the future. They demonstrate that in ND-CKD, renal transplant and PD patients IV iron is more efficacious and safe. In addition, a high-dose low frequency administration strategy in dialysis dependent chronic kidney disease (DD-CKD) patents is safe and improves outcomes in patients (63).

CKD ANEMIA TREATMENT

Erythropoiesis-Stimulating Agents (ESAs)

The first EPO analog available was epoetin α and short time later epoetin β. It is produced by recombinant DNA technology in cell cultures. Darbepoetin alfa (DA) and methoxy polyethylene glycol-epoetin beta where developed thereafter and presented a prolonged half-life. More recently, biosimilars of the original epoetin have been introduced in the market.

Not all ESAs are equal. They have different pharmacokinetic and pharmacodynamic properties, such as different half-lives and EPO receptor affinity, allowing a less frequent dosing and ease of administration for NDD CKD patients with long-acting ESAs. In addition, it is important to point out the fact that the conversion factor between short-acting and long-acting ESAs is likely not linear. In fact, at higher doses, long-acting ESAs are more dose-effective (64). However, based on efficacy and safety data, various Cochrane metaanalysis advocate for insufficient evidence to suggest the superiority of any ESA formulation or any ESA administration pattern (65, 66).

Some observational studies have shown conflicting results regarding such outcomes. For instance, the Study of the Japanese Registry of Dialysis that showed a 20% higher risk of mortality from any cause in patients treated with long-acting ESAs compared to those treated with short-acting ESAs (67). On the contrary, an Italian observational study in ND-CKD that

TABLE 1 | Summary of the key recommendations of the most recent anemia guidelines.

	Diagnosis of iron deficiency	Treatment initiation	Hb target under treatment with ESAs	SF and TSAT objectives in patients under treatment	FE oral vs. IV
NICE (2015)	**Test every 3 months (1–3 m in HD)** - Use %HRC > 6%, only if blood processing within 6 h. - if not possible, use CHr < 29 pg - If not, use a combi-nation SF < 100 ng/mL and TSAT < 20%	**Correct iron deficiency before ESA therapy.** - Patient-centered: discuss risks benefits of treatment options. Take into account the person's choice. Avoid Hb < 10 g/dL.	**Hb 10–12 g/dl**	**Avoid SF > 800 ng/mL** To prevent this, review iron dose if SF > 500 ng/mL	**ND-CKD with anemia and iron deficiency:** - offer a 3 months trial of oral iron therapy. - If it fails, offer IV iron therapy. **- DD-CKD:** Preference for IV iron - **If IV iron, consider high dose, low frequency** formulations for ND and DD-CKD patients.
KDIGO (2012)	**SF ≤ 100 ng/mL and TSAT ≤ 20%.**	**A trial with IV iron** if Hb increase or ESA dose reduction is desired and SF≤ 500 ng/mL and TSAT ≤ 30% **ND-CKD: When Hb < 10 g/dL:** Individualize decision based on the rate of fall of Hb, risks and symptoms. **DD-CKD:** When Hb 9-10 g/dL. Avoid Hb < 9 g/dl.	**Hb ≤ 11.5 g/dl** - **Target to Hb > 11.5 g/dl if QoL improve** is foreseen and patient accepts risks. Avoid Hb >13 g/dL	**Stop iron supplements** if SF > 500 ng/mL	**ND-CKD: Select route based on severity of ID, prior response, side effects, costs**, A trial of iv iron, or a 1–3 month trial of oral iron therapy. **- DD- CKD**: Preference for IV iron
ERBP (2009)	**SF < 100 ng/mL and TSAT <20% if ESA naïve. SF ≤ 300 ng/mL and TSAT ≤ 30% if ESA treated**	**Avoid Hb < 10 g/dL.** - If low risk patients or a benefit in QoL foreseen ESA could start at ↑ Hb (avoid Hb >12 g/dL) - In high risk patients with worsening heart disease, treatment initiation at Hb9-10 g/dL.	**Hb 10–12 g/dl** - High risk patients with asymptomatic disease: target Hb around 10 g/dL	**Avoid SF > 500 ng/ml and TSAT > 30%.**	**ND-CKD and mild-moderate anemia:** Oral iron as first line therapy for > 3 months. **ND-CKD and severe anemia or when oral iron ineffective:** IV iron as first choice.

SF, serum ferritin; TSAT, Transferrin saturation; %HRC, percentage of hypochromic red blood cells; CHr, hemoglobin content in reticulocytes; Hob, Hemoglobin; ND-CKD, Non dialysis dependent Chronic kidney disease; DD-CKD, dialysis dependent CKD; QoL, quality of life; IV, intravenous ESA erythropoiesis stimulating agent; Fe, iron.

showed a higher risk of progression to ESKD and mortality in patients receiving short-acting ESAs at high doses (68). These results should be taken cautiously due to the study design and the risk of bias. In contrast, a recent randomized controlled trial (RCT) comparing monthly administration of CERA with reference shorter-acting agents epoetin alfa/beta and DA, showed non-inferiority regarding Hb target achievement, major adverse cardiovascular events or all-cause mortality in NDD and DD-CKD. It was however observed that patients who did not achieve levels of Hb above 10 g/dL or those at the highest quartile of ESA dose, had a higher risk of CV events or death, independent of the assigned treatment (69). More RCTs are needed to assess the differences between different ESA formulations and administration patterns, particularly in patients requiring higher doses of ESA.

Individualizing Hb Target According to the Patient Profile

The target Hb concentration during ESA therapy is still controversial. Studies early after the appearance of rhuEPO demonstrated its efficacy in reducing the need for blood transfusions, the symptoms related to anemia and an improved quality of life (70, 71). Various landmark trials have dwelt on the convenience of a complete correction of anemia. Indeed, in the CHOIR trial the use of a target Hb level of 13,5 g/dl was associated with increased risk of suffering a composite of death, myocardial infarction, hospitalization for congestive heart failure and stroke, and no incremental improvement in the quality of life. CREATE trial did not observe an increase of the risk of cardiovascular events, but showed an increase in the necessity of dialysis among the group that targeted Hb in normal range (13–15 g/dl) The TREAT trial compared the use of darbepoetin alfa targeting Hb level of 13 g/dl vs. a rescue therapy when Hb level dropped below 9 g/dl in patients with diabetes, NDD-CKD and moderate anemia. The use of darbepoetin alfa did not reduce the risk of either of the two primary composite outcomes (either death or a cardiovascular event or death or a renal event), and was associated with an increased risk of stroke (65, 72–75). Yet, it is still unclear whether this increased risk is due to the higher ESA doses and the possible non-erythropoietic effects, whether it is due to the underlying systemic inflammation in patients with ESA-hyporresponsiveness, rather than due to the high Hb level itself (76).

Conversely, although some trials have demonstrated significant improvements in quality of life (QoL) in patients targeted to normal Hb levels (73, 75, 77) the clinical relevance

of these findings are questioned. The results of these trials were highly influential in changing the guidelines and clinical practice of anemia in NDD-CKD.

Current evidence, then, demonstrates a clear benefit in correcting Hb levels if they are below 10 g/dL, but also an increased risk when exceeding Hb 13 g/dl. The Hb target, then, appears to be somewhere in between 10 and 12 g/dl. Individualizing the Hb target relative to the patient's risks, basal conditions and preferences is advisable.

Iron Supplementation for Anemia in CKD

Guidelines acknowledge that the optimal strategy to manage iron metabolism remains unclear, and advocate for balancing the potential benefits and risks of iron supplementation (13, 62, 63). **Table 1** summarizes the principles and targets of the management of iron supplements of the KDIGO (13)—ERBP (62)—NICE (63) guidelines. In recent years some good quality pre-clinical studies, clinical trials and epidemiological studies have shed some light on the therapeutic approach regarding iron deficiency in CKD and will surely change clinical practice.

Intravenous (IV) iron has shown benefits both in DD-CKD and more recently in NDD-CKD, as it has proved to be more efficacious in rising ferritin and Hb levels, while reducing ESA and transfusion requirements. Specifically, in hemodialysis patients, oral preparations seem to be useless, maybe except for the phosphate binder ferric citrate (78). In addition, gastrointestinal intolerance and constipation reduce tolerance and compliance of oral iron formulations (79).

However, some concerns raised about IV iron formulation such as enhanced oxidative stress, endothelial dysfunction or the potential role in favoring infection. Further, IV iron administration has been associated with an increased risk of hypotension, headaches or hypersensitivity reactions. Labile iron, which is the iron that is freed into the circulation after administration and non-bound to transferrin, is an important cause of such adverse reactions.

IV iron supplements are non-biologic complex drugs. An iron core, covered by a complex structure of polysaccharides forms them. Indeed, the differences in the structure of the molecule among different IV iron formulations may be responsible for the differences in outcomes of each IV iron formulation. Some studies have even demonstrated differences in the attainment of Hb levels between the "original" brand and its generic form of iron sucrose (80).

On the other hand, there is growing evidence that oral compound can have a deleterious effect on gut microbiota which may worsen uremic dysbiosis (81, 82). Whether oral iron induced changes in gut microbiota, increases uremic toxins production and/or inflammation in CKD remain to be elucidated.

Dosing Patterns: The "Iron First-Approach" and the "High Dose-Low Frequency Approach"

As mentioned before, iron is essential for an adequate erythropoesis. In this sense, several trials have demonstrated that the correction of iron deficiency lessens the need for ESA in CKD patients (FIND:CKD) (49). Hence, results from TREAT study demonstrate that control group receiving only IV iron but no ESAs may increase Hb by 1 g/dl. The so called "iron fist approach" suggested by guidelines (CITA) is based in his efficacy for anemia correction. Unfortunately, we have no evidence of the effect on hard end-points. Moreover, the risk of Hb overshooting depends on high levels of EPO but no IV iron use, since iron is not a growth factor.

In addition, various studies carried out in patients with heart failure (HF) with reduced ejection fraction and iron deficiency demonstrated that IV iron supplementation [but not oral iron, (83)] have shown an improvement outcomes, HF symptoms, functional class and quality of life, (FAIR-HF, CONFIRM-HF, EFFECT-HF) (84–86). Further, a meta-analysis demonstrated that these benefits of IV iron therapy were independent of the presence of anemia. More recently, a reduction of hospitalizations for HF has also been demonstrated in a study among patients with acute heart failure and iron deficiency (AFFIRM_AHF) (87). Likewise, an improvement in renal function has been observed with IV ferric carboximaltose in a subanalysis of the FAIR-HF study (88). In a small study in patients with HF, CKD and anemia with iron deficiency, IV iron was associated with an improvement of myocardial function and of cardiac dimensions (89), similar to the observations in another pilot study (90). There is a considerable correlation between heart failure, iron deficiency and renal failure and each comorbidity reduces the survival of these patients (91). Large registry data show that CKD is present in 12 to 74% of HF cases (92) and that its prevalence increases as renal function declines.

The Anemia Working Group of the Spanish Society of Nephrology published a review advocating for this "iron first-approach" and recommending the administration of IV ferric carboxymaltose in patients with CKD, HF with reduced ejection fraction and iron deficiency, even in the absence of anemia, extrapolating the recommendations of the Heart Failure Guidelines of the European Society of Cardiology (93, 94).

Moreover, newer IV iron formulations are more stable and have safer profiles that allows the administration of higher doses of iron per session (95, 96). The recent PIVOTAL trial has confirmed the efficacy and safety of high-dose IV iron sucrose: it is a UK open label, randomized controlled trial among 2,141 incident hemodialysis patients, that compared a proactively administered high-dose IV iron regimen with a reactively administered low-dose regimen. The trial demonstrated that a proactive high-dose schema reduced the death of all causes or an aggregated of non-fatal cardiovascular events [HR de 0.85: IC 95% (0.73–1.0); $p < 0.001$ for non-inferiority and $p = 0.04$ for superiority]. In addition, the high-dose regimen was not associated with higher risks of death, major adverse cardiovascular events, or infection (97). These findings should lead to a change in clinical guidelines.

Iron Targets: How Much Iron Is Too Much Iron?

Iron overload is a condition of elevated body iron content associated with signs of organ dysfunction that is presumably

caused by excess iron. Some studies have demonstrated an increase in the liver iron content in hemodialysis patients, and an association between hepatic iron overload and hepatic steatosis has been recently described (98). However, its clinical relevance is still not known, and no deposits have been observed in other territories, such as cardiac or pancreatic (99–101). A metaanalysis of clinical trials and observational studies in the setting of hemodialysis suggests that patients that received higher doses of IV iron did not show a higher risk of mortality, infections or cardiovascular events (102). Nonetheless, the strength of the findings is limited by the small number of patients and of events in the clinical trials, and by the statistical heterogeneity in the observational studies included.

A recent epidemiological study has shown a slight higher mortality risk in patients with NDD-CKD and ferritin levels above 500 ng/ml, compared to patients with no iron deficiency, and patients with absolute or relative iron deficiency (40). These findings should be taken cautiously due to the presence of possible confounding factors. On the contrary, incident hemodialysis patients in the proactive high dose iron regimen in the PIVOTAL study showed a reduced risk in the primary end point (composite of death, MI, stroke, hospitalization and HF), as mentioned above in this article, and achieved higher mean ferritin levels (without exceeding 700 ng/ml, as per protocol). The upper limits of iron targets and the long-term safety of high doses of IV iron supplementation, specially of the accumulated high iron doses in hemodialysis patients, still needs to be clarified.

There has long been a concern whether iron supplements increased the risk of infections. A sub-analysis of PIVOTAL study did not show differences in infection episodes, hospitalization or death for infection between the proactive high dose regimen and reactive low dose-iron groups of patients (103).

Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitors

In recent years, new drugs have been developed for the treatment of anemia. These are the so called HIF-prolyl-hydroxylase inhibitors (HIF-PHIs). These drugs inhibit the action of prolyl-hydroxylase, which leads to an increase in the levels of HIF, and therefore, to an increase in endogenous EPO (24).There are currently four HIF-PHIs undergoing phase III clinical trials: roxadustat, daprodustat, vadadustat and molidustat. All of them are administered orally. However, they have differences in pharmacodynamics and pharmacokinetics, which probably determine differences in their interaction with the HIF system, and thus lead to differences in efficacy and safety profiles (104) (**Table 2**).

Other compounds such as desidustat (Zyan1; Cadila Healthcare), JNJ-42905343 (Janssen) or enarodustat (JTZ-951; Japan Tobacco/Akros Pharma), have completed phase II studies or are in early stages of development.

Efficacy of HIF Prolyl Hydroxylase Inhibitors

Roxadustat is the most advanced HIF-PHI under clinical development, which has already been approved in China and Japan. Two phase 3 studies were published in 2019 comparing roxadustat with placebo in NDD, and with epoetin alfa in DD-CKD patients in China. These studies had a relative small sample size a study population and of short duration. The former compared roxadustat with placebo, without adjuvant iron supplements, and demonstrated its efficacy in rising hemoglobin levels after 9 weeks (105). The latter compared roxadustat with epoetin alfa, with iron supplement only as a rescue therapy. After 26 weeks of follow up, the attained hemoglobin levels in the roxadustat group were non-inferior to those in the epoetin alfa-arm, and both groups had a similar safety profile (106). These results were similar to those found by a phase 3 study comparing roxadustat to ESAs in hemodialysis and peritoneal dialysis patients in Japan (107, 108).

The results of several phase III clinical trials were presented in the past 2019 and 2020 Annual Meetings of the American Association of Nephrology. The ROCKIES, PYRENES and SIERRAS studies compared roxadustat vs. epoetin alpha in prevalent HD patients (109–111). The HIMALAYAS study compared roxadustat vs. epoetin alpha in incident HD patients (112). In a pooled analysis ($n = 3.917$) roxadustat was significantly superior to EPO in anemia correction and the roxadustat group received fewer transfusions 9.5 vs. 12.8%; HR (95% CI) =0.82 (0.689, 0.99). In prevalent DD patients the risk of major cardiovascular events (MACE) was comparable between the two treatment arms, whereas there was a 16% reduction in the risk of MACE plus [HR = 0.84 (0.73, 0.97); $p = 0.02$] in the roxadustat group. (MACE+: Mace plus heart failure and thromboembolic events). Interestingly, patients receiving roxadustat had reduced iron needs, and those on roxadustat and an elevated C-reactive protein were able to increase Hb levels. Among 1.526 incident DD patients, there was a 30% reduction in the risk of MACE and a 34% reduction in the risk of MACE+: [HR (95% CI) = 0.70 (0.51, 0.97); $p = 0.03$] and [HR 0.66 (0.50, 0.89); $p = 0.005$] respectively, among patients receiving roxadustat (113).

The OLYMPUS, ALPS, and ANDES trials evaluated roxadustat vs. placebo in NDD-CKD patients (110, 114, 115). An integrated analysis ($n = 4.270$) showed that roxadustat was efficacious in achieving and maintaining Hb levels, with lower risk of rescue therapy. Regarding adverse events, both arms of treatment had comparable safety profiles regarding cardiovascular events and all-cause mortality (113).

The results of the DOLOMITES trial were presented i in the past ERA-EDTA congress in June 2020 (116) and in the 2020 ASN Annual Meeting (117). This phase 3, randomized, open-label, active-controlled study evaluated the efficacy and safety of roxadustat compared to DA in the treatment of anemia in NDD- CKD patients. The median time of follow up was 104 weeks and the study enrolled 616 adult anemic patients with CKD stages 3–5. Roxadustat was non-inferior to DA in the primary endpoint, which was the achievement of Hb response during the first 24 weeks of treatment. Regarding secondary efficacy endpoints, roxadustat was superior in decreasing low-density lipoprotein cholesterol and in time to first IV iron use. Roxadustat was non-inferior in blood pressure control and time to first occurrence of hypertension, in changes in Quality of life

TABLE 2 | Pharmacological characteristics and current knowledge status of different Hypoxia-inducible factor prolyl hydroxylase inhibitors.

	Roxadustat (FBG-4592)	Vadadustat (AKB-6548)	Daprodustat (GSK 1278863)	Molidustat (BAY-3934)
Affinity IC50 (uM)	0.027	0.029	0.067	0.007
PHD Isoform Selectivity	PHD 1-3	PHD 3>2	PHD 2-3	PHD 2< 1 and 3
HIFα selectivity	HIF1α and 2α	HIF2α > 1α	HIF1α and 2α	HIF1α and 2α
Inhibitory concen-tration IC 50 (μM)	>100	29	21	65
Half life humans (h)	12	4.5	4	Not available
Current status of development	2019 approval in Japan and China Phase III reported at ASN 2019	Ongoing phase III Preliminary report at ASN 2020	Ongoing phase III	Completed phase II
Phase III clinical trials	DD (HD/DP) and NDD Correction and maintenance	DD (HD/DP) and NDD Correction and maintenance	DD (HD/DP) and NDD Correction and maintenance	Not available

IC50, half maximal receptor inhibitory concentration; PHD, prolyl hydroxylase domain protein; HIF, hypoxia-inducible factor; ASN, American Society of Nephrology; DD, Dialysis Dependent; HD, Hemodialysis; DP, Peritoneal dialysis; NDD, Non-dialysis dependent.

scores, and in Hb change. The occurrence of treatment-emergent adverse events (TEAEs) was similar between the two groups, and the TEAEs leading to withdrawal of treatment were more frequent in the roxadustat group. They reported no significant differences between groups in adjudicated cardiovascular events. In all the Roxadustat studies the roxadustat patients presented an early and sustained LDL-reduction as a pleiotropic effect.

Another compound, vadadustat has also been approved in Japan in 2020. The results of two phase III trials comparing vadadustat vs. DA in Japanese HD and non-dialysis dependent patients were presented in past 2019 ASN annual meeting (NCT03439137 and NCT03329196, respectively). Over 300 patients were followed for 52 weeks in each study. In both trials, vadadustat showed non-inferiority in maintaining Hb levels within the target range and a similar safety profile. Additionally, vadadustat was associated with an increase in total iron binding capacity and a decrease in hepcidin in DD and NDD-CKD (118, 119). Furthermore, the results of the PRO2TECT study (NCT02648347) were presented at the 2020 ASN annual meeting. It consists of two randomized, phase 3, global, open-label, sponsor-blind, parallel-group, active-controlled non-inferiority trials comparing oral daily vadadustat to parenteral DA in NDD-CKD patients, already treated for anemia and non-previously treated (naïve), respectively. Vadadustat did not meet the pre-specified non-inferiority criterion compared to DA with regards to cardiovascular safety. Interestingly, cardiovascular safety was similar between the two arms of treatment in the regions were the Hb target was 10–11 g/dL, but the cardiovascular risk was higher in patients randomized to vadadustat in regions with a Hb target of 10–12 g/dL.

Daprodustat has also been approved recently for use in Japan, and various phase III clinical trials are currently ongoing: ASCEND-D (NCT02879305), ASCEND-ID (NCT03029208), ASCEND-TD (NCT03400033), ASCEND-ND (NCT02876835), and ASCENDNHQ (NCT03409107). Daprodustat has already demonstrated its efficacy in managing anemia of CKD both in DD and NDD-CKD patients in phase II studies.

Lastly, molidustat, which is structurally different to the other study drugs mentioned before, is currently being evaluated in small phase III studies (NCT03351166, NCT03543657, NCT03418168, NCT03350321, NCT03350347). Several phase II studies, which are part of the DIALOGUE program, compared molidustat with either placebo or ESA therapy in CKD patients, demonstrating the efficacy, safety and tolerability of the drug (120).

By the time of elaboration of the present manuscript, the Cochrane Kidney and Transplant Group

Has published the protocol for a systematic review on HIF-PHIs for the treatment of anemia of chronic kidney disease (121).

Safety of HIF Prolyl Hydroxylase Inhibitors

From a mechanistic point of view, the inhibition of prolyl-hydroxylases prevents HIF from degradation, leading to an increase of endogenous EPO within the physiological range, rather than the pharmacological levels achieved by current ESAs. Therefore, the rate of adverse events related to the high EPO levels should be expected to be lower than with ESAs. However, as mentioned before, HIF also modulates many other non-erythropoietic genes. This activity would explain the potential beneficial effects seen in pre-clinical and early clinical studies such as an improved iron utilization, HDL and LDL lowering effect, ischemia protection and a protective effect on CKD progression, improved neo-vascularization or better blood pressure control (122).

Nonetheless, potential deleterious side effects due to the modulation of other genes with this new class of drugs have also been postulated, notably tumor progression, enhanced vascular calcification, enhanced growth of renal cysts, worsening of retinopathy, or an increase in pulmonary artery pressure. In addition, whether these prolyl-hydroxylase inhibitors inhibit other di-oxygenases beyond HIF-PHIs and thus other pathways is unknown. Data from large phase III studies are still to be published and will surely help to answer these open questions.

In conclusion, in light of this evidence, HIF-PHIs, can be a viable alternative for the treatment of NDD and DD anemic CKD patients. However, more data is required regarding their long-term safety and their possible non-erythropoietic effects. In addition, the subset of patients that may benefit from these new agents still needs to be elucidated.

ANEMIA MANAGEMENT, ESAs AND COVID 19

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) has become a worldwide pandemic during 2020 and millions of cases have been reported worldwide. The coronavirus disease can lead to sepsis, acute kidney injury (AKI), multiple organ dysfunction and an atypical form of the acute distress respiratory syndrome. Anemia and a disturbed iron metabolism are common in COVID 19 patients. In an observational study Among 11,265 patients across 13 New York hospitals admitted between March 1 and April 27 2020, an elevation in D-dimer level was associated with a lesser median hemoglobin level and a greater serum ferritin level. And so are they in patients with COVID 19 suffering an AKI and in maintenance dialysis patients (123, 124). The exact mechanisms of COVID 19 are not completely known, but patients with a severe COVID 19 often present with an intense inflammatory phase and with a prothrombotic state. In these cases, the efficacy of ESAs is limited and they could even be potentially harmful. Fishbane et al. in a recent editorial article suggest avoiding ESA therapy (124). In the case of maintenance dialysis if the patient was already on that treatment, the authors recommend the continuation of ESA at the same dose but targeting a lower Hb targets (Hb 8–9 g/dL). Some other authors speculate on the potential role of HIF –PHDs as a protective agents against COVID. Indeed, the activation of the HIF1α pathway would decrease angiotensin convertase enzyme 2 (ACE2), which is the bound for COVID 19 membrane spike protein to enter the host cell, and therefore, decrease the invasiveness of SARS-CoV-2 (125).

Regarding iron supplementation, systemic inflammatory processes as happens with severe COVID 19 decrease the availability of iron. Furthermore, iron is also essential for viral replication (126). In addition, patients with viral infections and iron overload have a poor prognosis. Therefore, limiting iron supplements could be beneficial for patients with severe COVID 19 although more studies are need to shed light into this subject (127).

Anemia in CKD-Key Points

- The pathophysiology of CKD-anemia is multifactorial, thus requiring a holistic approach
- Not all ESA are equal and whether their different pharmacokinetics and pharmacodynamics is associated with different outcomes in CKD patients remains to be elucidated.
- Iron is essential for other physiologic process beyond erythropoiesis. Observational studies in NDD-CKD patients suggest that iron deficiency is associated with worse outcomes, paving the way to randomized controlled trials that demonstrate the benefit of correcting iron deficiency beyond anemia.
- The upper limits of ferritin and TSAT indicating iron overload and risk of developing adverse events are still not clear, especially in the long-term.
- HIF prolyl hydroxylase inhibitors are new drugs under clinical evaluation. The available data suggest that they are efficacious and safe alternatives to ESA for the treatment of anemia in NDD and DD-CKD patients with several potential advantages over current therapies. However, more data is required to confirm these findings.

AUTHOR CONTRIBUTIONS

All authors contributed to the writing article and approved the submitted version.

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Thrombotic Microangiopathy After Kidney Transplantation: An Underdiagnosed and Potentially Reversible Entity

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Thrombotic microangiopathy is a rare but serious complication that affects kidney transplant recipients. It appears in 0.8–14% of transplanted patients and negatively affects graft and patient survival. It can appear in a systemic form, with hemolytic microangiopathic anemia, thrombocytopenia, and renal failure, or in a localized form, with progressive renal failure, proteinuria, or arterial hypertension. Post-transplant thrombotic microangiopathy is classified as recurrent atypical hemolytic uremic syndrome or *de novo* thrombotic microangiopathy. *De novo* thrombotic microangiopathy accounts for the majority of cases. Distinguishing between the 2 conditions can be difficult, given there is an overlap between them. Complement overactivation is the cornerstone of all post-transplant thrombotic microangiopathies, and has been demonstrated in the context of organ procurement, ischemia-reperfusion phenomena, immunosuppressive drugs, antibody-mediated rejection, viral infections, and post-transplant relapse of antiphospholipid antibody syndrome. Although treatment of the causative agents is usually the first line of treatment, this approach might not be sufficient. Plasma exchange typically resolves hematologic abnormalities but does not improve renal function. Complement blockade with eculizumab has been shown to be an effective therapy in post-transplant thrombotic microangiopathy, but it is necessary to define which patients can benefit from this therapy and when and how eculizumab should be used.

Keywords: thrombotic microangiopathy, kidney transplantation, atypical hemolytic uremic syndrome, eculizumab, complement system activation

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INTRODUCTION

Thrombotic microangiopathy (TMA) is a life-threatening disease, characterized by endothelial dysfunction and the presence of thrombi in small blood vessels. As the thrombus forms, there is platelet consumption and mechanical disturbance of red blood cells, leading to thrombocytopenia and microangiopathic hemolytic anemia. Vessel occlusion results in tissue ischemia and organ damage, primarily affecting the kidneys, although other organs can be involved (1–4).

TMA syndromes can be classified according to the pathogenic mechanism (5, 6). Primary TMA syndromes include TMA whose etiology is known: thrombotic thrombocytopenic purpura (TTP), due to deficiency of the von Willebrand factor-cleaving protease ADAMTS13 (7); typical hemolytic uremic syndrome (HUS), caused by Shiga toxin-producing *Escherichia coli* (8); pneumococcal-associated HUS (9, 10); and atypical HUS (aHUS) caused by inherited

or acquired abnormalities in complement proteins leading to unregulated activation of the alternative pathway of the complement system and the formation of the membrane attack complex (MAC) (11, 12). Other genetic causes, such as diacylglycerol kinase ε, an endothelial cell and podocyte protein, and cobalamin C deficiency have been described as causes of primary aHUS, mainly in children (13, 14). Secondary TMA syndromes occur in the context of infections, organ transplantation (solid organ and hematopoietic stem cell transplantation), drugs (cancer chemotherapy, vascular endothelial growth factor [VEGF] inhibitors, immunosuppressants such as calcineurin inhibitors [CNIs] and mammalian target of rapamycin inhibitors [mTORis]), malignancies, pregnancy, malignant hypertension, and autoimmune diseases (systemic lupus erythematosus, antiphospholipid syndrome, scleroderma, and vasculitis) (3, 5, 6). The distinction between primary and secondary TMAs is not absolute because genetic variants have been identified in patients with secondary TMAs. Moreover, secondary TMAs are also called secondary aHUS, because a complement deregulation has been described in some of those conditions, suggesting an overlap between these categories (15).

Post-transplant thrombotic microangiopathy (PT-TMA) is a rare but devastating condition that can lead to poor patient and graft outcomes. It can occur as a *de novo* disease or as a recurrence of a previous aHUS (sometimes undiagnosed before kidney transplantation). *De novo* PT-TMA is caused by various pathogenic mechanisms, whereas aHUS recurrence is a consequence of complement system deregulation triggered by several activating conditions (**Table 1**). The primary aHUS normally requires a second hit for disease to develop. The aHUS triggers match several situations that can produce secondary TMA, making the distinction between the two entities difficult (16).

TABLE 1 | Causes of post-transplant thrombotic microangiopathy.

1. Caused by complement protein mutations: atypical hemolytic uremic syndrome
2. *De novo* post-transplant associated TMA or secondary aHUS
   a. Related to the type of donor and the organ procurement
      - Complement activation associated to DBD and CDC
      - Ischemia reperfusion injury
   b. Associated to post-transplant events
   - Drugs
      - Calcineurin inhibitors
      - mTOR inhibitors
   - ABMR
   - Infection
   - Viral: CMV, parvovirus, Nile fever
   - Funghi

   Antiphospholipid syndrome
   c. Other causes of TMA not related to the kidney transplant: malignancies, pregnancy, other drugs (anti VEGF, gemcitabine,…)

DBD, donor after brain death; DCD, donor after cardiocirculatory death; ABMR, antibody mediated rejection; CMV, cytomegalovirus.

EPIDEMIOLOGY OF POST-TRANSPLANT THROMBOTIC MICROANGIOPATHY

Post-transplant TMA is observed in 0.8–14% of kidney transplants (17, 18). (USRDS) *de novo* PT-TMA is much more frequent than recurrent aHUS (90 vs. 10% of all cases), but the risk associated with the development of PT-TMA is much higher (36.5 times; 29 vs. 0.8%) in patients with a history of aHUS (18).

CLINICAL MANIFESTATIONS OF POST-TRANSPLANT THROMBOTIC MICROANGIOPATHY

The manifestations of PT-TMA are quite variable and can range from a limited form confined to the kidney to a full-blown systemic variant (19, 20, 24). The systemic form is typically acute, consisting of the classic triad of thrombocytopenia, microangiopathic hemolytic anemia with increase in lactate dehydrogenase, reduced haptoglobin, and schistocyte formation, and acute kidney injury (AKI); this has been described in 18–62% of patients with PT-TMA (19, 21, 25, 26). The localized form can manifest as isolated AKI or as a chronic form with slowly progressive graft dysfunction, proteinuria, or difficult-to-control arterial hypertension, and it can only be diagnosed when a kidney biopsy is performed (5, 27).

Although aHUS recurrence and PT-TMA are clinically and pathologically indistinguishable, a personal and family history of aHUS, an abrupt onset, and a complete and systemic TMA are suggestive of aHUS recurrence (28). Extrarenal manifestations of aHUS apart from hemolytic anemia (29–34) are frequent in aHUS recurrence, but they are rarely observed in *de novo* PT-TMA (35).

PT-TMA and aHUS recurrence can appear at any time in the post-transplant course (17, 36), but they develop primarily in the first 3 months after transplantation (21, 37), in conjunction with the presence of more complement activating events (e.g., ischemia-reperfnusion injury, high immunosuppressive drug levels, higher infectious risk). Systemic TMA usually appears in the early post-transplant period, and the localized form can appear at all stages after transplantation.

HISTOLOGICAL CHANGES

In the kidney biopsy, the active lesions are intraluminal fibrin occlusive thrombi along with endothelial cell activation signs, such as endothelial swelling, fragmented red blood cells in capillaries, mesangiolysis and microaneurisms, myocyte necrosis, and intramural fibrin in arterioli. Immunofluorescence microscopy is negative, except for fibrinogen. Electron microscopy shows subendothelial widening by flocculent material ("subendothelial fluff"). In the chronic phase, the characteristic lesions are double contour formation in peripheral walls with hyaline deposits in arterioles and fibrous intimal thickening with concentric lamination (onion skin). By electron microscopy, new subendothelial basement membrane and widening of the subendothelial zone can be observed (15, 21, 25, 38). The lack of thrombi in the biopsy does not

TABLE 2 | Recurrence risk of aHUS after kidney transplantation in the preeculizumab era.

High (RR 80–90%)	- Previous recurrence - Pathogenic mutations in CFH - CFH/CFHR1 hybrid genes. >80 of recurrence rate. High graft loss risk (45, 114) - CFB > 80% of recurrence risk - TBHD = 80% (46, 47)
Moderate (RR 40–75%)	- Isolated CFI mutations. 40–60% recurrence risk (11, 39, 41, 44) - C3. 30–70%* - Detectable circulating Anti–CFH antibodies (48–50) - Two at risk CFH haplotypes (39) - Negative complement genetic study 30% (11) - Complement gene mutation of unknown significance
Low (RR <20%)	- Isolated MCP mutation+(41) - DGKε mutation (13) - Negative Anti–CFH antibodies at the time of transplantation (48–50)

**Recurrence risk varies depending on the different series. +Recurrences in patients with MCP account for patient with combined mutation/risk polimorphisms.*
RR, recurrence risk; CFH, complement factor H; CFHR1 complement factor H related protein 1; CFB, complement factor B; TBHD, thrombomodulin; CFI, complement factor I; MCP, membrane cofactor protein; DGKε, diacylglycerol kinase epsilon.

exclude TMA (15). The biopsy does not allow the identification of the etiology, although some changes might suggest certain etiologies, such as C4d deposition, peritubular capilaritis, and glomerulitis in antibody mediated rejection (ABMR) or intimal thickening, reduplication of the elastic lamina, and hyaline degeneration in arterial hypertension. In PT-TMA, histological characteristics can be influenced by the donor's previous injuries. Thus, the interpretation of chronic injuries can be more complex. The findings of any acute injuries in the biopsy, together with clinical and laboratory data, can lead us to suspect TMA activity and the need for active treatment.

After the diagnosis of TMA, the etiology of the native kidney ESRD should be investigated to rule out previously missed aHUS (38).

PROGNOSIS FOR POST-TRANSPLANT THROMBOTIC MICROANGIOPATHY

The overall prognosis for PT-TMA is quite poor for the allograft and for the patient, resulting in a graft loss rate of 33–40% in the first 2 years (17, 18, 20, 21, 23), and a patient survival of 50% at 3 years after TMA diagnosis in a previous series (18); recently, however, 97% 1-year patient survival has been reported (25). Recurrence of aHUS appeared in 60% of transplant patients with the disease, leading to 89–90% graft loss in the first year in the pre-eculizumab era (39). In case of PT-TMA, although poorer short-term graft survival had been described in patients with the systemic form of PT-TMA, reflecting a more severe disease with a higher incidence of dialysis-dependent AKI and plasma exchange (PE) needs (19), the prognosis in the long term is similar in both forms (19, 25, 26). The prognosis is also similar in early (<3 months) and late (>3 months after transplantation) presentation (25).

CAUSES OF POST-TRANSPLANT THROMBOTIC MICROANGIOPATHY

At the time of kidney transplantation, the coincidence of several mechanisms that activate the complement system can trigger the recurrence of aHUS in patients with a genetic background or the development of *de novo* PT-TMA.

aHUS Recurrence

In aHUS, the recurrence risk is determined by a genetic mutation in complement proteins (11, 15, 39–41, 48). Stratifying the risk according to the mutation is the key to making decisions about the post-transplant management of aHUS. A higher recurrence risk is considered in patients with recurrence in previous transplants and in carriers of pathogenic variants in CFH/CFB/CFH:CFHR1 rearrangements/TBHD; moderate risk in carriers of CFI variants/C3/anti-FH antibodies/homozygous for haplotypes CFH-H3/absence of variants (29, 39–48, 114); and low risk in those with isolated MCP/DGKE variants and with negative anti-FH antibodies at the time of transplantation (13, 40, 48–50) (**Table 2**). The use of prophylactic eculizumab reduced the post-transplant recurrence rate from 49 to 12% in patients with aHUS, reducing the probability of graft loss, and significantly increasing the number of patients with aHUS who receive a kidney transplant (51).

Secondary Thrombotic Microangiopathy

In the absence of previous aHUS, Chua et al. (52) showed activation of the classical and terminal complement in several transplant-associated conditions: in donors after brain death (DBD) or donors after circulatory death (DCD) (53–55), or associated with ischemia reperfusion injury (56), immunosuppressive drugs (mainly CNI or mTORi) (41, 42, 57–61), ABMR (62–68), viral and fungal infection (69–82), and recurrence of antiphospholipid syndrome (APS) (83–85). The laboratory tests needed to evaluate causes of PT-TMA are described in **Table 3**. *De novo* aHUS has also been described in patients with C3 glomerulopathy in the native kidneys (86, 87) and in 1 patient with TTP (88).

All these triggers appear in patients with or without a known genetic background; Le Quintrec reported 30% complement genetic mutations in a series of patients with *de novo* PT-TMA (29), indicating an overlap between recurrent and *de novo* TMA.

Complement Activation Related to the Donor and Procurement Process

Activation of the complement system can be observed from earlier stages of transplant. This activation can be related to the type of donor. Naesens et al. (53) showed a relevant increase in expression of complement factors, such as C1, C3, and CFB, in renal allograft pre-implantation and after transplantation biopsies in DBD compared with living donor (LD) biopsies. Ischemia reperfusion damage has been associated with early

TABLE 3 | Main laboratory tests to perform in PT-TMA.

Identify TMA		
	Anemia	- Dropping hematocrit
	Thrombocytopenia	- ≥25% drop from baseline
	Schystocytes	- Often absent. Fairly pathognomonic when present
	Elevated LDH	- ≥2 fold increase
	Descended haptoglobin	
	Rising creatinine (or absence of improvement in recent transplanted patients)	- 25% increase or slow and progressive increase in sCr
	Proteinuria	- >300 mg/d
Rule out	**TTP**	
	ADAMTS13 activity <10%	
	Plasmic score*	
	STEC-HUS	
	Stool culture for *E. coli*	
	PCR in stool sample for shiga toxin	
	CbIC deficiency	
	Elevated plasma homocysteine	
	Eleveated methylmalonic acid in urine or plasma	
	Abnormalities in *MMACHC* gene	
PT-TMA	**DITMA**	
	Immunosuppresive (CsA, TAC, EVE, SRL) plasmatic levels	
	Infections	
	Blood PCR for CMV, BK virus	
	Microbiological tests to discard Influenza, H1N1, HH6, HIV, HCV, Funghi	
	ABMR	
	Antibodies to Class I + II HLA (Luminex)	

**Plasmic score: a laboratory-derived scoring system to predict TTP. Five independent markers were identified as highly predictive of TTP, including a platelet count less than 30 × 10^9/L, serum creatinine level less than 2.0, INR less than 1.5, mean corpuscular volume (MCV) less than 90, and the presence of a hemolysis variable (reticulocyte count greater than 2.5 percent, undetectable haptoglobin, or indirect bilirubin greater than 2.0 mg/dL). Absence of active cancer, solid-organ transplant, or stem-cell transplant have high negative predictability and were also included in the score. Each of the seven factors is given a score of one point if present. Score = 0 predicts 4.3 risk of TTP. Score = 7 predicts 96.2 percent risk of TTP. An intermediate score of five to six has limited utility with a TTP risk prediction of 56.8 percent.*

injury of the renal allograft. Experimental data have suggested that, after ischemia reperfusion, complement is activated by the lectin pathway, and afterward, the alternative complement pathway could amplify the injury through the release of C3, C5, and the MAC. In addition, the damage to endothelial glycocalyx secondary to ischemia would reduce the union of factor H to the endothelial cells. In a similar manner, this complement activation after ischemia reperfusion would be higher in recipients of kidneys from DBDs than from LDs (54–56). The use of kidneys from DCDs has been associated with a higher risk of PT-TMA. The prolonged warm ischemia in this type of donor would aggravate the endothelial lesions in the graft and would increase complement activation and secondary damage (41, 112).

Drug-Induced Thrombotic Microangiopathy

Drug induced TMA (DITMA) is suspected when there is a sudden onset acute kidney injury, usually within hours or a few days after drug exposure, and resolution can be observed when the drug is stopped or reduced (57, 89, 113).

The association between CNIs and *de novo* PT-TMA has been well documented in the literature, with the risk higher with cyclosporine than with tacrolimus (41). Various mechanisms have been associated with the development of PT-TMA after CNI treatment. The loss of normal equilibrium between vasoactive peptides, with an increase in vasoconstrictor substances, such as angiotensin II, thromboxane A2, and endothelin, and a reduction of vasodilatory molecules, such as prostaglandin (PG) E2, prostacyclin (PGI2), and nitric oxide leads to arteriolar vasoconstriction and endothelial injury secondary to renal ischemia. CNIs also favor platelet aggregation and plasminogen activation, with a higher risk of thrombosis. Cyclosporine causes endothelial cells to release microparticles that activate the alternate complement pathway (58).

The diagnosis of CNI-related TMA is found in the early post-transplant period when the levels of these drugs are high. Recently, new advances in the understanding of TMA and its association with complement abnormalities have questioned the relevance of CNIs in this disease. It has been suggested that there must be some predisposing factors for PT-TMA development in patients taking CNIs, given that more than 95% of renal transplant recipients receive this treatment. Data from USRDS have shown a higher PT-TMA incidence in patients without CNI treatment. In addition, results from a French aHUS registry have not shown the association between CNIs and PT-TMA, and the lack of CNI use did not prevent the recurrence of aHUS in this study group (42). It was thought that mTORi could be a good alternative to CNIs for patients with aHUS. Unfortunately, various studies have not shown this protective effect. Data from USRSD showed a higher incidence of PT-TMA with sirolimus than with CNIs, and the French registry results highlighted a higher risk of recurrence post-transplant in patients with aHUS when mTORis were used. However, the fact that mTORis might have been used as a rescue therapy after diagnosis of PT-TMA limits the interpretation of these results (18, 41, 57). Inhibition of mTOR inhibition leads to the death of endothelial progenitor cells and the decrease in renal expression of vascular endothelial growth factor (VEGF), which also would lead to a reduction in Factor H synthesis. Other factors, such as an increased procoagulant and a reduced fibrinolytic state, are also believed to contribute to the pathogenesis of TMA in patients taking mTORis (59, 60). Recently, the combined use of CNIs with mTORis is increasing as an alternative treatment for renal transplant recipients, but this combination increases the risk of PT-TMA compared with single medication treatment, particularly when the blood levels of both drugs are high (22, 23, 61).

Antibody-Mediated Rejection-Associated Thrombotic Microangiopathy

The complement system plays an important role in ABMR. The donor-specific antibodies bind to human leukocyte antigens on the allograft endothelium and activate the classical complement pathway through C1q, leading to activation of C4 and C3. The deposit of C3b on the membrane of endothelial cells triggers the activation of the alternate complement pathway with the generation of the MAC, which produces cell lysis and an inflammatory infiltration (62). The histological finding of TMA in patients with ABMR has wide variability, between 4 and 46%, most likely as a consequence of the focal presentation of the disease (63, 64). A negative impact of TMA on ABMR has been described. Wu et al. (65) found lower graft survival in patients with ABMR and TMA compared with ABMR without TMA. Diagnosis of TMA in the sensitized kidney transplant recipient is predominantly confirmed by histological findings.

Infection-Related Thrombotic Microangiopathy

Viral infections can trigger PT-TMA due to the endothelial trophism of the virus, which induces the expression of adhesion molecules and the release of von Willebrand factor, causing platelet adhesion and microvascular thrombosis (71). CMV is the most frequently involved virus (69–73). In all CMV-related TMA cases, treatment with intravenous ganciclovir and plasma exchange (PE) resolved hemolysis; however, a TMA recurrence occurred in one case, which was resolved with valganciclovir and eculizumab (71). Parvovirus (74–76), hepatitis C virus, and its treatment (77–79) and fungal infections such as histoplasmosis PT-TMA have also been described (80, 81). Recently a case of ABMR and TMA associated with Nile Fever has been reported (82).

Other Causes of Post-transplant Thrombotic Microangiopathy

APS can cause ESRD due to large and small kidney vessel thrombosis and TMA. These symptoms can recur after transplantation, and eculizumab has shown a beneficial effect in patients with this disease, preventing and treating the recurrence of APS (83–85).

TREATMENT OF POST-TRANSPLANT THROMBOTIC MICROANGIOPATHY

Treatment of aHUS Recurrence

Classically, transplantation of patients with aHUS has shown low graft survival with a high rate of loss due to recurrence of the

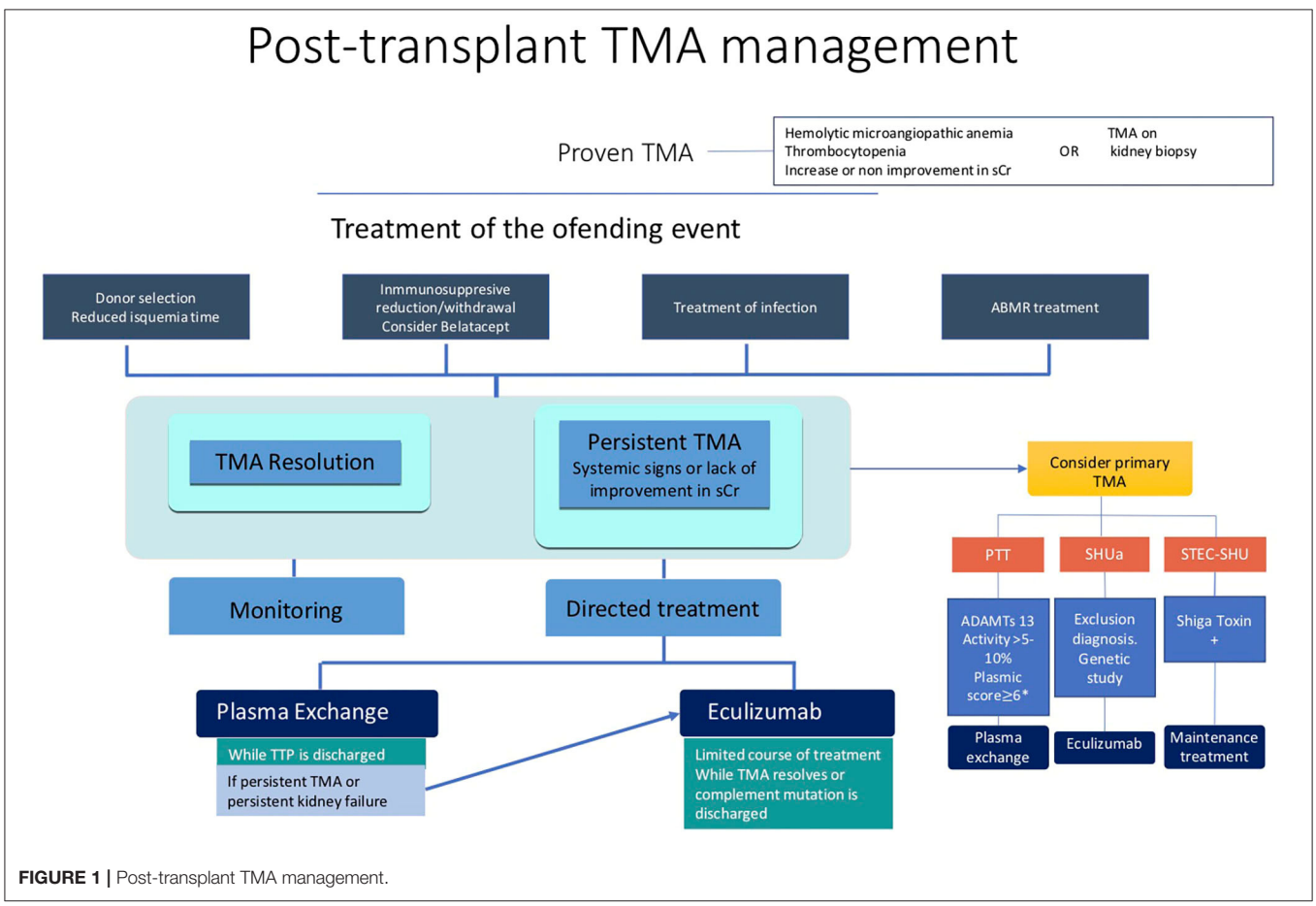

FIGURE 1 | Post-transplant TMA management.

disease. Prophylactic treatment or recurrence treatment are the two alternatives. Prophylactic treatment based on plasmapheresis does not effectively prevent recurrence in patients with high or moderate risk mutations, and subclinical complement activation have been reported in these patients. Excellent results with eculizumab as the first-line therapy have been reported; the usefulness of a first dose 1 h before reperfusion and a second dose 24 h after transplantation has been considered, both to reduce secondary complement activation due to ischemia-reperfusion. In the treatment of recurrence, little success has been achieved after plasmapheresis; nevertheless, good results have been observed with an early introduction of eculizumab after recurrence (42).

Treatment of *de novo* Post-transplant Thrombotic Microangiopathy

Treatment of *de novo* PT-TMA should be based on correcting the potential cause of the disease and varies depending on the time of onset. Given the extreme heterogenicity of the mechanisms related to the appearance of TMA, therapeutic maneuvers must be individualized. The first step is to avoid complement over-activation before donation, preventing renal hypoperfusion during organ procurement, and reducing cold ischemia time.

In cases of PT-TMA secondary to immunosuppressive medications, the first step is to reduce or stop the offending agent, by switching from a CNI to another CNI or to an mTORi. This approach can resolve the TMA, but the effectiveness of this strategy is controversial. Satoskar showed no difference in outcomes between changing immunosuppression or not (20). Belatacept, a cytotoxic T lymphocyte antigen 4-immunoglobulin fusion protein that inhibits T cell function, allows the minimization or discontinuation of endothelial toxic immunosuppressants such as CNIs and mTORis (90, 91). However, a higher risk of acute kidney transplant rejection compared with current standard immunosuppressive therapy has been observed after conversion to belatacept in kidney transplant rejection (92).

In ABMR-associated TMA the mainstay of treatment is plasmapheresis (PP), with or without IVIg and additional immunosuppression (65–68). Despite the implication of complement activation in ABMR, the efficacy of complement inhibitors for the prophylaxis or treatment of ABMR is difficult to assess with current clinical data and is yet to be established. Instead, eculizumab use is currently recommended as a rescue therapy in AMR-associated TMA when hemolysis persists despite maximal management including plasma exchange (PLEX) and in those with PLEX dependency (66–68).

PT-TMA that is unresponsive to the previous measures has typically been treated with PE. PE has been shown to reduce mortality in TTP patients (93, 94), and it was the first-line therapy for aHUS in the pre-eculizumab era. PE can remove vasoconstrictor molecules such as thromboxane A2 and mutant complement proteins and provides deficient factors such as PGI2-stimulating factor and normally functioning complement components (27, 36). Due to these effects in primary TMA, the use of PE was extrapolated to PT-TMA. In 2003, Karthikeyan et al. (36) reported a graft salvage rate of 80% with PE in addition to CNI withdrawal in 29 patients with biopsy-proven TMA. Epperla et al. (95) showed a 100% response rate in 5 patients using withdrawal of the suspected offending agent associated with PE in 4, eculizumab in 2, and rituximab in 1 patient. However, the use of PE in a large proportion of patients does not improve kidney function despite correcting the hematological abnormalities, and it is associated with a 20–42% risk of graft loss (97, 98).

TABLE 4 | Eculizumab in kidney transplant associated TMA*.

Cause	*n*	Previous management	Eculizumab duration	Outcomes after eculizumab	Comments
CAPS (83, 85)	8	Usual CAPS treatment	5w-Indefinite	3 pt: no CAPS relapse 5 pt: TMA resolution	CAPS prophylaxis in KT TMA treatment
Early CA (donor factors, Drugs) (53–61)	10	Treatment of the offending event PE	2 w−4 m**	7 pt: renal recovery and TMA resolution 1 pt: PRR 2 pt: graft loss	In all patients: more than one causal event 2 patients shared the same donor, indicating pre-tx CA
CMV (71)	1	Antiviral	1 yr	TMA resolution	Eculizumab+Valganciclovir
PT-TMA (97)	15	DW, DR PE 12 pt	2-52 w	TMA resolution Kidney function improvement	No graft loss No recurrence after ecu discontinuation
MAT PT (98)	16 Early 6 Late	DW,DR PE 13/16 pt PE 6/6 pt	3 w 11 w	8/11: CRR 2/11: PRR 1/6: CRR 2/6: PRR 3/6: Graft Loss	A shorter interval between TMA diagnosis and eculizumab leads to a better kidney function recovery

**Antibody mediated rejection related TMA is not included.*

***In one patient, the length of treatment is not described.*

CAPS, Catastrophic antiphospholipid syndrome; Pt, patients; KT, kidney transplant; CA, complement activation; PE, plasma exchange; CRR, complete renal remission, PRR, partial renal remission; DW, drug withdrawal; DR, drug reduction.

Schwimmer showed similar graft outcomes in a series of 742 PT-TMA transplants, regardless of whether they had received PE (19).

Eculizumab, a recombinant, fully humanized monoclonal antibody targeted against human complement protein C5, blocks generation of the lytic C5b-9 MAC. It has been shown to be effective in the treatment and prevention of recurrent aHUS after transplantation (99–103). In post-transplant TMA, a complement over-activation has been demonstrated (51–55, 104), even in patients without pathogenic complement proteins variants. Therefore, the inhibition of the complement system could be a suitable PT-TMA approach (**Figure 1**) (96).

The main risk associated with the eculizumab use is meningococcal infection; thus, vaccination against *Neisseria meningitidis* serogroups B, A, C, W135, and Y is mandatory. Vaccination should be administered 2 weeks before initiation of eculizumab treatment. But in PT TMA, usually it is not possible to delay eculizumab treatment two weeks, and prophylactic antibiotic should be used in the meantime. Given neither vaccines nor prophylaxis guarantee full protection against meningitis, patients and their families should be taught to recognize the alarm signs and symptoms of the infection (48).

Several case reports and small case series have been published documenting the efficacy of eculizumab in *de novo* PT-TMA refractory to previously mentioned treatments (**Table 4**) (53–61, 71, 83–85, 97, 98). Recently, the efficacy of eculizumab has been described in larger series. Cavero et al. (97) showed 15 kidney transplanted patients with PT-TMA; 14 patients were using tacrolimus, 4 of them combined with an mTORi. All but one withdrew the offending drug. Twelve patients also received PE (a mean of 5.46 sessions per patient, range 2–12) without improvements in serum creatinine (mean 4 mg/dl, 3.4–5.6). Eculizumab was started from 4 to 53 days after TMA detection, and a mean of 6.4 doses from 2 to 17 per patient were used. At the end of follow-up, mean serum creatinine was 1.7 mg/dl, with no graft losses or recurrences of TMA after eculizumab discontinuation. Portolés et al. (98) reported 22 patients with PT-TMA, 16 with early- (<1 month) and 6 with late- (>1 month post-transplant) onset TMA. All patients presented hematological TMA and most had a confirmatory biopsy. Some 77% of early and 100% of late patients with PT-TMA received PE, achieving a complete hematological response, but with an incomplete or an absent renal response in most cases. Eculizumab was then added, for an average of 21 days in the early TMA group and 83.5 days in the late TMA group. In the early group, 8 complete and 2 partial responses were observed, whereas in the late group, one patient had a complete response, two had a partial renal response, and the remaining patients lost their grafts. The patients with better responses had a shorter time lapse between diagnosis and the beginning of the treatment. Eculizumab was withdrawn in all cases, without relapses.

Eculizumab has been used more frequently in cases of lack of renal recovery after a short course of PE, obtaining an improvement of kidney function with few adverse events.

The duration of eculizumab treatment in cases of aHUS recurrence can be lifelong (depending on the genetic mutation); in secondary TMA, however, it is not clearly addressed. In previous reports, a short course of eculizumab was shown to be an efficient therapy to control TMA and to reduce the risk of graft loss due to this disease. Discontinuation of the therapy can be considered when complications of TMA have completely resolved, and on a case-by-case basis. It can also be considered when the kidney function has not improved after 3 to 6 months or when a lack of viability of the kidney graft is documented by means of a kidney biopsy or imaging tests (computed tomography, magnetic resonance imaging).

The current data are not strong enough to expand the recommendation for the use of eculizumab in all patients with PT-TMA, but in cases resistant to treatment of the offending event, eculizumab is a good therapeutic option. Although PE has been used, its efficacy is limited and it is important to note that in patients with PT-TMA, the sooner eculizumab starts, the better the renal function will be at the end of the follow-up (97, 98).

CONCLUSION

The incidence and the impact of PT-TMA, either *de novo* or recurrent, on allograft survival is underestimated. Several factors related to the donor and procurement process and to the recipient and events during the post-transplant period (immunosuppressive drugs, rejection, and infections), trigger the development of this disease. The treatment of PT-TMA includes management of the offending event, PE, and recently, eculizumab, with promising results. However, further studies are needed to establish which PT-TMA kidney transplant recipients are most likely to benefit from eculizumab therapy, including when and how to use it given the high economic burden associated with this approach.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

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Microalbuminuria After Kidney Transplantation Predicts Cardiovascular Morbidity

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Background: Microalbuminuria is a well-characterized marker of kidney malfunction, both in diabetic and non-diabetic populations, and is used as a prognostic marker for cardiovascular morbidity and mortality. A few studies implied that it has the same value in kidney transplanted patients, but the information relies on spot or dipstick urine protein evaluations, rather than the gold standard of timed urine collection.

Methods: We revisited a cohort of 286 kidney transplanted patients, several years after completing a meticulously timed urine collection and assessed the prevalence of major cardiovascular adverse events (MACE) in relation to albuminuria.

Results: During a median follow up of 8.3 years (IQR 6.4–9.1) 144 outcome events occurred in 101 patients. By Kaplan-Meier analysis microalbuminuria was associated with increased rate of CV outcome or death ($p = 0.03$), and this was still significant after stratification according to propensity score quartiles ($p = 0.048$). Time dependent Cox proportional hazard analysis showed independent association between microalbuminuria and CV outcomes 2 years following microalbuminuria detection (HR 1.83, 95% CI 1.07–2.96).

Conclusions: Two years after documenting microalbuminuria in kidney transplanted patients, their CVD risk was increased. There is need for primary prevention strategies in this population and future studies should address the topic.

Keywords: kidney transplantation, albuminuria, urine collection, cardiovascular morbidity, proteinuria

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INTRODUCTION

Increased urine protein excretion is a validated risk factor for end stage kidney disease (ESKD), and cardiovascular morbidity and mortality (1–3). microalbuminuria defined as urine excretion between 30 and 300 mg albumin per 24 h, has been used as an early marker for diabetic kidney disease (4, 5), and has been recognized as an important marker for cardiovascular disease (CVD) in the diabetic and the non-diabetic populations (6, 7).

Following kidney transplantation recipients become a unique chimera, in which the renal vasculature is different from that of the other organs. This is an interesting setting to explore the link between microalbuminuria and CVD, as the kidney endothelium, that facilitate most of the leakage of protein into the urine, represents the donor rather than the recipient. A paper published in 2007, was the first to describe in kidney transplanted patients the correlation between

albuminuria, mortality and eventually graft loss, stating that its prognostic strength surpasses that of renal function (8). The largest retrospective analysis published so far, implied that transplanted patients could manifest the same relationship between albuminuria and CVD. Weiner et al. showed that microalbuminuria, as evaluated by albumin creatinine ratio (ACR), had a non-significant trend for association with increased risk of CVD in patients after kidney transplantation (9). An analysis from our institute showed proteinuria, using urine dipstick, was significantly associated with an increased risk of major cardiovascular adverse events (MACE) (10). However, this association was not evaluated by the gold standard of timed urine collection. Our study sought to characterize the association between microalbuminuria, as evaluated by precise urine collection, and CVD risk in stable patients after kidney transplantation.

METHODS

The study cohort included adult kidney allograft recipients that participated in a study comparing the agreement between urine collection and albumin creatinine ratio for evaluation of urinary albumin excretion, termed UAE (11). Sixty-six patients were not included in the original report because their samples were intended to investigate albuminuria and obesity in transplanted patients, a study that was not completed. Our cohort included these patients, and the original cohort of 264 patients to create a cohort of 330 patients. Of these patients 16 had urinary albumin excretion of more than 300 mg per 24 h, 12 did not perform urine collection, three had no reliable data regarding collection timing or had inadequate collection and 13 had essential data missing. As a result, the final cohort of our study included 286 patients (**Figure 1**). Rabin medical center ethics committee approved the study. Informed consent was obtained from all participants and the study was conducted according to the declarations of Helsinki and Istanbul.

Study Procedure

Urine collection was evaluated once for each patient. Patients were asked to provide a 24 h urine collection and were instructed to accurately monitor the collection time. When the duration of the collection differed from 24 h, the urine albumin was divided by the collection exact duration and multiplied by 24 so urine albumin excretion ration (UAER) was expressed per 24 h. Exclusion criteria were an infectious disease, use of non-steroidal anti-inflammatory drugs or acute rejection during the 30 days before the collection. Blood pressure (BP) was measured on the morning following the urine collection; BP is the mean of 3 measurements, taken by a trained personal following 5 min of complete rest, using an electronic oscillometric device. Gender, age, body weight and height were also recorded. Blood was drawn from a peripheral vein for serum creatinine measurement. The urinary albumin concentration was determined by a solid-phase, competitive chemiluminesence enzyme immunoassay (Immulite Analyzer, DPC). We revisited this cohort, 5 years after it was created, to evaluate time dependent CVD risk in relation to microalbuminuria.

Outcomes

The primary outcome was the composite of MACE and all-cause mortality. The secondary outcomes included death censored MACE and the separate CV outcomes; coronary artery disease (CAD), cerebrovascular disease, peripheral vascular disease (PVD) and CV mortality. All-cause mortality was specified as primary outcome since the exact cause of death is sometimes complex, and the contribution of CV disease might be significant even when the cause of death is attributed to other factors. In addition, including all-cause mortality in the composite outcome reduced the risk of bias by competing risks.

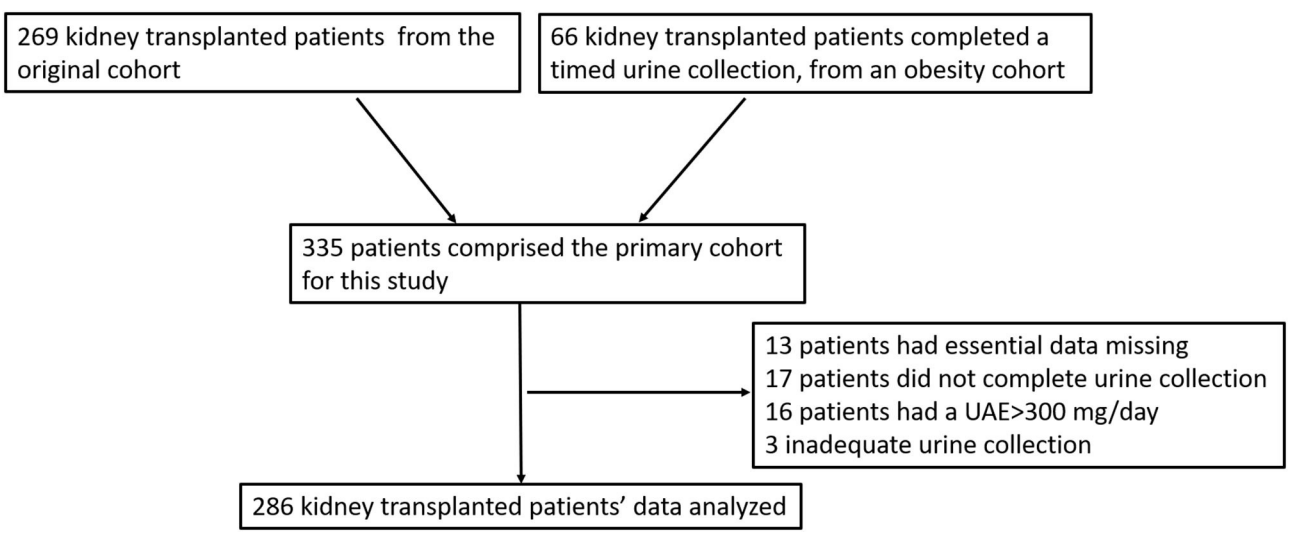

FIGURE 1 | Consort diagram describing addition and reduction of patient to create the analyzed cohort.

CAD was defined as myocardial infarction, acute coronary syndrome and coronary artery revascularization percutaneously or by surgery. Cerebrovascular disease included cerebrovascular accident (CVA), transient ischemic attack (TIA), or carotid or cerebral revascularization, documented by emergency department referral or imaging. Peripheral vascular disease was defined as hospitalization due to peripheral ischemia, need for revascularization or need for amputation due to limb ischemia. Congestive heart failure (CHF) was defined according to echocardiogram evaluating new onset systolic heart function (or hospitalization attributed to CHF). Cardiovascular mortality was defined as mortality attributed to CVA, PVD, CAD, arrhythmia, or CHF.

All outcomes were collected from the patients' electronic health records (EHR) that included complete reports of all hospitalizations during the study period. Study researcher (DB) evaluated all reports for outcome events, in case of any doubt another researcher (BRZ) evaluated the report and in case of disagreement third researcher (RR) decided between the different opinions.

Statistical Analysis

Data were analyzed using SPSS (version 25). For normally distributed variables results are expressed as mean and standard deviation (SD), and differences between the means of different groups were analyzed using standard *t*-test. To describe non-normally distributed variables we used median and interquartile range, and Mann-Whitney to show differences between groups. Comparison of frequency distribution was estimated by the $\chi 2$ test.

We used propensity stratified (PS) analysis to overcome confounding caused by differences in baseline characteristics. Propensity score was used by performing a forward stepwise logistic regression model with microalbuminuria as outcome. Variables considered in the model were gender, systolic blood pressure, donor type (living or deceased), immunosuppressive drug, LDL cholesterol, blood creatinine, anti-hypertensive medications [calcium channel blockers (CCB), beta blockers (BB), and angiotensin converting enzyme inhibitors (ACE I) or angiotensin receptor blockers (ARB)] and creatinine clearance.

For survival analysis we used Kaplan-Meier survival curve with log rank test for significance level. For adjusted analysis we stratified the analysis according to PS quartile.

We also used univariable and multivariable Cox proportional hazard model. The proportionality of hazard assumption was evaluated by examining the interaction between each variable and time. Multivariable analysis included traditional risk factors for CVD and albuminuria: age, gender, systolic blood pressure, LDL cholesterol, HbA1C, BMI, ACE I, or ARB use, blood creatinine and donor type.

The proportional hazard assumption for albuminuria was rejected. As a result, we used time varying Cox analysis investigating the first 2 years and the rest of the follow up time separately. This time point was chosen because the Kaplan Meier curve showed different hazard ratios before and after 2 years following the urine collection. The proportional hazard assumption for albuminuria was valid when evaluated for each time period separately.

For evaluation of subgroup interaction with the association between the exposure and the outcome, we used Cox analysis with microalbuminuria as time varying variable, and the interaction term between the subgroup and microalbuminuria and evaluated for significance of the interaction term.

RESULTS

Between December 2007 and February 2012, 330 urine collections were performed of them 286 patients were included in the analysis, their characteristics are presented in **Table 1**.

The mean 24 h urinary albumin excretion was 48.9 ± 63.3 mg [median 23 mg interquartile range (IQR) 9–56 mg]. Microalbuminuria was present in 121 patients (42.5%) and was associated with higher systolic blood pressure, use of antihypertensive medications, hyperlipidemia, higher blood creatinine, higher BMI, a living donor, and the use of mTOR inhibitors. By multivariable analysis all these factors, in addition to gender, were associated with microalbuminuria and used to calculate the propensity score.

Prevalence of primary renal disease was evaluated in both groups. However, since our institution serves as a tertiary referral center we rely on primary care physician investigation and diagnosis. We assume that glomerular disease, that tend to recur in the transplant, are usually diagnosed by biopsy. Other diseases such as PKD or diabetic nephropathy do not mandate invasive procedures unless diagnosis is unclear. There was no difference in the distribution of the different etiologies for renal failure between the groups (**Table 1**). As a result, we did not include this variable in the regression model.

Association of MIA and Primary Outcome: CV Including All-Cause Mortality

Six patients (2.1%), of the 286 patients included in the cohort, were lost to follow up and hence excluded from the survival analysis.

We calculated the propensity score for microalbuminuria using the variables described above. The area under the receiver operating characteristic (ROC) curve for this model was 0.79 (0.73–0.84). The ROC curve analysis and Box plot of the four quartiles according to microalbuminuria status are presented in **Supplementary Figures 1, 2**, respectively.

During median follow up time of 8.3 years (IQR 6.4–9.1) 144 outcome events occurred in 101 patients. The outcome events are described in the next section. By Kaplan-Meier analysis microalbuminuria was associated with increased rate of CV outcome or death ($p = 0.03$, **Figure 2**), the association was still significant after stratification according to PS quartiles ($p = 0.048$). By univariable time varying Cox proportional hazard analysis there was no association between microalbuminuria and CV outcomes during the first 2 years [Hazard Ratio (HR) 1.03, 95% Confidence Interval (CI) 0.43–2.43] but significant

TABLE 1 | Demographic, clinical, transplantation related, and laboratory characteristics of kidney transplanted patients who completed a timed urine collection.

	All (286)	MIA (121)	No MIA (165)	*p*-value
Men *n* (%)	191 (67%)	83 (68%)	108 (65%)	0.613
Age (years)	53.0 ± 12.7	53.6 ± 12.6	52.6 ± 12.8	0.515
Diabetes *n* (%)	98 (34.3%)	42 (34.7%)	56 (33.9%)	0.9
HD *n* (%)	78 (27.3%)	36 (29.8%)	42 (25.5%)	0.424
CVA *n* (%)	33 (11.5%)	11 (9.1%)	22 (13.3%)	0.349
Living donor *n* (%)	192 (67.1%)	72 (59.5%)	120 (72.9%)	0.019
Primary renal disease				0.895
Glomerular	84 (29%)	32 (26.4%)	52 (31.5%)	
Diabetic nephropathy	39 (13.6%)	18 (14.9%)	21 (12.7%)	
Tubulointerstitial	34 (11.9%)	14 (11.6%)	20 (12.1%)	
Pkd	45 (15.7%)	19 (15.7%)	26 (15.8%)	
Nephrosclerosis	15 (5.2%)	8 (6.6%)	7 (4.2%)	
Unknown	69 (24.1%)	30 (24.8%)	39 (23.6%)	
Smoking *n* (%)	25 (8%)	9 (7.4%)	14 (8.5%)	0.828
Time from Tx (years)	1.2 (0.4–5.2)	1.5 (0.3–5.7)	1.2 (0.4–4.2)	0.253
Patients evaluated 1-year post transplantation	158 (55%)	69 (57%)	89 (53.9%)	0.632
Medication				
CCB *n* (%)	69 (24.1%)	38 (34.1%)	31 (18.8%)	0.017
Beta blockers *n* (%)	121 (42.3%)	61 (48.4)	60 (37.5%)	0.064
Duretics *n* (%)	37 (12.9&)	18 (14.9%)	19 (11.5%)	0.4
ACEI/ARB *n* (%)	104 (36.4%)	54 (44.6%)	50 (30.3%)	0.018
Tacrolimus *n* (%)	243 (85%)	97 (80%)	146 (88.5%)	
Cyclosphorine *n* (%)	14 (4.9%)	6 (5%)	8 (4.8%)	
MTOR inhibitors *n* (%)	29 (10.1%)	18 (15%)	11 (6.7)	0.071
Indexes				
Waist circumference (cm)	97.5 ± 16.6	99.5 ± 16.9	96 ± 16.2	0.087
BMI (kg/m^2)	27.1 ± 5.1	27.9 ± 5.5	26.4 ± 4.7	0.014
Systolic BP (mmHG)	134.9 ± 18	140.3 ± 18.3	130.9 ± 16.9	<0.001
Diastolic BP (mmHG)	77.3 ± 12.7	78.1 ± 14.3	76.7 ± 11.4	0.367
Laboratory				
LDL (mg/dl)	89.2 ± 26.1	93.2 ± 27.3	86.3 ± 24.8	0.031
HDL (mg/dl)	52.3 ± 16.2	52.5 ± 15.2	52.1 ± 16.9	0.84
Creatinine clearance (ml/min)	76.3 ± 27.1	75.4 ± 30.9	77 ± 24.1	0.63
HbA1C (mg/dl)	6.1 ± 1	6.2 ± 1.1	6.1 ± 0.9	0.21

Patients were grouped according to the presence of microalbuminuria. Percent relate to column, p-value describes the significance of statistical difference between groups according to a certain variable. Tx, transplantation.

association appeared thereafter (HR 2.01, 95% CI 1.34–3.29). The association between microalbuminuria and CV outcomes after 2 years was significant even after stratification according to PS quartiles (HR 1.77, 95% CI 1.14–2.93). These results were not influenced by multivariable analysis for age, gender, systolic blood pressure, LDL cholesterol, HbA1C, BMI, blood creatinine, ACE I or ARB use and donor type (HR 1.76, 95% CI 1.05–2.96), which are traditional risk factors for CVD and albuminuria.

Subgroup analysis for the main outcome of CV events and mortality is presented in **Figure 3** and **Supplementary Figure 1**. There were some subgroups such as women, non-diabetics and patients with history of heart disease that exhibited an effect of microalbuminuria on survival, however no subgroup had significant interaction with the association between the main outcome and microalbuminuria ($p = 0.078$, $p = 0.295$, $p = 0.376$, $p = 0.803$, $p = 0.451$, $p = 0.366$ and 0.245 for interaction with gender, Diabetes Mellitus (DM), history of heart disease, hypertension, time from transplantation, living donor, and median age (56 years), respectively.

Association of Microalbuminuria and Secondary Outcome Without All-Cause Mortality

Microalbuminuria was associated with increased risk for CV outcomes, without mortality, according to Kaplan-Meier curve ($p = 0.008$, **Supplementary Figure 3**), an association that was still significant after stratification according to PS quartiles ($p = 0.045$). This association became significant according to time varying analysis after 2 years from documentation of albuminuria, by simple and PS stratified analyses [(HR 2.05, 95% CI 1.26–3.33) and (HR 1.79, 95% CI 1.05–3.08)], respectively. It was still significant using multivariable analysis adjusted for widely accepted CV risk factors including gender, systolic blood pressure, LDL cholesterol, HbA1C, blood creatinine and donor type (HR 1.74, 95% CI 1.04–2.91).

Association of Microalbuminuria and Secondary Outcomes: CAD, CVA, PVD, and All-Cause Mortality

Supplementary Table 1 depicts the different components of secondary outcome in relation to microalbuminuria. During follow up there were 53 episodes of CAD, 24 (14.9%) in the patients without microalbuminuria and 29 (24.3%) in the microalbuminuria group. Microalbuminuria was associated with CAD 2 years after timed urine collection by simple and PS stratified analyses (HR 2.36, 95% CI 1.29–4.342) and (HR 1.79, 95% CI 1.05–3.08), respectively, according to time dependent Cox analysis. During the first 2 years of follow up there was no increased risk of CAD.

There were 25 episodes of CVA, 10 (8.3%) in the microalbuminuria group and 15 (9.1%) in the non-microalbuminuria group. By Cox model there was no difference in the hazard for CVA between the groups (HR 1, 95% CI 0.55–2.25).

In contrast, PVD occurred in 12 (9.9%) patients in the microalbuminuria group and 4 (2.4%) in the non-microalbuminuria group. Simple cox model revealed a significantly increased risk for PVD associated with microalbuminuria (HR 4.17, 95% CI 1.34–12.93). However, after stratification according to PS quartiles the association was no longer significant (HR 3.15, 95% CI 0.95–10.58).

During follow up there were 42 events of all-cause mortality, 22 of them (18.6%) in the microalbuminuria group and 20

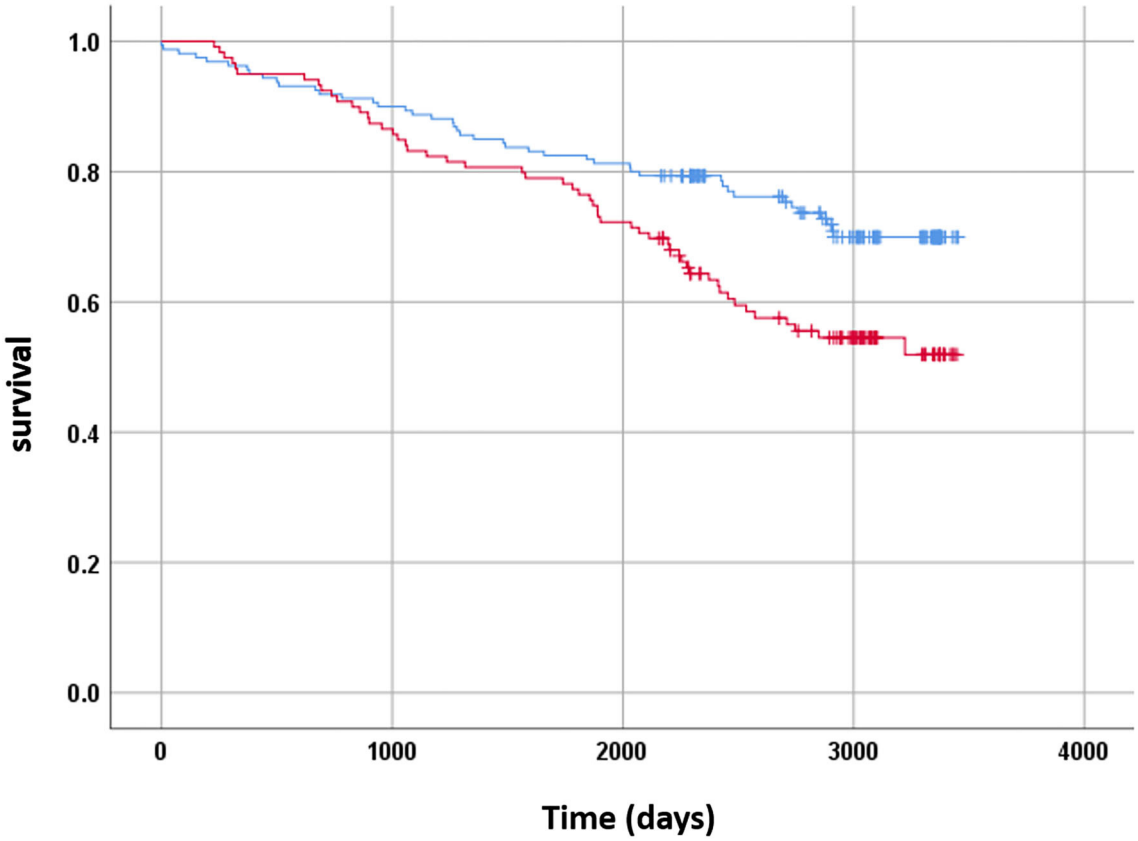

FIGURE 2 | Kaplan-Meier analysis showing rates of CV outcome or death in relation to microalbuminuria. X axis shows days since urine collection, Y axis shows cumulative survival. Blue curve—patients without microalbuminuria, red curve—patients with microalbuminuria.

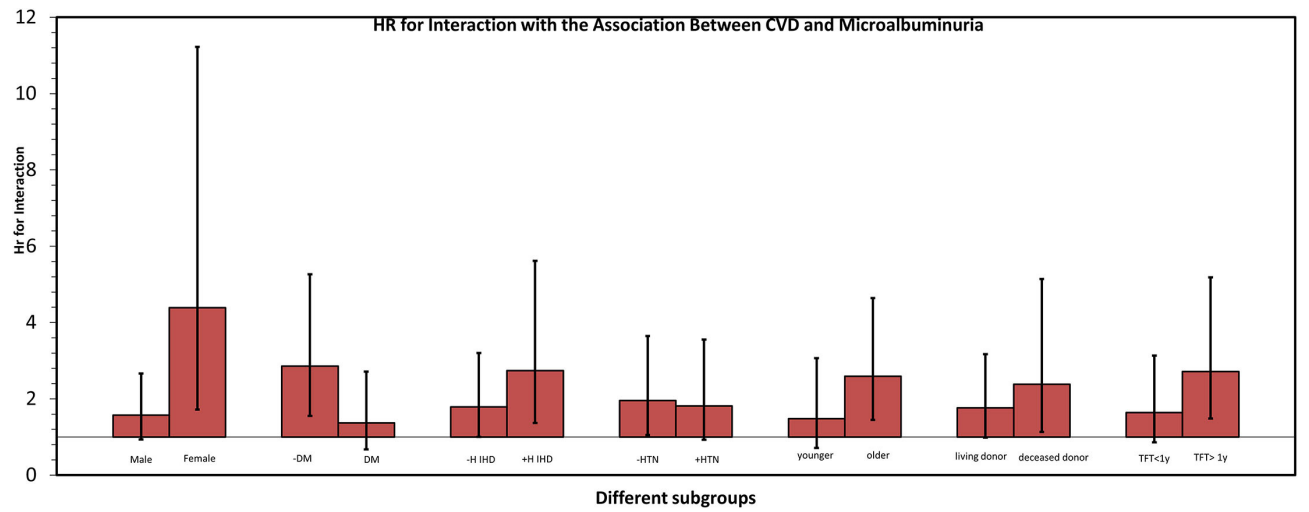

FIGURE 3 | Subgroup analysis of CVD risk according to presence of microalbuminuria. Each couple presents a certain subpopulation according to a certain risk factor. Each column illustrates the relation between Hazard Ratio (HR) (Y axis) and presence of a risk factor (X axis). Black line across each column represents Confidence Interval for HR. None of the interactions were significant with $p = 0.078$, $p = 0.295$, $p = 0.376$, $p = 0.803$, $p = 0.451$, $p = 0.366$, and 0.245 for interaction with gender, Diabetes Mellitus (DM), history of heart disease, hypertension, time from transplantation, living donor, respectively, and median age (56 years). DM, Diabetes Mellitus; TFT, time from transplantation, >/< 1 y, more or less than a year; H IHD, history of ischemic heart disease. Older/younger—above and below median age (56.6 years).

(12.4%) in the control group. There was no statistically significant association between microalbuminuria and mortality (HR 1.44, 95% CI 0.79–2.65).

DISCUSSION

We evaluated the association between microalbuminuria, as measured by accurate urine collection, and the composite outcome of CVD and all-cause mortality, following kidney transplantation. We found time varying association between microalbuminuria and CVD morbidity and mortality, with increased risk emerging around 2 years post documentation of albuminuria. The results were similar for the secondary outcome of CVD excluding non-cardiovascular death. The increased risk of CV events in the presence of albuminuria was driven mainly by CAD and PVD cases, while CVA incidence was similar between groups.

Our results are in accordance with previous studies in various populations that showed an association between microalbuminuria and CVD. The first hint to this phenomena in kidney transplanted patients was observed was in a paper describing microalbuminuria as a cause of graft loss and death (8). A few years later, Weiner and colleagues were the first to design a study dedicated to this hypothesis. They evaluated the association between CVD and various degrees of albuminuria as assessed by albumin-creatinine ratio. Even though they did not reach statistical significance, probably due to short follow-up, they raised awareness to the rising risk in this special population (9). A similar project published this year form our institute exemplifies the importance of controlling proteinuria to reduce CVD risk (10).

The attenuated effect described by Weiner et al. might result from the use of ACR, which could be confounded by creatinine excretion, a measure affected by gender, BMI and age. This study also assessed incidence of CVD close to specimen collection, hence did not have the perspective of long-term follow-up.

Attention should be given to other contributing factors and possible confounders that arise during analysis. In a recent paper by Rahamimov et al. BMI was associated with microalbuminuria by univariate logistic regression analysis, yet after multivariate analysis this relation lost significance. BMI was also associated with increased odds ratio [OR] for microalbuminuria (12), as well as serum creatinine and treatment with mTOR inhibitors.

Multivariate logistic regression analysis, in our cohort, showed that treatment with mTOR inhibitor, systolic blood pressure, serum creatinine, and BMI were associated with increased OR for microalbuminuria. BMI was still significantly associated with increased rate of urinary albumin excretion after introducing DM status into the model. The influence of adiposity on albuminuria, if it occurs, is expected to be time-related and to develop after prolonged exposure to an obese environment provided by the recipient. Risk of albuminuria was shown to correlate with high BMI 3 years following transplantation (12), however this influence was not evident in our cohort because of the shorter time line between transplantation and urine collection. When BMI was included in the multivariate analysis it did not affect the association between microalbuminuria and CVD.

Several mechanistic explanations were suggested to elucidate in the late period, BMI is an independent predictor of albuminuria and microalbuminuria after adjustment, amongst others, for arterial pressure and the presence of DM. The association between microalbuminuria and cardiovascular risk. One of them poses albuminuria as a marker of diffuse atherosclerosis, a major cause for CVD in transplanted patients (13–15). As atherosclerosis develops over years and most patients included in the study had relatively short follow up, this explanation falls in short. Another optional mechanism is that albuminuria activates neuro-hormonal response in the kidney that may increase the CV risk (16). This mechanism is possible as the timeline for nerve regeneration in transplanted kidneys that causes hypertension is 2 years (17), a similar period was observed by us as the lag between albuminuria and CVD morbidity. Nerve density reaches values observed in native kidney arteries after 2 years and is associated with hypertension-related arteriolar lesions in transplanted kidneys (17).

One possible contributory factor could be glomerular hyperfiltration. The solitary functioning kidney carries the metabolic burden alone, and more so in the presence of increased weight and BMI as seen in the MIA group. Among 9,515 healthy participants from the CARTaGENE trial (18), 473 had glomerular hyperfiltration. Compared with the normal filtration rate, glomerular hyperfiltration was associated with an increased cardiovascular risk (HR 1.88). The cardiovascular risk of the highest eGFR percentiles was similar to that of participants with CKD3a. These findings suggest that glomerular hyperfiltration is independently associated with increased cardiovascular risk in middle-aged healthy individuals. Yet, it is hard to evaluate hyperfiltration in a transplanted kidney as the baseline potential of the transplanted kidney is variable, and this hypothesis should be addressed in future studies. Yet, there was a difference in the donor type between groups, with a deceased donor more prevalent among patients with microalbuminuria. This difference might be due to mismatch between the transplanted kidney and the recipient metabolic demand. However, this explanation is speculative and should be address in future studies.

One reasonable pathway linking albuminuria with CVD could be endothelial dysfunction, that might link both phenomena. Endothelial dysfunction was characterized in diabetic patients, as well as the general population (19), by various tests including endothelial function plasma markers (20) and vasodilation in response to agonists such as cholinergic factors and increase in flow (21). However, as our study didn't evaluate any measure of endothelial dysfunction, this is a purly hypothetical association and further research is required to evaluate the role of endothelial dysfunction after kidney transplantation.

The reason for the time varying nature of this association is not clear and further studies are needed to answer

this question. A possible explanation is that there is a different threshold of damage accumulation, needed for each manifestation. Common biological pathways can cause both microalbuminuria and CVD but at different time course, i.e., rapid development of microalbuminuria and slower for CVD. Since urine collection was performed chronologically soon after transplantation (median time of 2.8 years) this could be a reasonable interpretation. Longitudinal studies evaluating change in urine albumin excretion over time, and its association with cardiovascular risk, might shed some light on this important observation.

Our study has several important strengths. First, for albuminuria evaluation, we used very accurate timed urine collection, in a research setting and under strict observation. This prevented the confounding effects of both adjustment to creatinine as mentioned above and imprecise collection that is very common in the clinical setting. Another advantage we employed was the use of EHR as a reliable information source to report cardiovascular events, and their evaluation by experienced physicians. The use of EHR also enabled detailed analysis of the different CV outcomes. Last, our cohort was followed for an extensive time period, enabling the discussion of causality between a known risk factor and disease progression. These characteristics are unique and provide both insight and strength to our findings, and reduce bias resulting from incomplete reporting.

This study has also some limitations. First, our findings suggest different association for the various components of the secondary outcome. Unlike CAD and PVD that were associated with microalbuminuria this was not the case for CVA. Composite CV outcome are a common and powerful tool, but there is some difference in the pathophysiology of the different components and detailed analysis might be of value. These differences should be evaluated in larger studies and in other populations, in search for mechanistic distinction.

Second, our small size population attenuates the study power. As accurate urine collection is cumbersome and time consuming, it is hard to use in large sample groups. Due to that, subgroup analysis was limited and valuable data regarding interactions between microalbuminuria and CVD could not be interpreted. Another limitation is that this is a single center study which might limit its external validity especially regarding non-Caucasians populations that were not represented in the analysis.

Changes in GFR over time with progressive CKD, could account for the increase rate of MACEs rather than microalbuminuria. Unfortunately, serial GFR evaluations were no available for our cohort. However, a recently published evaluation of the effect of time dependent proteinuria on CVD found no independent association between serial GFR measurements and MACE (10).

Of note, prevalence of primary renal disease was similar between groups, yet as a tertiary referral center, we rely on primary care physician evaluation and diagnosis and primary renal disease prevalence should be interpreted cautiously.

Last, the interaction of CVD with time was not pre specified and was discovered when the proportionality assumption was evaluated before performing Cox analysis. Thus, this observation should be interpreted cautiously until verified by other studies. Nevertheless, the time varying nature might give important insights regarding the association of microalbuminuria and CVD, as a continuum of the same pathological process.

According to our results, and studies evaluating other populations, microalbuminuria is an independent risk factor for CVD, and a marker of it (22–24). Chronic kidney disease is a risk factor for CVD (25–27) and following kidney transplantation it is added to the use of medication endangering with hypertension and diabetes, thus increasing the risk. Clinicians should perceive microalbuminuria as a risk equivalent when evaluating their patients' CVD risk and initiate primary or secondary preventive measures.

Further research is needed in order to extend our understanding of the association between albuminuria and CVD. These should rely on the association between urine albumin excretion using urine collection to verify our results. large scale studies are not expected due to the relative complexity of accurate timed urine collection, but even small series can be pooled together to generate statistical power. Another important aspect that should be explored is the dynamic of urine albumin excretion and its association with CVD. These kinds of studies are especially important due to the time varying nature of the association observed in our study. Furthermore, a mechanistic explanation should be sought to reveal the relation between albuminuria and different time points after kidney transplantation, by using urine exosome extraction technique. Since the origin of the endothall differ, from recipient vs. donor, this could be a non-invasive manor to assess pathology emergence.

CONCLUSION

We show that microalbuminuria is associated with increased risk of CVD in kidney transplanted patients, 2 years after a timed urine collection documenting its appearance. Therefore, newly documented albuminuria after kidney transplantation should be considered risk equivalent for CVD.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: deidentified database will be provided from the corresponding author upon a specified request. Requests to access these datasets should be directed to benayarz@gmail.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Rabin Medical Center ethics committee approved the study. Informed consent was obtained from all participants and the study was conducted according to the declarations of Helsinki and Istanbul. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DB performed data collection, decision making regarding clinical outcome per patient, designed the figures, and wrote the manuscript with BR. RR, BZ, and LA-G performed research conduction and contributed to the final version of the manuscript. AC was involved in data collection and analysis of the baseline data. BR conceived the presented idea, performed the statistical analysis, designed the figures, and wrote the manuscript with DB. All authors discussed the results and contributed to the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.635847/full#supplementary-material

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REVIEW
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A Nephrologist Perspective on Obesity: From Kidney Injury to Clinical Management

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Obesity is one of the epidemics of our era. Its prevalence is higher than 30% in the U.S. and it is estimated to increase by 50% in 2030. Obesity is associated with a higher risk of all-cause mortality and it is known to be a cause of chronic kidney disease (CKD). Typically, obesity-related glomerulopathy (ORG) is ascribed to renal hemodynamic changes that lead to hyperfiltration, albuminuria and, finally, impairment in glomerular filtration rate due to glomerulosclerosis. Though not only hemodynamics are responsible for ORG: adipokines could cause local effects on mesangial and tubular cells and podocytes promoting maladaptive responses to hyperfiltration. Furthermore, hypertension and type 2 diabetes mellitus, two conditions generally associated with obesity, are both amplifiers of obesity injury in the renal parenchyma, as well as complications of overweight. As in the native kidney, obesity is also related to worse outcomes in kidney transplantation. Despite its impact in CKD and cardiovascular morbility and mortality, therapeutic strategies to fight against obesity-related CKD were limited for decades to renin-angiotensin blockade and bariatric surgery for patients who accomplished very restrictive criteria. Last years, different drugs have been approved or are under study for the treatment of obesity. Glucagon-like peptide-1 receptor agonists are promising in obesity-related CKD since they have shown benefits in terms of losing weight in obese patients, as well as preventing the onset of macroalbuminuria and slowing the decline of eGFR in type 2 diabetes. These new families of glucose-lowering drugs are a new frontier to be crossed by nephrologists to stop obesity-related CKD progression.

Keywords: obesity, adiposity, chronic kidney disease, insulin resistance, kidney transplantation

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INTRODUCTION

Obesity is a major global public health problem, as the World Health in 1997 (1). Obesity is one of the epidemics of our era. Its prevalence is higher than 30% in the U.S. (WHO 2008) and it is estimated to increase by 50% in 2030. Obesity is defined by excessive body fat accumulation and its incidence is growing, as a result of changes in food habits and physical activity. Recent data indicate there are about 600 million people with obesity worldwide (2) and this number is supposed to increase. Obesity is a chronic and metabolic disease that has an important impact on several specialties such as endocrinology, cardiology and nephrology. As its incidence grows, obesity-related health problems will also be more common in medical specialties.

In Nephrology, obesity-related complications are more than just obese-related glomerulopathy that included glomerulomegaly and focal segmental glomerulosclerosis (FSGS). Several mechanisms have been associated with the obesity-related kidney disease that would mainly be summarized in three groups: the hemodynamic, the adipose tissue-related and the insulin resistance pathways. Increased cardiovascular disease risk and high blood pressure are also health problems associated with obesity that nephrologists have to face. Furthermore, obesity is very common in patients who are in the kidney transplant waiting list, and its presence may be associated with worse allograph transplant function and prognosis. In this article, we will review the main pathophysiological pathways that link obesity and kidney injury, cardiovascular risk and diabetes. In addition, we will also focus on principal strategies in kidney transplantation and pharmaceutical therapies in this population.

PATHWAYS INVOLVED IN OBESITY-RELATED KIDNEY DISEASE

Obesity is an independent risk factor for the development of chronic kidney disease (CKD) and it has been related to a decreased glomerular filtration rate (GFR) and albuminuria (3–5). The development of CKD is closely related to other comorbidities that appear in conjunction with weight increase, namely hypertension or insulin-resistance and diabetes. Obese patients with kidney disease show glomerulomegaly and mesangial expansion (6, 7), which are a consequence of the increased blood flow and hyperfiltration that glomeruli suffer. Hyperfiltration in turn produces albuminuria which, together with other damaging mechanisms, leads to a progressive decline in GFR. Therefore, obesity is initially associated with hypertension and hyperfiltration, that produces albuminuria and subsequent loss of GFR.

Fat mass increase and weight gain activate several harmful pathways that simultaneously damage both the glomeruli and the tubules. Over time, the chronic activation of these pathways leads to progressive kidney injury, the development of albuminuria and CKD (8). Numerous mechanisms have been implied in obesity-related kidney disease, but many of them could be summarized within three main groups: the hemodynamic pathway, the adipose tissue-related pathway and the insulin resistance—hyperinsulinemia pathway (**Figure 1**). The sole purpose of this last classification is to facilitate the understanding of the mechanisms involved. During the development of kidney injury, the three pathways interact between them and their effect are modulated by other factors associated with obesity and the patient himself, such as diabetes, hypertension, age or sex.

Regarding the hemodynamic pathway, it is proven that obesity increases tubular sodium reabsorption (7, 9). It leads to volume overload and facilitates the development of hypertension, which later contributes to glomerular hyperfiltration and renal microvascular damage. Renin-angiotensin system (RAS) activation is also described in obese patients and animal models (8, 10). RAS activation participates in hypertension development and sodium reabsorption. Moreover, it is a critical step that promotes glomerular hyperfiltration. Some studies postulate that in obesity, sodium reabsorption occurs initially in the first segments of the tubule, namely proximal tubule and the ascending loop of Henle (7, 9). Thus, sodium delivery to distal segments of the tubule is diminished and sensed by macula densa cells, activating tubuloglomerular feedback and inducing renin secretion by juxtaglomerular apparatus (7). This mechanism is similar to that which contributes to hyperfiltration in diabetic kidney disease. Studies performed in 1993 already demonstrated that dogs fed with high-fat diet showed increases renin secretion (9). Different mechanisms have been implied in this initial reabsorption in proximal tubular segments. Obesity induces insulin resistance which produces a compensatory increase in insulin secretion, and the increase in insulin availability has been implicated in increased sodium reabsorption in the proximal tubule and the ascending loop of Henle (9, 11). Recently, the interaction between the gut and the kidney has been described, where secreted gastrin after sodium ingestion acts in the kidney and increases sodium excretion in proximal tubular cells through NHE3 and Na^+/K^+-ATPase inhibition (12). In obese patients, an inappropriate diet rich in fats is usually accompanied by increased sodium ingestion, which may interfere with the described gastro-renal axis (8).

The adipose-tissue related pathway is linked to the increase of fat mass that occurs in obese patients. Adipocytes are active cells capable of secreting cytokines named adipokines (13). The increase of adipose tissue modulates adipokine secretion and larger adipocytes are associated with an increased inflammatory adipokine secretion (13). In obesity-related kidney disease, several adipokines have been implied such as leptin, adiponectin, tumor necrosis factor α (TNF-α) or interleukin 6 (IL-6) (7). Leptin secretion by adipose tissue is augmented in obese patients. Leptin has been related to increased activity of the sympathetic nervous system that contributes to hypertension, and leptin infusion in rats raised mean blood pressure (14). In addition, leptin promotes fatty acid oxidation, increasing oxidative stress and the secretion proinflammatory cytokines like monocyte chemoattractant protein 1 (MCP-1) (15). Conversely, adiponectin levels are decreased in obese patients (8). Lower adiponectin levels are associated with insulin resistance and impaired glucose and fatty acids metabolism (13). Besides, adiponectin is involved in glomerular filtration barrier structure regulation. In adiponectin knock-out mice podocyte foot process effacement has been evidenced which leads to an increase in albuminuria, and albuminuria is partially reversed with exogenous adiponectin administration (16). Moreover, in cultured podocytes adiponectin administration reduced permeability to albumin and oxidative stress through AMPK activation (16). Adipose-tissue also regulates RAS. In adipocytes, aldosterone and angiotensinogen secretion have been identified (17). Aldosterone secreted by fat tissue participates in adipocyte differentiation and is implicated in obesity-related hypertension (11, 18).

Insulin resistance is another pathway closely related to obesity-induced kidney disease. Insulin resistance is dependent

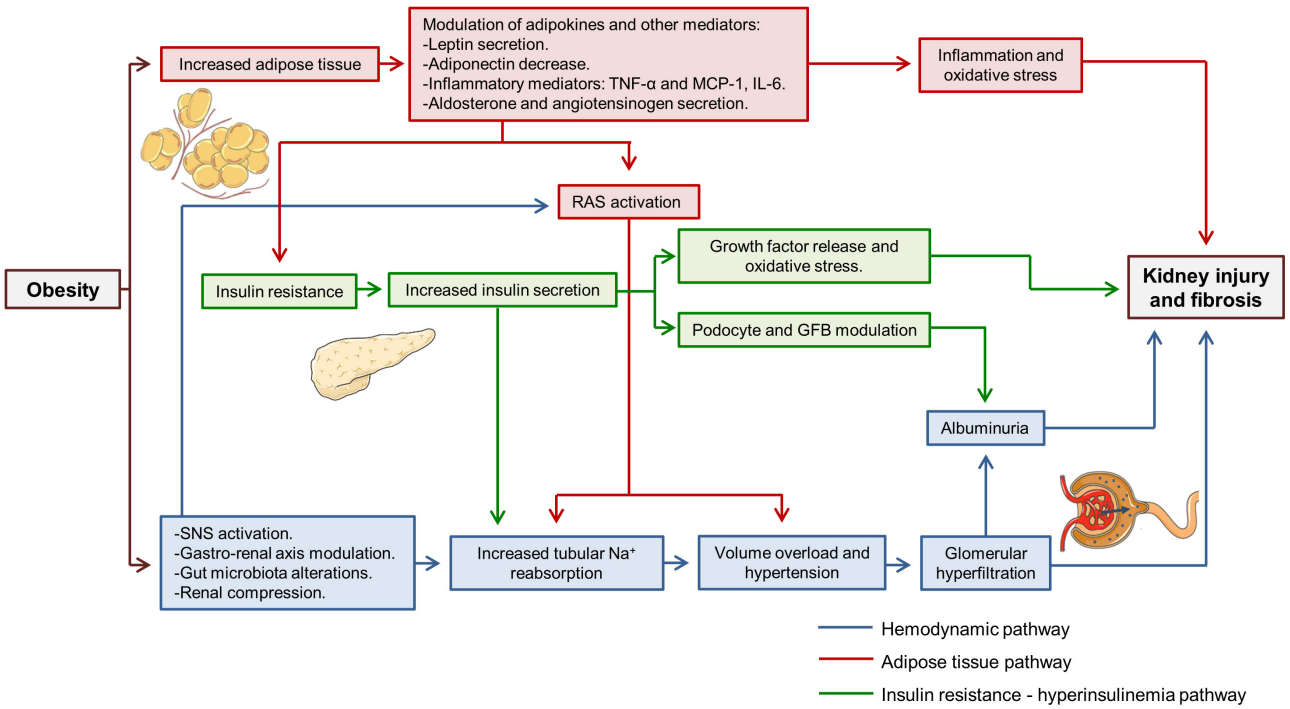

FIGURE 1 | Pathways involved in obesity-related kidney disease. Three main pathways involved (hemodynamic, adipose tissue related and insulin resistance—hyperinsulinemia pathways) have been highlighted in different colors. It can be observed how three pathways interact simultaneously to produce kidney injury. TNF-α, tumor necrosis factor α; MCP-1, monocyte chemoattractant protein 1; IL-6, interleukine 6; RAS, renin-angiotensin system; GFB, glomerular filtration barrier; SNS, sympathetic nervous system; Na^+, sodium.

on the increase in fat mass and the modulation of adipokines that occurs as a consequence of the latter (8). Insulin resistance promotes compensatory insulin secretion and insulin by itself has different effects on the kidney. Insulin acts directly on podocytes through insulin receptors themselves. AKT/mTOR intracellular pathway or glucose transporter 4 (GLUT4) have been related to its signaling (19). Insulin is implied in podocyte function and actin cytoskeleton modulation (20). Thus, augmented insulin secretion affects glomerular filtration barrier selectivity leading to proteinuria. In both rat models and cultured podocytes, insulin administration increases albumin permeability (21). Furthermore, insulin promoted oxidative stress in podocytes through NADPH oxidase activation (21). Insulin also acts on tubules and in cultured renal proximal tubular cells, it promotes TFG-β and collagen IV formation that eventually lead to tubulointerstitial fibrosis (22).

OBESITY AND CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is an important cause of death and disabilities worldwide. Clinical manifestations of CVD such as acute myocardial infarction, stroke or peripherical vasculopathy are attributed to atherosclerosis. The pathophysiological alterations of atherosclerosis are associated with chronic inflammation of the vessel wall that results from the accumulation of cholesterol-rich atherogenic Apo-B lipoproteins (VLDL, IDL, and LDL) in vascular intima. Lipoprotein accumulation leads to subsequent infiltration of macrophages and T cells of the arterial wall, producing atherosclerotic plaques (23, 24).

Obesity is considered as a major modifiable risk factor for different CVD, even after adjustments for the co-existing risk factors (25). Obesity itself has a deleterious effect on most of the major CVD risk factors: it increases plasma lipid levels, raises blood pressure and impairs glycaemic control in diabetes (26, 27). An increase of body weight, principally abdominal fat accumulation, is associated with a greater atherosclerosis progression. Post-mortem studies have described that both thickness of the adipose panicle and body mass index are associated with more extensive fatty streaks and raised lesions in the right coronary artery (28). In this regard, visceral obesity is also associated with an increased risk for cerebrovascular disease (29). Heart failure (HF) is frequent in obese patients, with approximately 11% of HF in men and 14% in women attributable to obesity (30). It is worth mentioning that an increase of every unit in body mass index (BMI) raises the risk of HF 5% in men and 7% in women (31). Strategies to achieve weight loss in obese patients may be beneficial to reduce the risk of CVD. Among all weight interventions, bariatric surgery has demonstrated beneficial effects on CVD morbidity and mortality (32). Recently, new pharmacologic therapeutics targeting the glucagon-like peptide-1 receptor such as semaglutide are promising for weight loss in adults with obesity or overweight (33).

OBESITY, INFLAMMATION, LIPID ABNORMALITIES, AND HYPERTENSION

As mentioned above, one of the mechanisms implied in the increased risk of CVD in obese patients is chronic inflammation driven by the adipose tissue. Adipose tissue is an active endocrine organ capable to synthesize and release into the circulation different types of hormones, peptides, and inflammatory molecules that promote endothelial dysfunction contributing to the development of atherosclerosis (34). Obesity-induced inflammation is marked by an increased infiltration and activation of innate and adaptive immune response. Macrophages are the predominant immune cells infiltrating the adipose tissue in obese individuals. They are polarized into proinflammatory M1 macrophages that secrete proinflammatory cytokines such as TNF-α, interleukins, and C-reactive protein, while simultaneously suppress anti-inflammatory cells and reduce the production of adiponectin, predisposing to increase oxidative stress (35). The inflammatory triggers are still unknown, however, obesity-induced adipose tissue remodeling provides many signals such as adipocyte death, hypoxia and mechanical stress that are capable of initiating an inflammatory response (36).

Lipid abnormalities as elevated triglyceride, VLDL, Apo B, and non-HDL-C levels are typically seen in obesity (37). There is an increase of small dense LDL and it is considered to be more pro-atherogenic than large LDL particles (38). Approximately 60–70% of obese patients are dyslipidemic while 50–60% of patients who are overweight are dyslipidemic (37), increasing risk for cardiovascular disease.

These abnormalities are produced by the greater delivery of free fatty acids to the liver from increased total and visceral adiposity, insulin resistance, and a pro-inflammatory state (37, 39). Adipokines, such as adiponectin and resistin are implicated in lipid metabolism. Levels of adiponectin are decreased in obese patients and are associated with increase in serum triglyceride and decreases in HDL-C levels (40). High levels of resistin have been observed in obesity and it is directly correlated with plasma triglyceride levels (41). Moreover, resistin has been shown to stimulate hepatic VLDL production and secretion due to an increase in the synthesis of Apo B, triglycerides, and cholesterol (41, 42). Finally, resistin is associated with a decrease in HDL-C and Apo A-I levels (42). Additionally, the pro-inflammatory cytokines such as tumoral necrosis factor stimulate lipolysis in adipocytes increasing circulating free fatty acid levels, which increases production of triglyceride by liver and also stimulate *de novo* production of VLDL and triglycerides (43). At higher levels the pro-inflammatory cytokines decrease the expression of lipoprotein lipase and increase the expression of angiopoietin like protein 4, an inhibitor of lipoprotein lipase (43, 44), delaying the clearance of triglyceride rich lipoproteins. Pro-inflammatory cytokines also affect HDL metabolism, decreasing the production of Apo A-I, the main protein constituent of HDL. These changes induced by pro-inflammatory cytokines result in a decrease in reverse cholesterol transport that plays a key role in preventing cholesterol accumulation in macrophages thereby reducing atherosclerosis (45).

Hypertension is frequent in obesity (46), and the mechanisms implicated are under investigation. Endothelial disfunction constitutes the initial alteration that initiates atherosclerotic disease. In this regard, it has been previously described in obese children and adolescents, that the number of circulating endothelial cells, a type of cells that reflect endothelial damage, are related with greater body fat percentage and hypertension. This suggests an association between body fat, endothelial dysfunction and hypertension (47). Obesity-induced inflammation, insulin resistance and high plasma leptin promote an impairment of synthesis of nitric oxide (NO) producing an imbalance between vasodilatation and vasoconstriction (48), favoring arterial stiffness and vascular remodeling what promotes arterial hypertension. It has also been observed that sympathetic activation plays an important role in obese-related hypertension. High intake of fat stimulates peripheral α1 and β-adrenergic receptors, increasing sympathetic activity and hypertension (49, 50). Moreover, parasympathetic activity is also altered, probably related to the presence of atherosclerotic plaque in large arteries and an increase of central stiffness impairing the baroreflex sensitivity (46). High levels of plasma renin activity, plasma angiotensin, angiotensin II and aldosterone are also associated with obesity (51–53). Renin, angiotensin II, angiotensinogen and angiotensin II receptors are found in AT suggesting that RAS is settled at adipocyte cells (54). RAS activation leads in turn to a salt-sensitive hypertension and vasoconstriction.

In this context, obesity-related hypertension treatment should be multidisciplinary. The treatment program should include an important lifestyle intervention that includes a low caloric diet with low intake of fat, increase of physical activity, reduced salt intake and pharmacological treatment (55). The first option of antihypertensive drugs in obese patients are angiotensin receptors blockers (ARB) and angiotensin converting enzyme inhibitors (ACEi) because both are associated with a lower incidence of diabetes (56), favorable effects on left ventricular hypertrophy and reduce risk of obese-related glomerulopathy.

INTERSECTION BETWEEN DIABETES AND OBESITY: DIABESITY

The term "diabesity" was first described in the 1970s to name the strong relationship that exists between these two entities, diabetes and obesity. Both of them, together with high blood pressure and dyslipidemia, constitute the cluster of conditions known as "metabolic syndrome," which is associated with a higher CVD risk (57). In addition, both obesity and diabetes are increasing their prevalence worldwide (58), mainly explained by changes in lifestyle and by genetic susceptibility (2).

It is known that overweight and obesity increase the risk of type 2 diabetes (59, 60). This risk is proportional to body mass index increase and it is higher in the presence of abdominal fat. This abdominal fat is related to insulin resistance and has been identified as an independent risk factor for the development of type 2 diabetes in obese patients (61). In fact, the pathophysiology that connects obesity and diabetes is based on two main factors: insulin resistance and insulin deficiency (62) (**Figure 2**). In obese patients, circulatory levels

of free fatty acids are increased, inhibiting glucose transport and promoting the use of lipids by the muscle, which causes insulin resistance. On the other hand, the decrease in glucose use by the muscle develops chronic hyperglycemia that perpetuates insulin resistance and in turn promotes increased insulin secretion. This hyperinsulinemia is also perpetuated due to the lipotoxicity of the liver cells derived from the accumulation of lipids in the liver and pancreatic β-cells rather than obesity itself (63). Thus, hyperinsulinemia and liver and pancreatic lipotoxicity promote an insulin deficit. Furthermore, obese patients present a systemic pro-inflammatory state that promotes insulin resistance leading to established diabetes along with insulin deficiency (64).

Interestingly, there are certain abnormalities in metabolism that play a role in the development of diabetes and obesity. It has been shown that oxidative stress, inflammation and advanced glycation end-products can induce microvascular damage and a deficit in tissue perfusion (65). Epidemiological studies have found an association between short periods of sleep and increased diabetes or obesity. This lack of sleep has been linked to a decrease in leptin and an increase of hunger and intake of carbohydrates and food with high quantity of calories (66). It has been also demonstrated that hormones, namely testosterone, also play a role in diabesity. In men, an inverse relationship has been evidenced between testosterone concentration and visceral fat accumulation. In women, an increase in testosterone has been associated with glucose intolerance and insulin resistance (67). Vitamin D has been reported to inhibit fat accumulation, increase insulin secretion, preserve pancreatic β-cells, decrease insulin resistance and reduce appetite (68). Finally, the gut microbiota also contributes to the development of diabetes and obesity: a reduction in Bacteroidetes and an increase in Firmicutes has been associated with obesity (69).

In relation to DM, obesity and their common pathways it is important to mention sodium-glucose cotransporters (SGLT), that are widely distributed in the body, especially in the renal tubules, where glucose is reabsorbed. There are two subtypes of these cotransporters: SGLT-1 and SGLT-2. Most of the glucose is reabsorbed by the SGLT-2. In patients with DM there is an over expression of SGLT-2 in the hyperfiltration phase (70). Nowadays, exists a newly line of drugs to inhibit these SGLT2, increasing the excretion of glucose through the urine in these patients. Thus, the glycemic control improves with a reduction in glycosylated hemoglobin of 0.66%, weight loss of 1.8 kg and a decrease in systolic blood pressure of 4.45 mmHg compared to placebo (71). These benefits are promising in diabesity patients. In addition, beneficial results of said treatment have been shown in terms of cardiovascular mortality and morbidity and improvement in renal prognosis. One of the most frequently observed adverse effects is an increase in urinary tract infections and fungal genital infections derived from increased glycosuria (72).

IMPACT OF OBESITY BEFORE AND AFTER KIDNEY TRANSPLANTATION

Obesity is not considered to be a contraindication for kidney transplantation according to most clinical practice guidelines (73, 74). However, the reality is that many centers avoid transplanting obese patients (75), considering a BMI>35 Kg/m^2 a relative contraindication to kidney transplantation (76). Once on the waiting list, obese patients experience longer waiting times and fewer chances to access to a transplant compared to non-obese patients (77, 78). In a large population-based study using the United Network for Organ Sharing (UNOS) data on 132,353 patients on the waiting list, revealed that the probability of receiving kidney transplantation decreased with the increase of BMI. Furthermore, obese patients were more likely excluded when an allograft was available as the BMI increased (79). Importantly, evidence shows that kidney transplantation in obese patients offers better survival than remaining on dialysis. In a registry study using the United States Renal Data System (USRDS) data in which 7,443 obese patients were included, the mortality rate for those who received a kidney transplant was half of those who stayed on the waiting list (3.3 deaths vs. 6.6 deaths per 100 patients-year), and the mortality rate was even lower in the subgroup of patients who received a living donor transplant. However, this survival advantage was not achieved on patients with BMI ≥40 Kg/m^2(80). In a more recent study using also the USRDS data that included 208.986 patients, a survival benefit was reported in all grades of obesity for both living and deceased donor kidney transplant recipients, although the benefit observed was lower in patients with BMI ≥ 40 Kg/m^2 (78). In this study, better patient survival was also demonstrated in the subgroup of patients who received a kidney from an expanded criteria donor, and the only subgroup in which a survival benefit could not be demonstrated were Black patients with BMI ≥ 40 Kg/m^2.

There are conflicting results regarding the association between patient and graft survival with obesity. While there are studies that have shown a negative impact of obesity on both patient and graft survival (81–85), others have found no association (86–89). In a systematic review and meta-analyses that included 138,081 kidney transplant recipients, obesity was associated with increased graft failure but no differences were found between obese and non-obese patients in terms of survival (90). A large registry study that included more than 50,000 patients, reported worse graft and patient outcomes in patients with very low and very high BMI (82). A recent systematic review that included data from more than 200,000 kidney transplant recipients found worse patient and graft survival at one, two and three years post-transplant in obese compared to non-obese patients (91). In contrast, the ANZDATA registry-based study (n = 5,864) did not show an independent association between obesity and patient or graft survival, although obese patients were more likely to experience delayed graft function (DGF) and acute rejection (AR) (92). Other single-center retrospective studies have not shown any impact on patient survival or graft loss when comparing outcomes between obese and non-obese patients (88, 93, 94). Interestingly, in a single-center retrospective study, patient and graft survival in obese patients was comparable to non-obese patients when those suffering from surgical wound complications were excluded from the analysis (95).

Delayed graft function is associated with recipient BMI in many retrospective studies (89, 91, 92, 96), and a UNOS registry-based study showed a significant gradual increase in the risk of DGF according to the obesity grade (83). The association

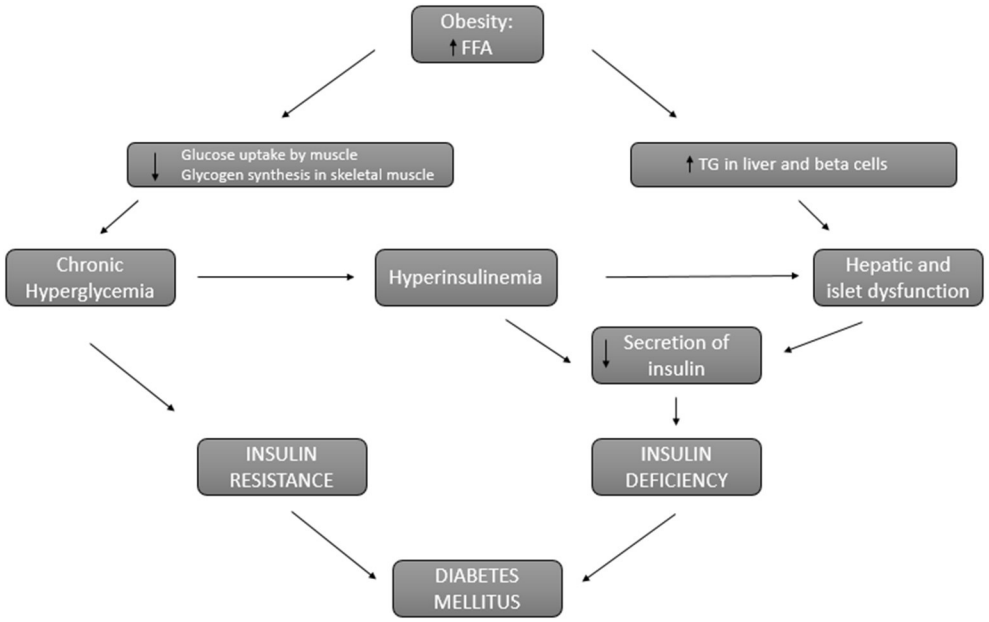

FIGURE 2 | Connection between obesity and diabetes. Pathways involved in insulin resistance and insulin deficiency that lead to diabetes in obese patients. FFA, free fatty acids; TG, triglycerides.

between obesity and rejection is controversial. Some studies have shown an increased risk of rejection in this population (85, 87, 92, 94, 97), while others have not (86, 98–101). There is an intrinsic problem in obese patients regarding the difficulty on achieving and adequate exposure to immunosuppression, with frequent overexposure resulting into calcineurin-inhibitor toxicity, or otherwise, underexposure, placing these patients at a higher risk of rejection (99). Surgical interventions in obese patients are complex, technically more difficult and result in an increased risk of surgical complications (91, 95, 100, 102). Obese patients are more likely to have an increased risk for surgical wound infections, which have been reported to range from 20–30% in patients with BMI 30–40Kg/m^2, and up to 40% in patients with BMI $\geq$ 40 Kg/m^2 (95, 103). Reducing surgical complications becomes important if we consider that there is evidence showing that outcomes are similar between obese and non-obese patients in the absence of such complications (95).

Weight gain after transplantation is very common (81, 104), and a significant increase in weight after transplantation has been associated with decreased patient survival (81). Obesity has also been associated with an increased risk of cardiac events and congestive heart failure after transplantation (105), as well as with the development of diabetes mellitus (106, 107). Although there is conflicting data regarding the impact of obesity on kidney transplantation, it undoubtedly represents a risk factor for surgical complications, and it probably has a real impact on outcomes. Obese patients cannot be excluded from transplantation because of their BMI, since survival after transplantation is better than remaining on dialysis. Thus, management of these patients must include multidisciplinary strategies to lose weight, including the possibility of bariatric surgery in selected candidates as well as pharmacological treatments (85, 108).

PHARMACOLOGICAL MANAGEMENT OF OBESITY

The management of a patient with obesity must be holistic. Medical treatments are only a piece in a puzzle that includes physical activity, nutrition, behavioral therapy, pharmacological treatment, and, sometimes, bariatric surgery (109). Thus, anti-obesity pharmacological therapies should always be established together with nutritional and behavioral assessment and physical activity advice. Generally, anti-obesity drugs are prescribed by qualified clinicians in obesity units. However, some of them are commonly used in nephrological patients, since they are not obesity-exclusive treatments. Pharmacological management should be quickly considered in patients who do not lose weight with lifestyle modifications or are not able to maintain weight reductions.

At the time this article is written, four drugs have been approved for long-term (>12 weeks) obesity treatment by the U.S. Food and Drug Administration (FDA): orlistat, naltrexone extended-release (ER)/bupropion ER, phentermine/topiramate controlled-release (CR), and liraglutide (110). Phentermine/topiramate has not been approved for long-term use by the European Medicines Agency (110). Recommendations for starting anti-obesity drugs differ from U.S. and Asian guidelines: in the U.S. are recommended for patients with BMI $\geq$30 or $\geq$27 kg/m^2 with comorbidities (diabetes, hypertension, dyslipidemia, sleep apnea) (111) while in Asia are recommended with lower BMI ($\geq$25 or $\geq$23 kg/m^2) to patients who present at least one weight-related comorbidity (1).

Orlistat works as a gastrointestinal lipase inhibitor, avoiding fatty acids intestinal absorption and weight loss is about 5% (112, 113). Due to its mechanism of action, flatus is an important side effect, but orlistat has been also related to malabsorption, especially of fat-soluble vitamins. It has been associated with kidney stones and contraindications include malabsorption syndrome and cholestasis. Pancreatitis and liver disease are very rare. Data on patients with CKD are scarce. A study including 32 patients with CKD stages 3–5 showed significant weight loss and no serious adverse events in this population (75). Furthermore, Son JW et al. recommended that both orlistat and liraglutide can be carefully used in CKD patients with obesity (114).

Liraglutide is a glucagon-like peptide-1 (GLP-1) receptor agonist that was firstly approved for the treatment of type 2 diabetes. It is 97% similar to human GLP-1 but it has a longer action time (115). It works by reducing appetite, delaying gastric emptying and balancing insulin and glucagon secretion time (115). At the highest dose of 3 mg per day, liraglutide has shown benefits in obese patients, with 5–10% body weight loss and an increased time of onset of diabetes in prediabetic obese patients (116). Liraglutide has also shown benefits in terms of glycemic control, blood pressure, lipid levels (116). The dose of 1.8 mg has been related to a decreased risk of death from cardiovascular disease, non-fatal myocardial infarction, and non-fatal cerebral infarction in diabetic patients (117). Main side effects are gastrointestinal: nausea and vomiting, constipation and diarrhea. Slowly increasing of dose could help to diminish them (117). Liraglutide has also been associated with pancreatitis and should not be used in patients with a previous history of pancreatitis (118). It also should not be used in patients with a personal or family history of medullar thyroid cancer or multiple endocrine neoplasia (119).

Bupropion is used for smoking cessation and depression. It inhibits appetite *via* the reward system and increases energy consumption. Naltrexone is an opioid antagonist that also inhibits food intake. Their combination has a synergistic effect (120). Weight reduction seems to depend on the dose: 6.1% for naltrexone/bupropion 32/360 mg and 5% for the 16/360 mg group according to the Contrave Obesity Research (COR)-I trial. The combination of both drugs has shown improvement in glycemic control, insulin resistance, and lipid profiles (121). The main side effect is nausea, which makes that some patients discontinue the treatment. Emotional or psychological disorders could appear in patients without psychiatric disease and the risk of suicidal ideation in young patients taking bupropion has been reported by FDA. Combination of naltrexone/bupropion could increase the risk of seizures, especially in patients with a previous history or with excessive alcohol intake or cocaine, as FDA notes. This drug should not be used in patients with end-stage CKD, since there are no studies in this population.

Combination of phentermine (sympathomimetic amine, anti-obesity drug) and topiramate (used to treat seizures and migraine headaches) drives weight reduction through increased satiety and energy waste, reducing caloric intake and taste abnormalities (122). Weight loss is about 5–10%. As well as other anti-obesity drugs, it has shown improvements in cardiovascular risk factors (123). A metanalysis recently published showed that phentermine/topiramate has the greatest weight loss effect among the currently available anti-obesity drugs (124). A pregnancy test must be performed before starting phentermine/topiramate since topiramate can cause birth defects. It also could cause depression, anxiety, sleep disorder, suicidal ideation, and difficulties in concentration. Topiramate has been related to inhibition of carbonic anhydrase activity and subsequently, metabolic acidosis, hypokalemia, renal stones, angle-closure glaucoma could be side effects. In patients with CKD this treatment should be avoided. Of note that this drug has not been approved for obesity treatment in Europe.

As Son JW et al. mentioned, the weight loss effect of these anti-obesity drugs can be ranked as follows: phentermine/topiramate CR > liraglutide 3.0 mg > naltrexone ER/bupropion ER > orlistat. New clinical trials are ongoing to study new anti-obesity drugs, such as sodium-glucose cotransporter two inhibitors, other GLP-1 receptor agonists, dopamine reuptake inhibitors or mineralocorticoid receptor agonists (114). Obesity therapies goal is to treat adipose tissue dysfunction, excessive body fat and diseases that are secondary to increased body fat and its adverse consequences, as renal impairment. It is worth mentioning that some drugs that are frequently used by nephrologists are also associated to body weight gain, such as insulin (125), glucocorticoids (126) and some β-blockers (atenolol, metoprolol) (127).

CONCLUSIONS

We reviewed the obesity-related kidney disease regarding the involved pathophysiologic mechanisms that include the hemodynamic, the adipose tissue-related and the insulin resistance—hyperinsulinemia pathways. Advances on the knowledge of the link between obesity, cardiovascular risk and diabetes in CKD patients including the crucial importance of obesity pre/post kidney transplantation are of great interest for the management of renal patients with obesity. The discussed studies provide insights on new frontiers regarding the fact that new pharmacological strategies are promising in a future for treating and management of obesity.

AUTHOR CONTRIBUTIONS

CG-C, AV, SB, MA, JS, and MS have conceptualized and written the manuscript. All authors contributed to the article and approved the submitted version.

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The Effect of Coronary Angiography Timing on Cardiac Surgery Associated Acute Kidney Injury Incidence and Prognosis

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Introduction: Acute kidney injury has been identified as a common complication of cardiac surgery. To date, the effect of the time interval from coronary angiography to cardiac surgery on postoperative acute kidney injury is still controversial. The aim of this study was to investigate the relationship between the timing of coronary angiography and cardiac surgery associated acute kidney injury.

Methods: Eight hundred thirteen patients who underwent coronary angiography and cardiac surgery successively from January 2017 to December 2018 were included in this retrospective cohort study. We applied multivariate logistic regression, propensity score analysis, and subgroup analysis to evaluate the association between the time interval and postoperative acute kidney injury incidence and prognosis. Meta-analysis was conducted to verify the results.

Results: The overall incidence of the cardiac surgery associated acute kidney injury was 28.8%. Age (OR = 1.046, 95%CI: 1.017–1.075), cardiopulmonary bypass (OR = 3.439, 95%CI: 1.316–8.986) and diabetes (OR = 2.522, 95%CI: 1.439–4.417) were found to be independent risk factors of postoperative acute kidney injury in multivariate logistic regression and propensity score analysis. Undergoing cardiac surgery within 7 days after coronary angiography was not associated with increased incidence of postoperative acute kidney injury or worse prognosis. Meta-analysis obtained consistent results.

Conclusions: The time interval shorter than 7 days had no influence on cardiac surgery associated acute kidney injury incidence and prognosis. The decision of delaying the surgery should be made after comprehensive evaluation of the patient.

Keywords: coronary angiography, cardiac surgery, acute kidney injury, contrast media, interval time

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INTRODUCTION

Cardiac surgery associated acute kidney injury (CSA-AKI) is a serious and common complication after cardiac surgery. Because of variable definitions, the incidence of CSA-AKI ranges from 9 to 40%, with 1–7% of cases requiring dialysis. CSA-AKI is one of the strongest predictors of mortality, and can delay recovery in up to 30% of patients (1–3). The etiology and pathophysiology

of AKI after cardiac surgery are complex and not fully understood. It is well-established that the underlying mechanisms include oxidative stress, ischemia-reperfusion injury, inflammation, and nephrotoxins (4). As there are not sufficient effective interventions to improve the prognosis, early identification, and modification of risk factors are especially critical.

Cardiac surgical procedures often follow diagnostic coronary angiography (CAG). However, contrast agent is a recognized risk factor for contrast-induced acute kidney injury (CI-AKI), which has an incidence of 7–10% (5, 6). To date, it is uncertain whether coronary angiography prior to cardiac surgery may enhance the risk of CSA-AKI. According to previous literatures, many predisposing factors are associated with the CSA-AKI, include age, diabetes, cardiac insufficiency, pre-existing kidney disease, and cardiopulmonary bypass (CPB) (7–10). One of the great challenges is that most of the risk factors are inherent and unmodifiable. But the time interval from CAG to cardiac surgery can to some extent be adjusted by clinicians. Del Duca et al. found that performing cardiac surgery within 5 days after CAG increased the risk of postoperative AKI (9), Although this was contradicted by another report (10). In other words, the optimal timing of surgery following angiography remains controversial.

To clarify relationship between the timing of surgery after CAG and CSA-AKI, we conducted a study in a large cohort of Chinese patients. In the meanwhile, we performed a comprehensive meta-analysis to validate our findings.

MATERIALS AND METHODS

Study Population and Perioperative Management

All 2,112 adult patients undergoing invasive CAG and elective cardiac surgery at Jiangsu province hospital from January 1st, 2017 to December 31th, 2018 were screened for this retrospective cohort study. Patients were excluded if they met the following criteria: (1) <18 years old; (2) received renal replacement therapy (RRT) before the surgery; (3) had a time interval from CAG to surgery >30 days; (4) underwent percutaneous coronary intervention; (5) underwent emergency or salvage surgery; (6) had vital signs that were not stable (e.g., in the case of shock) or required mechanical ventilation before surgery; and (7) had missing data. Ultimately, a total of 813 patients were included in the analysis. All data were retrieved from the hospital information system (HIS), which includes electrical medical record (EMR), laboratory information system (LIS), anesthesia information management system (AIMS), and nursing information system (NIS). The institutional review board and Ethics Committee of Jiangsu province hospital approved this study.

Perioperative management, CAG procedure, anesthetic, and surgical techniques were standardized for all patients. Surgical options and the use of cardiopulmonary bypass were decided by a same professional team of cardiac surgeons. All enrolled patients were advised to avoid potentially nephrotoxic drugs during hospitalization and were routinely given adequate hydration with intravenous normal saline at a rate of 1 ml/kg/h for 12 h after angiography. All patients received iso-osmolar contrast media (CM) at an iodine concentration of 320 mg/ml (Iodixanol, Visipaque®, GE Healthcare, Ireland) and amount of CM was determined in light of each patient's weight. For patients with baseline serum creatinine (SCr) >133 umol/L, the amount of CM was restricted, and N-acetylcysteine (1,200 mg) was administered every 12 h, 1 day before and after angiography.

End Points and Definitions

The primary endpoint was the incidence of cardiac surgery associated acute kidney injury. CSA-AKI was defined according to Kidney Disease Improving Global Outcome (KDIGO) Clinical Practice Guidelines: increase in SCr by ≥26.5 umol/L within 48 h; or increase in SCr to ≥1.5 times baseline that is known or presumed to have occurred within the prior 7 days (11). Urinary output criteria in defining AKI were not considered as there was frequent use of diuretics in perioperative period, which would make it unreliable. Patients with AKI were staged on the basis of KDIGO classification. Severe AKI was defined as stage 2 or 3 AKI. Baseline laboratory values were collected at admission or immediately before surgery when CAG was performed on the same operation day. Estimated creatinine clearance (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (12).

Statistical Analysis

Continuous variables were examined for normal distribution by the Kolmogorov-Smirnov test and summarized as either mean ± standard deviation or medians with interquartile range if the distribution of variables was skewed. Unpaired Student *t*-test or Mann-Whitney *U*-test were used to compare between groups. With regard to dichotomous variables, between-group differences were tested by Pearson chi-square or Fisher exact test.

We collected five types of variables that could potentially influence the occurrence of CSA-AKI: demographic information, previous history, preoperative data, intraoperative situation, and postoperative outcome. Basic patient information included age, gender, smoking history, and significant comorbidities such as hypertension, diabetes mellitus, cerebrovascular disease (CVD), anemia, and chronic obstructive pulmonary disease (COPD). Kidney and cardiac functions were assessed by SCr, eGFR, New York Heart Association (NYHA) classification, left ventricular ejection fractions (LVEF), and pulmonary arterial pressure (PAP). Perioperative parameters included important laboratory tests, American Society of Anesthesiologists (ASA) grade, operating time, CPB time, cross-clamp time, and fluid infusion.

First, the interval between CAG and cardiac surgery was deemed as a continuous variable and modeled as restricted cubic splines with 4 data-driven knot locations to account for the possible non-linear regression relationship with CSA-AKI. According to the restricted cubic splines and previous reports, we divided the patients into two time interval groups using 7 days as the cutoff value. We defined the operation on the same day of the CAG as interval 0. Subsequently, univariable analysis for the occurrence of CSA-AKI was carried out and statistically significant variables were tested in an adjusted

logistic regression model. Calibration and discrimination of the multivariable model were evaluated by the Hosmer-Lemeshow goodness-of-fit test and the area under the receiver operating characteristic curve, respectively. In order to verify the stability of the adjusted logistic regression model, the time interval was reincorporated into the regression model as a continuous or multiple categorical variable. Furthermore, to control for selection bias related to covariates and make the results more reliable, a propensity score analysis was performed. The method generated a propensity score within 0 to 1 and the patients in ≤7 days group were matched 1:1 to patients in >7 days group, using the nearest neighbors matching algorithm. The propensity score was created based on variables that were same as those that were adjusted in the multivariate logistic regression. Absolute standardized differences showed by love plot were estimated to evaluate the prematch imbalance and postmatch balance and the differences < 10% were considered inconsequential (13, 14). Lastly, subgroup analyses stratified by CPB, age, gender, diabetes mellitus, operation type, cardiac function, and kidney function were also conducted.

Meta-Analysis

A meta-analysis was further performed to validate the association between the interval time and CSA-AKI. We searched the electronic databases PubMed, Embase, and the Cochrane Library up to February 29th, 2020 using the keywords: "catheterization or angiography or percutaneous coronary intervention" and "cardiac surgery" and "acute kidney injury or AKI or renal failure" without restrictions on the baseline conditions of study objects. Included studies met the following criteria: (1) the study investigated the risk of AKI and 7-day interval between CAG and cardiac surgery; (2) the study provided sufficient information to calculate odds ratios (ORs) and 95% confidence intervals (CIs); and (3) the study was a randomized controlled trial, cohort or case-control study. Two authors (M Li and K Liu) extracted the following data: first author, publication year, country, sample size, gender ratio, NYHA, operation type, AKI definition, and the number of patients suffering from AKI, severe AKI, RRT and death. Disagreements were resolved by discussion or consensus with a third reviewer (L Li). The study contained detailed data of patients undergoing on-pump or off-pump cardiac surgery was regarded as two separate studies. The quality of studies was assessed by the Newcastle-Ottawa scale (NOS) and those with scores of six stars or greater were considered to be of high-quality studies (15).

Summary ORs with their corresponding 95% CIs were used to assess the strength of the association. Between-studies heterogeneity was evaluated by the chi-square-based *Q*-test and $P < 0.10$ indicated the existence of heterogeneity. The pooled OR estimate of each study was calculated with the random-effects models using the DerSimonian and Laird method when these studies were heterogeneous (16). Sensitivity analysis was performed to determine whether a particular study or studies would result in heterogeneity and assess the stability of the results. Begg's funnel plot and Egger's regression test were applied to examine publication bias. All tests in this study were two-sided with the alpha level set at 0.05 for statistical significance. Statistical analyses were performed with Stata, version 15.1 (StataCorp LP, College Station, TX, USA).

RESULTS

Baseline Characteristics

The baseline demographics, clinical characteristics, intraoperative, and postoperative variables of the 813 included patients are shown in **Table 1**. The mean age was 62.5 ± 8.9 years and there were 493 (60.6%) males. The overall incidence of the CSA-AKI was 28.8%. The majority of patients suffered from stage 1 AKI (62.4%) and 27 required dialysis (3.3%). There was no significant difference in AKI incidence between two groups. Compared to patients who underwent cardiac surgery after 7 days, those who had surgery within 7 days of CAG had fewer comorbidities such as hypertension, diabetes mellitus, and cerebrovascular disease and had a higher ejection fraction, albumin level, and hematocrit value. In terms of surgical procedure, the median operation time, blood loss volume, ultrafiltration volume were higher and the proportions of CPB, platelet infusion were increased in the shorter interval (≤7 days) cohort. Nonetheless, the number of patients requiring RRT and in-hospital mortality rate were similar between the two groups.

Association Between the Time Interval and CSA-AKI

Univariate logistic regression analysis was performed to identify the predictors of CSA-AKI (**Table 2**). Older age, diabetes, cerebrovascular disease, worse renal function, lower levels of hemoglobin and albumin, CPB, prolonged operation time, large bleeding volume, and ultrafiltration volume might increase the hazard of CSA-AKI. However, we failed to observe any correlation between the time interval and CSA-AKI. It is worth noting that AKI had a marked impact on patient prognosis, resulting in longer hospital stays and higher mortality.

In the multivariate analysis, age, diabetes, CPB, cerebrovascular disease, and baseline creatine level were risk factors of CSA-AKI after adjusting for potential confounders (**Table 3**). However, time interval ≤7days was not associated with the incidence of AKI. The result was the same regardless of whether the time interval was considered as a continuous or a multiple categorical variable, or whether age and Scr were substituted with eGFR (**Supplementary Table 1**). In an attempt to control for the selection bias related to covariates, the propensity score method was applied. As shown in **Table 1**, the two groups were well-matched, with no significant differences with respect to the variables. Older age (OR = 1.046, 95%CI: 1.017–1.075), CPB (OR = 3.439, 95%CI: 1.316–8.986), diabetes (OR = 2.522, 95%CI: 1.439–4.417) but not time interval ≤7days (OR = 1.045, 95%CI: 0.676–1.615) played an important role in the occurrence of CSA-AKI.

Subgroup Analysis and Prognosis Analysis

We divided the patients into different subgroups according to CPB, age, diabetes, operation type, NYHA classification, eGFR, and gender to probe the potential association between the interval time and CSA-AKI in specific populations. Performing

TABLE 1 | Baseline and perioperative characteristics of the patients stratified by the CAG interval.

Variables	Prematch			Postmatch		
	>7days ($n = 367$)	≦7days ($n = 446$)	*P*	>7days ($n = 216$)	≦7days ($n = 216$)	*P*
Demographic data						
Age (year)	63.6 ± 8.8	61.6 ± 8.8	**<0.001**	62.9 ± 8.5	62.9 ± 8.8	0.791
Male	242 (65.9)	251 (56.3)	**0.005**	136 (63.0)	141 (65.3)	0.616
Previous history						
Smoking	131 (64.3)	131 (70.6)	0.055	73 (33.8)	79 (36.6)	0.546
Hypertension	212 (57.8)	200 (44.8)	**<0.001**	121 (56.0)	114 (52.8)	0.499
Diabetes mellitus	87 (23.7)	55 (12.3)	**<0.001**	40 (18.5)	41 (19.0)	0.902
COPD	5 (1.4)	8 (1.8)	0.623	1 (0.5)	4 (1.85)	0.372
Cerebrovascular disease	53 (14.5)	42 (9.4)	**0.026**	32 (14.9)	24 (11.1)	0.244
Anemia	18 (4.9)	24 (5.4)	0.760	10 (4.6)	14 (6.5)	0.401
Kidney and cardiac status						
Serum creatinine (umol/L)	73.7 (61.4, 84.1)	70.3 (59.6, 81.5)	**0.029**	74.0 (59.2, 84.7)	73.2 (61.7, 82.9)	0.875
eGFR (ml/min/1.73m^2)	90.9 (79.2, 101.9)	93.0 (80.6, 102.4)	0.102	89.6 (78.0, 101.4)	91.0 (80.4, 101.1)	0.762
NYHA class I-II	226 (61.6)	264 (59.2)	Ref	139 (64.3)	126 (58.3)	Ref
NYHA class III-IV	140 (38.4)	182 (40.8)	0.459	77 (35.7)	90 (41.7)	0.199
Ejection fraction (%)	62.1 (53.7, 64.6)	62.7 (58.7, 65.1)	**0.006**	62.7 (57.9, 65.2)	62.2 (55.9, 64.8)	0.229
PAP (mmHg)	33 (27, 41)	33 (28, 40)	0.726	34 (28, 41)	31 (27, 40)	0.255
Preoperative data						
Hemoglobin (g/L)	131.5 ± 16.1	131.2 ± 16.6	0.800	133.0 ± 16.6	131.1 ± 16.9	0.397
Albumin (g/L)	38.3 ± 4.00	39.2 ± 3.8	**0.004**	39.0 ± 3.9	38.8 ± 3.8	0.737
Hematocrit (%)	37.4 ± 6.6	38.9 ± 7.0	**<0.001**	38.4 ± 6.6	38.2 ± 7.6	0.808
Intraoperative data						
Operation type						
Isolated CABG	197 (53.7)	94 (21.1)	**<0.001**	84 (38.9)	83 (38.4)	1.000
Isolated valve	112 (30.5)	251 (56.3)		88 (40.7)	89 (41.2)	
CABG+valve	39 (10.6)	65 (14.6)		28 (13.0)	28 (13.0)	
Other types	19 (5.2)	36 (8.0)		16 (7.4)	16 (7.4)	
ASA Grading	3 (3, 3)	3 (3, 3)	**0.006**	3 (3, 3)	3 (3, 3)	0.909
CPB	177 (48.2)	354 (79.4)	**<0.001**	141 (65.3)	135 (62.5)	0.548
Operating time (min)	257 (217, 336)	277 (233, 343)	**0.003**	276 (225, 357)	264 (220, 330)	0.235
CPB time (min)	143 (117, 190)	141 (111, 178)	0.301	140 (117, 190)	143 (110, 188)	0.694
ACC time (min)	103 (76, 139)	103 (76, 134)	0.933	105 (78, 142)	108 (75, 138)	0.832
Blood transfusion						
Red blood cells	317 (86.4)	370 (83.0)	0.180	185 (85.6)	182 (84.3)	0.686
Plasma	50 (13.6)	63 (14.1)	0.837	31 (14.3)	27 (12.5)	0.572
Platelets	162 (44.1)	321 (72.0)	**<0.001**	127 (58.8)	125 (57.9)	0.845
Fluid balance						
Blood loss (ml)	500 (500, 800)	700 (500, 1,000)	**<0.001**	600 (500, 800)	600 (500, 800)	0.828
Ultrafiltration (ml)	2,000 (0, 2,500)	2,100 (1,500, 2,700)	**<0.001**	2,000 (1,300, 2,600)	2,000 (1,000, 2,500)	0.776
Postoperative data						
Acute kidney injury	104 (28.3)	130 (29.2)	0.800	67 (31.0)	67 (31.0)	1.000
KDIGO stage 1	65 (17.7)	81 (18.2)		46 (21.3)	40 (18.5)	
KDIGO stage 2	22 (6.0)	28 (6.3)		17 (7.9)	16 (7.4)	
KDIGO stage 3	17 (4.6)	21 (4.7)		4 (1.8)	11 (5.1)	
RRT	13 (3.5)	14 (3.1)	0.757	2 (0.9)	7 (3.2)	0.175
In-hospital mortality	15 (4.1)	25 (5.6)	0.319	7 (3.2)	15 (6.9)	0.080
Length of hospital stay (day)	23 (19, 31)	19 (16, 23)	**<0.001**	25 (19, 31)	18 (15, 22)	**<0.001**

eGFR, estimated glomerular filtration rate (CKD-EPI formula); NYHA, New York Heart Association; PAP, pulmonary artery pressure; CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; ACC, Aortic cross clamp; KDIGO, Kidney Disease: Improving Global Outcomes; RRT, renal replacement therapy.
Bold characters are used to express P-values that are statistically significant (< 0.05).

TABLE 2 | Preoperative, operative, and postoperative univariate analysis of AKI.

Variables	No AKI ($n = 579$)	AKI ($n = 234$)	*P*
Demographic data			
Age (year)	61.7 ± 9.0	64.4 ± 8.3	**<0.001**
Male	347 (59.8)	147 (62.8)	0.418
Previous history			
Smoking	187 (32.3)	75 (32.1)	0.946
Hypertension	290 (50.1)	122 (52.1)	0.597
Diabetes mellitus	86 (14.9)	56 (23.9)	**0.002**
COPD	8 (1.4)	5 (2.1)	0.537
Cerebrovascular disease	56 (9.7)	39 (16.7)	**0.005**
Anemia	25 (4.3)	17 (7.3)	0.086
Kidney and cardiac status			
Serum creatinine (umol/L)	70.6 (59.4, 82.4)	74.9 (61.8, 84.3)	**0.008**
eGFR (ml/min/1.73m^2)	93.1 (81.2, 102.9)	88.9 (75.3, 99.0)	**<0.001**
NYHA class I-II	362 (62.5)	129 (55.1)	Ref
NYHA class III-IV	217 (37.5)	105 (44.9)	0.051
Ejection fraction (%)	62.7 (57.9, 64.8)	61.9 (53.6, 65.0)	0.175
PAP (mmHg)	33 (28, 41)	33 (27, 40)	0.849
Preoperative data			
Hemoglobin (g/L)	132.2 ± 16.0	129.2 ± 17.1	**0.022**
Albumin (g/L)	39.0 ± 3.9	38.2 ± 3.8	**<0.001**
Hematocrit (%)	38.1 ± 7.0	38.5 ± 6.5	0.732
Interval time (day)	7 (4, 11)	7 (5, 10)	0.723
CAG interval≦7d	316 (54.6)	130 (55.6)	0.800
Intraoperative data			
Operation type			
Isolated CABG	255 (44.0)	66 (28.2)	**<0.001**
Isolated valve	255 (44.0)	108 (46.2)	
CABG+valve	57 (9.8)	47 (20.1)	
Other types	12 (2.2)	13 (5.5)	
ASA Grading	3 (3, 3)	3 (3, 3)	0.188
CPB	357 (61.7)	174 (74.4)	**0.001**
Operating time (min)	263 (224, 325)	289 (232, 373)	**0.001**
CPB time (min)	138 (111, 174)	151 (120, 203)	**<0.001**
Aortic cross clamp time (min)	101 (74, 130)	110 (80, 148)	0.078
Blood transfusion			
Red blood cells	487 (84.1)	200 (85.5)	0.628
Plasma	73 (12.6)	40 (17.1)	0.094
Platelets	324 (56.0)	159 (68.0)	**0.002**
Fluid balance			
Blood loss (ml)	600 (500, 800)	700 (500, 1,000)	**0.005**
Ultrafiltration (ml)	2,000 (1,200, 2,500)	2200 (1,500, 3,000)	**0.004**
Postoperative data			
In-hospital mortality	10 (1.7)	30 (12.8)	**<0.001**
Length of hospital stay (day)	20 (16, 25)	23 (18, 29)	**<0.001**

eGFR, estimated glomerular filtration rate (CKD-EPI formula); NYHA, New York Heart Association; PAP, pulmonary artery pressure; CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass.

Bold characters are used to express P-values that are statistically significant (< 0.05).

the surgery 7 days after CAG was merely found to mitigate the risk of AKI in patients with diabetes (OR = 0.452, 95%CI: 0.209–0.977; **Figure 1**). We analyzed prognosis in order to establish the relationship between 7-day interval and CSA-AKI. Detailed outcomes data were shown in **Supplementary Table 2**. Undergoing cardiac surgery 7 days after CAG did not reduce the risks of RRT (OR = 0.97, 95%CI: 0.44–2.13) or mortality (OR = 0.54, 95%CI: 0.27–1.09) in the multivariate analysis. Performing the surgery after 7 days was also not associated with the risks of RRT (OR = 0.40, 95%CI: 0.10–1.91) or mortality (OR = 0.45, 95%CI: 0.12–1.62) in the subgroup analysis of diabetic patients.

Results of Meta-Analysis

A total of six papers with scores more than six stars including 6,841 patients were included in the meta-analysis to further verify the association between 7-day interval and the risk of postoperation AKI (17–21) (**Supplementary Figure 1**). Detailed characteristics of the studies are listed in **Table 4**, **Supplementary Table 2**. Pooled analysis of the studies revealed that AKI risk did not differ between a time interval ≤7 days vs. >7 days in the on-pump (OR = 1.16, 95%CI: 0.79–1.70; **Figure 2**) and off-pump (OR = 1.13, 95%CI: 0.96–1.34) subgroups. We also carried out subgroup analyses stratified by ethnicity, AKI definition, and operation type and found not significant associations (**Supplementary Table 3**). The heterogeneity across studies was low except for the on-pump, Asian and KDIGO subgroups. The sensitivity analysis showed that the results were statistically robust. Moreover, Egger's test revealed no evidence of significant publication bias ($P = 0.493$). The meta-analysis of prognosis exhibited no heterogeneity and no significant differences in mortality, incidence of severe AKI, or risk of RRT (**Figure 3**).

DISCUSSION

AKI has been identified as a common complication of cardiac surgery (22). Even less-severe AKI is associated with long in-hospital stay and increased mortality, which imposes a considerable burden on healthcare systems (23). As there is a lack of therapeutic methods to treat CSA-AK, in the past decade attention has shifted from treatment to optimization of perioperative management and avoidance of risk factors. Recently, some researchers made a point that the time interval from CAG to cardiac surgery might be an interventional risk factor for CSA-AKI. For example, Kramer et al. enrolled 668 non-emergent adult cardiac surgical cases and suggested discharge and readmission as a potential strategy to reduce the postoperative incidence of AKI (24). The main evidence supporting for this conclusion is that contrast media may bring about the kidney injury and the underlying pathophysiological mechanisms include a deleterious reduction in renal arteriolar blood flow and glomerular filtration rate, as well as the direct toxicity to tubular epithelial cells (25). The CAG procedure likely induces a pre-injury state in the kidney which can be described as acute kidney stress, such that subsequent cardiac surgery can accelerate the development of AKI. However, in the present retrospective cohort study, we observed that 7-day

TABLE 3 | Multivariable logistic analysis for acute kidney injury in the study patients.

Variables	Unadjusted for Propensity score[a]		Adjusted for Propensity score[a]	
	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Age	1.038 (1.017–1.060)	**<0.001**	1.046 (1.017–1.075)	**0.002**
CPB	3.194 (1.536–6.643)	**0.002**	3.439 (1.316–8.986)	**0.012**
Diabetes mellitus	2.712 (1.736–4.236)	**<0.001**	2.522 (1.439–4.417)	**0.001**
CVD	2.020 (1.255–3.252)	**0.004**	1.672 (0.893–3.217)	0.108
Serum creatine	1.009 (1.001–1.016)	**0.030**	1.008 (0.998–1.019)	0.113
Hemoglobin	0.993 (0.982–1.003)	0.167	0.994 (0.981–1.008)	0.431
Albumin	0.972 (0.929–1.017)	0.226	0.940 (0.883–1.002)	0.054
Operating time	1.000 (0.999–1.001)	0.245	1.000 (0.998–1.002)	0.729
Platelet transfusion	0.870 (0.444–1.704)	0.685	0.580 (0.231–1.455)	0.246
Blood loss	1.000 (0.999–1.001)	0.111	1.000 (0.999–1.001)	0.203
Interval ≤7days	1.007 (0.713–1.422)	0.967	1.045 (0.676–1.615)	0.844

CI, confidence interval; CPB, cardiopulmonary bypass; CVD, cerebrovascular disease.
[a]Propensity score developed for the likelihood of receiving CAG interval ≤7 days.
Bold characters are used to express P-values that are statistically significant (< 0.05).

interval between CAG and cardiac surgery was not a risk factor for the postoperative occurrence of AKI.

Consistent with the findings of our study, a number of previous studies reported similar results. An investigation of 644 patients reported no association between the contrast-to-surgery time interval and occurrence of CSA-AKI, even after increasing the postoperative period over which AKI was defined or including only patients undergoing elective surgery. Borde DP and colleagues analyzed 900 patients who underwent off-pump coronary artery bypass surgery (CABG) after contrast exposure and suggested that performing the surgery within 7 days did not increase the risk of post-operative AKI compared to patients who underwent the operation after 7 days. The first possible explanation is that the influence of CM on kidney function is exaggerated (26). A meta-analysis of 13 studies involving 25,950 patients showed no significant difference in AKI risk between patients with and those without exposure to CM (OR = 0.79, 95%CI = 0.62–1.02) (27). A nationwide analysis of data for about 6 million patients in the U.S. demonstrated AKI rates were nearly identical no matter patients received CM or not, which proved that risk for AKI attributable to CM was modest (28). Secondly, non-nephrologists have become increasingly aware of renal adverse events associated with the use of contrast agents and monitor creatinine frequently, which could to some extent influence the determination of surgery time and reduce the incidence of CSA-AKI. In addition, the use of iso-osmolar CM and advances in CAG should be taken into consideration. In this context, it is not surprising that AKI risk was not increased in cardiac surgical patients who accepted a same-day coronary angiography (29).

It is widely shared that several risk factors acting at pre-, intra-, and postoperative levels play a role in the occurrence of CSA-AKI. In our study, we found age, CPB, diabetes, cerebrovascular disease, and basal renal function but not time interval were contributing factors of CSA-AKI in the multivariate logistic regression analysis. This is consistent with the risk variables reported in previous studies, which emphasizes the multifactorial etiology of CSA-AKI and the need for a comprehensive assessment to establish AKI risk in each patient (4). It should be noted that several previous studies with positive results existed latent selection bias; for instance, some studies included critically ill patients undergoing emergency operation who were susceptible to CSA-AKI (9). Additionally, in some studies the baseline characteristics of patients in the long and short time interval cohorts were not comparable. The exact association between the time interval and CSA-AKI might be misestimated or not properly detected. Given the above, propensity score matching method was adopted to confirm that a shorter time interval did not contribute to a reduced incidence of CSA-AKI. However, age, diabetes, and CPB were found to be independent predictors of CSA-AKI. It is well-known that AKI is often a continuum of kidney injury rather than a single-hit. The CPB procedure may disrupt hemodynamics by ischemia-reperfusion injury, resulting in low cardiac output and hemodilution (4, 30). Senility and diabetes also aggravate renal microvascular dysfunction (31, 32). These risk factors, which can be considered as first hits, predispose the kidney to further injury, with subsequent cardiac surgery being the second hit that causes further deterioration of renal function rather than recovery. In order to clarify the potential correlation between the time interval and CSA-AKI in some specific populations, we conducted subgroup analysis and perceived that undergoing cardiac surgery after 7 days mitigated the risk of AKI merely in patients with diabetes. This finding was actually in agreement with the results of multivariate analysis. We speculate that shortening the time interval worsens renal function, which increases the risk of AKI; preexisting diabetes increases susceptibility to rather than mediates CSA-AKI (33). Finally, the meta-analysis stratified by ethnicity, AKI definition, CPB, and operation type supported the results of our single center study. Notably, heterogeneity was still observed in some subgroups. Our study population had the lowest incidence of CSA-AKI among published studies; differences in perioperative treatment strategies, proportions of operation types, and skill levels in the operation and in

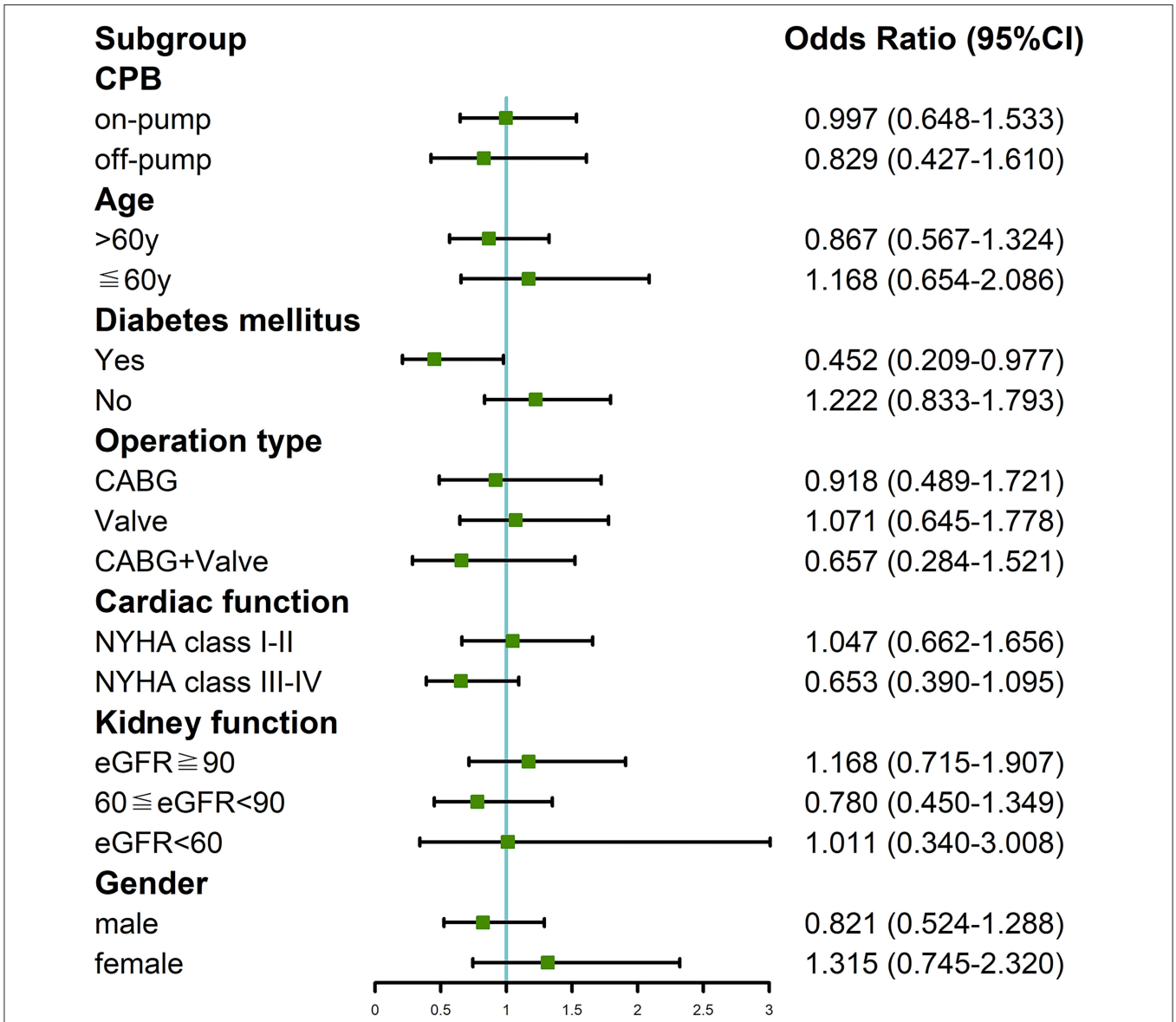

FIGURE 1 | Subgroup analysis of the association between 7-day interval from CAG to cardiac surgery and incidence of CSA-AKI in our cohort study.

nursing across studies could account for this variability. Small sample sizes in the published studies prevented us from further examining the sources of heterogeneity.

Clinicians often focus more on the prognosis of a disease. CSA-AKI remains an important cause of patient morbidity and mortality, which bring it into limelight. A series of studies have shown that postoperative AKI has a moderately poor prognosis (34, 35). In the current study, in-hospital mortality was significantly higher in patients with CSA-AKI than in those without kidney injury. Further analysis indicated that 7-day interval was unrelated to hemodialysis rate and mortality, even in diabetic patients. This result appeared to be contradictory with the interrelation between the time interval and CSA-AKI incidence. We suppose that the correlation does not actually exist, or that the time interval merely affects the incidence of stage 1 AKI, which has a relatively favorable prognosis in most cases. To monitor creatinine level, discontinue nephrotoxic drugs, and stabilize hemodynamics are crucial measures for stage 1 AKI and renal function generally recover without invasive interventions (36, 37). Previous studies uncovered that the nominal increments in creatinine levels used to define AKI were observed in patients exposed to CM (33). As a consequence, a shorter time interval from CAG to cardiac surgery might give rise to a slight elevation in serum creatinine without affecting prognosis. Since there were relatively few cases of RRT and death, we further conducted a meta-analysis of published studies, and the pooled ORs validated our results that the time interval was not an essential factor in the occurrence of severe AKI, RRT, and death.

TABLE 4 | Main characteristics of all studies included in the meta-analysis.

Study	Year	Country	Sample size	Male	NYHA III-IV	Main operation	CPB	AKI definition	AKI incidence	Mortality
McIlroy et al. (17)	2012	USA	644	61.2%	–	Mixed[a]	Both[b]	AKIN	21.9%	3.0%
Kim et al. (18)	2016	Korea	701	73.3%	–	CABG	On-pump	KDIGO	48.9%	1.1%
			1,670	77.0%			Off-pump		36.8%	
Dayan et al. (19)	2017	Uruguay	1,044	54.0%	33.2%	Valve	On-pump	AKIN	27.5%	5.6%
Jiang et al. (20)	2018	China	1,069	62.4%	75.2%	Mixed[a]	Both[b]	KDIGO	38.5%	0.3%
Borde et al. (21)	2019	India	900	78.7%	54.1%	CABG	Off-pump	KDIGO	23.8%	1.0%
This study	2019	China	531	54.4%	43.9%	Mixed[a]	On-pump	KDIGO	32.8%	4.3%
			282	72.3%	31.6%		Off-pump		21.3%	6.0%

NYHA, New York Heart Association; CPB, cardiopulmonary bypass; AKI, acute kidney injury; CABG, coronary artery bypass grafting; AKIN, Acute Kidney Injury Network; KDIGO, Kidney Disease: Improving Global Outcomes.

[a]*Mixed: CABG, Valve and CABG+Valve.*

[b]*Both: On-pump and Off-pump.*

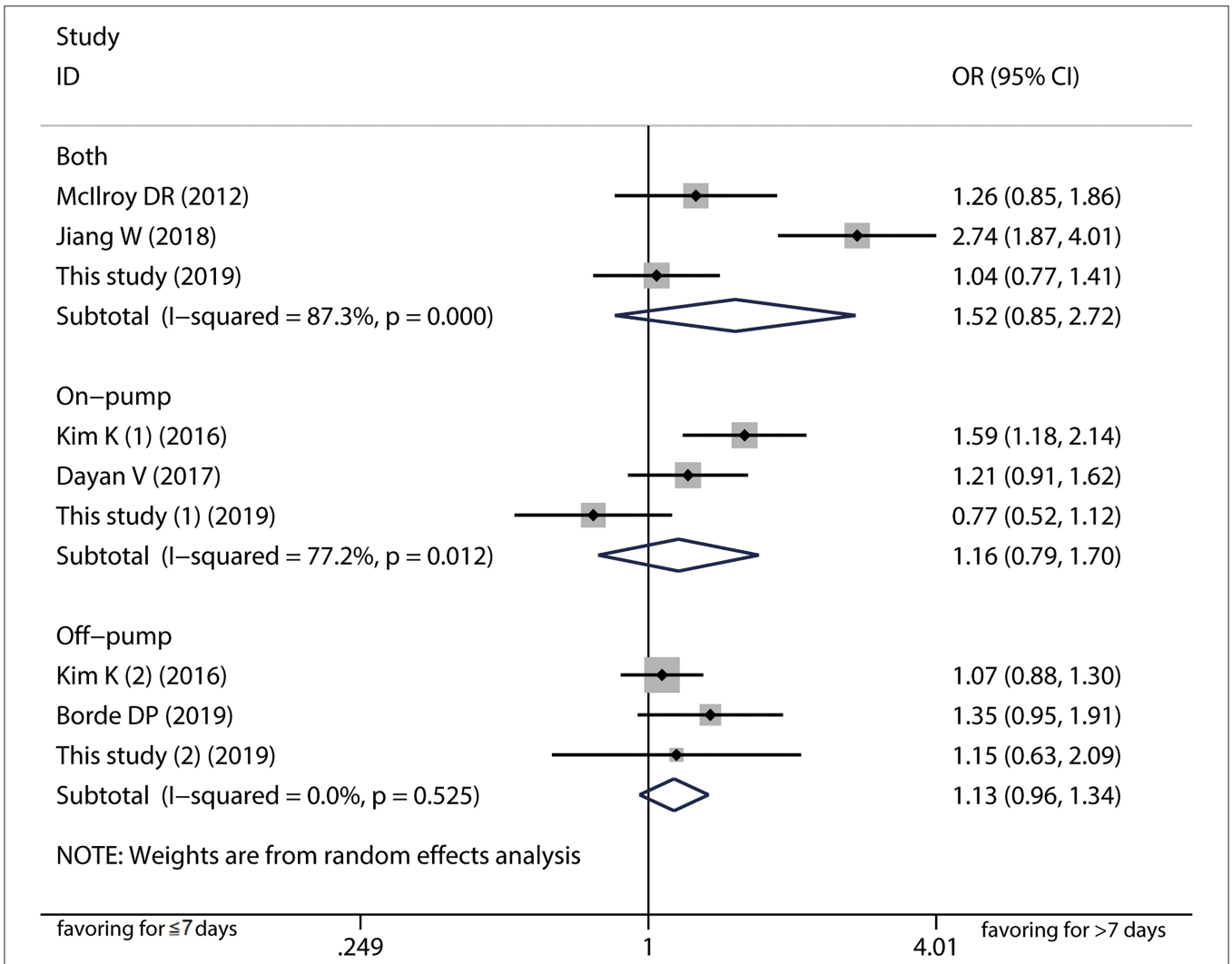

FIGURE 2 | Forest plot comparing ≤7-day and >7-day intervals stratified by cardiopulmonary bypass in the meta-analysis. The results indicated AKI risk did not differ between a time interval ≤7 days vs. >7 days in the on-pump, off-pump, and both groups.

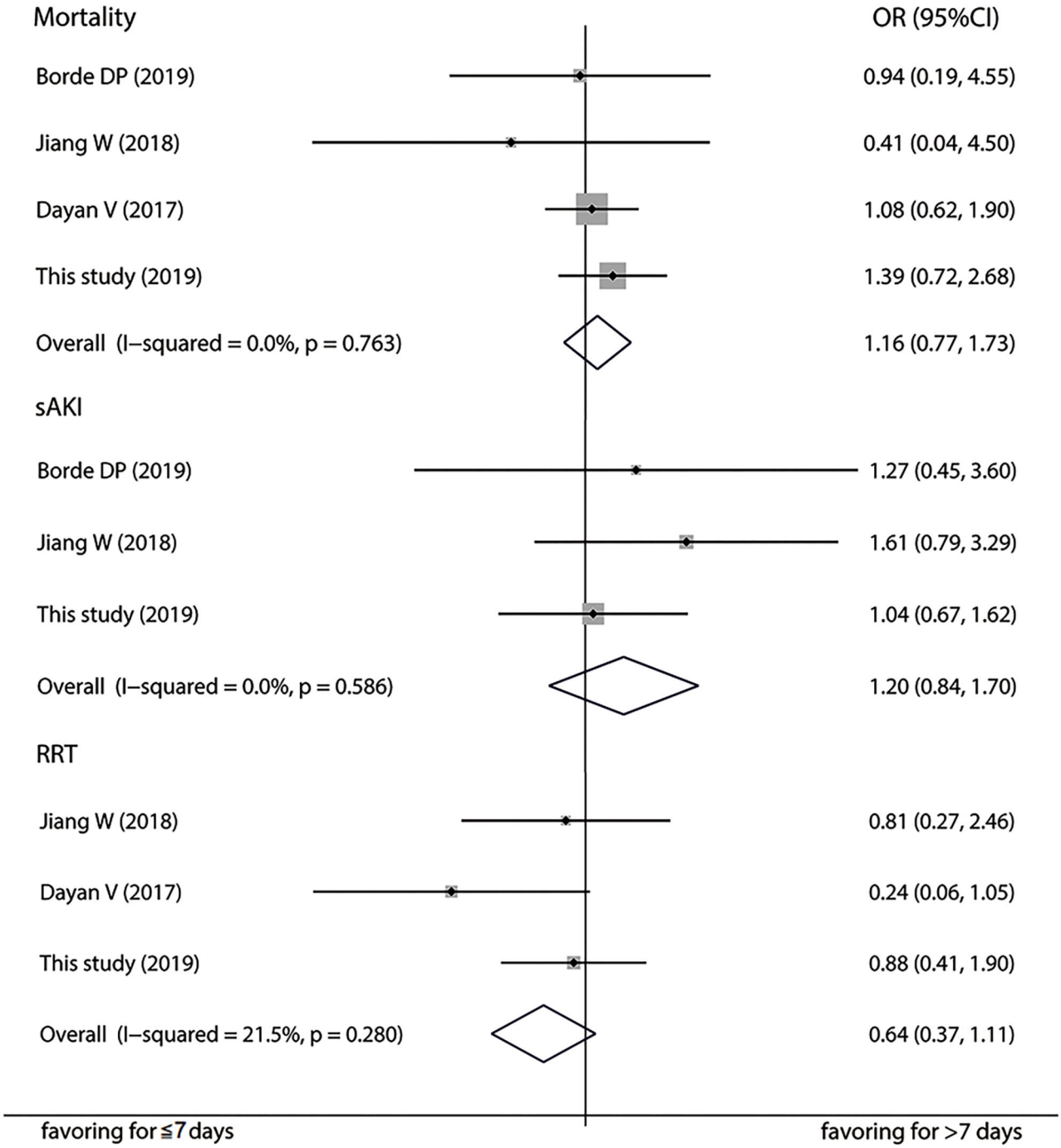

FIGURE 3 | Forest plot comparing ≤7-day and >7-day intervals in terms of mortality, severe acute kidney injury, and renal replacement therapy in the meta-analysis.

According to the results of this investigation, delaying cardiac surgery for 7 days after CAG to avoid the development of CSA-AKI is unwarranted. In clinical practice, clinicians should instead focus on the patient profile including comorbidities as well as the surgical procedure. In most patients, contrast-associated acute kidney injury may be transient and reversible in nature. For patients at increased risk of AKI and those with impending AKI, the decision of delaying the surgery should be made after a comprehensive evaluation. Likewise, shortening the time interval should be weighed against the expected benefit. Unfortunately, we do not have any effective tool to help choose the timing of CAG before the surgery so far. In other words,

it is unable to obtain an optimal time interval on the basis of our results. This is the most important limitation of this study. It is worth mentioning that scientists have identified several novel biomarkers (e.g., NGAL, KIM 1, IL-18, TIMP2*IGFBP7, cystatin C, and GST) released from the kidney during injury or filtered by the kidneys. These biomarkers offer several theoretical advantages over serum creatinine, which are in increasing use to determine the nature of kidney injury, and consequently assess the result of therapy and improve patient management (38, 39). Thus, a well-designed prediction model based on clinical data and biomarkers may be a promising solution. Our study also had several other limitations. Firstly, we included all cardiac surgery patients regardless of the type and mode of surgery, which made the conclusions more applicable to the general population. However, individual differences were neglected. The analyses with refined and detailed evaluation parameters in patients of specific surgical types should be conducted in further large sample size study. Secondly, data on creatinine in the time interval between CAG and surgery were missing for some patients, making it difficult to distinguish between contrast-induced AKI and CSA-AKI in these patients. Thirdly, data of detailed dose of CM, postoperative fluid infusion and some other immeasurable variables were not collected, which might confound the results. We could not rule out the possibility that negative results were due to a type II error. Finally, this was a single center study with a retrospective design. Although we performed subgroup and propensity score analyses as well as meta-analysis to confirm our results, the limited sample size and heterogeneity could undermine the robustness of results. A further large prospective, multicentric, randomized, controlled study is still needed to validate our findings.

CONCLUSION

To sum up, undergoing cardiac surgery within 7 days after CAG was not associated with an increased risk of CSA-AKI or worse prognosis. The optimal timing of CAG before surgery should be weighted with reference to the severity of cardiovascular disease and risk factors for CSA-AKI in patients who are susceptible to acute kidney injury. To carry out a well-designed prospective study with large sample size and establish a practical prediction model based on clinical indices and novel renal biomarkers are worthwhile directions in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

KL, HM, and CX: project design. ML and LL: data collection. KL and BW: statistic analysis. XX and YG: supervision and validation. KL, ML, and HM: writing and revision. All authors: read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.619210/full#supplementary-material

Supplementary Figure 1 | Flow diagram of study selection for the meta-analysis.

Supplementary Table 1 | Multivariable logistic analysis for acute kidney injury in the study patients in different models.

Supplementary Table 2 | Detailed data of all studies included in the meta-analysis.

Supplementary Table 3 | Subgroup analyses of Meta-analysis.

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MINI REVIEW
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Clinical Approach to Vascular Calcification in Patients With Non-dialysis Dependent Chronic Kidney Disease: Mineral-Bone Disorder-Related Aspects

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Chronic kidney disease (CKD) is associated with a very high morbimortality, mainly from cardiovascular origin, and CKD is currently considered in the high- or very high risk- cardiovascular risk category. CKD-mineral and bone disorders (CKD-MBDs), including vascular and/or valvular calcifications, are also associated with these poor outcomes. Vascular calcification (VC) is very prevalent (both intimal and medial), even in non-dialysis dependent patients, with a greater severity and more rapid progression. Simple X-ray based-scores such as Adragão's (AS) are useful prognostic tools and AS (even AS based on hand-X-ray only) may be superior to the classic Kauppila's score when evaluating non-dialysis CKD patients. Thus, in this mini-review, we briefly review CKD-MBD-related aspects of VC and its complex pathophysiology including the vast array of contributors and inhibitors. Furthermore, although VC is a surrogate marker and is not yet considered a treatment target, we consider that the presence of VC may be relevant in guiding therapeutic interventions, unless all patients are treated with the mindset of reducing the incidence or progression of VC with the currently available armamentarium. Avoiding phosphate loading, restricting calcium-based phosphate binders and high doses of vitamin D, and avoiding normalizing (within the normal limits for the assay) parathyroid hormone levels seem logical approaches. The availability of new drugs and future studies, including patients in early stages of CKD, may lead to significant improvements not only in patient risk stratification but also in attenuating the accelerated progression of VC in CKD.

Keywords: CKD, CKD-MBD, calcification, vascular calcification, phosphate, calciprotein particles

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INTRODUCTION: CHRONIC KIDNEY DISEASE AND CARDIOVASCULAR DISEASE

Chronic kidney disease (CKD) has become a major public health problem because of the associated high mortality and elevated cost of treatment for dialysis patients. Worldwide, it is estimated that about 500 million adults suffer from CKD (1), but prevalence varies widely between countries (2). In Spain, CKD prevalence is 15.1% (3), is more common in men, in patients with cardiovascular disease (CVD) (39.8%), and increases with age (37.3% in those ≥65 years) (3). Given this significant link between CKD and CVD, CKD is currently considered an independent cardiovascular risk factor (4). A similar scenario is described for diabetes (5), a leading cause of CKD. Moreover, an independent, graded association was observed between a reduced estimated glomerular filtration rate (eGFR) and the risk of death, CVD, and hospitalization in a huge community-based population (6). Consequently, the European Society of Cardiology and European Atherosclerosis Society (ESC/EAS) recently considered moderate (eGFR 30–59 ml/min/1.73m^2) and severe (eGFR<30 ml/min/1.73m^2) CKD to be in the high- and the very high risk category of cardiovascular risk factors (7).

Since the seminal report by Foley et al. (8), who noted an extremely high cardiovascular mortality even in young dialysis patients, the existence of non-traditional cardiovascular risk factors, including mineral metabolism parameters, has caught the attention of many. Subclinical atherosclerosis is very frequent in CKD patients, and its evolution is also closely linked with CKD progression and related to several CKD-associated factors (9, 10). Thus, bone was recently described as a new endocrine organ "at the heart" of CKD and mineral-bone disorders (CKD-MBD) (11). Subsequently, the presence (12, 13) and progression (14, 15) of vascular and valvular calcification (16), which are also associated with "normal" aging (17), were clearly linked to prognosis (18–22), including in non-dialysis CKD (ND-CKD) patients, especially if the coronary artery calcification (CAC) score was evaluated (20–24). Therefore, CKD could be viewed conceptually as an accelerator of traditional cardiovascular risk factors and vascular calcification (VC) (17). Thus, in this review we will focus on the CKD-MBD aspects of VC including potentially helpful treatment considerations.

CHRONIC KIDNEY DISEASE-MINERAL AND BONE DISORDERS (CKD-MBD) AND VASCULAR CALCIFICATION

The term CKD-MBD was first coined in 2006 (25). It described a systemic disorder due to CKD, manifested by mineral and bone abnormalities and/or extraskeletal calcifications (25). Several studies have demonstrated the high prevalence of cardiovascular calcifications in CKD, even in ND-CKD patients (20, 21, 26–30), with a greater severity (20, 31) and more rapid progression (32, 33). Furthermore, it has been suggested that these cardiovascular calcifications may be not only a useful prognostic tool but also relevant in guiding therapeutic interventions (19, 34, 35), as it will be reviewed in section Treatment Implications. However, we will first let readers understand the different diagnostic methods and the important contribution of CKD-MBD-related aspects in the pathophysiology of VC in CKD patients (section Pathophysiology of CKD-MBD-related Vascular Calcification).

In clinical studies, VC is frequently detected by multisliced computed tomography (CT) and less often by electron-beam CT (20, 36–38), multiterritorial vascular ultrasound (9), intravascular ultrasound (IVUS) or Virtual Histology® IVUS (39, 40), arteriography, or even positron emission tomography (PET) scans (41, 42). Thus, the prevalence of CAC has been recently systematically reviewed and authors found a variable prevalence ranging from 28 to 93% in predialysis patients (59% pooled prevalence) (20). High heterogeneity was present, at least partially related to ethnic and population differences (such as age or CKD stages) (20). However, simple X-rays may also be used despite their much lower sensitivity and variable associations with CAC (43). In fact, guidelines suggest that a lateral abdominal radiograph can be used to detect VC and an echocardiogram can be used to detect valvular calcifications, as reasonable alternatives to CT-based imaging (35, 44). These guidelines also suggest that patients with CKD G3a–G5D with known vascular or valvular calcification be considered at highest cardiovascular risk (evidence 2A) and that it is reasonable to use this information to guide CKD-MBD management (35, 44).

Lateral abdominal X-ray is helpful not only to semiquantify an aortic risk score such as Kauppila's (KS) (45) (**Figure 1**) but also to detect unnoticed vertebral fractures, with potential therapeutic renewed interest in CKD-associated osteoporosis guidelines (35, 44, 46, 47). Spanish CKD-MBD guidelines also suggest use of the simpler Adragão's score (AS) (19, 48) (**Figure 1**). In their original study in hemodialysis patients, an AS≥3 was independently associated with coronary, peripheral, and vascular disease (48). Importantly, patients with an AS≥3 had a 2–4-fold higher risk of cardiovascular mortality, hospitalizations, and fatal or non-fatal cardiovascular events. In another study, it was found that an AS>3 was also significantly associated with pulse wave velocity (PWV), pulse pressure, and lower adjusted cumulative survival[hazard ratio (HR) = 3.31)] (19).

In the observational multicentre OSERCE-2 study (26), we prospectively analyzed VC in a cohort of 742 ND-CKD G3–G5 patients. VC was present in 79%, and VC was severe (AS≥3 or KS>6) in 47%. Age, phosphate (P) levels, and diabetes were independently associated with AS≥3. After a median follow-up of 35 months, AS≥3 but not KS>6 was independently associated with all-cause (HR = 2.07) and cardiovascular (HR = 3.46) mortality, and a shorter hospitalization event-free period (HR = 1.14). Moreover, only AS based on hand-X-ray only (AS-hands) (**Figure 1**) showed a significant correlation with parathyroid hormone (PTH) levels and renal function. Interestingly, the group AS≥3 showed more than double the risk of all-cause mortality, whereas the risk increased 5-fold using AS-hands. Moreover, AS≥3 but *not* KS>6 independently predicted all-cause, cardiovascular mortality and hospitalization, and AS-hands ≥1 was also an independent predictor of cardiovascular mortality and hospitalization-related outcomes. Nevertheless, other authors have described significant associations with

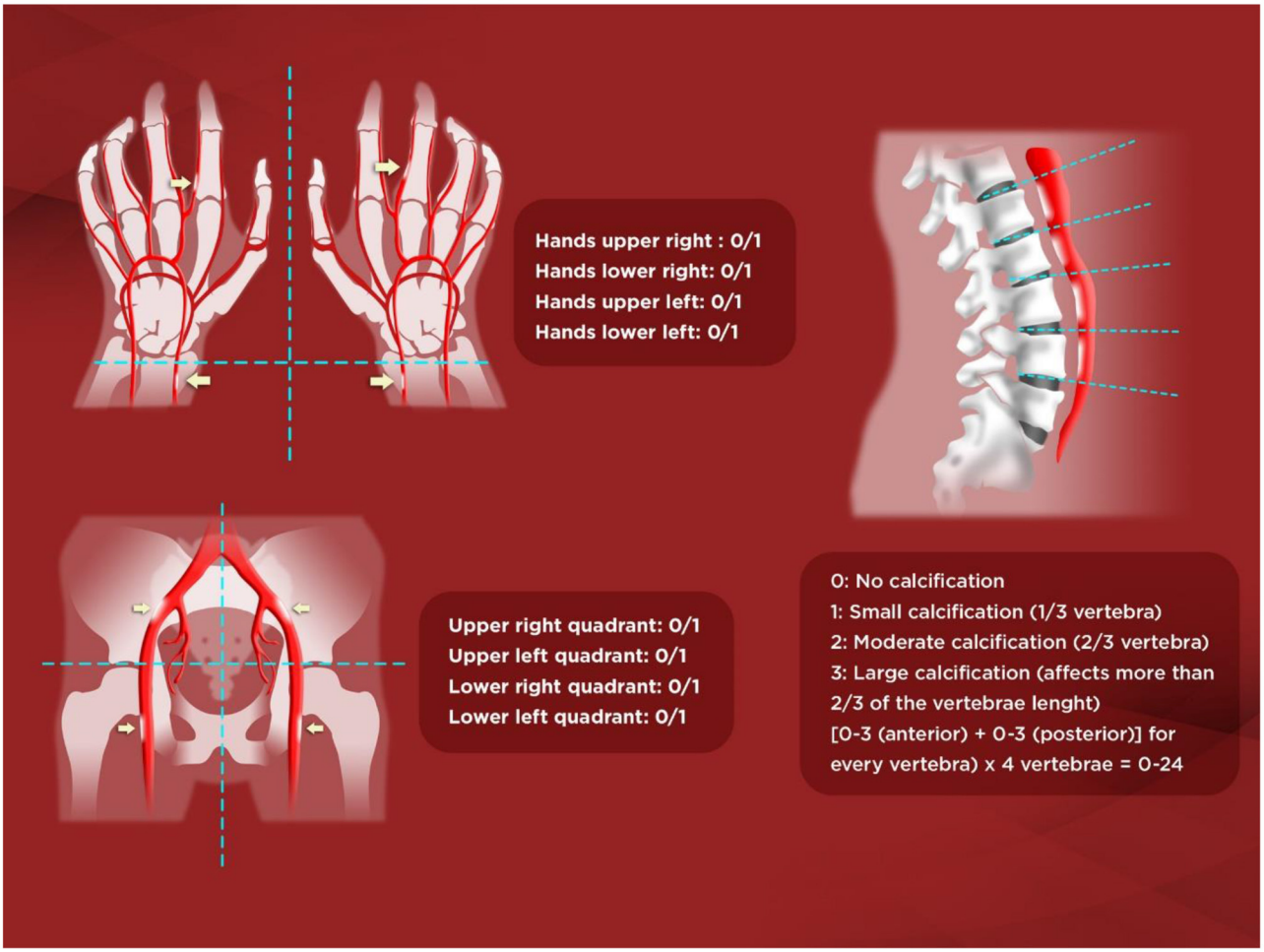

FIGURE 1 | Adragão and Kauppila scores. ***Kauppila score (KS)***. The score is assigned from 1 to 3 [1: small calcification (1/3 of the vertebral length), 2: moderate (2/3), 3: large (> 2/3)] depending on the length of each plaque detected. The anterior and posterior part of the aorta shall be taken into consideration, associating them with the place where they are located, in front of the L1, L2, L3, or L4 vertebrae. With KS, a final score is achieved between *0 and 24 points*. ***Adragão score (AS)***. The score is determined by the sum of the absence of calcification (0 points), or the presence of unilateral (1 point) or bilateral (2 points) linear calcification in each section. AS analyzes the calcification of iliac, femoral, radial, and digital arteries. The final value ranges from 0 to 8 points (0–4 in the pelvis and 0–4 in the hands).

hard-outcomes in ND-CKD patients evaluated for aortic, pelvic or even breast artery calcifications, and significant differences were also present among distinct vascular beds and associated factors (43, 49–52).

We therefore confirmed the potentially better predictive ability of simple X-rays with the AS (vs. KS), and extended its value into the ND-CKD population. Moreover, the even simpler AS-hands demonstrated an important predictive ability, probably because the radial and cubital arteries are muscular arteries with a greater tendency to calcification of the medial layer (arteriosclerosis), especially affected in CKD, as opposed to the elastic abdominal aorta, which may be more prone to intimal calcification (atheromatosis) (26, 48, 53).

Medial and intimal VC lead to different comorbidities: medial calcification (Mönckeberg's disease) induces arterial stiffness and consequently an increased PWV, left ventricular hypertrophy and heart failure, whereas intimal calcification is associated with ischemic episodes and is pathophysiologically characterized by endothelial damage, lipid accumulation, and infiltration of inflammatory cells. These data support the concept that VC (especially in CKD patients) is not just a single entity but the consequence of a wide range of different biological processes (54), affecting both intimal and medial layers, and it is difficult to study separately because common radiological techniques do not allow clear distinction between them (55, 56). Reliable differentiation between medial and intimal VC can only be achieved pathomorphologically (57). Moreover, VC is not exclusive to CKD and is especially prevalent in diabetes and during the aging process (also accelerated in CKD patients) (17).

Nevertheless, while the high prevalence and prognostic value of VC in CKD patients are now well-established, the clinical usefulness of early screening is still controversial since it is far from proven that early diagnosis permits beneficial actions that

improve outcomes (e.g., regarding different P binders or vitamin D (VD) derivatives).

PATHOPHYSIOLOGY OF CKD-MBD-RELATED VASCULAR CALCIFICATION

CKD-MBD-related factors are obviously not the only mechanisms responsible for VC but, as mentioned before, CKD acts as an accelerator of the VC process (17). Moreover, VC is not only a passive process of excess calcium (Ca) and P deposition (enhanced in CKD due to the loss of excretory function) but also an active process where transdifferentiation of vascular smooth muscle cells (VSMCs) from a contractile to a secretory calcific phenotype plays an important role (53, 54). This is probably magnified by CKD-MBD-related factors (11, 17). These VSMCs can also display adipogenic and macrophagic features (58–60). Moreover, adventitial Gli1+ mesenchymal stem-cells serve as progenitors of VSMC which migrate into the media and neointima during arteriosclerosis and atheromatosis (61).

Multiple physiopathological mechanisms and pathways are involved in the process of VC, but a comprehensive review is beyond the goals of this article, and we therefore refer interested readers to other references (62–67). Interestingly, some critical factors might play a double and sometimes opposite roles in terms of vascular health, such as the effects of high and low PTH levels on stem-cells (68, 69), or the roles of fibroblast growth factor-23 (FGF23) (70), VD (71), and calcimimetics (72). Importantly, in addition to a genetic background, the altered balance between calcification contributors (73–80) (**Figure 2**) and circulating or tissue calcification inhibitors (81–88) (**Figure 2**) makes CKD patients more or less susceptible to accelerated VC. Different comorbid pathologies associated with intimal atheroma calcification (ischemic events), arteriosclerosis (arterial stiffening), and heart valvular calcification may be overlapping, and CKD predisposes to all these types of VC (54, 64). Finally, the revolution in omics technology (genomics, epigenomics, transcriptomics, proteomics, and metabolomics) has resulted in the accumulation of cellular and molecular-level data (63, 67) which will provide novel approaches on the mechanisms associated with VC (63, 67). Although clinical application is still in its early stages, CKD may become an interesting model to analyse (17, 63), especially considering that as many as 17% of dialysis patients show no calcification (89).

Central to the increasing knowledge on mechanisms involved in VC is the discovery that calcifying extracellular vesicles act as active precursors of cardiovascular microcalcification in diverse vascular beds (90). Novel *in vitro* assays may quantify the propensity for VC in serum (as a composite measure capturing a global effect) by evaluating the semimaximal transformation time (T_{50}) from the so-called primary to secondary calciprotein particles (CPP) when challenged with additional Ca and P (91, 92). Primary CPP are amorphous accumulations of Ca-P, and their transformation to secondary CPP, composed of crystalline Ca-P, may provide information about the contributor/inhibitor balance (91, 93). T_{50} has been associated not only with more severe CAC and progression in patients with CKD G2–G4, but also with cardiovascular and all-cause mortality in individuals with ND-CKD, hemodialysis and transplant patients (94–97). T_{50} could also reflect factors that promote intimal calcification (98) since secondary CPP stimulates inflammation and apoptosis of macrophages, which may promote ectopic calcification (99, 100). Actually, early stages of CKD were associated with local up-regulation of proinflammatory and pro-osteogenic molecules in the vascular wall and calcification of the aortic media layer (101). Moreover, Ca×P product correlated well with markers of inflammation, but not with calcification itself (101). In fact, Takx et al. (102) recently conducted a retrospective study including CKD-G3 patients and matched controls who underwent fluorodeoxyglucose-PET or CT imaging and concluded that moderate CKD is associated with arterial inflammation, independently of subclinical atherosclerosis. On the other hand, Joshi et al. (103) demonstrated the presence of VC at baseline to be associated with progressive calcification and suggested that VC can be the *inducer* of inflammation and not only its consequence. Of note, hyperphosphatemia, among other mineral metabolism parameters, has also been independently associated with inflammation and VC in CKD (104, 105). Thus, T_{50} may not directly reflect the initial pathophysiological pathway of VC but could theoretically help in clinical decisions on CKD-MBD-related treatments and/or identifying high-risk patients (97). Similarly, the so-called fetuin-A (VC inhibitor) reduction ratio was also evaluated as a potential parameter to measure extraosseous calcification stress (97, 106, 107).

TREATMENT IMPLICATIONS

Although VC is a surrogate marker and is not yet considered a treatment target in CKD (108), the presence of VC may have important therapeutic implications (34, 35, 44), unless all patients are treated with the mindset of reducing the incidence or progression of VC (i.e., using all minimization strategies such as reducing exogenous Ca and P).

Phosphate Binders

Several studies have shown that non-Ca-based P binders, mainly sevelamer in dialysis patients, attenuate the progression of valvular and VC as compared to Ca-based P binders (109–112). Non-Ca-based P binders have also been associated with improved survival (109, 113–115), although certainty of evidence is low (116). Russo et al. (117) randomized 90 non-diabetic ND-CKD G3-G5 patients without a history of CVD to three groups: low P-diet only, Ca-based P binders, or sevelamer. They monitored patients for an average of 2 years and found that CAC significantly increased in patients on the low-P diet alone, to a lesser extent in Ca-carbonate-treated patients, and not at all in sevelamer-treated patients (117). Moreover, Di Iorio et al. found in 212 patients with CKD G3-G4 that sevelamer provided benefits in all-cause mortality and in the composite end point of death or dialysis inception (118). Interestingly, considering only patients with CAC>0, a significant regression of CAC was observed in more patients treated with sevelamer than Ca-carbonate (24

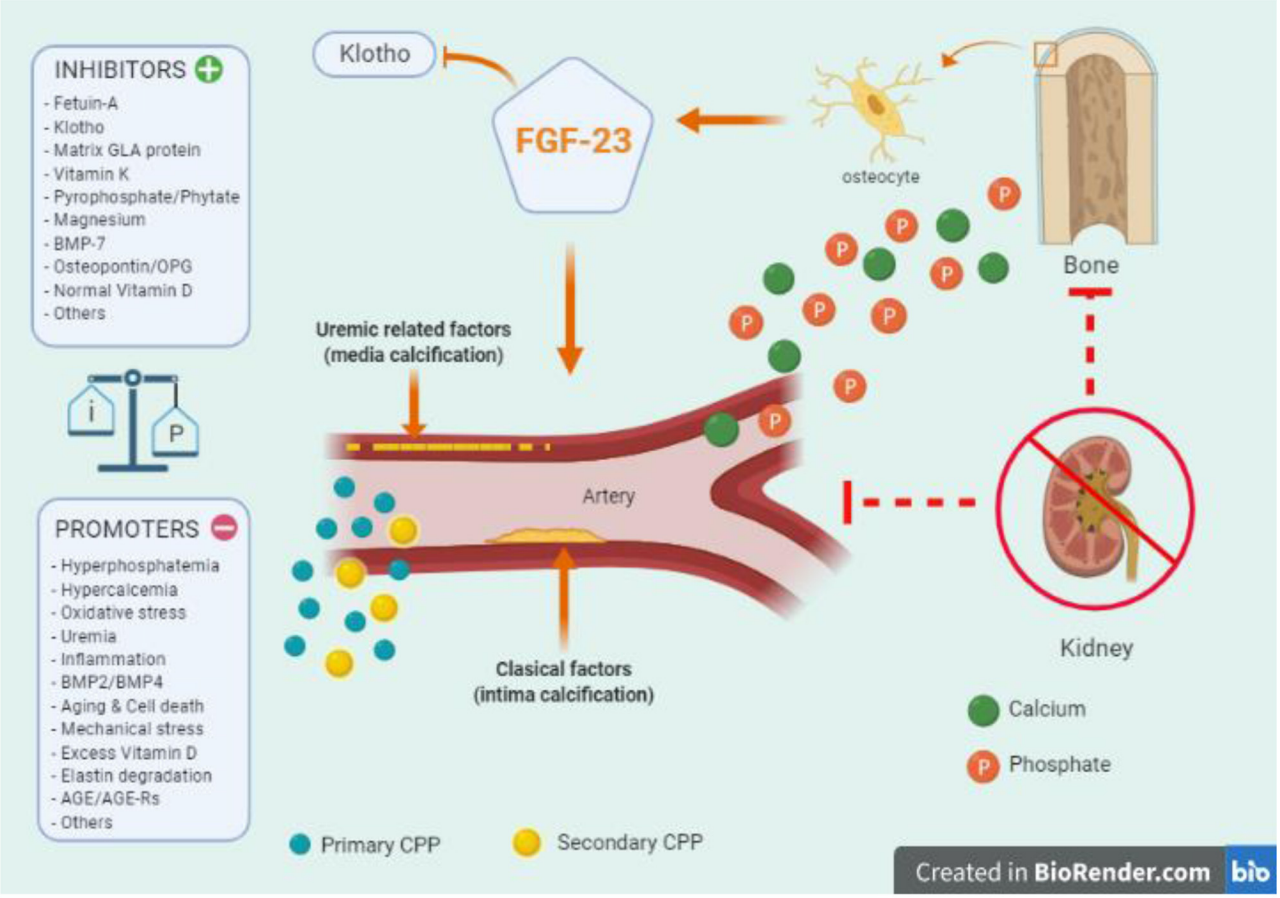

FIGURE 2 | Key aspects of vascular calcification (VC) in chronic kidney disease (CKD): the important balance between calcification contributors and inhibitors (tissue or circulating factors) in the pathophysiology of vascular calcification. The altered balance between calcification contributors and inhibitors makes CKD patients more or less susceptible to accelerated calcifications. Of note, CKD patients exhibit both types of VC, intimal atheroma calcification, and medial arteriosclerosis. A key role in the initiation and propagation are calcium (green) and phosphate (orange) ions. The passive formation of calciprotein nanoparticles (CPP) act as active precursors of micro-calcification. Initially, the small calcium/phosphate complex can be removed by Fetuin-A, which can eventually become saturated leading to primary CPP (light blue) that can develop secondary CPP particles (yellow). Later, phosphate entries into vascular smooth muscle cells producing osteochondrocytic transdifferentiation (vascular "ossification"). Many of the contributors may also promote this phenotypic osteochondrocytic transdifferentiation. Among them, tissue non-specific alkaline phosphatase (TNAP), aldosterone, parathyroid hormone, activin-A, collagen I, osteocalcin, osteonectin, oxidized-low density lipoproteins, have been associated with vascular calcification. On the other hand, parathyroid hormone related-peptide, collagen IV, high density lipoproteins, nitric oxide, and others, have been described as potential inhibitors. At the bone level, the role of FGF-23 and the co-factor Klotho are relevant in all the process, and a genetic background (e.g., proteins derived from Ank, Npps, ENPP1 genes) should also be considered. BMP, Bone Morphogenetic Proteins; TNF-α, Tumor Necrosis Factor- α; IL, Interleukin; AGE, Advanced-Glication End-products; AGE-Rs, AGE-receptors.

vs. 2). Over 24 months, the final cumulative percentage of *de novo* onset of CAC was 12.8 and 81.8%, respectively (118). Consequently, recent guidelines (35, 44) increased the degree of evidence from 2C to 2B, suggesting that in adult patients with CKD G3a–G5D the dose of Ca-based P binders should be restricted.

In these guidelines, lowering elevated P levels only toward the normal range is also suggested, mainly because of an absence of data supporting the benefit of efforts to maintain P in the normal range (e.g., in G3a–G4 patients) and also some safety concerns (35, 119). Therefore, guidelines now suggest that treatment should only aim at overt hyperphosphatemia and emphasize that early "preventive" P-lowering treatment is currently not supported by data (35). Nevertheless, recent studies have analyzed the potential of "preventive" treatment of early P loading in CKD patients (120, 121). Bouma-de Krijger et al. (120) found that sevelamer carbonate for 8 weeks did not induce a significant reduction in PWV and that serum FGF23 did not decrease despite a decline in 24-h urine P excretion. These findings challenged to some extent the concept that "preventive" P binder therapy is a useful approach, at least over this short period. Interestingly, in a subgroup of patients with absent or limited abdominal VC, treatment did result in a statistically significant reduction in adjusted PWV, suggesting that PWV is amenable to improvement in this subset (120, 122). On the other hand, Toussaint et al. (121) recently reported that

lanthanum carbonate did not affect arterial stiffness or VC in CKD G3b-4 patients; however, an absence of a significant decline in 24-h urine P excretion seems difficult to explain. Therefore, interpretation of the scarce and heterogeneous observations described in early CKD remains difficult, and the possibility of beneficial effects of a "preventive" treatment may not yet be completely disregarded (122). Finally, significant beneficial effects of a fixed dose of ferric citrate in advanced ND-CKD patients merit further study (123), since it was not a placebo-controlled randomized clinical trial (RCT), about 1/3 of patients in the "standard of care" arm took Ca-based P binders, and the outcome was a composite end-point.

Treatment for Hyperparathyroidism

Recent guidelines also state that in patients with ND-CKD G3a–G5, the optimal PTH level is not known (35, 44). However, an association between improvement of PTH levels with all-cause mortality has not only been described in dialysis but also in ND-CKD patients (124–126). Guidelines also suggest that patients with intact PTH levels progressively rising or persistently above the assay's upper normal limit should be evaluated for modifiable factors, including hyperphosphatemia, hypocalcemia, high P intake, and VD deficiency (evidence 2C) (35). In fact, low calcidiol levels represent a novel cardiovascular risk marker and have been directly associated with the presence and progression of VC and decreased survival (127, 128). VD may have atheroprotective effects; however, clinical studies do not show improved survival with VD administration (129).

Supplementation with native (nutritional) VD has been shown to have beneficial effects on several biological parameters, even in dialysis patients (130, 131), but a significant RCT with positive hard-outcomes is lacking. On the other hand, in adult patients with ND-CKD G3a–G5, recent guidelines (35) suggest that calcitriol and VD analogs should *not* now be routinely used (evidence 2C), now considering reasonable to reserve them for patients with CKD G4–G5 with severe and progressive hyperparathyroidism (35). Recent RCTs of VD analogs failed to demonstrate improvements in clinically relevant outcomes and an increased risk of hypercalcemia (132, 133). However, very high doses of paricalcitol and/or Ca-based P binders were frequently used in those studies (132, 133). All guidelines agree that modest increases in PTH may represent an appropriate adaptive response in CKD and "progressively rising" PTH levels (trends) rather than PTH "above the upper normal limit" should be considered for treatment (35, 44). Nevertheless, other guidelines do not consider it acceptable to wait until *severe* hyperparathyroidism is present, and physicians are then advised to avoid hypercalcemia and/or hyperphosphatemia but *not* to aim for complete normalization of PTH (44, 134). In experimental animals, low clinically relevant dosages of calcitriol and paricalcitol seem to protect against CKD-related VC (135). Paricalcitol and calcitriol at equipotent doses also showed different effects on VC (136, 137), and distinct positive pleiotropic effects and survival benefits were described in retrospective studies in dialysis patients (138, 139); however, they have not been demonstrated in RCTs.

On the other hand, calcimimetics are important contributors to the achievement of biochemical treatment goals (especially Ca and PTH, but also P) in CKD-MBD (140, 141). Calcimimetics have been shown to decrease VC in experimental uremic animals (142–145) and, in the ADVANCE study, Raggi et al. demonstrated that in hemodialysis patients with secondary hyperparathyroidism, cinacalcet plus low-dose VD may attenuate cardiac valve and VC (37). In fact, calcimimetics do not only correct PTH hypersecretion but also modulate the vascular Ca-sensor activity (72). However, cinacalcet has not been approved in ND-CKD patients, although it can be prescribed in patients with *primary* hyperparathyroidism (with or without CKD) as an alternative or bridging therapy to treat hypercalcemia (146–148).

Finally, the fact that a statin paradox exists (statins promoted coronary atheroma calcification but improved clinical outcomes) (149) means that the implication of VC may be reconsidered (150). Nevertheless, many experimental and/or preliminary clinical studies on VC with different compounds are ongoing (151–160). Thus, other factors (directly or indirectly related with CKD-MBD) such as magnesium, vitamin K, inhibitors of intestinal P transporters, bisphosphonates, denosumab, sodium thiosulfate, alkaline phosphatase inhibitors, apabetalone, sotatercept, recombinant BMP-7, as well as metformin, antioxidants, spironolactone, senolytics, zinc, and the recently tested SNF472 (35, 161, 162) have been shown to potentially attenuate VC. However, as it has been mentioned before, prospective RCTs on hard-outcomes are still lacking with any treatment.

CONCLUSION

CKD-MBD is a common complication and contributes to the high morbimortality (mainly cardiovascular) in CKD patients. The prevalence of VC (intimal and medial), even in early stages of CKD, is very high and progresses rapidly. Although VC may not be a direct cause of CVD, it may reflect underlying pathophysiologic derangements and does have deleterious hemodynamic consequences linked to a negative prognosis. Several common treatments may increase or attenuate its natural progression; therefore, CKD-MBD treatment requires an integral approach, addressing all its components, although clear evidence that this strategy improves hard outcomes is lacking. Meanwhile, it seems logical to avoid P loading, restrict Ca-based P-binders, avoid high doses of VD, and avoid normalizing PTH levels. The availability of new drugs and future studies including early CKD patients may lead to significant improvements not only in patient stratification but also in slowing the natural progression of VC.

AUTHOR CONTRIBUTIONS

JB was invited as an expert in the field as co-coordinator of the Spanish Nephrology guidelines and experience in the field of chronic kidney disease and mineral and bone disorders (pathophysiology and treatment), contributed to the conceptualization and writing and editing of the manuscript. AA contributed to the conceptualization, search and selection of the most important references, and writing and editing of

the manuscript, as well as creation of illustrative **Figure 1**. CA contributed to the search and selection of the most important references and editing of the manuscript. PM, ML, JO, GB, YG-M, and NR contributed to the conceptualization and review and editing of the manuscript. LD'M contributed to the conceptualization and review and editing of the manuscript, as well as the creation of illustrative **Figure 2**. JG contributed to the conceptualization, review and editing of the manuscript, and he is the first author of the main contribution to the field of vascular calcification in non-dialysis dependent CKD patients.

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REVIEW
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Cardiorenal Fat: A Cardiovascular Risk Factor With Implications in Chronic Kidney Disease

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There is a growing interest in the potential role of adipose tissues in cardiac and renal pathophysiology, and determining the mechanisms by which fat compartments around the heart and kidneys influence cardiovascular disease is of clinical importance in both general and high-risk populations. Epicardial fat and perirenal fat have been associated with adverse outcomes in chronic kidney disease (CKD) patients. Epicardial fat is a rich source of free fatty acids and is capable of secreting inflammatory and pro-atherogenic cytokines that promote atherosclerosis through a local paracrine effect. Recent evidence has demonstrated that perirenal fat has a closer correlation with kidney diseases than other visceral fat deposits in obesity or metabolic disturbances. Moreover, perirenal fat has been reported as an independent risk factor for CKD progression and even associated with cardiorenal dysfunction. Accordingly, these forms of organ-specific fat deposits may act as a connecter between vascular and cardiorenal disease. This review explores the possible links between epicardial and perirenal fat and its significant role as a modulator of cardiorenal dysfunction in CKD patients.

Keywords: chronic kidney disease, adipose tissue, cardiovascular disease, cardiorenal disease, renal failure

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INTRODUCTION

Interest in the potential impact of organ-specific adipose tissue on cardiac and renal pathophysiology is growing, and it is clinically important to determine the mechanisms by which fat compartments around the heart and kidneys influence cardiovascular disease (CVD) in both general and high-risk populations (1, 2). In these sense, epicardial fat (EF) and perirenal fat (PF) have been associated with adverse outcomes in chronic kidney disease (CKD) patients (3–5). EF and PF tissues accumulate with overweight and obesity (6), which are associated with microalbuminuria (7, 8), and thus with worse prognosis in CVD patients (9).

The close anatomical proximity and intercommunication between EF, coronary arteries and myocardium has been reported as a key factor in atherosclerotic disease development and progression (10). As a rich source of free fatty acids capable of secreting inflammatory and pro-atherogenic cytokines, EF is believed to promote atherosclerosis of the intra-epicardial branches of coronary arteries through a local paracrine effect. Interestingly, local secretion of certain adipocytokines in inflamed peri-coronary adipose tissue may have adverse consequences on myocardial contractility and vascular calcification (11). PF, the adipose tissue surrounding the kidneys, was originally thought to provide only mechanical kidney support, yet studies have shown have a closer association of PF with kidney diseases than other visceral fat deposits in obesity

or metabolic disturbances (12). PF has recently been identified as an independent risk factor for CKD progression and is even associated with cardiorenal dysfunction (13). Finally, these forms of organ-specific fat deposit may act as a connector between vascular and cardiorenal disease. This review outlines the evidence of possible links between epicardial and PF and its significant role as a modulator of cardiorenal dysfunction under pathologic conditions in patients affected by renal disease.

EPICARDIAL FAT

EF represents adipose tissue located below the visceral pericardium in direct contact with the epicardial coronary arteries (14). EF originates in the splanchnopleuric mesoderm, without fascia separating it from myocardium and arteries, which allows a direct flow of fatty substances to the coronary arteries and myocardium (15). Moreover, EF can also be found amid myocardial fibers, generally next to the intra-myocardial branches of the coronary arteries (16, 17).

Although the functional role of EF is complex and currently remains undefined, this tissue participates in multiple areas, such as mechanical, metabolic, thermogenic and endocrine/paracrine/autocrine functions (18). EF accompanies the principal branches of the coronary arteries along their atrioventricular course to provide mechanical protection against excessive deformation during the cardiac cycle (19). Moreover, EF is actively involved in energy production and lipid homeostasis, and has a higher rate of free fatty acid (FFA) release and absorption than other fat deposits (18). Since myocardial metabolism is dependent on FFA oxidation, EF supports myocardial energy needs, especially during periods of high demand (19). Like PF, brown adipose tissue is rich in mitochondria with significant production of the uncoupling protein-1 (UCP1) needed to produce heat in response to cold exposure. Evidence has shown that expression of UCP1 and related genes is higher in EF and other fat deposits such as abdominal or subcutaneous adipose tissue (20). These findings indicate a role for EF in heat production to protect the heart from hypothermia.

Studies in animals and humans have shown that EF secrete an abundance of cytokines (**Table 1**), which are implicated in control of endothelial function, coagulation, and inflammation (11, 16). These numerous bioactive molecules produced by EF can protect or negatively affect the myocardium and coronary arteries (21). Under physiological conditions, EF secretes cytokines with anti-inflammatory and anti-atherosclerotic properties, such as adiponectin and leptin (22). Secreted by adipocytes, adiponectin has antioxidant, anti-inflammatory and anti-atherogenic actions (23): it stimulates the oxidation of fatty acids *via* a protein kinase pathway to reduce lipid storage in the myocardium, and also inhibits production of inflammatory mediators to maintain an anti-inflammatory environment in the cardiovascular system. Conversely, under pathological conditions adiponectin production by EF drops, while production and secretion of pro-inflammatory or pro-atherogenic cytokines (such as leptin, vinfatin, or apelin) are increased (11, 21). Studies have linked leptin with pro-atherogenic effects, hypertension, endothelial dysfunction, inflammation, oxidative stress, and proliferation of vascular smooth muscle cells (24, 25), yet surprisingly, adipocyte-derived factors such as adiponectin and leptin remain controversial in the setting of renal dysfunction.

PERIRENAL FAT

PF represents fat located in the retroperitoneal space surrounding the kidneys. Renal sinus fat is also a component of PF and this adipose tissue is surrounded by fascia tissue. Regardless of its pre-adipocyte origin, PF undergoes an unusual progressive transition from brown to white adipose tissue after birth (26). In adults, PF is composed of a combination of white and brown adipose tissue (27). Anatomically, this fat is extensively vascularized by abdominal aorta branches including the inferior adrenal, dorsal, and gonadal arteries (28).

TABLE 1 | Pathophysiological effects of adipocytokines produced in organ-specific adipose tissue.

Adipokine	Metabolism in CKD		Cardiorenal effects					
	Accumulation	Outcome	Oxidative stress	Ischemia/ reperfusion	LV hypertrophy	Remodeling	Inflammation	Proteinuria
Adiponectin	Yes	Inflammation/CVD	⇩	⇩	⇩	⇩	⇩	⇧
Leptin	Yes	Inflammation/CVD	⇧	⇩	⇧	⇧	⇧	⇧
Visfatin	Yes	Endothelial damage/lipid dysregulation/CVD	⇧	⇧	⇩	⇩	⇧	⇧
Apelin	Yes	Inflammation/CVD	⇩	⇩	U	U	⇩	⇩
Resistin	Yes	Endothelial damage/inflammation/CVD	⇧	⇧	⇧	⇧	⇧	⇧
Omentin	Yes	Endothelial damage/inflammation/CVD	⇩	⇩	U	U	⇩	⇩

CKD, chronic kidney disease; CVD, cardiovascular disease; LV, left ventricle; U, unknown. Adapted from D'Marco et al. (11).

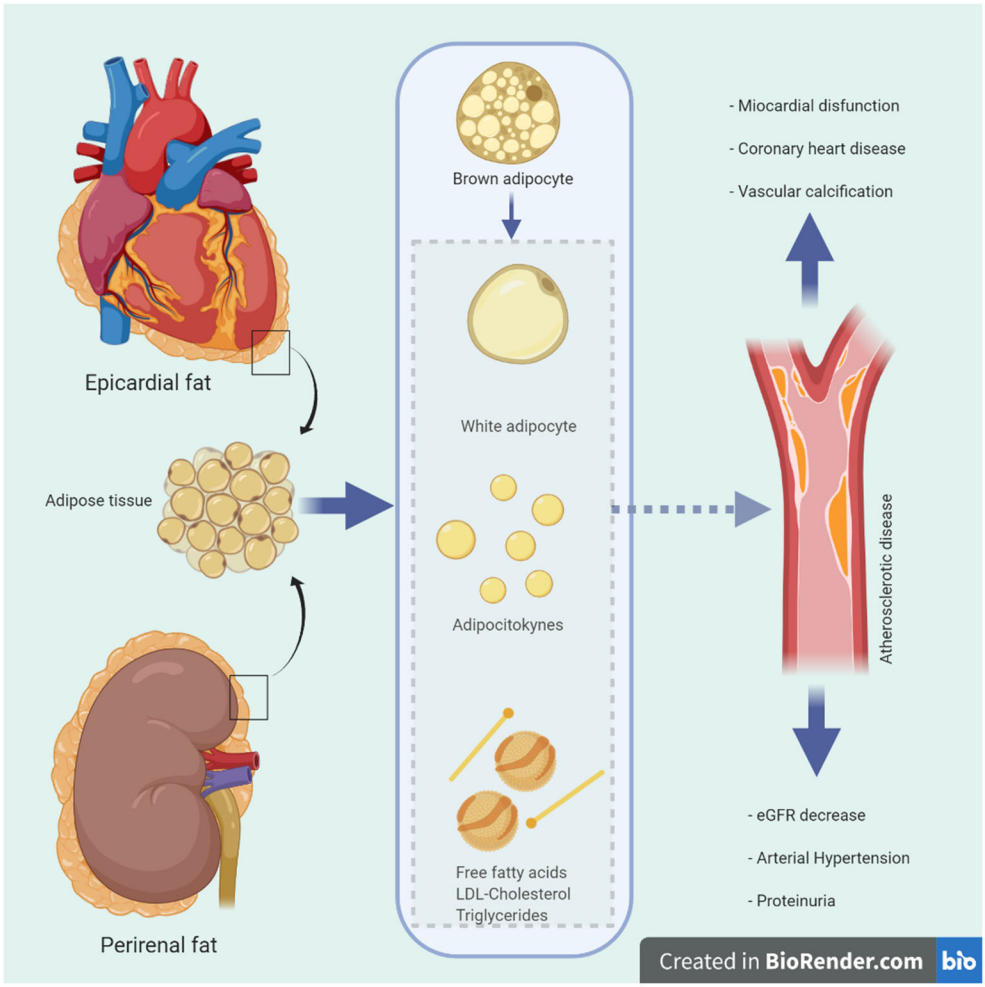

FIGURE 1 | Possible physiological and pathological mechanisms of epicardial and renal fat in vascular beds. Schematic diagram illustrating the anatomical location of epicardial and perirenal fat around the heart and kidneys, its various pathophysiological functions and proposed pathways of damage with adverse clinical consequences. eGFR, estimated glomerular filtration rate; LDL, low density lipoprotein.

Research has focused on the connections between the kidneys and PF, as well as between this tissue and bodily function. As observed in EF, PF produces and secretes adipokines and pro-inflammatory cytokines, including adiponectin, leptin, visfatin, resistin, tumor necrosis factor-α and interleukin-6 (IL-6), among others (13, 29) (**Table 1**). Moreover, these substances pass near to the kidneys and assist in regulating renal function through endocrine and paracrine pathways (30). PF has therefore been linked to direct lipotoxic effects such as increasing hydrostatic pressure in the glomerulus, activating the renin-angiotensin-aldosterone system, and accelerating CKD progression (31, 32). In this regard, an association between PF and abdominal obesity and albuminuria has been detected in obese patients (33, 34). Although studies have reported an association of PF with CKD progression, new evidence on the presence and accumulation of PF is warranted in this risk group (35, 36).

ORGAN-SPECIFIC FAT MEASUREMENT TECHNIQUES

Several non-invasive techniques have been used to measure body fat distribution, especially *via* computed tomography (CT) and magnetic resonance imaging (MRI) (37, 38), although their use is hindered by the high cost and exposure to radiation. Studies have demonstrated that ultrasound is a practical and reasonable alternative to CT or MRI, given that this imaging technique involves no radiation exposure and is cheap and reproducible (39). Other studies have explored the use of abdominal ultrasound to determine PF burden (40). EF has also been measured with echocardiography techniques; however, these imaging methods offer only a partial approach to organ-specific adipose tissue, since measurement is limited to tissue (41). In contrast, CT and MRI techniques allow more spatial resolution with extended measures such as the total volume

TABLE 2 | Different studies showing epicardial and perirenal fat with different outcomes in CKD populations.

References	Technique	Study population	*N*	Outcomes
Perirenal Fat				
Shen et al. (34)	Ultrasound	T2DM patients with Albuminuria	89	Increased PF was associated with albuminuria in patients with T2DM
D'Marco et al. (5)	Ultrasound	CKD patients in all stages (non-dialysis)	103	PF thickness was associated with metabolic risk factors in CKD patients
Fang et al. (35)	Ultrasound	Patients with T2DM and reduced eGFR	171	This study confirmed a negative independent relationship between PF and eGFR in patients with T2DM
Geraci et al. (36)	Ultrasound	Hypertensive patients	216	PF significantly correlated with eGFR with no differences in groups divided by sex, diabetes, or BMI.
Lamacchia et al. (44)	Ultrasound	T2DM subjects	151	PF was an independent predictor of eGFR decrease and altered renal resistance index and hyperuricemia
Koo et al. (45)	CT	Asymptomatic subjects, hypertension and dyslipidemia.	3.919	PF was independently associated with vascular calcifications.
Epicardial fat				
Turkmen et al. (43)	CT	ESKD patients on dialysis + 27 healthy subjects.	80	EF significantly correlated with MIA syndrome in ESKD patients
Karohl et al. (3)	CT	CKD stages 4-5 patients awaiting kidney transplantation	411	EF was the best predictor of abnormal myocardial perfusion in CKD patients.
D'Marco et al. (4)	CT	HD patients	95	Each 10 cc increase in EF volume was associated with a significant 6% increase in risk of death during follow-up
Erdur et al. (42)	CT	ESRD patients on dialysis + 42 healthy subjects	76	Age and BMI are independent predictors of EF.
Ko et al. (46)	CT	HD patients	109	EF progression slows significantly with non-calcium-based resins (Sevelamer)
Karatas et al. (47)	Echocardiography	CKD and HD patients + healthy subjects	111	EF is significantly high in HD patients compared to pre-HD and healthy subjects.
Ayan et al. (48)	Echocardiography	CKD all stages, dialysis, and healthy subjects	97	No correlations between EF thickness and inflammatory markers were found.
Cano Megías et al. (49)	CT	CKD and HD patients	104	A higher EF was associated with increased mortality and lower free survival time to fatal and non-fatal cardiovascular events

T2DM, type 2 diabetes mellitus; PF, perirenal fat; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; BMI, body mass index; CT, computer tomography; ESKD, end stage kidney disease; EF, epicardial fat; MIA, malnutrition inflammation atherosclerosis; HD, hemodialysis.

of fat surrounding these organs. Finally, although DEXA and Bioimpedance techniques offer interesting quantification of total body fat, as yet no specific organ compartments can be measured by these devices.

ORGAN-SPECIFIC FAT AS CAUSATIVE FACTOR OF CARDIORENAL DISEASE

Recent evidence has shown that region-specific adipose tissue is an important cardiovascular risk marker in the general and high-risk population. For example, there appears to be a connection between EF and CVD in conditions as common as high blood pressure, metabolic syndrome, diabetes, and cardiorenal disease (31, 32). Recognition of the adipocyte as a cell type with complex functioning including endocrine, paracrine and autocrine capacity has aroused research interest in disciplines such as nephrology, cardiology, and endocrinology, and it has become a fertile ground for scientific debate. Its wide heterogeneity of location, functions, proteomics, and metabolomics (1) are particularly important in adiposity, where changes in function and volume are associated with increased cardiorenal and metabolic risk (**Figure 1**).

Although studies evaluating EF in CKD are still ongoing, some research has reported a relationship between EF and vascular disease in patients with renal involvement (42, 43) (**Table 2**). In a study carried out in a cohort of more than 400 patients with CKD stage 4-5 and 5 on dialysis EF emerged as a risk factor for alterations in myocardial perfusion as well as appearance of vascular calcification (3), and was also independently associated with vascular calcification in predicting myocardial perfusion defects in this study. Karatas et al. recently demonstrated that EAT thickness measured by echocardiography was significantly greater in hemodialysis patients than in those in pre-dialysis stages or in healthy subjects (47). Similarly, in a sub analysis of the RIND study (Renagel in a new dialysis trial), which initially evaluated the presence of coronary artery calcifications (CAC) and alterations in calcium and phosphorus in patients starting dialysis treatment, it was observed that each 10 cc increase in EF volume raised mortality by 6% (4). Other factors such as CAC and age were also associated with increased mortality. Further publications have reported that patients with CKD have higher EF thickness and volume compared to control subjects (50).

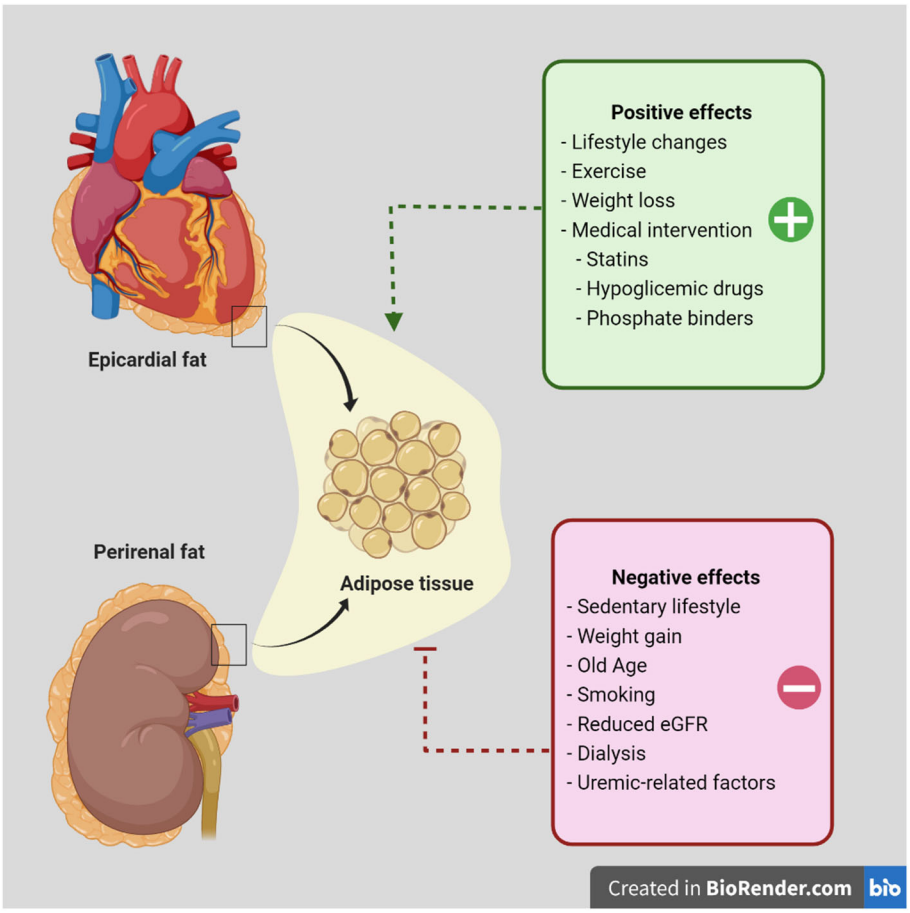

FIGURE 2 | Proposed factors that improve or worsen epicardial or perirenal fat tissue accumulation in patients with chronic kidney disease. Diagram showing adipose tissue around the heart and kidney. In a pathological environment, this adipose tissue becomes a cardiovascular risk factor in susceptible populations. Current evidence has been published regarding the effect of lifestyle changes and weight loss or exercise. Moreover, medical intervention has supported the use of hypoglycemic agents and other drugs to reduce adipose tissue accumulation and/or dysfunction. Although further evidence regarding perirenal fat intervention is warranted in future studies, controlling metabolic abnormalities such as hyperglycemia, dyslipidemia or hyperuricemia may improve clinical outcomes.

Conversely, in other research EF was not confirmed as an isolated risk predictor but rather as a cofactor associated with body mass index (BMI) and other obesity indices.

Recent studies have identified excess PF as an emerging risk marker of CVD, independently of common metabolic risk factors (13, 44). Our previous analysis showed that PF thickness was associated with metabolic risk factors in non-dialysis-dependent patients (5). Other investigations found PF thickness to be positively correlated with high blood pressure in overweight patients and hypertension (6, 51). Moreover, higher levels of PF have been linked with carotid intima-media thickness, indicating that excess PF is associated with atherosclerosis and CVD (52).

As a CVD-related risk factor, diabetes or dyslipidemia-associated PF may also indirectly contribute to CVD. A recent study showed that excess PF was independently associated with hyperinsulinemia and insulin resistance in obese patients, irrespective of other anthropometric or metabolic parameters, suggesting PF as a strong marker of insulin resistance (6). Moreover, PF development and remodeling have also been associated with other metabolic parameters in patients with CKD. Hence, PF thickness was significantly correlated with abnormal triglycerides and uric acid levels, in which patients with stage 4 and 5 CKD had the greatest PF thickness (5). Of interest, excess PF is associated with a reduced glomerular filtration rate, regardless of other indices of adiposity, in patients with hypertension and or diabetes (36). A more recent study reported a positive correlation between PF and albuminuria among type 2 diabetes patients (35).

The physio-pathological processes and association of these specific cardiac and renal adipose tissues allow the hypothesis that an excess of epicardial and PF contributes to CVD. PF is potentially related to EF as both exhibit mesothelial layers like visceral organs enriched in white adipose tissue progenitors which produce adipocytes (12, 53). This finding bolsters the hypothesis of PF as a risk predictor for CVD in the renal-affected population, and likewise, EF has been considered a CVD risk factor that predicts cardiac dysfunction in CKD patients (48, 49). Regarding this latter proposal, these adipose tissues are linked to proven cardiovascular indicators such as carotid intima-media thickness and vascular calcifications indexes (4, 45, 52),

and they also express browning fat markers with interactions between both tissues through complex endocrine pathways with differential behaviors to those observed in other adipose tissues (54–56).

ADIPOSE TISSUE AND BONE MINERAL AXIS WITH CARDIORENAL IMPLICATIONS

Elevated serum levels of fibroblast grow factor 23 (FGF23) are strongly associated with increased CVD in CKD patients. Hence, FGF23 levels rise as kidney function declines, with the result that in patients with end state kidney disease (ESKD), FGF23 contributes to hypertension, vascular calcification, and left ventricular hypertrophy by distinct mechanisms (57). It is currently accepted that adipocytes are capable of storing vitamin D, and beyond this activity new evidence has revealed crosstalk between energy storage organs such as adipose tissues and bone. Recent reports show that adiponectin and leptin directly stimulate FGF23 expression in the skeleton (58), thus suggesting a link between these adipokines and FGF23. Moreover, a recent investigation found adiponectin to be a strong modulator of FGF23 response to vitamin D receptor (VDR) activation in CKD patients (59). Of note, mice overexpressing the most abundant adipose tissue protein, adiponectin, exhibit a substantially enhanced FGF23 response to phosphate load compared with wild-type and adiponectin knock-out mice. Additionally, VDR activation markedly upregulates FGF23 gene expression and substantially increases circulating levels of FGF23 in healthy subjects with vitamin D deficiency and CKD patients. Accordingly, the association between FGF23 and fat mass was correlated with higher serum leptin levels in animal models, which adds support for leptin regulation of FGF23, and thus FGF23 was also associated with cardiac and endothelial damage (60). Beyond the correlation between EF and coronary calcification, insulin resistance and inflammation (IL-6), FGF23 has showed an association with EF in stage 3-5 CKD patients (61).

We postulated that local peri-vascular expression of adiponectin and leptin may dysregulate local FGF-23 production in calcified endothelial cells, suggesting the possibility of an undercover regulatory pathway in a uremic environment. This mechanism may explain why EF has been reported as an independent risk factor for CAC and myocardial dysfunction in CKD patients (3).

THERAPEUTIC APPROACHES

Organ-specific adipose tissue reduction has been achieved with lifestyle changes and pharmacological interventions (**Figure 2**). Intriguingly, a study showed a 2% regression in EF volume in patients who lost 5% of their body weight, compared to a 23% increase in those who gained weight (62). Moreover, other reports describe decreased EF volume after intensive lipid-lowering therapy with statins (63, 64). This effect may be related to the known anti-inflammatory activity and inhibition of vasa-vasorum proliferation shown by statins (65).

In a recent study, Couselo-Seijas et al. showed that treatment with sodium-glucose co-transporter 2 inhibitor (SGLT2i) reduced EF (66), and use of an SGLT2i (Dapagliflozin) thus improved glucose metabolism with no lipogenesis-involved gene regulation or lactate production, mainly in patients with CAC. Interesting findings from another study provide insights into the distinctive role of the PPARα/adiponectin/SGLT2 pathways in regulating sodium and glucose homeostasis in PF (67). This work showed that excessive sodium intake also increased plasma level of adiponectin, as well as its expression in both PF and renal cortex. Other research indicated that glucagon-like peptide-1 (GLP-1) analogs Exenatide and Liraglutide are effective in reducing EF in obese patients with diabetes mellitus, effects that are mostly weight-loss dependent (68). In diabetic patients, adding a dipeptidyl peptidase-4 (DPP-4) inhibitor to standard care therapies produced a significant decrease in EF volume over 2-year follow-up (41). Similar benefits have been observed with pioglitazone in patients with metabolic syndrome (69).

In patients with ESKD, use of non-calcium-based phosphate binding agent sevelamer reduced serum cholesterol and markers of inflammation (70). Additionally, sevelamer has shown to be effective in slowing down CAC progression and EF in dialysis patients (46). The effect of weight loss and regular exercise on accumulation of visceral adipose tissue in CKD patients remains to be elucidated.

CONCLUSIONS

Renal-affected patients express extremely high incidence of CVD usually attributed to classical and uremic-related conditions. This suggests that specific adipose tissues may represent an additional cardiovascular risk factor in CKD patients. Finally, organ-specific adipose tissue, specifically EF and PF, represents an interesting and underexplored field to consider in this population.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Sodium-Glucose Co-transporter-2 Inhibitors and Nephroprotection in Diabetic Patients: More Than a Challenge

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Diabetic nephropathy is the most common cause of end-stage renal disease worldwide. Control of blood glucose and blood pressure (BP) reduces the risk of developing this complication, but once diabetic nephropathy is established, it is then only possible to slow its progression. Sodium-glucose cotransporter-2 inhibitors (SGLT2is) are a novel class of oral hypoglycemic agents that increase urinary glucose excretion by suppressing glucose reabsorption at the renal proximal tubule. SGLT2is lower glycated hemoglobin (HbA1c) without increasing the risk of hypoglycemia, induce weight loss and improve various metabolic parameters including BP, lipid profile, albuminuria and uric acid. Several clinical trials have shown that SGLT2is (empagliflozin, dapagliflozin canagliflozin, and ertugliflozin) improve cardiovascular and renal outcomes and mortality in patients with type 2 diabetes. Effects of SGLT2is on the kidney can be explained by multiple pathways. SGLT2is may improve renal oxygenation and intra-renal inflammation thereby slowing the progression of kidney function decline. Additionally, SGLT2is are associated with a reduction in glomerular hyperfiltration, an effect which is mediated by the increase in natriuresis, the re-activation of tubule-glomerular feedback and independent of glycemic control. In this review, we will focus on renal results of major cardiovascular and renal outcome trials and we will describe direct and indirect mechanisms through which SGLT2is confer renal protection.

Keywords: CKD, type 2 diabetes, renal risk, cardiovascular risk, clinical trials, review, SGLT2i

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INTRODUCTION

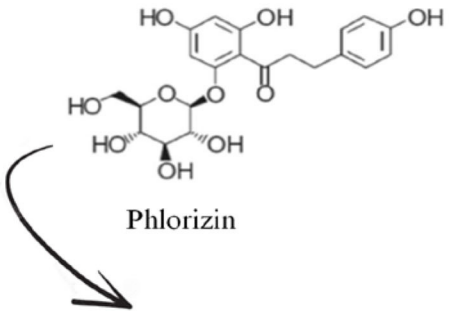
Diabetes mellitus (DM) is a rapidly increasing disease. In 2030, it is estimated that about 7.7% of the total adult population will be affected by diabetes (1). An important complication of DM is chronic kidney disease (CKD), which occurs in about a third of diabetic patients, and represents one of the main causes of mortality and End-Stage-Kidney-Disease (ESKD) (2). The 2012 Kidney Disease Improving Global Outcomes (KDIGO) guidelines define CKD as chronic kidney damage characterized by albuminuria ($\geq$ 30 mg/die) and/or reduced glomerular filtrate (eGFR) <60 ml/min/1.73 m^2, persistent for at least 3 months (3). It has been widely demonstrated that eGFR <60 ml/min/1.73 m^2 and the presence of albuminuria are two independent risk factors for all-cause and cardiovascular (CV) mortality (4, 5). The predictive ability of albuminuria and eGFR on cardiovascular events is similar to that of traditional risk factors, such as blood pressure (BP) or smoking habit. This lead some authors to consider CKD as one of the criteria defining a higher risk of future coronary events (6). Monitoring albuminuria is, thus, a crucial point of management of CKD patients, particularly in those also suffering from diabetes. In fact, Minutolo et al. (7) highlighted that the presence of mild or moderate albuminuria was associated with a higher risk of mortality and CV events in diabetic CKD compared with non-diabetic CKD patients, whereas the ESKD risk was correlated with the severity of albuminuria, regardless of the presence of diabetes. Diabetic nephropathy is an important microvascular complication that occurs in about 30% of patients with type 1 diabetes and 40% of those with type 2 diabetes (8). Diabetic nephropathy is related to the duration of diabetes and the quality of glycemic control. It is known that hyperglycemia plays a key role in developing kidney damage. Hyperglycemia causes glomerular hypertension and induces intra- and extra-cellular alterations, which lead to loss of glomerular barrier selectivity and expansion of the mesangial and interstitial matrix, resulting in glomerulosclerosis and interstitial tubular fibrosis (9). Over the past few decades, a number of randomized clinical trials have been carried out with the ambitious goal of reducing CV and renal risk in patients with CKD and type 2 diabetes (10). These studies have allowed to uncover the efficacy of drugs interfering with the Renin-Angiotensin-Aldosterone system, namely the Renin-Angiotensin-Aldosterone system inhibitors (RAASi), in relenting CKD progression and conferring CV protection to diabetic CKD patients (11, 12). Nevertheless, despite the wide and beneficial use of RAASi among clinicians of different specialties, these drugs have been shown to reduce but not delete the high risk for these patients (13–15). Hence, clinical research has focused resources and efforts in finding novel effective therapeutic strategies which may improve the standard-of-care. To this end, striking results have been provided by the sodium glucose co-transporter 2 inhibitors (SGLT2is), a new class of orally active drugs used in the management of type 2 diabetes that promotes glucose excretion in the kidney. SGLT2is not only improve fasting plasma glucose and HbA1c, but are able to induce BP reduction and weight loss and an improvement in kidney damage as well (16). The mechanisms by which SGLT2is promote such an improvement are not fully understood yet. This review provides an overview regarding the effects of these medications on the kidney and the most relevant findings derived from recent randomized clinical trials.

SGLT2 INHIBITOR

The first SGLT2i discovered was phlorizin, a molecule found in the root bark, leaves, shoots and fruit of the apple tree (17–19), whose use failed due to poor oral bioavailability, absence of selectivity for SGLT2 and gastrointestinal side effects (20). Several drugs called gliflozins were subsequently developed, retaining the active center of phlorizin and implementing structural changes in order to increase its bioavailability, its selectivity for SGLT2 and to decrease its side effects (21). To date, of the 9 existing SGLT2i molecules, only 4 have been approved by the Food and Drug Administration (FDA) for the treatment of type 2 diabetes namely dapagliflozin, empagliflozin, canagliflozin and ertugliflozin (**Figure 1**). The other ones are ipragliflozin, luseogliflozin, tofugliflozin, sotagliflozin, and remogliflozin. Dapagliflozin, the first SGLT2i approved for the treatment of type 2 diabetes (in Europe in 2012) (22), is a selective orally active SGLT2i (23), with a selectivity for SGLT2 > 1,400-fold greater than that for SGLT1 (24). The initial dose is 5 mg, which can be increased to 10 mg. Its half-life (T1/2) is 13 h (25, 26). Empagliflozin, approved in 2014, is the drug with the highest selectivity for SGLT2 over SGLT1 (>2,500-fold). The suggested dose is 10 mg and can be titrated up to 25 mg once daily (25). Its T1/2 is 13 h (24). Canagliflozin has 250-fold selectivity for SGLT2 over SGLT1. It is administered at an initial dose of 100 mg, that can be up titrated to 300 mg daily, with a T1/2 of 11 h and 13 h (for the 100 mg dose and the 300 mg dose), respectively (26). Ertugliflozin is the newest SGLT2i approved by the FDA. It has a 2,000-fold increase in selectivity for human SGLT2 over SGLT1 (27). The initial suggested dose is 5 mg once daily, and it can be titrated up to 15 mg once daily (25). Its elimination T1/2 is 16.6 h (28).

KIDNEY METABOLISM OF GLUCOSE: PHYSIOLOGICAL ROLE OF THE SODIUM-GLUCOSE COTRANSPORTERS

The kidneys play a key role in glucose homeostasis being involved in both glucose reabsorption *via* sodium co-transporters (SGLTs) and endogenous glucose production *via* gluconeogenesis as well as in glucose utilization (29–32). Reabsorption is mediated by SGLTs and Glucose transporters (GLUTs) (33); in particular SGLTs are expressed at the luminal brush border and GLUTs at the basolateral membrane of the epithelial cells (34). There are two types of SGLT co-transporters. The first one, SGLT2, is a high-capacity, low-affinity co-transporter; while the second one, SGLT1, is a high-affinity transporter expressed in the more distal part of the proximal tubules (35, 36). SGLT2 proteins are part of the SGLT family that include six different isoforms with different substrate specificity and localization. SGLT2 is mainly expressed in the kidney. In contrast, SGLT1 is mainly expressed



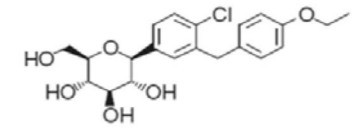
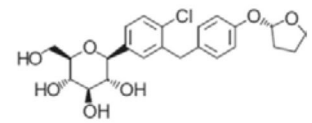
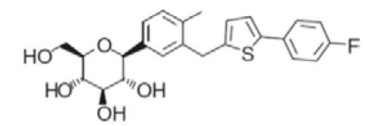
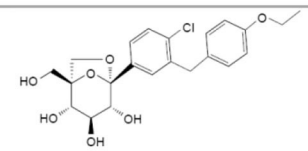
	Drug	Selectivity for SGLT2	Dose	Half-life
	Dapagliflozin	>1,400	5-10 mg	13 h
	Empagliflozin	>2,500	10-25 mg	13 h
	Canagliflozin	250	100-300 mg	11 h (100 mg) 13 h (300 mg)
	Ertugliflozin	2,000	5-15 mg	16.6 h

FIGURE 1 | Molecular and pharmacokinetics characteristics of the principal SGLT2 inhibitors. Phlorizin was the first SGLT2 inhibitor discovered. Later, several gliflozins have been developed, keeping the active center of phlorizin and implementing structural changes in order to achieve a better bioavailability, selectivity for SGLT2 and decrease their side effects. Only four molecules have received Food and Drug Administration approval for the treatment of type 2 diabetes namely dapagliflozin, empagliflozin, canagliflozin, and ertugliflozin.

in the intestine with a lower density being detectable in the kidneys, heart and skeletal muscles (37). In healthy individuals, all filtered plasma glucose is reabsorbed in the renal tubules, and the tubular maximum glucose absorptive capacity (TmG) is about 375 mg/min (29, 38, 39). When the filtered glucose exceeds the renal threshold, defined as glucose concentration at which the TmG is exceeded, glycosuria appears (40). In diabetic patients the TmG is higher than healthy individuals, this contributing to the worsening of hyperglycemia (41). The increase in absorptive capacity is due to upregulation or hyperactivation of GLUTs and SGLTs (30, 42). This mechanism, which may seem protective, leads to an increase in glycemia as it reduces the excretion of excess filtered glucose in the urine (30). The kidneys filter 160–180 g of glucose per day in healthy individuals (43) and ∼97% of the filtered glucose is reabsorbed in the early proximal tubule by SGLT2; the remaining glucose is reabsorbed by SGLT1

in the late proximal renal tubule (44). The exact mechanism combines the reabsorption of sodium and glucose through the SGLTs (34–36). In particular, on the apical membrane of the renal tubule, sodium is transported along with glucose into the cell through the SGLTs and then the glucose enters in circulation through an active transport mechanism mediated by GLUT2, while sodium is actively exchanged through a Na+/K+ pump (31, 45). The sodium: glucose coupling ratio is 1:1 for SGLT2 and 2:1 for SGLT1 (46). The high affinity of SGLT1 for glucose together with the transport molar ratio of 2:1 leads to a complete reabsorption of the filtered glucose in the S3 segment of the proximal renal tubule. Another important mechanism contributing to renal glucose control is gluconeogenesis. In particular, after prolonged fasting, the kidney is able to produce about 20–25% of the glucose released in circulation, while, post-prandial renal gluconeogenesis increases by 100% and accounts for 60% of endogenous glucose release (47–49). This process could be explained by the use of endogenous glucose as a source for the constitution of liver glycogen (31). Renal gluconeogenesis, as well as hepatic gluconeogenesis, contributes to the worsening of hyperglycemia in diabetic patients (**Figure 2**) (31). Finally, the glucose utilization by kidneys is, in the fasting state, about 10% while in the post-prandial state increases by 3-fold. This mechanism is upregulated in diabetic patients in which there is increased renal glucose utilization (49–51). These drugs, causing glycosuria, lead to caloric loss and diuresis with a reduction of body weight and BP, and improve beta cell function, insulin sensitivity and consequently favor better glycemic control without hypoglycemia (50).

DIABETIC KIDNEY DISEASE: ROLE OF SGLT2 INHIBITORS

Initially, diabetic nephropathy is characterized by an increase of the GFR, a phenomenon known as glomerular or renal hyperfiltration (according to guidelines it is defined as a GFR of more than two standard deviations above the mean GFR of healthy subjects). Two major hypotheses are proposed to explain this phenomenon: the hemodynamic hypothesis, including the myogenic, neurogenic and hormonal factors which determine vasoconstriction of the afferent arteriole; and the tubular hypothesis, namely the early changes in diabetic kidneys involving proximal tubular reabsorption (52). Physiologically, tubules and glomeruli work together to maintain an adequate extracellular volume, through two mechanisms: glomerulo-tubular balance, that modulates renal proximal tubular reabsorption related to GFR changes, and tubule-glomerular feedback (TGF), which through the *macula densa* keeps constant the filtered load of solutes that reaches the renal distal tubule (53). Some studies in the rat showed that in the early stages of diabetes, TGF has a major role, in fact hyperglycemia causes proximal tubular hypertrophy, inducing an increase in renal proximal tubular reabsorption and in sodium/glucose cotransport. These alterations decrease the amount of solutes reaching the *macula densa* and so the TGF is deactivated, causing an increase in GFR (54). Data from the RIACE cohort shows that renal hyperfiltration is an independent predictor of death from any cause in patients with type 2 diabetes (55). In the study of Rigalleu et al. (56), kidney hypertrophy was correlated with disease progression in patients with overt diabetic nephropathy. Furthermore, renal tubular glucose active transport plays a key role in diabetic nephropathy (32). As previously described, glucose is mainly reabsorbed via the SGLT2 (57). SGLT2is reduce glucose reabsorption in the renal proximal tubules, thus promoting glucosuria. The degree of induced glucosuria is proportioned to the glycemic control, being greater in patients with higher circulating levels of glucose, as well as to the dose of the drug administered (58). The reduction in blood glucose levels is an important pharmacodynamic effect of SGLT2is. Moreover, this pattern of action is independent of insulin secretion and this means that these drugs also work in patients with reduced pancreatic β-cell function. Intriguingly, by blocking the SGLT2, these agents cause a decrease in sodium reabsorption. This pattern of action leads to two important consequences. The first one is the natriuretic effect, which results in reduction of intravascular volume and BP (59). The second one is the increase of sodium delivery to the *macula densa* that is followed by a re-activation of TGF, and a prompt reduction in single-nephron GFR. This effect is mediated by the vasoconstrictive adenosine and mitigates the renal hyperfiltration that is in turn responsible for deleterious long-term effects on the renal parenchyma (59).

SGLT2is have been shown to elicit several other nephroprotective effects. They improve endothelial dysfunction and reduced oxidative stress and inflammation, all of which are largely related to the effect of glucose on renal vascular and tubular cells (60). Furthermore, they have been shown to decrease the renal resistive index (RI), a marker of intrarenal vascular resistance that is also associated with a worse individual risk profile and prognosis. The effect of the SGLT2i dapagliflozin occurred after a short period (8-weeks) of treatment in a study enrolling type 2 diabetic patients (61, 62). Interestingly, SGLT2is seem to improve the regulation of extracellular matrix deposition, by decreasing the epithelial-to-mesenchymal transition, a mechanism which involves metalloproteinases and predisposes to renal fibrosis over time (63, 64). SGLT2is significantly lower albuminuria, thus reducing its toxic effects on renal tubules (65). The reduction in albuminuria levels exerted by the SGLT2is is largely dependent on the reduction of intraglomerular pressure (66). In fact, SGLT2is have positive effects on both the afferent and efferent arteriolar tone. Previous studies in patients with type 1 diabetes showed that empagliflozin lowers glomerular hyperfiltration through the increase of afferent renal resistance (67, 68) with a mechanism that may involve the release of adenosine and its effect on adenosine A1 receptors. In type 2 diabetes, the main mechanism by which SGLT2is reduce the hyperfiltration is more likely due to the post-glomerular vasodilation via the activation of TGF and the effect of adenosine on A2 receptors (66). Other beneficial mechanisms that lead to the reduction of albuminuria have been reported. In animal models, SGLT2is reduce blood levels and tissue expression of inflammation, oxidative stress and fibrosis (69, 70). Such non-hemodynamic effects may potentiate the hemodynamic effects on renal inflammation and fibrosis which is *per se*

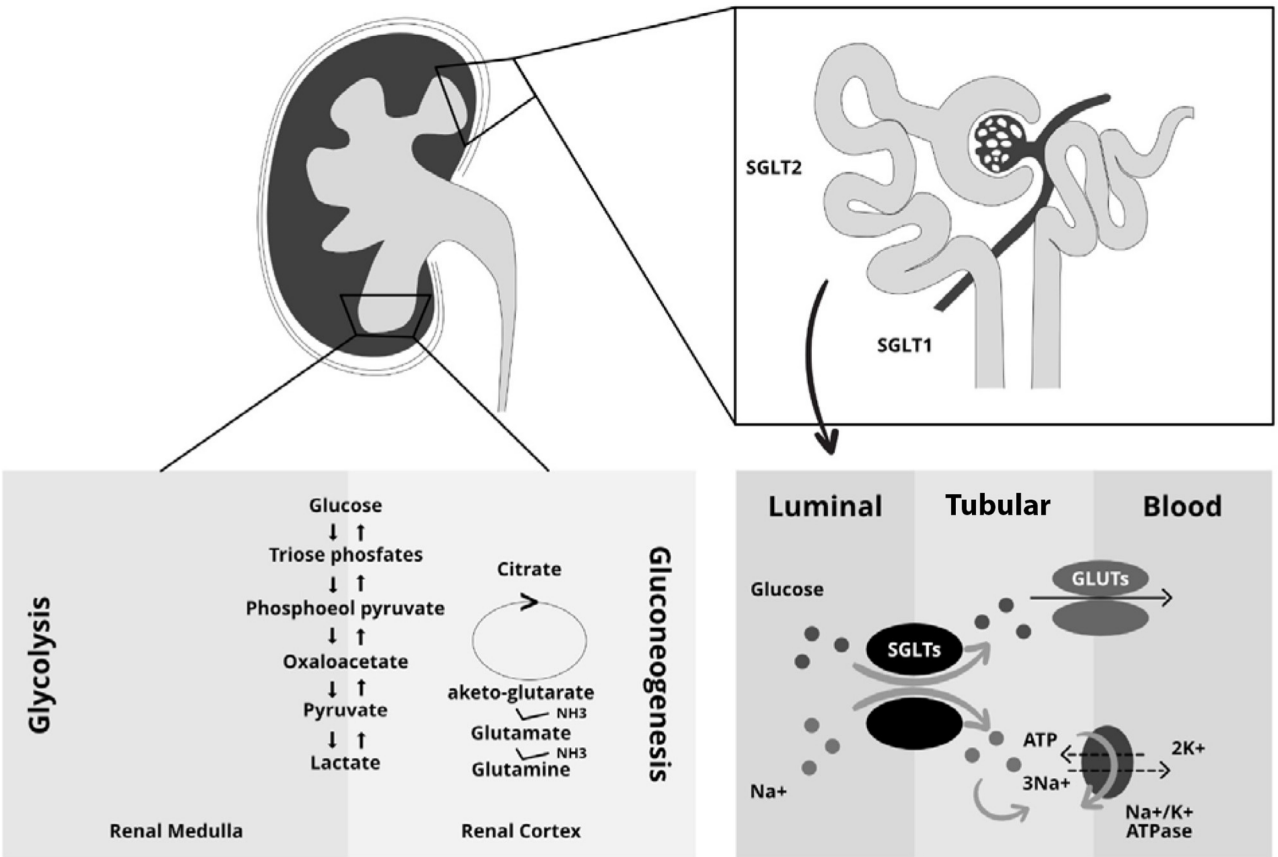


FIGURE 2 | Central role of the kidney in glucose metabolism. The kidneys intervene in glucose homeostasis regulation through three main mechanisms: the glucose reabsorption via sodium co-transporters (SGLTs), the gluconeogenesis with endogenous glucose production and the utilization of glucose. The right side of the figure shows the mechanism of sodium and glucose reabsorption through the SGLT and GLUT transporters in the kidney. SGLTs, which are mainly located on the brush border (luminal side) of epithelial tubular cells, enables the transfer of both sodium and glucose from the lumen into the tubular cells. Sodium is transported along with glucose through the SGLTs. Next, glucose enters blood circulation through an active transport mechanism mediated by GLUTs, which are located on the basolateral membrane of the epithelial tubular cells. Sodium is actively exchanged between tubular cells and blood circulation through a Na+/K+/ATPase pump. The different phases of renal gluconeogenesis and glycolysis are depicted on the left side of the figure.

determined by the reduction in glomerular hyperfiltration. Altogether, although it is often complex to distinguish between the weight of hemodynamic and non-hemodynamic patterns, all these mechanisms confer a renoprotection and significantly reduce albuminuria.

RENAL OXYGENATION

As previously discussed, glomerular hyperfiltration is a key pathogenetic step in the determination of diabetic kidney disease. It has been shown that renal hyperfiltration is associated with increased renal O_2 consumption and with reduced availability of O_2 in the renal cortex and medulla (71). This phenomenon has been attributed to the increase in reabsorption of Na^+ via the SGLT that leads to a raised activity of the Na^+/K^+ATPase pump in the proximal kidney tubules. O'neill et al. have interestingly shown, in a rat model of diabetic kidney disease, that the inhibition of SGLT with the non-selective agent phlorizin reversed renal cortical hypoxemia via the reduction of glomerular hyperfiltration and sodium reabsorption (72). More specifically, phlorizin reduced glomerular filtration by 4 mL/min on average in diabetic rats included in this study and O_2 consumption was significantly reduced in diabetic rats but not in control rats. Conversely, the increase of fractional sodium excretion caused an overload of Na^+ to the distal part of the nephron raised O_2 consumption and worsened hypoxia in the renal medulla. All these findings suggested a strict connection between glomerular filtration, Na^+ uptake and renal hypoxia. The peculiar mechanism of action of SGLT2is may predispose to hypoxic damage of a portion of the renal parenchyma, namely the medulla, that is *per se* more susceptible to this pathogenic insult. Indeed, while the renal cortex physiologically receives enough O_2 to supply the metabolic needs, the renal medulla is supplied with only about 10% of the total blood renal flow (73). Such low partial pressure of O_2 in the medulla reflects limited

regional blood flow that enables the generation of high medullary osmolality, required for the urine concentrating capacity. The degree of tubular activity, and thus the O_2 consumption, is regulated by several substances including adenosine, nitric oxide and prostaglandins which modulate the distal tubular transport through complex mechanisms that are impaired in the presence of CKD. Moreover, the presence of type 2 diabetes *per se* contributes to the low oxygenation amount of renal parenchyma (74). As a consequence of the hypoxic effect on the renal medulla, SGLT2is causes an increase in hematocrit (75–77). In a randomized study evaluating the diuretic effects of dapagliflozin, hydrochlorothiazide (HCT) vs. placebo, Lambers Heerspink et al. found an increase of hematocrit by 2.2% in patients treated with dapagliflozin compared with those treated with placebo after 3 months of treatment (75). Moreover, the increase in hematocrit in this trial was also accompanied by an increase in the mean reticulocytes and serum erythropoietin (EPO), thus suggesting a role of enhanced erythropoiesis. Erythropoietin is released from peritubular interstitial cells in response to reduced parenchymal O_2. The EPO gene is upregulated by the hypoxia-inducible factors (HIFs) a heterodimer composed of α and β subunits. Hypoxia prevents the proteasomal degradation of the HIF-α subunit. Hence, it has been hypothesized that SGLT2is, by reducing renal oxygenation at the corticomedullary junction, lead to the stabilization and accumulation of HIF-α, permeating its binding to the beta subunit and the induction of HIF-mediated genes (73, 77). Hence, SGLT2is seem to exert an opposite effect toward the cortical and medullary portion of the kidney. In a recent clinical study enrolling patients with Acute Kidney Injury (AKI) while taking SGLT2is, Darawshi et al. found that, in this specific patients population, the biomarkers of distal renal tubular injury such as serum and urine Neutrophil Gelatinase-Associated Lipocalin (NGAL) levels were higher than the levels found in patients without AKI, whereas biomarkers of proximal renal tubular injury namely serum and urine kidney injury molecule-1 (KIM-1) levels were not significantly different between the two groups (78). Such important findings suggest that SGLT2is may mitigate hypoxic injury in cortical parenchyma and, at the same time, may trigger hypoxic injury in the renal medulla, this being particularly in high risk conditions such as the presence of diabetes or CKD (or both clinical entities).

SGLT2 INHIBITORS AND ACUTE KIDNEY INJURY

The novel drug family of SGLT2is has rapidly captured the attention of clinicians given their efficacy in slowing CKD progression and in reducing CV risk in patients with CKD and diabetes. This notwithstanding, since their introduction in clinical practice several studies raised concerns regarding their safety, particularly with regards to the risk of AKI (79, 80). The mechanisms underlying the onset of AKI during treatment with SGLT2is are represented by their actions on glomerular hemodynamics, the effect of volume depletion and the hypoxic effect (73). The initiation of treatment with SGLT2is is followed by a variable eGFR reduction of 2–6 mL/min/1.73m^2 lasting for about 2 weeks (81). This initial dip is followed by a subsequent reduction in eGFR decline over time (82–84). Hence, a biphasic trajectory of eGFR following SGLT2is start has been reported. The initial eGFR decline mainly reflects the reduction of glomerular hyperfiltration which is a crucial pathogenetic mechanism of diabetic kidney disease. This means that patients who present the initial dip in eGFR would be those who will experience the greater protection from eGFR decline in the long follow-up. Moreover, it has been shown that the biphasic trajectory is shared by almost all SGLT2is and it has been reported for both higher and lower basal eGFR levels (85, 86). Due to these pieces of evidence, De Nicola et al. hypothesized this initial dip of eGFR to be a "check-mark sign" of SGLT2is activity (80). Data surrounding the true incidence of AKI after SGLT2is are controversial. In a meta-analysis of randomized trials evaluating the adverse renal outcomes (eGFR decline up to renal failure) in patients with type 2 diabetes, dapagliflozin but not empagliflozin was associated with a high risk for adverse renal events compared with placebo (87). Conversely, in the CANVAS trial, canagliflozin was not significantly associated with the risk of AKI (88). The reports submitted to the FDA for the period March 2013-October 2015, signaled 101 cases of AKI during treatment with canagliflozin or dapagliflozin and principally occurred in patients with CKD, volume depletion, old age, concomitant use of diuretics, concomitant treatment with RAASi or non-steroidal anti-inflammatory drugs (89). However, data regarding the true incidence of AKI are limited and further studies are needed to clarify whether this phenomenon is present and whether it is associated with all SGLT2is or to a specific one.

EXTRA-KIDNEY EFFECTS OF SGLT2 INHIBITORS

The effects of SGLT2is are not limited only to glycemic control and/or to improve CKD. They have also shown extra-kidney beneficial effects that can also contribute to the improvement of renal hemodynamics.

Cardiac Effects

Cardioprotective effects of SGLT2is have been evaluated in several cardiovascular outcome trials (CVOTs) (88, 90–92). Three SGLT2is, empagliflozin, canagliflozin, and dapagliflozin have so far been evaluated (88, 90–92). In patients with type 2 diabetes and established atherosclerotic CV disease, SGLT2is showed a significant reduction of major cardiovascular events (MACE), the composite of non-fatal myocardial infarction (MI), stroke and CV death (93). Moreover, these antidiabetic agents reduced by 30–35% the rates of hospitalization for heart failure, both in patients with and without CV disease (88, 90–92). The cardioprotective mechanisms of SGLT2is have been largely studied but, to date, not yet proven. They cannot be explained entirely by the glycemic control and the improvement of traditional CV risk factors (BP, body weight, lipid profile, arterial stiffness) as they take a long time to give cardiovascular protection (94, 95). Indeed, beneficial effects were observed after a short time interval, indicating the presence of a hemodynamic,

rather than anti-atherosclerotic effect (96). Similar results were observed in a clinical trial evaluating the effect of dapagliflozin in patients with HF (96).

Blood Pressure

In the renal proximal tubule, the inhibition of SGLT leads to natriuresis, osmotic diuresis and a consequent extracellular volume contraction. This results in a decrease of BP, which is more marked for higher baseline systolic BP values (93). Other factors implicated in the decrease of BP may be weight loss, sympathetic nervous activity reduction and inhibition of myocardial sodium-hydrogen exchanger 3 (NHE3), which modulates sodium reuptake (97) and seems to concur to oxidative stress and HF (98). Conversely, improvement in glycemic control seems not to affect BP.

Uric Acid

SGLT2is increase renal uric acid excretion, thereby reducing serum uric acid concentrations (99). SGLT2is raise glucose concentration in the renal tubules, the site where glucose competes with urates for the Glucose transporter 9b (GLUT9b). This leads to a reduction in urate reabsorption (99). This reduction became modest or absent in patients with CKD (100).

Ketone Bodies

SGLT2is cause a reduction in blood glucose levels which, in turn, lead to a shift in cellular metabolism. The result of such change is the increased use of fatty acid oxidation and lipolysis as energy sources. Lipid oxidation generates acetyl-CoA that is converted in ketone bodies when glucose oxidation is reduced. Lower plasma glucose level stimulates glucagon secretion and suppression of insulin production leading to an increase in the glucagon: insulin ratio. As previously discussed, SGLT2is induce natriuresis and volume depletion. It has been demonstrated that SGLT2is increase renal tubular reabsorption of acetoacetate from the proximal tubule during volume depletion, thus contributing to the pathogenesis of hyperketonemia (101, 102). Moreover, SGLT2is, like phlorizin, can decrease renal clearance of ketone bodies. These metabolic changes overall promote ketogenesis (103). Oxidation of ketone bodies produces more amounts of ATP per molecule of oxygen than glucose or fatty acids oxidation does and may provide a more efficient energy source for the myocardium (103). Moreover, ketones generate a minor production of reactive oxygen species (ROS) and therefore they can contribute to prevent mitochondrial dysfunction. In fact, enhanced production of ketone bodies has been proposed as one mechanism driving protection from cardiovascular death and HF observed during SGLT2is therapy. On the other hand, the increase in concentrations of ketone bodies leads to the feared diabetic ketoacidosis (DKA) (104).

Oxidative Stress and Inflammation

Oxidative stress is involved in the development of diabetic complications and plays a key role in the progression of atherosclerosis (105, 106). SGLT2is have recently showed to protect against oxidative stress by direct and indirect mechanisms, such as glucose and free radical generation lowering. Prior studies reported that SGLT2is prevent mitochondrial dysfunction by improving redox state (107), they modulate expression and/or activity of pro-oxidant enzymes (108) and decrease advanced glycation end products (AGEs) generation by reducing glycaemia (109). In addition, SGLT2is may decrease oxidative damage also by inhibiting inflammatory pathways. Indeed, treatment with SGLT2is was associated with a suppression of serum inflammatory markers, such as IL-6 and Tumor Necrosis Factor-α (TNF-α) (110), and with a reduction of MCP-1, p65, toll-like receptor 4 andosteopontin expression (111).

Arterial Stiffness and Endothelial Dysfunction

In several studies the chronic or acute use of SGLT2is improved arterial stiffness and endothelial dysfunction (7, 60, 112), independent of changes in BP.

Adipokin Levels

A reduction in serum leptin levels and an increase in serum adiponectin, which has an anti-inflammatory effect, has been observed during SGLT2i therapy (110). To date, it is unclear whether SGLT2is alters adipokine levels with direct or indirect action in adipose tissue.

Clinical Trials on SGLT2is and Renal Outcomes

Several placebo-controlled clinical trials have demonstrated that SGLT2is could prevent the development of chronic kidney disease or decrease the progression of kidney disease in patients with type 2 diabetes. The nephroprotective effects of SGLT2is have been demonstrated in five major CVOTs: EMPA-REG OUTCOME (empagliflozin), CANVAS and CANVAS-R (canagliflozin), DECLARE-TIMI 58 (dapagliflozin) and VERTIS CV (ertugliflozin). All these trials were designed to investigate CV outcomes in patients with type 2 diabetes and established atherosclerotic CV disease or multiple CV risk, and the renal endpoints were evaluated only as secondary outcome. Most recently, two clinical trials, the CREDENCE trial (canagliflozin) and DAPA-CKD trial (dapagliflozin), were designed to investigate renal outcomes as the primary endpoint. In addition, several clinical studies evaluated the effects of SGLT2 on eGFR and urinary albumin to creatinine ratio (UACR) in patients with type 2 diabetes.

Empagliflozin

EMPA-REG OUTCOME was a randomized, double-blind, placebo-controlled trial involving patients with type 2 diabetes, almost all with established CVD. Patients had a significant history of atherosclerotic heart disease including MI, coronary artery bypass grafting and/or multiple vessel disease. The mean eGFR at baseline was 74.1 mL/min/1.73 m^2 and the median UACR was 18 mg/g.

The EMPA-REG OUTCOME trial showed that compared with placebo, empagliflozin was able to improve a primary composite endpoint including CV death, MI or stroke. Moreover, it improved all-cause mortality and significantly

reduced the rates of hospitalization for HF. Composite renal outcomes in the EMPA-REG OUTCOME trial included incident or worsening nephropathy and incident albuminuria (86). Worsening nephropathy (defined as progression to macroalbuminuria, doubling of serum creatinine, initiation of renal replacement therapy or death from renal causes) was significantly lower in patients treated with empagliflozin when compared with placebo.

Furthermore, *post-hoc* analysis of the EMPA-REG-OUTCOME trial showed that empagliflozin significantly slowed the decline in eGFR (113). Interestingly, the results observed in this study were obtained also when only modest reduction of A1c in the treatment arm were observed. Moreover, it is not clear if renal benefits observed in this population with high cardiovascular risk may also be obtained in patients with low cardiovascular risk and/or without diabetes. At present there is an ongoing study assessing the renal effects of Empagliflozin in patients with CKD with or without type 2 diabetes, namely The Study of Heart and Kidney Protection With Empagliflozin (EMPA-KIDNEY, Clinical-Trials.org identifier NCT03594110).

Canagliflozin

Similar results were observed with canagliflozin in the CANVAS and CANVAS RENAL (CANVAS Program) studies. These studies were designed to evaluate the efficacy and the safety of canagliflozin in a population of diabetic patients with different levels of CV risk. The primary composite endpoint included CV death, non-fatal MI or non-fatal stroke. The rate of the primary outcome was lower in patients in treatment with canagliflozin than with placebo (HR, 0.86; 95% CI, 0.75–0.97; $P < 0.001$ for non-inferiority; $P = 0.02$ for superiority). When death from any cause and death from cardiovascular causes, were evaluated individually, significant differences between two arms of treatment were not found. Canagliflozin significantly reduced the rates of hospitalization for HF [HR, 0.67 (95% CI, 0.52–0.87)] and the benefit appeared to be similar for patients with reduced ejection fraction (HFrEF) and patients with preserved ejection fraction (HFpEF). Renal outcomes in the CANVAS trials were progression of albuminuria or regression of albuminuria and the renal composite comprising a 40% reduction in eGFR sustained for at least two consecutive measures, the need for renal-replacement therapy (dialysis or transplantation), or death from renal causes. Progression of albuminuria was defined as more than a 30% increase in albuminuria and a change from either normal-albuminuria to microalbuminuria or macroalbuminuria or from microalbuminuria to macroalbuminuria. Regression of albuminuria was defined using criteria comparable to those defined for category progression. Progression of albuminuria occurred less frequently among patients in therapy with canagliflozin than among those assigned to the placebo arm and the effects were greater in CANVAS-R (HR, 0.64; 95% CI, 0.57–0.73) than in CANVAS (HR, 0.80; 95% CI, 0.72–0.90) ($P = 0.02$ for homogeneity). Similarly, regression of albuminuria occurred more frequently among those assigned to canagliflozin than among those assigned to placebo. The composite renal outcome was also reduced in patients treated with canagliflozin than among those in the placebo group. No significant difference in this outcome was seen between CANVAS and CANVAS-R (88).

The EMPA-REG OUTCOME, CANVAS, and CANVAS-R trials, as well as the DECLARE-TIMI-58 (Dapagliflozin Effect on Cardiovascular Events-Thrombolysis in Myocardial Infarction 58) were clinical trials in which efficacy and safety of SGLT2is inhibitors on CV outcomes were evaluated in patients with type 2 diabetes and established or at high risk of atherosclerotic heart disease. Most patients enrolled in these trials showed variable degrees of diabetic kidney disease but generally with an estimated glomerular filtration rate (eGFR) of >60 mL/min/1.73m^2 whereas the safety and efficacy of SGLT2is in patients with more severe CKD still remain undefined.

The CREDENCE (Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation) trial was a double-blind, randomized trial, that enrolled patients with T2D and albuminuric chronic kidney disease to receive canagliflozin or placebo. All the patients had an eGFR of 30 to <90 ml per minute per 1.73 m^2, and albuminuria (ratio of albumin [mg] to creatinine [g]) of >300–5,000 and were treated with renin–angiotensin system blockade. The CREDENCE trial represents therefore the first dedicated renal outcomes trial with an SGLT2 inhibitor. Indeed, the primary outcome was a composite of end-stage kidney disease (dialysis for at least 30 days, transplantation, or a sustained estimated GFR of <15 ml per minute per 1.73 m^2, for at least 30 days), a doubling of the serum creatinine level, or death from renal or CV causes. The CREDENCE trial has been prematurely stopped after achievement of the renal primary endpoint composite during the interim analysis. The relative risk of the primary composite outcome was significantly lower in patients in treatment with canagliflozin than in the placebo group (HR, 0.70; 95% CI, 0.59–0.82; $P = 0.00001$) (92).

Similarly, the relative risk across renal components, including the doubling of the serum creatinine level and the outcome of dialysis, kidney transplantation, or renal death was significantly lower in the canagliflozin group than in the placebo group (HR, 0.60; 95% CI, 0.48–0.76; $P < 0.001$ and HR, 0.72; 95% CI, 0.54–0.97, respectively). The nephroprotective effects of canagliflozin were also observed in the subgroup of patients with more advanced kidney disease and very low eGFR (eGFR 30–45 mL/min/1.73 m^2) (HR, 0.75; 95% CI, 0.59–0.95). Moreover, patients treated with canagliflozin also had a lower risk of several secondary CV outcomes including the composites of CV death or hospitalization for HF (HR, 0.69, 95% CI, 0.57–0.83; $P < 0.001$), CV death, MI, or stroke (HR, 0.80, 95% CI, 0.67–0.95; $P < 0.01$) and hospitalization for HF (HR, 0.61, 95% CI, 0.47–0.80; $P < 0.001$).

Interestingly, these results have been obtained although very low and non-significant differences in blood glucose levels were observed between the group treated with canagliflozin and the placebo. This supports the hypothesis that the effects induced by SGLT2is on renal function are likely independent of glucose levels. Moreover, the observed benefits were obtained in patients that received stable doses of angiotensin-converting enzyme inhibitor (ACE-i) or angiotensin II receptor blocker (ARB) therapy, which are other medications that can induce nephroprotective effects in type 2 diabetes (92).

Dapagliflozin

Dapagliflozin has been the first SGLT2 inhibitor approved for the treatment of patients with type 2 diabetes. Safety and efficacy of dapagliflozin on major adverse cardiovascular events (MACE) were evaluated in the DECLARE-TIMI-58, a randomized, double-blind, placebo-controlled trial including people with type 2 diabetes and atherosclerotic CV disease or multiple CV risk factors. In this study, Dapagliflozin resulted in a significantly lower rate of CV death and hospitalization for HF than placebo but failed to achieve superiority to reduce MACE (HR 0.93, 95% CI 0.84–1.03; $P < 0.001$ for non-inferiority and $P = 0.17$ for superiority). Similarly, no significant difference in the rate of death from any cause was observed between the two groups. Dapagliflozin showed a significant decrease in the renal composite outcome (>40% eGFR reduction, new ESKD or renal or cardiovascular death) when compared with placebo (HR 0.76, 95% CI 0.67–0.873) (91).

More recently, the efficacy and safety of dapagliflozin on progression of chronic kidney disease has been evaluated in patients with chronic kidney disease and with and without type 2 diabetes. In the Dapagliflozin and Prevention of Adverse Outcomes in Chronic Kidney Disease (DAPA-CKD) study, patients with and without diabetes, an eGFR of 25 to 75 mL/min/1.73 m^2 and UACR of 200–5,000 milligrams, were enrolled. All the participants received a stable dose of an ACE-i or ARB. The primary outcome was a composite of a sustained decline in the eGFR of at least 50%, the onset of end-stage kidney disease (dialysis for at least 28 days, transplantation, or a sustained eGFR of <15 ml per minute per 1.73 m^2, for at least 28 days), or death from renal or CV causes. Patients who received dapagliflozin had a significantly lower risk of the primary composite outcome than those that received placebo (HR, 0.61; 95% CI, 0.51–0.72; $P < 0.001$) and the effects were similar in patients with type 2 diabetes (HR, 0.64; 95% CI, 0.52–0.79) and without type 2 diabetes (HR, 0.50; 95% CI, 0.35–0.72). Moreover, patients in dapagliflozin group also had a lower risk for the composite of CV death or hospitalization for HF (HR, 0.71, 95% CI, 0.55–0.92). As previously reported in other studies with SGLT2 inhibitors, also in the DAPA-CKD trial in the first 2 weeks of treatment, there was a greater reduction in eGFR in patients in treatment with dapagliflozin than in placebo group, but thereafter the decline in the eGFR was slower in the dapagliflozin arm than in the placebo arm (114). The benefits of dapagliflozin on some markers of renal function (eGFR and/or urinary UACR) have also been explored in other clinical studies. The outcomes and the results of the DERIVE, DELIGHT, and DIAMOND studies are summarized in the **Table 1**.

Ertugliflozin

Efficacy and safety of ertugliflozin on cardiovascular and renal outcomes were assessed in the eValuation of ERTugliflozin EffIcacy and Safety Cardiovascular Outcomes Trial (VERTIS CV), a multicenter, randomized, double-blind, placebo-controlled trial including people with T2D and established atherosclerotic cardiovascular disease. The primary composite outcome included CV death, non-fatal MI or non-fatal stroke. Secondary outcomes were a composite of death from CV causes or hospitalization for HF; death from CV cause and a composite of death from renal causes, doubling of the serum creatinine level or renal replacement therapy. Unlike previous CV outcomes trials with SGLT2 inhibitor, ertugliflozin was non-inferior to placebo with regard to the primary composite outcome but failed to achieve superiority to reduce major adverse CV events (death from cardiovascular causes, non-fatal MI or non-fatal stroke). Similarly, no significant benefit was observed in the secondary composite renal outcome (115, 120). These data are in contrast with those obtained in other trials with SGLT2is, although the population of patients with atherosclerotic cardiovascular disease enrolled in VERTIS-CV study was broadly similar to those of previous trials performed with other SGLT2is. However, recently, has been demonstrated that when used in addition to standard of care medications, ertugliflozin is associated with a decrease in the risk of a sustained 40% decline in eGFR, with preservation of eGFR over time and a reduction of UACR in individuals with established atherosclerotic CVD and type 2 diabetes (121).

The results of major CVOTs, renal outcomes trials and clinical studies are summarized in **Table 1**.

SUMMARY RESULTS OF THE CLINICAL TRIALS ASSESSING THE EFFICACY OF SGLT2is ON RENAL ENDPOINTS

A number of large clinical trials have demonstrated improvement in renal outcome in high-risk patients with CKD and DM (88, 90–92, 114). The EMPA-REG, CANVAS, and DECLARE studies have shown as positive class-effects of the SGLT2is a significant reduction in albuminuria progression and preservation of eGFR decline over time (88, 90–92). The CREDENCE trial was specifically designed to evaluate renal outcomes in diabetic kidney disease patients. This study showed that, when added to the standard nephroprotective treatment, namely the maximum tolerated RAAS inhibition, canagliflozin exerted a striking 30% risk reduction for the renal endpoint (composite of ESKD, that is, dialysis for at least 30 days, transplantation, or a sustained eGFR of <15 mL/min/1.73 m^2 for 30 days, doubling of the serum creatinine for at least 30 days, or death from renal or cardiovascular disease). More importantly, this effect was independent of the baseline eGFR, being true also for patients with more advanced CKD (92). Once again, also in the CREDENCE trial, the long-term risk reduction was associated with a significant reduction in albuminuria in the canagliflozin group. The DAPA-CKD study extended these evidences to the more general population of CKD, with or without diabetes, testifying that SGLT2is act on pathophysiologic patterns that are active in CKD *per se* (114).

FUTURE PERSPECTIVES: THE USE OF SGLT2is IN THE PERSONALIZED MEDICINE ERA

The therapeutic *armamentarium* for the management of patients with CKD is undergoing an important revolution. If the early

TABLE 1 | Studies comparing risk renal outcomes among patients with CKD treated with SGLT2i.

Study	Population	Sample size	Intervention	Outcome	Results
EMPA-REG OUTCOME study (86)	T2DM at high risk for cardiovascular events	7,020 empagliflozin 10 mg ($n = 2,345$), 25 mg ($n = 2,342$), or matching placebo ($n = 2,333$)	empagliflozin 10 mg vs. empagliflozin 25 mg vs. placebo	Incident or worsening nephropathy (progression to macroalbuminuria, doubling of the serum creatinine level, initiation of renal-replacement therapy, or death from renal disease) and incident albuminuria.	Improvement of incident or worsening nephropathy (such as doubling of serum creatinine) for empagliflozin vs. placebo (12.7% vs. 18.8%, HR 0.61, 95% CI 0.53–0.70; $p < 0.001$), decrease of progression to macroalbuminuria (11.2 vs. 16.2%, $p < 0.001$)
CANVAS study (88)	T2DM at high risk for cardiovascular events	10,142 canagliflozin ($n = 5,795$) vs. placebo ($n = 4,347$	canagliflozin 100, 300 mg vs. Placebo	Progression of albuminuria	Canagliflozin was also associated with a lower rate of progression of albuminuria ($p < 0.05$)
DECLARE-TIMI 58 (91)	T2DM and established CV disease or risk factors for atherosclerotic CV disease	17,160 dapagliflozin 10 mg ($n = 8,582$) or placebo ($n = 8,578$).	dapagliflozin 10 mg vs. Placebo	≥40% decrease in eGFR to <60 mL/min/1.73 m^2 or new end-stage renal disease or death from renal/CV cause	The incidence of the renal composite outcome was 4.3% in the dapagliflozin group and 5.6% in the placebo group (hazard ratio, 0.76; 95% CI, 0.67–0.87).
VERTIS CV study (115)	T2DM and established CV disease	8,246 ertugliflozin 5 mg ($n = 2,752$), 15 mg ($n = 2,747$), or placebo ($n = 2,747$)	Ertugliflozin 5, 15 mg vs. Placebo	Renal death or dialysis/transplant or doubling of serum creatinine from baseline	Renal composite (renal death, dialysis/transplant, doubling of serum creatinine) not achieve statistical significance (3.2 vs. 3.9%, $p = 0.08$.
CREDENCE study (92)	CKD and T2DM	4,401 canagliflozin 100 mg daily ($n = 2,202$) or placebo ($n = 2,199$)	canagliflozin Vs. Placebo	Composite of ESRD (dialysis, transplantation, or sustained estimated GFR of <15 mL/min/1.73 m^2), doubling of the serum creatinine, or death from renal or cardiovascular causes.	ESRD, doubling of serum creatinine, renal or cardiovascular (CV) death, for canagliflozin vs. placebo, was 43.2 vs. 61.2 per 1,000 patient-years (P-Y) ($p = 0.00001$)
DELIGHT study (116)	T2DM and chronic kidney disease of moderate to severe grade	1,187 dapagliflozin group ($n = 145$), dapagliflozin–saxagliptin group ($n = 155$), placebo group ($n = 148$)	dapagliflozin 10 mg only, dapagliflozin 10 mg and saxagliptin 2.5 mg, or placebo once-daily	Worsening nephropathy and progression of albuminuria	Dapagliflozin with or without saxagliptin, in addition to ACE-i or ARBs, slows the progression of kidney disease in patients with diabetes and chronic insufficiency from moderate to severe.
CANTATA-SU (117)	T2DM patients already treated with metformin	1,450 Canagliflozin 100 mg ($n = 483$), Canagliflozin 300 mg ($n = 485$), Glimepiride ($n = 482$)	canagliflozin 100 mg or 300 mg/day vs. glimepiride 6–8 mg/day	Decrease in eGFR and progression of albuminuria.	It showed a reduction in eGFR: −0.5 (canagliflozin 100 mg), −0.9 (canagliflozin 300 mg), −3.3 (glimepiride) mL/min/1.73 m^2 at 2 years; and a reduction of albuminuria: −31.7% (canagliflozin 100 mg), −49.3% (canagliflozin 300 mg) relative to glimepiride.
DERIVE study (118)	Type 2 diabetes and chronic kidney disease in stage IIIA	302 dapagliflozin 10 mg ($n = 160$) or placebo ($n = 161$)	dapagliflozin vs. placebo	This study assessed the efficacy and safety of dapagliflozin 10 mg vs. placebo in patients with T2DM and moderate renal impairment (estimated glomerular filtration rate [eGFR], 45–59 mL/min/1.73 m^2; chronic kidney disease [CKD] stage 3A)	This study underlines the positive benefit and poor risk profile of dapagliflozin for the treatment of patients with T2D and CKD 3A.
DIAMOND study (119)	CKD in patients with non-diabetic kidney disease	58 dapagliflozin then placebo ($n = 27$) and placebo then dapagliflozin ($n = 26$)	dapagliflozin vs. Placebo	Change in 24-hr proteinuria and GFR in patients with non-diabetic kidney disease	This study showed that 6-week treatment with dapagliflozin did not affect proteinuria in this sample, but induced an acute and reversible reduction of eGFR.

(Continued)

TABLE 1 | Continued

Study	Population	Sample size	Intervention	Outcome	Results
DAPA-CKD study (85)	CKD in patients with non-diabetic kidney disease	4,304 dapagliflozin 10 mg (n = 2,152) placebo (n = 2,152)	Dapagliflozin vs. Placebo	Occurrence of one of the components of the composite: ≥50% sustained decline in eGFR or reaching End Stage Kidney Disease or CV death or renal death	The risk of a composite endpoint was significantly lower with dapagliflozin than with placebo, regardless of the presence or absence of T2DM.

2000's was the period of the demonstration of efficacy of RAASi agents in lowering CV and renal risk in CKD patients, then the 2020s' have been marked to be another step forward (10). During this period, several randomized studies have been completed and published (114, 122, 123). The Study of Diabetic Nephropathy with Atrasentan (SONAR) showed that the endothelin receptor antagonist (ERA) atrasentan confers protection against renal events (ESKD/doubling of serum creatinine) in patients with CKD and type 2 diabetes. The risk for the renal endpoint in the SONAR trial was 35% lower in the atrasentan arm compared with placebo (122). Furthermore, the finerenone in Reducing Kidney Failure and Disease Progression in Diabetic Kidney Disease (FIDELIO-DKD) study evaluated the efficacy of finerenone, a novel non-steroidal Mineralocorticoid Receptor Antagonist (MRA), vs. the onset of both renal and CV events (123). Finerenone, in the FIDELIO study, caused a significant CV and renal risk reduction and also showed the great advantage of being associated with a low rate of adverse events. None of the patients included in the finerenone arm had to discontinue the treatment due to hyperkalemia, which is a feared, potential life-threatening adverse event mainly attributed to the classic MRAs (e.g., spironolactone, eplerenone) (124). Hence, a great effort has been made to expand the therapeutic opportunities for reducing the high burden and residual risk of CKD patients (125–127). Interestingly, both the MRA and ERA agents show synergistic and additive effects if combined with the SGLT2is. With respect to ERA, they suffer a major adverse event: namely fluid (water and sodium) retention due to the stimulation of ENaC channels in the renal collecting duct. This effect is opposed by the SGLT2is that increase natriuresis in renal tubules. For this reason, a new clinical trial has been started combining the ERA zibotentan and the SGLT2i dapagliflozin in CKD patients (ZENITH trial: Protocol D4325C00001). ERAs and SGLT2is may potentiate their nephroprotective effects, such as the albuminuria reduction, mitigating the adverse events of each other. Moreover, MRA, ERA, RAAS is and SGLT2is share similar beneficial effects on renal parenchyma, reducing the oxidative stress, inflammation and fibrosis over time. Thus, one interesting future perspective would be to start more studies testing whether the combination of more drugs (including SGLT2is) with different mechanisms of action would warrant a better prognosis in CKD patients and what type of CKD patients would benefit from these combinations. Such a strategy may overcome the limit of current nephroprotective interventions and the high residual CV and renal risk of CKD patients (128). Moreover, although SGLT2is determine an initial dip of eGFR (check-mark sign) and a significant reduction in albuminuria, the magnitude of the short-term eGFR decline and the thresholds of albuminuria reduction that are associated with a protection in the long term are still undetermined. This initial eGFR dip is not only manifest after SGLT2is initiation but also as a hemodynamic response to low salt diet, RAAS inhibition, and antihypertensive treatments. Nevertheless, according to the first analysis the relationship between the eGFR dip and the following normalization of eGFR slope seems to be stronger for SGLT2is than for the other pharmacological and non-pharmacological treatments (80). Future studies should provide more insights into this topic given that diabetic kidney disease patients are likely to be at lower risk of hard renal endpoints mainly after a positive response in terms of albuminuria and eGFR during the 1st weeks of treatment, as shown in previous positive and even negative randomized trials from the past decades (129). SGLT2is would also be part of the novel trial designs or personalized medicine. The future direction will consider, with growing interest, to evaluate the drug's efficacy in small trials of similar patients (e.g., CKD patients with IgA Nephropathy or Membranous Nephropathy), rather than large trials including all patients with general eGFR/albuminuria thresholds. Examples of the new clinical trials designs are represented by the Master Trials Protocol (MTP) (130). These include the "Umbrella" trials, where patients are assigned to different treatments on the basis of the presence of a specific biomarker or other individual features. Contrariwise, MTP could be planned to evaluate a single treatment in different diseases, e.g., by including CKD patients with glomerular disease, DKD, hypertensive nephropathy as primary renal disease. These latter designs are defined as "Basket" trials. A third type, even more sophisticated than MTP, are the "Platform" trials (131). The Platform trial has a standing structure, with no predefined stopping date (hence the diction "Eternal" trial), during which patients can be started or withdrawn with a specific treatment without leaving the protocol in case the first treatment used is not effective. At the trial start, patients are included in an experimental cohort, namely a large database, whether they meet general inclusion criteria following the principle that disparate trials, with different treatments and outcomes, will be started. After the inclusion, patients are screened on the basis of clinical features, biomarkers or any other potential relevant information, including histology. Next, patients with strictly similar characteristics are randomized to an experimental treatment vs. the standard-of-care. If the treatment is not able to provide any benefit it is stopped and patients would be assigned to another treatment in the future. Conversely, the new treatment could replace the standard-of-care if it has been shown to improve the outcomes in that subgroup of

patients. The advantages of MTP, in reference to traditional RCT, are that these studies enable to evaluate what is the best treatment for a disease by comparing multiple treatments as well as to find the best drug combination for a specific subgroup of patients with possibly the same mechanisms of the disease. Hence, the current general tendency is to include in randomized studies a small number of patients with similar characteristics rather than a large number of patients with more general inclusion criteria. These novel designs have been prompted in nephrology, and a "CKD-Platform," the Global Kidney Patient Trials Network (ClinicalTrials.gov Identifier: NCT04389827) has been started in 2020 including 140 centers worldwide to identify patients eligible for clinical trials and to optimize treatment of CKD patients. SGLT2is, given their excellent results provided thus far, will be the best candidate to enter these novel trial strategies in the future.

CONCLUSION

The SGLT2is are a new class of orally active drugs used in the management of type 2 diabetes that promotes glucose excretion in the kidney. SGLT2is not only improve fasting plasma glucose and HbA1c but are able to induce blood pressure reduction and weight loss, improvement of CV outcomes and an improvement in chronic kidney disease. The nephroprotective effects of SGLT2is have been demonstrated in several major intervention studies. However, all these trials were designed to investigate CV outcomes in patients with type 2 diabetes and established atherosclerotic CV disease or multiple CV risk and the renal endpoints were evaluated only as a secondary outcome. Most recently, two clinical trials, the CREDENCE trial (canagliflozin) and DAPA-CKD trial (dapagliflozin), were designed to investigate renal outcomes as primary endpoint.

These studies have demonstrated that SGLT2is are able to delay renal disease progression in patients with and without type 2 diabetes and chronic kidney disease.

Interestingly, the effects induced by SGLT2is on renal function are likely independent of glucose levels and thus these medications could also be used in patients with CKD without type 2 diabetes. However, beyond the important results reported thus far, future studies should be performed to clarify the mechanisms of cardiorenal protection of SGLT2is. Clinical studies assessing how to combine SGLT2is with other nephroprotective treatments are also eagerly expected.

AUTHOR CONTRIBUTIONS

MP, MCP, MA, and FA: conceptualization, validation, and formal analysis. MP, MCP, and FA: methodology, writing—review and editing, and supervision. MP, MCP, IZ, BT, RP, MR, RS, AS, AM, MA, and FA: investigation, data curation, and writing—original draft preparation. All authors contributed to the article and approved the submitted version.

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REVIEW
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Management of Chronic Hyperkalemia in Patients With Chronic Kidney Disease: An Old Problem With News Options

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Hyperkalemia is one of the main electrolyte disorders in patients with chronic kidney disease (CKD). The prevalence of hyperkalemia increases as the Glomerular Filtration Rate (GFR) declines. Although chronic hyperkalemia is not a medical emergency, it can have negative consequences for the adequate cardio-renal management in the medium and long term. Hyperkalemia is common in patients on renin-angiotensin-aldosterone system inhibitors (RAASi) or Mineralocorticoid Receptor Antagonists (MRAs) and can affect treatment optimization for hypertension, diabetes mellitus, heart failure (HF), and CKD. Mortality rates are higher with suboptimal dosing among patients with CKD, diabetes or HF compared with full RAASi dosing, and are the highest among patients who discontinue RAASis. The treatment of chronic hyperkalemia is still challenging. Therefore, in the real world, discontinuation or reduction of RAASi therapy may lead to adverse cardiorenal outcomes, and current guidelines differ with regard to recommendations on RAASi therapy to enhance cardio and reno-protective effects. Treatment options for hyperkalemia have not changed much since the introduction of the cation exchange resin over 50 years ago. Nowadays, two new potassium binders, Patiromer Sorbitex Calcium, and Sodium Zirconium Cyclosilicate (SZC) already approved by FDA and by the European Medicines Agency, have demonstrated their clinical efficacy in reducing serum potassium with a good safety profile. The use of the newer potassium binders may allow continuing and optimizing RAASi therapy in patients with hyperkalemia keeping the cardio-renal protective effect in patients with CKD and cardiovascular disease. However, further research is needed to address some questions related to potassium disorders (definition of chronic hyperkalemia, monitoring strategies, prediction score for hyperkalemia or length for treatment).

Keywords: hyperkalemia, chronic kidney disease, RAASi, potassium binders, patiromer, zirconium

INTRODUCTION

Potassium regulates many biological processes and plays a main role in human physiology. Among the total body electrolyte composition, 2% potassium resides in extracellular compartment and 98% in the intracellular space. Vital physiological processes are based on the balance of potassium regulation, including acid-base homeostasis, systemic blood pressure control and vascular tone, hormone secretion, carbohydrate metabolism, gastrointestinal motility, fluid and electrolyte equilibrium, the resting cellular-membrane potential and action potentials in neuronal, muscular, and cardiac tissue (1, 2). An average adult has approximate 50 mmol of potassium per kg, meaning 3,500 mmol of total body potassium. The normal kidney maintains normal potassium homeostasis regardless of the total amount of dietary intake. Approximately 90% of the daily potassium intake is excreted in the urine; the rest is excreted by the gastrointestinal tract, leading a <10% of variations in the plasma potassium level during the course of a day. Fresh fruits and vegetables, are considered healthy choices for most people and they are associated with a less renal complications except for hyperkalemia (3). Nevertheless, among patients with chronic kidney disease (CKD), a higher dietary potassium intake may be associated with a higher risk of kidney disease progression (4).

Potassium disorders are common in patients with CKD, due to low glomerular filtration rate (GFR) and tubular disorders and represent a potentially fatal condition. Hyperkalemia represents a frequent complication of CKD progression, limiting the therapeutic strategies options recommended for treatment and prevention of cardiovascular disease (CVD), including renin-angiotensin-aldosterone inhibitors (RAASi) (5). An important CKD-related condition that contributes to hyperkalemia is metabolic acidosis, which causes a shift of potassium from the intracellular to the extracellular space (6). An unmet need exists for hyperkalemia management in the daily setting of special populations as CKD patients. The use of potassium binders has been controversial due to tolerability and safety profile, therefore new released, Patiromer and Zirconium, rather than the classic Sodium Polystyrene Sulfonate (SPS) could represent a strategy for the optimal manage of potassium disorders.

CLASSIC FEEDBACK REGULATION AND FEEDFORWARD CONTROL: TWO MECHANISMS OF POTASSIUM REGULATION

Dietary potassium intake initiates both increased potassium excretion and sequestration in liver and skeletal muscle, an effect drived by insulin, catecholamines, alkalosis, and mineralocorticoids. Postprandial insulin not only regulates the serum glucose concentrations but also shifts dietary potassium into cells until the kidney starts potassium excretion. Kaliuresis is driven by two mechanisms, depending on the plasma potassium level (the classic feedback regulation) or independent of the plasma potassium level (feedforward regulation) (5). The healthy kidney has the capacity to excrete high amounts of potassium. In the absence of CKD, humans can intake very large amounts without developing clinically significant hyperkalemia. If the quantity of released potassium is sufficient to increase the plasma potassium level, the feedback system is activated. The potassium is freely filtered by the glomerulus and almost completely reabsorbed in the tubule, just a small portion reaches the distal nephron. A high potassium dietary intake is related to a inhibitory effect on sodium reabsorption in the proximal tubule and the thick ascending limb, facilitating increased delivery of Na^+ to the aldosterone-sensitive distal nephron (ASDN), running a potassium fine regulation (1). A potassium sensor in the distal tubule has also been described as complementary mechanism in the potassium regulation.

Two mechanisms have been proposed as the major drivers for the proximal tubule reabsorption of 60–75% of filtered potassium are solvent drag and electrodiffusion. Several co-transporters and ion channels are involved in the complex regulatory reabsorption system of the 15–20% of filtered potassium in the thick ascending limb of the loop of Henle. The best characterized is the sodium potassium chloride cotransporter also known as NKCC2 (7). NKCC2 has one of the highest overall reabsorptive capacities in the kidney. The distal convoluted tubule mediates reabsorption of 5–10% of filtered potassium. In the early segment of the distal convoluted tubule, potassium transport is driven exclusively by the thiazide-sensitive sodium chloride cotransporter, whereas in the later segment of the ASDN, the epithelial sodium channel also participates. Three main factors regulate potassium secretion in the ASDN: (1) sodium load, (2) fluid flow rate, and (3) aldosterone and catecholamines (7).

Potassium homeostasis is regulated by renal and extrarenal mechanisms. To enhance the ability to eliminate potassium, there is a complementary mechanism called the "feedforward control." As the GFR declines, potassium excretion is compensated in the remnant functional kidney by enhancing its ability excreting this excess of potassium (1). It is also well-described that hyperkalemia does not appear until GFR reaches the threshold of 15 ml/min, earlier if aldosterone dysfunction is associated. Beyond this adaptive response, extra-renal contribution to potassium handling, especially colonic excretion, becomes critical to prevent acute or chronic hyperkalemia and makes a substantial contribution to potassium homeostasis in patients with CKD (8). Potassium homeostasis in the bowel is modulated due to an enteric sensing system (gut factor), that enhances kaliuresis once potassium enters the intestine, the feedforward system initiated at splanchnic receptors provides maintenance of total body potassium levels within narrow limits after the ingestion of a meal (2, 5) (**Figure 1**). There are two compensatory mechanisms in the colon in response to elevated serum potassium levels in patients with CKD: passive secretion which is responsible for net colonic potassium secretion, basically in the distal colon, and active secretion, that occurs throughout the colon and mechanistically follows the "pump leak" model (basolateral uptake of potassium via Na/K pump, Na/K/Cl cotransporter and efflux of potassium through BK channels) (9) (**Figure 2**). Conversely, during fasting, potassium is released from intracellular stores (liver, muscles). Both feedback and feedforward control work together to maintain potassium

and sodium homoeostasis (10). Daily potassium intake, renal management and enteric sensor are not the only players in the potassium homeostasis. There is a central circadian clock that regulates the nightly and daily kaliuresis. This rhythm synchronizes the renal tubule cells with the brain, and increases excretion during the active daylight phase and diminishes it during the inactive nighttime phase, regardless from activity, posture or dietary intake (2).

Disorders of potassium balance are common due to changes in dietary intake, GFR, or physiological management (renal and gastrointestinal) among different clinical settings, as CKD patients, renal transplantation (RT), resistant hypertension, or cardiorenal syndrome. The use of otherwise recommended medication like RAASi or MRAs could be critical in the incidence and severity of hyperkalemia.

Traditional dietary recommendations to CKD patients limit the intake of fruits and vegetables because of their high potassium content. However, there is a controversy based on to the benefits derived from a fundamentally vegetarian diet (3). Western diets are largely acid-producing since they are deficient in fruits and vegetables and rich in animal proteins that can induce metabolic acidosis in CKD patients. Thus, diets rich in vegetables and fruits might lower the dietary acid load and induce similar beneficial results as an alkali therapy in CKD patients (11, 12).

CHRONIC HYPERKALEMIA IN PATIENTS WITH KIDNEY DISEASE

Hyperkalemia in Patients With CKD Non-dialysis

Studies in patients with CKD have shown a remarkable frequency in hyperkalemia in advanced CKD stages, hyporreninemic hypoaldosteronic diabetic patients, renal transplantation (RT), and patients with RAAS inhibition (4, 6). Available information shows prevalence percentages ranging from 5 to 20% depending on the stage of CKD (13). The burden of hyperkalemia is remarkable not only in terms of prevalence, but also in terms of patient survival even for serum potassium levels only moderately increased (14, 15). Compensatory mechanisms may help improving tolerance to hyperkalemia in patients with CKD and a J-shaped correlation was found between serum potassium and overall mortality risk in non-dialysis patients (16). Moreover, new onset or persistence of mild-to-moderate hyperkalemia (potassium 5.0–6.0 mmol/L) during 12 months of observation significantly increased by 30% the risk of End Stage Renal Disease (ESRD) (4, 17, 18). It has been suggested that CKD patients adapt to elevated potassium concentrations through modifications in gastrointestinal secretions which may favor intracellular potassium storage, or by increasing insulin-mediated intracellular potassium uptake in splanchnic and peripheral muscle tissues (19). On the other hand, relevant are also the related economic costs that double in the presence of persistent hyperkalemia (20). In a recent retrospective cohort study of 1,499 patients with chronic hyperkalemia and CKD, heart failure or diabetes mellitus followed up for 36 months, the annual healthcare cost per patient with severe hyperkalemia has calculated as double compared to mild hyperkalemia (21).

Hyperkalemia in Patients With Renal Transplantation

Hyperkalemia is a common complication in RT with a reported incidence ranging from 25 to 44% in RT on calcineurin inhibitors (CNIs) (22). It is a life-threatening complication that may increase the length of stay (23). Hyperkalemia in the RT is usually seen in association with renal tubular acidosis and can occur even without insulin deficiency, metabolic acidosis, decreased eGFR or decreased distal sodium delivery. Insulinopenia or insulin resistance can disturb the shifting of potassium and glucose from the extracellular to the intracellular compartment and developing hyperkalemia in the post-transplant setting, especially in insulin dependent diabetics (22). Also the medications used post-transplant has been described as a major cause for post-transplant hyperkalemia in RT, even in those with a well-working graft. As mentioned above, CNIs are considered the major players in the development of hyperkalemia in the RT (24). The use of trimethoprim in standard doses can contribute to hyperkalemia by ENaC blockade. The use of RAASi is recommended after transplantation associated to a better patient and graft survival in RT, but the risk of life-threatening hyperkalemia is twice as the risk for recipients not on these medications (25). **Table 1** shows the most recent options of treatment of hyperkalemia in RT, highlighting the scarce number of patients included in various studies, especially with the new potassium binders.

Hyperkalemia in Patients With Resistant Hypertension

Resistant hypertension (RHT) is defined by the failure of the recommended treatment strategies (at least three drugs, including a diuretic) to reduce systolic (SBP) and diastolic blood pressure (DBP) values to $<$140 mmHg and/or $<$90 mmHg (30). The PATHWAY-2 randomized trial suggested that RHT is commonly a salt-retaining state, most likely due to inappropriate aldosterone secretion (31). In accordance with these findings, in recent years, an increasing body of evidence has shown a beneficial effect of MRAs, such as eplerenone and spironolactone, in improving BP control in patients with RHT. A recent meta-analysis based on data from multiple RCTs provides the evidence that add-on use of spironolactone in patients with RHT is effective in lowering SBP and DBP (32). Nevertheless, the hyperkalemic effect of MRAs in the treatment of hypertension limits their use in patients with advanced CKD. The latest guidelines from the European Society of Hypertension and the American Heart Association established that, besides optimal doses or best tolerated doses of an optimal therapy, the fourth-line treatment should involve a blockade of aldosterone through the use of MRAs (30, 33). However, it is suggested that the use of spironolactone must be restricted to patients with an eGFR $>$ 45 mL/min and a potassium $<$4.5 mmol/L. For these reasons, it is important to provide the nephrologist with tools to control potassium while implementing the cardiology guidelines in patients with CKD and RHT. Recently, the AMBER study

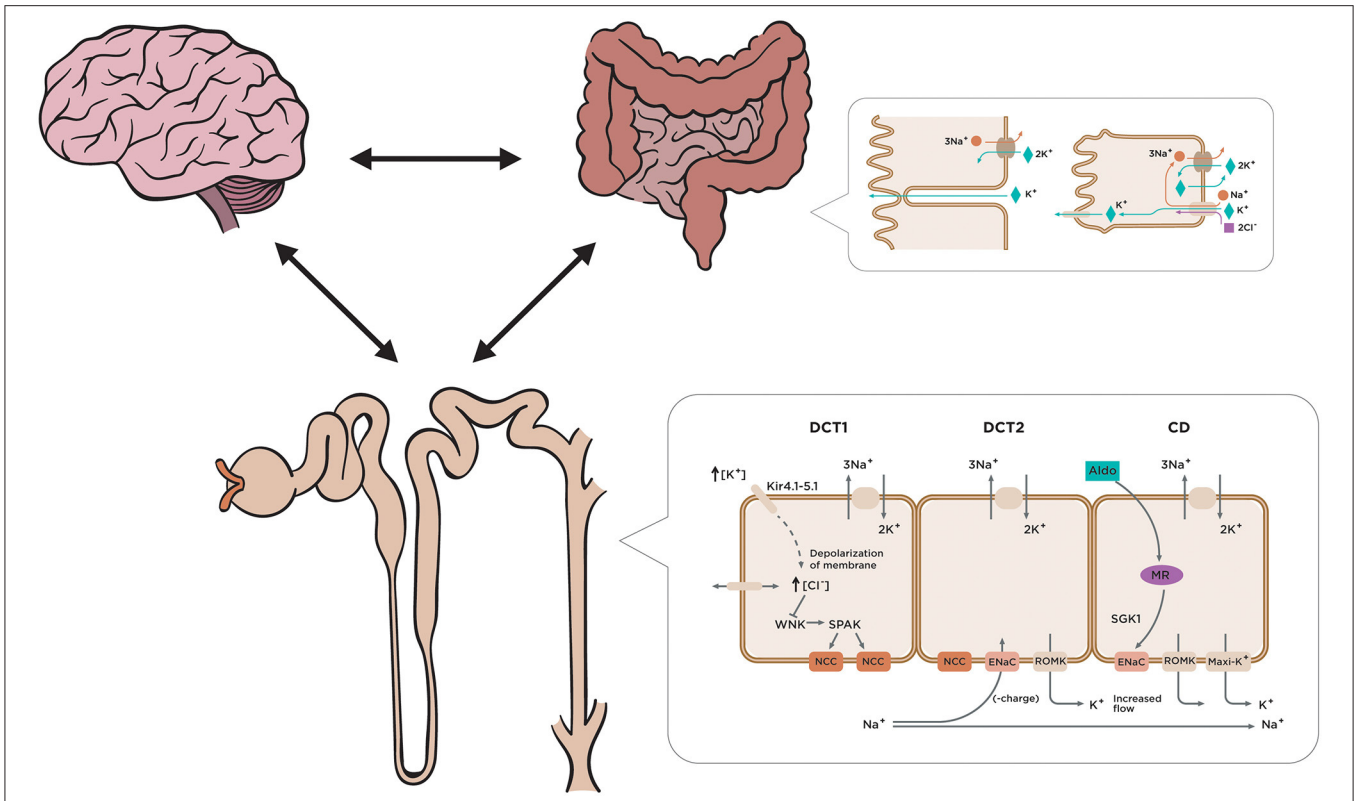

FIGURE 1 | Regulation of external and internal potassium balance. So-called feed-forward control refers to a potassium control pathway that responds to a pre-determined signal from the organism and is highly relevant to the mechanism of additional potassium regulation. The brain generates a regulatory circuit with the kidney and the colon that anticipates the presentation of food. Gastrointestinal-renal signals with a kaliuretic effect are generated, which will be able to mediate renal potassium elimination independent of changes in serum potassium and aldosterone concentration. Several co-transporters and ion channels are involved in the complex regulatory system of potassium reabsorption. The distal convoluted tubule mediates reabsorption of 5–10% of filtered potassium. Increased plasma K^+ concentration depolarizes cells in the proximal portion of the distal convoluted tubule (DCT1) through effects dependent on the potassium. Increased Na^+ delivery and flow to the downstream distal portion of the DCT where aldosterone sensitivity begins (DCT2, connecting tubule, and collecting duct) along with increased aldosterone levels drive potassium secretion.

has suggested that Patiromer enables the use of spironolactone, which effectively lowers systolic blood pressure in patients with RHT and CKD. Persistent spironolactone enablement in this population of patients has clinical relevance for the treatment of RHT (34).

WHAT ARE THE CONSEQUENCES OF DECREASING OR DISCONTINUING RAAS INHIBITORS?

The renoprotective effects of RAASi should be balanced against the associated risk of hyperkalemia, especially in patients with CKD. Although these drugs have shown an important benefit in patients with CKD, diabetic renal disease, HF and reduced ejection fraction (HFrEF) (35) and RT, hyperkalemia limits its use contributing to treatment withdrawal or underprescription (6, 36).

Current nephrology guidelines recommend RAASi as the primary therapeutic tool in patients with urine albumin excretion >300 mg/24 h or proteinuria >500 mg/24 h based on the proven renoprotective efficacy of these agents in proteinuric patients (37). RAASi enable preserving kidney function and delay the progression to ESRD in CKD (37, 38). Nevertheless, side effects limit their use, particularly associated to diuretics (39), dual blockade of RAAS ["Ongoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial," ONTARGET (40), or "Aliskiren Trial in Type 2 Diabetes Using Cardiorenal Endpoints," ALTITUDE] (41, 42), or neprylisin inhibitors (43). Hyperkalemia is more common than acute kidney injury (AKI) with an incidence ranging from 5 to 40%, and, moreover, patients who would otherwise benefit from RAASi either do not receive these medications, receive suboptimal doses, or discontinue therapy (44). An observational retrospective Japanese cohort study showed that 54% of patients discontinued RAASi after hyperkalemia (45). Controversy came up with the VA-NEPHRON-D trial that showed a strong trend to lowering the risk of renal disease progression with dual RAAS blockade vs. monotherapy (40). In the same way, MRA therapy could offer an additive proteinuria lowering effect but, like the dual blockage, increases the risk of hyperkalemia (46). Mortality rates are higher with

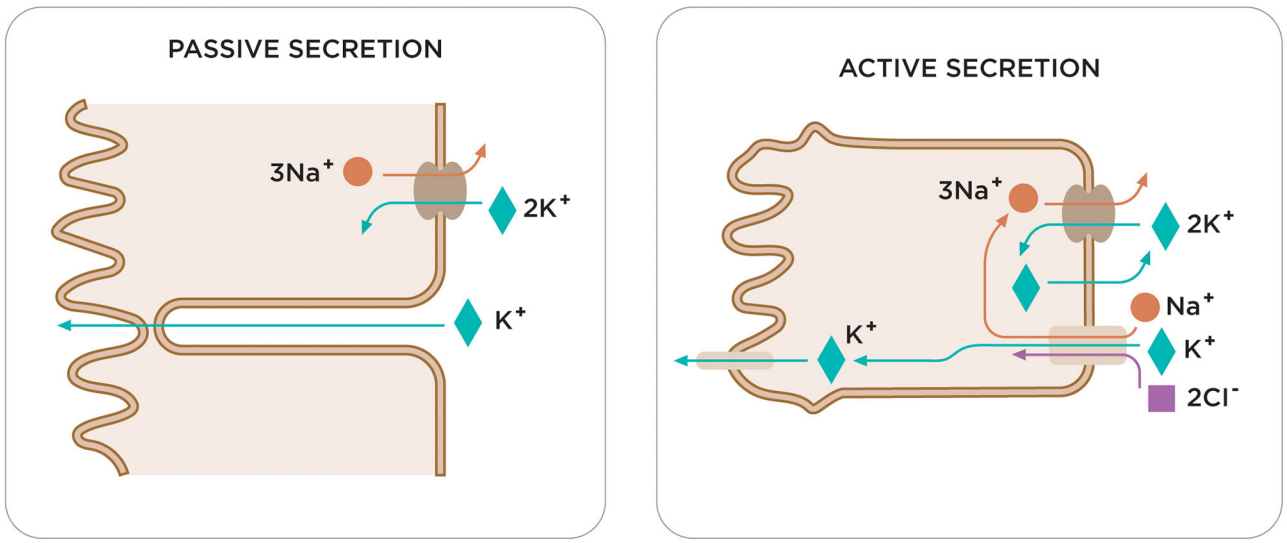

FIGURE 2 | Two compensatory mechanisms in the colon respond to elevated serum potassium levels in patients with CKD. Current studies highlight the existence of a feed-forward control in the regulation of potassium homeostasis, capable of causing rapid changes in renal potassium excretion. Among the different elements of this feed-forward control, the colon plays a fundamental role in the regulation of potassium. It is worth noting the different transport capacity of the potassium in the various segments of the colon or the different expression or activity of potassium channels on the membrane apical of the colon (BK channels). Although the role of the colon in excretion of potassium is not well-known yet, recent studies have found that in CKD the colon is responsible for a considerable increase in potassium removal, which is attributed to an increase in activity of the BK channels. There are two compensatory mechanisms in the colon in response to elevated serum potassium levels in patients with CKD: passive and active secretion.

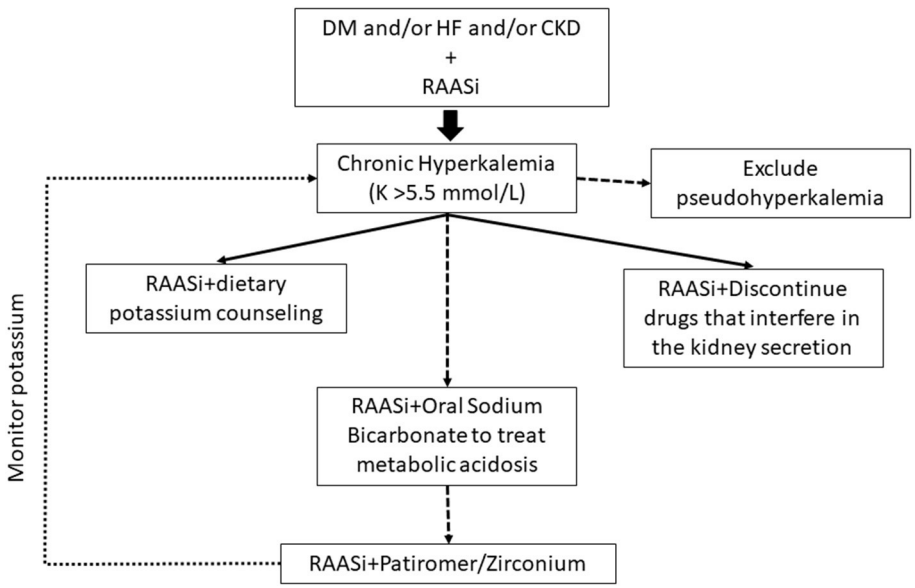

FIGURE 3 | Treatment algorithm for chronic hyperkalemia. Current recommendations regarding the management of chronic hyperkalemia (long-term elevated serum potassium) include the management of diuretics, modification of RAASi dose, treatment of metabolic acidosis with sodium bicarbonate, and removal of other hyperkalemia-causing medications. A team approach for chronic hyperkalemia, primary care physicians, nurses, pharmacists, or dietitians is optimal. The initiation of potassium binding agents should be considered in patients with chronic hyperkalemia despite optimized diuretic therapy and correction of metabolic acidosis. CKD, chronic kidney disease; DM, Diabetes Mellitus; HF, hearth failure; K, potassium; RAASi, renin-angiotensin-aldosterone system inhibitors.

suboptimal dosing/discontinuation of RAASi among patients with CKD, diabetes or HF compared with those with full RAASi dose (19, 47).

In routine clinical practice, we faced the conundrum of prescribing RAASi assuming the likelihood of hyperkalemia (up to 54%) or avoiding/discontinuing beneficial RAASi therapies.

TABLE 1 | Clinical studies of Patiromer and SZC in transplant patients.

Study	Drug	Population	Primary outcomes
Schnelle et al. (26)	Patiromer 8.4 g daily	SOT: kidney 73%, liver 21%, kidney-pancreas 3%, lung 3% *N* = 37	Moderate reduction in Potassium levels at week 4 and 12. Increase of TC levels.
Lim et al. (27)	Patiromer 8.4–16.8 g daily	Kidney transplants *N* = 17	K<5.2 mmol/l at last follow-up (84%). Seven patients required TC dose reduction.
Rattanavich et al. (28)	Patiromer 8.4–16.8 g daily	2 kidney transplants	Patiromer is effective and does not affect TC levels.
Winstead et al. (29)	SZC	SOT: kidney 45.7%, liver 40%, heart 5.7%, kidney-liver 5.7%, kidney-heart 2.9% *N* = 35	Potassium levels decreased by −1.3 mmol/l from day 0 to day 7. TC −0.54 ng/ml.

SOT, Solid Organ Transplantation; SZC, Sodium Zirconium Cyclosilicate; TC, Tacrolimus.

Although hyperkalemia has long been regarded as a reason for RAASi non-prescription, down titration, or discontinuation, it has been disregarded as a major topic in the literature (47). Nevertheless, the gap in information on the incidence of hyperkalemia between the real world and the world of clinical trials is well-known. A recent Portuguese study in patients with HFrEF highlighted their higher risk of developing hyperkalemia associated to comorbidities such as CKD, diabetes, or the use of dual or triple therapy including RAASi and concurrent HF therapy. In the same way the initiation of RAASi compared with calcium channel blockers may confer kidney and cardiovascular benefits among patients with advanced CKD from the Swedish Renal Registry (48). This point emphasizes the need to carry out studies on the impact of the reduction or suspension of RAASi on the prognosis of these patients (41). This significant body of literature highlights the need for a change of therapeutic strategy in maintaining the renal and cardio protection of RAASi in selected population as CKD patients, HF patients or diabetic patients, and the new potassium binders become essential treating hyperkalemia (**Figure 3**).

TREATMENT OF CHRONIC HYPERKALEMIA: SOMETHING OLD

In a recent meta-analysis of 1,217,986 participants in 27 diverse cohorts, hyperkalemia (serum K $\geq$ 5.0 mmol/L) was associated with significantly higher long-term risk of all-cause and CV mortality, and of ESRD. Although the definition of hyperkalemia is debated, ideal outcomes were observed with serum potassium concentrations of 4–4.5 mmol/L (15). Chronic management of hyperkalemia usually starts by dietary education and recommendation of a low potassium intake. However, it is well-known the renoprotective effect of potassium supplementation or intake by fruit and vegetable in CKD (49). It is well-known the renoprotective effect of potassium supplementation or intake by fruit and vegetable in CKD. Although the acknowledgment that dietary potassium restriction is a valid strategy to treat acute hyperkalemia, it's been hypothesized that potassium restriction as a general strategy to prevent hyperkalemia in persons with CKD may deprive patients of the beneficial effects associated with potassium-rich diets (9). The "KDIGO 2020 Executive Conclusions on Potassium Hemostasis and Disorders," did not find evidence that increased potassium intake, or liberalization of potassium restrictions, in patients with advanced CKD would be safer (9). The position statement of the Italian Society of Nephrology for Hyperkalemia management in CKD patients state that potassium $\geq$5.0 mmol/L must be considered pathologic in CKD and require careful follow-up and implementation of preventive and therapeutic strategies aimed at maintaining it in the optimal clinical range (50). On this regard, it is important to highlight the role of new potassium binders to overcome potassium restrictions in CKD (51, 52).

In healthy subjects the gastrointestinal tract contribution to potassium excretion is minimal (about 10% of the total), while in the case of kidney disease it may increase until it accounts for 50% of the total potassium excretion in patients on dialysis. The kidney (feedback control) and the colonic potassium handling (feedforward control) are strictly related, and it has been shown that the potassium enteral load may influence renal excretion (gut-dependent kaliuresis sensor, mentioned above) (53). This potassium secretory capacity makes of the colon a potential target for therapies aimed at treating and preventing hyperkalemia in patients with advanced CKD. Potassium binders make potassium unavailable in the distal colon for absorption by trapping it within the binder molecule, which is then excreted with the feces. Historically, the only options for promoting potassium elimination by the gastrointestinal tract have been limited to the "old" sodium cation-exchanging resin, sodium polystyrene sulfonate (SPS, Kayexalate; Sanofi-Aventis US LLC, Bridgewater, NJ). SPS was approved in 1958 but despite its common use, there is limited evidence demonstrating the effectiveness and safety in controlled studies (**Table 2**). The mixture with sorbitol at high concentrations, moreover, carries a risk of colonic necrosis and other serious GI adverse events (60, 61). A significant increase in the incidence of hospitalization for serious adverse GI events has been recently described in a large cohort of SPS users, when compared with matched non-users (62). Moreover, SPS initiation in adults with CKD stages has been associated with a higher

TABLE 2 | Clinical studies of sodium and calcium polysterene sulfonate (54).

Study	Drug	Population	Primary outcomes
Phase 4, randomized, double-blind, placebo controlled; (55)	SPS 30 g or placebo QD	Pre-dialysis (stages 3–5), EGFR<40 ml/min and potassium 4.5–5.5 mmol/L *N* = 33	Mean change in serum Potassium was superior to placebo in reducing serum potassium over 7 days vs. placebo: −1.04 mmol/l (−1.37 to 0.71 mmo/L)
Effect of SPS in CKD; (56)	4 single-dose SPS and placebo on 5 different test days	Patients with CKD *N* = 6	No significant effect of SPS on total potassium output
Randomized and crossover design; (57)	CPS vs. SPS therapy for 4 weeks	Pre-dialysis CKD 4–5 and Potassium >5 mmol/L *N* = 20	CPS safer for the treatment of hyperkalemia in pre-dialysis patients, because it did not induce hyperparathyroidism or volume overload
Randomized Control trial; (58)	CPS vs. SPS therapy	CKD stages 1–4 and Potassium >5.2 mmol/L *N* = 97	Both CPS and SPS can be used effectively for reducing hyperkalemia of CKD. CPS showed fewer side effects as compared to SPS
Prospective, Randomized, Crossover Study; (59)	CPS 3-week × 5 g/day	HD patients and Potassium >5.5 mmol/L *N* = 58	CPS decreases serum levels of potassium and phosphorus in HD patients with interdialytic hyperkalemia. CPS does not induce volume overload or disrupt electrolyte balance.

BB, Beta blockers; CKD, Chronic Kidney Disease; DM, Diabetes mellitus; CPS, Calcium polystyrene sulfonate; HD, Hemodialysis; HF, Heart Failure; HT, Hypertension; RAASi, Renin Angiotensin Aldosterone System Inhibitors; SPS, Sodium polystyrene sulfonate; QD, quaque die.

incidence of severe gastrointestinal adverse events, mainly ulcers and perforations, possibly in a dose-dependent manner (63).

In contrast, calcium polystyrene sulfonate (CPS) has long been used for patients with advanced CKD in some parts of the world. It avoids sodium retention and supplements calcium and may have an advantage over SPS. However, few clinical studies have evaluated the efficacy of CPS in the treatment of hyperkalemia (64) (**Table 2**).

TREATMENT OF CHRONIC HYPERKALEMIA: SOMETHING NEW

Two new colonic potassium binders have shown efficacy in lowering plasma potassium in recent clinical trials. Sodium Zirconium Silicate (SZC) and Patiromer have been introduced to manage hyperkalemia and promise to be more effective than SPS. In short and long-term studies involving patients on concomitant RAAS therapy, both SZC and Patiromer significantly lowered plasma potassium compared to placebo (**Tables 3**, **4**). By facilitating fecal potassium excretion, these new binders are likely to open new horizon for the treatment and prevention of hyperkalemia in high-risk patients, such as those in therapy with RAASi and/or MRAs. These new potassium binders may allow extending these therapies to patients, in whom concerns with hyperkalemia have limited their use. Patiromer was approved for the treatment of hyperkalemia by the FDA in 2015. Patiromer is a cross-linked polymer of 2-fluoro acrylic acid (91%), with divinylbenzenes (8%) and 1,7-octadiene (1%). It is used in the form of its calcium salt (ratio 2:1) and with sorbitol, a combination called Patiromer sorbitex calcium. Patiromer works by binding the free potassium ions in the gastrointestinal tract, mainly in the distal colon lumen, and releasing calcium ions for exchange, thus lowering the amount of potassium available for absorption and increasing the amount that is excreted with the feces (41, 79).

Under *in vitro* conditions mimicking the pH and potassium content of the colon, Patiromer binds 8.5–8.8 mmol of potassium per gram of polymer (80). In healthy volunteers, Patiromer administered for 8 days three times a day, caused a dose-dependent increase in fecal potassium excretion, with a corresponding dose-dependent reduction in urinary excretion (81).

The PEARL HF study explored the safety/efficacy profile of Patiromer in a large population of patients with HF and either a history of hyperkalemia resulting in the discontinuation of RAASi or MRA, or CKD treated with one or more HF therapies. Patiromer was associated with a significantly lower serum potassium levels, a lower incidence of hyperkalemia, and a higher proportion of patients on spironolactone after 4 weeks (65) (**Table 3**). Patiromer was also tested for 4 weeks in hyperkalemic CKD stage 3–4 patients undergoing stable treatment with one or more RAASi in the OPAL-HK study (66), showing a stable potassium lowering levels in two phases, regardless of age, gender, baseline potassium levels, diabetes, HF and maximal/not maximal RAASi dosage (82). An interesting

TABLE 3 | Clinical studies of patiromer (54).

Study	Drug	Population	Primary outcomes
PEARL-HF; phase 2, randomized, double-blind, placebo-controlled; (65)	Patiromer 15 g or placebo BID (+spironolactone 25 mg/d)[a]	CKD, HF, indication to initiate spironolactone, potassium of 4.3–5.1 mEq/L, receiving RAASi or BB *N* = 105	Mean change in serum Potassium: −0.22 mmol/l with Patiromer −0.23 mmol/l with placebo Mean difference vs. placebo: −0.45 mmol/L
OPAL-HK; phase 3, 2 stages: (1) treatment, single-group, single-blind (2) withdrawal, randomized, single-blind, placebo controlled; (66)	Patiromer 4.2 g (mild hyperkalemia) or 8.4 g (moderate to severe hyperkalemia) BID	CKD (stage 3–4), eGFR 15 to <60 ml/min, receiving RAASi and serum potassium levels of 5.1 to <6.5 mmol/L *N* = 237	Treatment stage: Mean change in Potassium at week 4: Mild hyperkalemia −0.65 mmol/L Moderate to severe hyperkalemia −1.23 mmol/L Withdrawal stage: Median change in potassium week 4: 0 mmol/l with patiromer +0.72 mmol/l with placebo
AMETHYST-DN; phase 2, randomized, open-label; (67)	Mild hyperkalemia: Patiromer 4.2, 8.4, or 12.6 g BID[b] Moderate hyperkalemia: patiromer 8.4, 12.6, or 16.8 g BID[b]	Type 2 DM and CKD (eGFR 15–60 ml/min) and serum potassium <5 mmol/l with RAASi *N* = 306	Mild hyperkalemia: Mean change in serum potassium: −0.35 mmol/l with Patiromer 4.2 g −0.51 mmol/l with Patiromer 8.4 g −0.55 mmol/l with Patiromer 12.6 g Moderate hyperkalemia: Mean change in serum potassium: −0.87 mmol/l with Patiromer 8.4 g −0.97 mmol/l with Patiromer 12.6 g −0.92 mmol/l with Patiromer 16.8 g
AMBER; phase 2, randomized, double-blind, placebo-controlled; (34)	Patiromer 8.4 g or placebo QD (+open-label spironolactone 25 mg/d)[c]	Uncontrolled resistant HT and CKD (eGFR 25–45 ml/min) and serum potassium 4.3–5.1 mmol/L *N* = 295	Patients remaining on spironolactone: 86% with Patiromer 66% with placebo More patients in Patiromer vs. placebo with serum potassium ≤5.5 mmol/L
DIAMOND; Phase 3 Patiromer for the Management of Hyperkalemia in Subjects Receiving RAASi for the Treatment of HF; (3)	Patiromer	Low ejection fraction heart failure (with or without CKD), receiving beta blocker, with either current hyperkalemia at screening or a history of hyperkalemia in the past year *N* = 2,400	Ongoing (NCT03888066)
PEARL-HD; Phase 4 Patiromer Efficacy to Reduce Hyperkalemia in ESRD; (68)	Patiromer	ESRD treated HD, two measured pre-dialysis K >5.5 mmol/l or one K >6.0 mmol/L *N* = 40	Ongoing (NCT03781089)
Single-center, randomized, open-label convenience sample pilot study in the ED; (69)	SOC or one dose of 25.2 g oral Patiromer plus SOC	Adult patients with ESRD and a serum potassium level of ≥6.0 mmol/L	2 h post treatment serum potassium with Patiromer was lower than SOC (5.90 vs. 6.51 mmol/L) and also 0.61 mmol/L lower than baseline
TOURMALINE: Open Label study, effect of Patiromer in hyperkalemic patients taking and not taking RAASi; (41)	Patiromer, 8.4 g/d to start, adjusted to achieve and maintain serum potassium of 3.8–5.0 mmol/L.	Hyperkalemia (potassium>5.0 mmol/L), receiving RAASi, BB or diuretics, CKD stages 1–5, HF and DM and/or HT *N* = 112	From baseline to week 4, the change in serum potassium was −0.67 mmol/l in patients taking RAASi and −0.56 mmol/l in patients not taking RAASi

CKD, Chronic Kidney Disease; BB, Beta blockers; DM, Diabetes mellitus; ED, Emergency Department; HF, Heart Failure; ESRD, End-Stage Renal Disease; HT, Hypertension; RAASi, Renin Angiotensin Aldosterone System Inhibitors; SOC, Standard of care; quaque die; BID, Bis in die.

[a]Spironolactone dosage increased to 50 mg/d after 2 weeks in patients with serum K >3.5 to ≤5.1 mmol/L. [b]Patiromer was titrated to achieve and maintain serum K≤5.0 mmol/L. [c]Spironolactone dosage increased to 50 mg/d after 2 weeks in patients with serum K >3.5 to ≤5.1 mmol/L.

finding of OPAL-HK study was the reduction of aldosterone levels independent of plasma renin activity, hyperkalemia or use of RAASi, followed by a reduction in blood pressure and albuminuria. This finding comes up with the idea that Patiromer may improve cardiovascular risk beyond the reduction in potassium levels (83).

The recent AMBER study, evaluated the use of Patiromer in patients with RHT and CKD. Patiromer allowed to 86% of the patients to remain on spironolactone with less hyperkalemia after 12 weeks, what has a critical relevance in the treatment of RHT (34). Finally, the ongoing DIAMOND study, that will end in 2022, will determine whether Patiromer treatment of HF subjects with hyperkalemia while receiving RAASi allows to continue RAASi considering not only safety or efficacy endpoints but primary "hard" endpoints (time to the first occurrence of cardiovascular death or hospitalization) (84).

Based on the above-mentioned large trials, Patiromer is recommended at a starting dosage of 8.4 g once daily,

TABLE 4 | Clinical studies of SZC (54).

Study	Drug	Population	Primary outcomes
Phase 2 study randomized, double-blind, placebo-controlled to assess safety and efficacy of SZC; (70)	0.3, 3, or 10 g of SZC three times daily for 2 Days or placebo	CKD (eGFR 30–60 ml/min) and moderate hyperkalemia (5–6 mmol/L), DM, HF, and HT *N* = 90	From baseline, mean serum potassium was significantly decreased by 0.92 ± 0.52 mmol/l at 38 h. Urinary potassium excretion significantly decreased with 10 g SZC as compared to placebo at Day 2
DIALIZE; phase 3b, randomized, double-blind, placebo-controlled; (71)	SZC 5, 10, or 15 g or placebo QD on non-dialysis days for 4 weeks.	HD 3 times, predialysis serum K>5.4 mmol/l (long interdialytic) and K>5 mmol/l (short interdialytic) *N* = 196	Maintenance of predialysis serum potassium 4.0–5.0 mmol/l during ≥ 3 of 4 hemodialysis sessions after long interdialytic interval without requiring rescue therapy: 41% with SZC 1% with placebo
ENERGIZE; phase 2, randomized, double-blind, placebo-controlled; (72)	SZC 10 g (3 doses in 10 h) or placebo	Emergency Department with potassium level >5.8 mmol/l *N* = 70	Mean change in serum Potassium at 4 h: −0.41 mmol/l with SZC −0.27 mmol/l with placebo Mean difference vs. placebo: −0.13 mmol/l
HARMONIZE; phase 3, 2-stage, randomized, double-blind, placebo controlled; (73)	Initial phase (open-label): SZC 10 g TID for 48 h Maintenance phase (double-blind): SZC 5, 10, or 15 g or placebo QD for 28 days	CKD <30 ml/min and hyperkalemia (potassium 5.1 mmol/L) *N* = 258	Initial phase (open-label): Mean change in serum potassium over 48 h: −1.1 mmol/l vs. baseline Maintenance phase (double-blind): 4.8 mmol/l with SZC 5 g QD 4.5 mmol/l with SZC 10 g QD 4.4 mmol/l with SZC 15 g QD 5.1 mmol/l with placebo vs. placebo for each dose
Phase 3 randomized, double-blind, placebo-controlled trial; (74)	Daily SZC (5, 10, or 15 g) or placebo for 28 days	HF patients with potassium ≥5.1 mmol/L *N* = 94	Compared with placebo, all three SZC doses lowered potassium and effectively maintained normokalemia for 28 days without adjusting RAASi
Phase 3 randomized, double-blind, two stages, placebo-controlled trial; (75)	SZC (1.25, 2.5, 5, or 10 g) or placebo three times daily for 48 h Initial phase and maintenance phase	CKD stage 3, serum potassium level 5–6.5 mmol/L *N* = 735	SZC showed a significant reduction in potassium levels at 48 h, with normokalemia maintained during 12 days of maintenance therapy as compared with placebo
Phase 2–3, randomized, double-blind, placebo-controlled, dose-response study; (76)	SZC 5, 10g, or placebo three times daily for 48 h	Japanese adults with hyperkalemia (Potassium>5.1 mmol/L) *N* = 103	At 48 h, the proportions of patients with normokalemia were 85.3, 91.7, and 15.2% with SZC 5 g, SZC 10 g, and placebo, respectively
ZS-005; phase 3, 2-stage, open-label; (77)	Correction phase: SZC 10 g TID for 24–72 h Maintenance phase: SZC 5 g QD	Hyperkalemia (two consecutive Potassium >5.1 mmol/L) *N* = 751	Correction phase: 78% of patients had serum Potassium 3.5–5.0 mmol/l at 72 h Maintenance phase: 88% of patients had serum Potassium <5.1 mmol/l over 3–12 mo
Post-hoc analysis of an open-label, single-arm trial compared SZC efficacy and safety; (78)	SZC 10 g TID for 24–72 h until normokalemia followed by once daily SZC 5 g for 12 months	Hyperkalemia (potassium >5.1 mmol/L) and CKD (4 and 5) vs. those CKD (1–3) >12 months *N* = 751	Correction Phase: 82% of patients achieved normokalemia in both eGFR within 24 h, 100 and 95% with eGFR <30 and ≥30 mL/min, respectively, within 72 h
PRIORITIZE-HF: Phase 2	SZC compared to placebo.	Patients with HF taking RAASi *N* = 182	Ongoing (NCT03532009).

BB, Beta blockers; CKD, Chronic Kidney Disease; DM, Diabetes mellitus; HF, Heart Failure; HT, Hypertension; RAASi, Renin Angiotensin Aldosterone System Inhibitors.

administered orally, which can be increased by 8.4-g increments per week, titrated up to a maximum of 25.2 g once daily (79).

Sodium Zirconium Cyclosilicate (SZC) is the most recently approved potassium binding agent with efficacy and safety established in phase 2 and 3 clinical trials of patients with hyperkalemia and CKD, HF, and/or diabetes or those receiving RAASi (**Table 4**). SZC is a non-absorbed, insoluble, inorganic crystal that selectively entraps potassium in the GI tract in exchange for sodium and hydrogen. Because of its high selectivity for potassium, SZC may bind it throughout the entire GI tract, and may produce a rapid potassium lowering effect. It has been estimated that 1 g of SZC binds about 3

mmol of potassium, and its activity begins within 1 h after taking (85).

SZC has been evaluated in several randomized trials and open-label long-term observational studies (**Table 4**). In the HARMONIZE study, hyperkalemic patients with CKD, HF, or diabetes received SZC for 48 h and showed a significant reduction in potassium levels. Ninety-eight percentage of patients achieved normokalemia after a week. Then, after achieving normokalemia, SZC reduced potassium during days 8 through 29 in a dose-dependent way (73, 78). The ENERGIZE trial tested SZC in patients admitted at the Emergency Department (ED) with acute hyperkalemia. SZC was administered up to three times during 10-h, in association with insulin and glucose compared to placebo. Reductions in potassium levels at 1 h with SZC or the placebo were similar, probably due to the predominant potassium-lowering effect of the concomitant insulin and glucose treatment, but a greater reduction in mean potassium was observed in the SZC compared with the placebo at 2 h suggesting the beneficial role of SZC in the emergency treatment of hyperkalemia (72).

The ZS-005 trial tested the long-term efficacy and safety of SZC in 751 outpatients with hyperkalemia. Ninety-nine percentage of the patients achieved potassium of 3.5–5.5 mmol/L in the correction phase, while more than 90% of the patients achieved a normal potassium level after 12 months of treatment (77).

In hyperkalemic patients undergoing hemodialysis, once-daily SZC on non-dialysis days effectively maintained pre-dialysis serum potassium levels on three out of four dialysis treatments, after long interdialytic period without rescue treatment over 8 weeks in the DIALIZE study. Interestingly, adverse effects, including interdialytic weight gain, were similar between the two groups (71). The ongoing PRIORITIZE-HF trial (estimated to end in 2020) (NCT03532009) will evaluate the effect of SZC compared to placebo in patients with HF taking RAASi.

SZC is dosed 10 mg TID in the acute phase and dropped to 5–10 mg daily thereafter. Given that SZC is insoluble, is not systemically absorbed and does not expand on contact with water, it is very well-tolerated.

Safety and Tolerability of News Potassium Binders

Patiromer and SZC are generally well-tolerated. Overall, Patiromer related adverse events occurred in ∼20% of the patients enrolled the major trials (54). These events include electrolyte disorders, such as hypomagnesemia and hypokalemia, and mild gastrointestinal symptoms, such as constipation (8%), diarrhea (5%), nausea and flatulence (65). Monitoring serum magnesium is recommended, considering supplementations for patients who develop hypomagnesemia while on patiromer (86). *In vitro* studies indicated that Patiromer may interact with some medications like ciprofloxacin, levothyroxine and metformin (87). Therefore, the administration of other oral medications at least 3 h before or 3 h after Patiromer is recommended.

SZC has not been associated with any serious adverse effects in RCTs. The most common were hypokalemia (5.8% of patients enrolled in different studies) and a dose-dependent increase in edema (88) cause because its sodium content. Monitoring signs of edema, especially in patients at risk of fluid overload, such CKD and CHF patients is recommended, and adjusting dietary salt intake and the dose of diuretics is probably required (89). In phase 2 and 3 trials, the incidence of gastrointestinal adverse events (nausea, constipation, vomiting or diarrhea) was similar between the treated group and the placebo group. However, as for patiromer, SZC should also not be used in patients with severe constipation, bowel obstructions or impaction, including abnormal postoperative bowel motility disorders. Because SZC may affect absorption of other oral medications with pH-dependent solubility due to a transient increase in gastric pH, SZC administration should be separated from these medications by at least 2 h (85),

SUMMARY

Hyperkalemia is a common complication in patients with comorbidities (CKD, diabetes, HF) and among those taking certain critical medications (RAASi and MRAs). The frequency and severity of hyperkalemia increases during CKD progression, and is associated with higher mortality. As RAAS inhibition augments the risk of hyperkalemia, improvement in potassium control could allow enhancing RAAS inhibitor use in patients with an evidence-based indication. Cation exchange resins used to treat hyperkalemia are unsuitable for long-term use owing to gastrointestinal side effects. Newer potassium-lowering therapies (Patiromer and SZC) can effectively and safely correct hyperkalemia and maintain normokalemia in patients with co-morbidities receiving RAASi therapy. The long-term efficacy and safety of newer potassium-binders remains to be ascertained. However, their use for cardiovascular and renal risk reduction in combination with RAASi therapy holds promise for renal and cardiovascular protection in non ND-CKD patients. Nowadays this represents one of the most important news released to the renal community.

AUTHOR CONTRIBUTIONS

EM, PC, and JM contributed equally in the writing and reviewing the paper. All authors contributed to the article and approved the submitted version.

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Effects of Sacubitril-Valsartan in Heart Failure With Preserved Ejection Fraction in Patients Undergoing Peritoneal Dialysis

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Aims: The effect of the angiotensin receptor–neprilysin inhibitor (ARNI) sacubitril-valsartan in patients with heart failure with preserved ejection fraction (HFpEF) remains unclear, and data on ARNI treatment in peritoneal dialysis (PD) patients are lacking. The present study was designed to assess the efficacy and safety of sacubitril-valsartan in patients with HFpEF undergoing peritoneal dialysis.

Methods and Results: End-stage kidney disease (ESKD) patients undergoing PD for 3 months with New York Heart Association (NYHA) class II–IV heart failure, ejection fraction of 50% or higher, and elevated levels of N-terminal pro–B-type natriuretic peptide (NT-proBNP) were assigned to receive sacubitril-valsartan. Patients were followed up regularly after medication treatment. The alterations in clinical and biochemical parameters before and after taking sacubitril-valsartan (generally 50–100 mg b.i.d) were investigated, and safety was also assessed. Twenty-one patients were recruited in this study. Compared with baseline levels, NT-proBNP levels [9769.0 (3093.5–21941.0) vs. 3034.0 (1493.2–6503.0), $P = 0.002$], and heart rate [80.0 (74.5–90.5) vs. 75.0 (70.3–87.0), $P = 0.031$] were markedly decreased after treatment with sacubitril-valsartan. Signs and symptoms of heart failure (21/21 vs. 15/21, $P = 0.021$) were obviously alleviated, NYHA classification and E/e' ratio showed a notable trend of improvement after 3–12 months of follow-up. None of the patients showed adverse drug reactions.

Conclusions: The present data suggested that sacubitril-valsartan treatment in patients with HFpEF undergoing PD was effective and safe.

Keywords: sacubitril-valsartan, heart failure, preserved ejection fraction, peritoneal dialysis, NT-ProBNP

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INTRODUCTION

Heart failure (HF) with preserved ejection fraction (HFpEF), also termed diastolic HF, is commonly defined as ejection fraction (EF) > 50% according to the criteria defined by the European Society of Cardiology (1); HFpEF accounts for approximately half of the cases of HF and is associated with substantial morbidity and mortality (2–4). Cheng et al. (5) showed that patients hospitalized

with HFpEF experience more readmission after discharge, demonstrated as 20% readmittance within 30 days and >50% within 1 year. Although HFpEF has a poor prognosis, there are no effective medications to treat HFpEF except for diuretics, which is a stark difference from HF with reduced EF (HFrEF) (6). HFpEF is a heterogeneous clinical syndrome that could be caused by varied aetiological factors, including ageing, obesity, coronary heart disease, diabetes, hypertension, and renal impairment (7). The pathophysiology of HFpEF remains incompletely understood, and cardiomyocytes, extracellular matrix, inflammation, and peripheral vasculature may contribute to the aetiology of diastolic HF (7, 8). In particular, patients with chronic kidney disease (CKD) are at increased risk of HF and are associated with worse outcomes (9, 10). Researchers (11) have found that CKD was more common in HFpEF than in HF with mid-range EF (HFmrEF) and HFrEF. Wang et al. (12) also showed that HFpEF is common in peritoneal dialysis (PD) patients (accounting for ∼55% of all HF), and is associated with an increased risk of mortality and poor cardiovascular outcomes in these patients compared with PD patients without HF.

Recently, PARADIGM-HF Clinical Trials demonstrated that the angiotensin receptor–neprilysin inhibitor LCZ696 was superior to enalapril in reducing the risks of death and hospitalization for patients with HFrEF (13). Notably, subgroup analysis demonstrated that sacubitril-valsartan also led to a slower decrease in eGFR in CKD patients with HFrEF (14). However, the effect of sacubitril-valsartan on HFpEF remains controversial. The PARAMOUNT-HF trial demonstrated that ARNI resulted in a lower level of N-terminal pro–B-type natriuretic peptide (NT-proBNP), a larger reduction in left atrial size, and greater improvement in the New York Heart Association (NYHA) functional class than valsartan (15). In contrast, recent data from the PARAGON-HF trial did not demonstrate a positive protective role of sacubitril/valsartan on hospitalizations for HF and death from cardiovascular causes among patients with an EF of 45% or higher (16). At present, data on patients with severe renal insufficiency treated with sacubitril/valsartan are lacking, since patients with severe renal insufficiency with a glomerular filtration rate (GFR) below 30 ml/min/1.73 m^2 of body surface area are usually excluded from trials. Thus, we undertook this study to investigate the effects of sacubitril/valsartan on patients with HFpEF undergoing PD.

METHODS

Study Design and Patients

This was a retrospective, self-controlled, observational study. Eligible patients in this study were 18 years or older with chronic HFpEF who underwent PD, and were referred to the Department of Nephrology in the PD Center of Sun Yat-sen Memorial Hospital between January 2018 and December 2019. The patients with end-stage kidney disease (ESKD) received percutaneous PD catheter insertion in our hospital and had been undergoing PD for more than 3 months. The PD modality was continuous ambulatory PD (CAPD) using glucose-containing dialysis fluid, which was exchanged four or five times daily. Inclusion criteria must simultaneously meet with following conditions: ESKD patients with residual renal function and undergoing CAPD > 3 months, experienced one or more episode of HF that required hospitalization. The exclusion criteria were as follows: acute coronary syndrome and pulmonary-associated disease including asthma attack, pulmonary embolism, or chronic obstructive pulmonary disease, inadequate PD, including irregular dialysis, overt hypervolemia, symptomatic hypotension or systolic blood pressure <100 mmHg at screening, and poor compliance with follow-up during the period of sacubitril-valsartan treatment. Patients with signs and symptoms of HF, NYHA class II–IV, an EF of 50% or higher within the previous 6 months, and elevated levels of NT-proBNP were prescribed sacubitril-valsartan. Sacubitril-valsartan was administered after consultation with the cardiologist and after informed oral consent of the patients together with previously prescribed medication for complications related to ESKD, including diabetes, hypertension, anaemia, and secondary hyperparathyroidism. Furthermore, ACE inhibitors were required to be discontinued 36 h before prescribing sacubitril/valsartan, and ARBs were discontinued except for three patients who received a low dose of valsartan simultaneously. No adverse reactions such as hyperpotassium and hypotension occurred in the above three patients. In this study, patients prior prescribed with aldosterone-antagonists continued to take as usual. Sacubitril-valsartan was progressively titrated, starting from a low dose to a tolerable maximum (generally 50–100 mg b.i.d), and no patients discontinued the drug during the follow-up. All patients were required to undergo serum potassium and creatinine tests once a week until stabilization, and were followed up for recurrent hospitalizations for HF and death from cardiovascular causes. The study protocol was submitted to our hospital's ethics committee and approved.

Data Collection

At baseline prior to drug administration, demographic and clinical parameters including age, sex, body mass index (BMI), duration of PD, Kt/V (weekly fractional clearance index for urea), primary renal disease, medical history, laboratory data, and medication use were obtained from the medical records and local laboratory analysis. Clinical parameters including blood pressure, heart rate, signs, and symptoms (defined as dyspnoea on effort, paroxysmal nocturnal dyspnoea, orthopnoea, oedema, rales, and third heart sound), and 24 h urine volume were collected.

Cardiac structure and function were assessed by two-dimensional echocardiography and NYHA functional class. There were two observers who carried out the echocardiographic measurements. The echo reports were cross-checked by two independent investigators. Parameters included left ventricular EF, E/e' ratio, TR (tricuspid regurgitation peak velocity), aortic dimension (AOR), ascending aorta (AAO), left atrium (LA), left ventricular diastolic diameter (LVDd), interventricular septum diastolic thickness (IVSd), and right ventricular diastolic diameter (RVDd). Additionally, cardiac biomarkers, including NT-proBNP, creatine kinase MB (CK-MB), cardiac troponin I and cardiac troponin T, were analysed. Chest radiography indexes were also obtained, such as cardiomegaly, interstitial or alveolar oedema, pleural effusion, vascular prominent hilum, and haziness of pulmonary vessels.

Adverse effects included hypotension (defined as a systolic blood pressure <100 mmHg), elevation of serum creatinine or decreased estimated GFR, hyperkalaemia, and angio-oedema.

The clinical parameters were collected in the same manner as above after sacubitril-valsartan prescription for at least 3 months.

Statistical Analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows (SPSS Inc., Chicago, IL, United States). Descriptive results of continuous variables are presented as medians and interquartile ranges (IQRs), and categorical variables are reported as percentages and numbers. For normally distributed quantitative data, paired samples *t*-test was employed to compare self-matching data, and for non-parametric data, the Wilcoxon matched-pair signed-rank (two samples) test was applied. Qualitative data were analysed using the chi-square text (Fisher's exact test). All tests were two-tailed, and a $P < 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics of the Study Subjects

From January 2018 to December 2019, 21 PD patients were recruited to participate in this study, and their baseline demographic, clinical, and laboratory characteristics are shown in **Table 1**. The mean age was 55.0 (38.0–61.0) years, male/female proportion was 14/7, mean BMI was 23.9 (21.0–26.2) kg/m^2, and the mean duration of PD was 16 (6–23) months. The underlying kidney diseases were chronic glomerulonephritis (38.1%), diabetic kidney disease (23.8%), hypertensive nephropathy (14.3%), obstructive nephropathy (9.5%), and others (14.3%).

TABLE 1 | Baseline characteristics of PD patients initially presenting before sacubitril-valsartan treatment.

Variables	All patients ($n = 21$)
DEMOGRAPHICS	
Age, years	55.0 (38.0–61.0)
Gender, male/female	14/7
BMI, kg/m^2	23.9 (21.0–26.2)
Duration of PD, months	16 (6–23)
Weekly Kt/V	1.8 (1.7–2.0)
CAUSES OF ESKD	
Chronic glomerulonephritis	8 (38.1)
Diabetic kidney disease	5 (23.8)
Hypertensive nephropathy	3 (14.3)
Obstructive nephropathy	2 (9.5)
Others	3 (14.3)
MEDICAL HISTORY	
Hypertension	21 (100)
Diabetes mellitus	5 (23.8)
Stroke	3 (14.3)
Previous myocardial infarction	2 (9.5)
Coronary heart disease	3 (14.3)
LABORATORY VALUES	
Cholesterol, mmol/L	4.47 (3.7–5.0)
Triglyceride, mmol/L	1.3 (1.1–1.7)
LDL-C, mmol/L	2.9 (2.2–3.2)
HDL-C, mmol/L	1.0 (0.8–1.2)
Apolipoprotein A, mmol/L	1.1 (0.9–1.3)
Uric acid, mmol/L	419.0 (352.0–517.5)
Albumin, g/L	29.3 (26.1–32.6)
HbA1c, %	5.1 (5.0–6.3)
MEDICATION USE	
Calcium channel blocker	21 (100)
ACE inhibitor or ARB	6 (28.6)
Beta-blocker	11 (52.4)
Diuretics	10 (47.6)
MARs	7 (33.3)
α-blocker	10 (47.6)

Values are proportion or median and interquartile range (IQR). PD, peritoneal dialysis; ESKD, end-stage kidney disease; Kt/V, weekly fractional clearance index for urea; ACE inhibitor, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blocker.

Comparison of the Characteristics of PD Patients Before and After Initiating Sacubitril-Valsartan

Twenty-one PD patients completed the self-comparison in terms of starting sacubitril-valsartan treatment. After 3–12 months of follow-up, compared with baseline levels, signs and symptoms of HF, including dyspnoea, paroxysmal nocturnal dyspnoea and orthopnoea, were obviously alleviated (21/21 vs. 15/21, $P = 0.021$), and heart rate was significantly lower than before starting sacubitril/valsartan ($P = 0.031$) (**Table 2** and **Figure 1**). Moreover, NYHA classification showed a notable trend of improvement after 3–12 months of follow-up, although it was not statistically significant, possibly because of the small sample size (**Table 2** and **Supplementary Figure 2**). Most importantly, NT-proBNP levels were markedly reduced after treatment with sacubitril-valsartan ($P = 0.002$) (**Table 2**, **Figure 2**, and **Supplementary Figure 1**). No significant differences existed, including systolic BP, diastolic BP, serum creatinine, serum potassium, phosphorus, eGFR, and echocardiography parameters, including LVEF (63 vs. 66%), E/e' (17.3 vs. 14.0), TR (257 vs. 237), AOR (21 vs. 21), LA (37 vs. 38), RVDd (20 vs. 21), IVSd (12 vs. 12), and LVDd (52 vs. 50), among patients before and after drug initiation.

Safety of Sacubitril-Valsartan

None of the PD patients showed adverse drug reactions such as hypotension, hyperkalaemia or angio-oedema. Additionally, there was no significant change in renal function estimated by eGFR [4.6 (3.9–6.5) vs. 4.4 (3.7–6.8), $P = 0.552$] (**Table 2** and **Supplementary Figure 3**), and serum creatinine [945.0 (790.0–1091.0) vs. 945.0 (674.0–1218.0), $P = 0.326$] (**Table 2**).

DISCUSSION

To our knowledge, this is the first report about treatment with sacubitril-valsartan in PD patients with HFpEF. Our findings demonstrated that sacubitril-valsartan significantly improved

TABLE 2 | Comparisons of the characteristics of PD patients before and after initiating sacubitril-valsartan with observation period of 3–12 months.

Variables	Before sacubitril-valsartan	After sacubitril-valsartan	*P*-value
CLINICAL PARAMETERS			
SBP, mmHg	144.0 (138.0–157.0)	151.0 (124.2–162.0)	0.925
DBP, mmHg	87.0 (75.5–101.5)	88.5 (76.5–102.8)	0.975
Heart rate, b.p.m	80.0 (74.5–90.5)	75.0 (70.3–87.0)	0.031
Signs and symptoms	21/21	15/21	0.021
24 h urine volume, ml	700.0 (225.0–1050.0)	700.0 (362.5–875.0)	0.061
LABORATORY VALUES			
Creatinine, μmoL/L	945.0 (790.0–1091.0)	945.0 (674.0–1218.0)	0.326
eGFR, ml/min/1.73 m^2	4.6 (3.9–6.5)	4.4 (3.7–6.8)	0.552
UACR, mg/g	2190.5 (1514.1–3933.0)	1670.4 (1217.2–5019.5)	0.345
iPTH, pg/ml	438.0 (211.0–597.0)	443.0 (215.0–914.0)	0.834
Calcium, mmoL/L	2.0 (1.9–2.2)	2.1 (1.9–2.3)	0.244
Hemoglobin, g/L	81.0 (74.0–92.5)	92.0 (79.2–102.5)	0.079
Phosphorus, mmoL/L	1.8 (1.3–2.2)	1.6 (1.5–2.3)	0.802
NT-proBNP, ng/ml	9769.0 (3093.5–21941.0)	3034.0 (1493.2–6503.0)	0.002
Cardiac troponin I, μg/L	0.0 (0.0–0.4)	0.0 (0.0–0.0)	0.655
Cardiac troponin T, pg/ml	86.5 (50.2–173.9)	72.6 (34.1–136.5)	0.799
Creative kinase MB, U/L	12.0 (9.0–16.0)	12.0 (8.5–15.5)	0.929
CARDIAC STRUCTURE AND FUNCTION			
NYHA functional class			0.656
I	0	2	
II	7	8	
III	13	10	
IV	1	1	
LVEF, %	63.0 (54.5–68.0)	66 (49.0–71.0)	0.875
E/e' ratio	17.3 (10.3–58.0)	14.0 (10.3–36.7)	0.291
e', cm/s	5.5 (3.0–10.0)	4.0 (3.0–7.0)	0.707
TR, cm/s	257.0 (218.8–331.0)	237.0 (197.0–299.0)	0.436
LVDd, mm	52.0 (49.0–60.0)	50.0 (47.0–61.0)	0.159
AOR, mm	21.0 (20.0–22.0)	21.0 (19.8–22.3)	1.000
AAO, mm	33.0 (30.5–35.0)	33.5 (30.8–35.3)	0.173
LA, mm	37.0 (35.5–40.5)	38.0 (35.0–41.0)	0.950
RVDd, mm	20.0 (19.5–22.0)	21.0 (19.0–24.0)	0.472
IVSd, mm	12.0 (10.0–13.0)	12.0 (10.0–14.0)	0.480
CHEST RADIOGRAPHY			
Cardiomegaly	5/21	3/18	0.702
Interstitial or alveolar edema	0/21	0/18	–
Pleural effusion	7/21	3/18	0.290
Vascular prominent hilum	1/21	1/18	1.000
Haziness of pulmonary vessels	7/21	6/18	1.000
ADVERSE EFFECTS			
Hypotension	0 (0.0)	0 (0.0)	–
Hyperkalaemia	0 (0.0)	0 (0.0)	–
Angioedema	0 (0.0)	0 (0.0)	–

Values are proportion or median and interquartile range (IQR). PD, peritoneal dialysis; SBP, systolic blood pressure; DBP, diastolic blood pressure; BUN, blood urea nitrogen; eGRF, estimated glomerular filtration rate; UACR, urinary albumin creatinine excretion rate; EF, ejection fraction; TR, Tricuspid regurgitation peak velocity; AOR, aortic dimension; AAO, ascending aorta; LA, left atrium; RVDd, right ventricular diastolic diameter; IVSd, interventricular septum diastolic thickness; RVDd, right ventricular diastolic diameter.

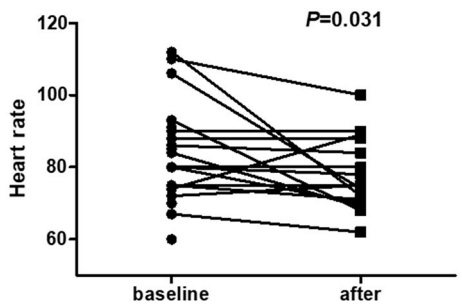

FIGURE 1 | Heart rate of PD patients before and after initiating sacubitril-valsartan with an observation period of 3–12 months. Paired samples *t*-test was employed to compare self-matching data on heart rate levels. Results show that compared with baseline levels, heart rate is markedly decreased after treatment with sacubitril-valsartan [80.0 (74.5–90.5) vs. 75.0 (70.3–87.0), $P = 0.031$].

and stabilized the cardiac function of CAPD patients with HFpEF, which is supported by clinical presentation and laboratory parameters, including strengthened exercise ability, fewer signs and symptoms of HF, and decreased NT-proBNP levels and heart rate.

Substantial evidence has confirmed that HFpEF is the most common form of HF in ageing people, which accounts for a growing proportion of patients with HF and is associated with high morbidity and mortality (17, 18). Epidemiological findings have shown that HFpEF causes almost one-half of the five million cases of HF in the United States (19). Similarly, HFpEF accounts

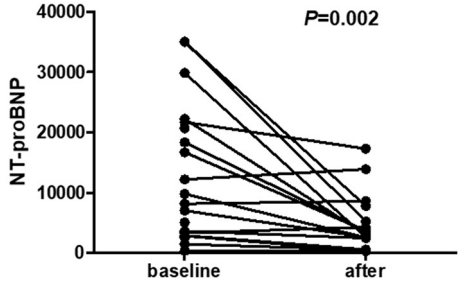

FIGURE 2 | NT-proBNP levels of PD patients before and after initiating sacubitril-valsartan with an observation period of 3–12 months. Wilcoxon matched-pair signed-rank (two samples) tests were applied to compare self-matching data on NT-proBNP. Results show that compared with baseline levels, NT-proBNP levels are markedly decreased after treatment with sacubitril-valsartan [9769.0 (3093.5–21941.0) vs. 3034.0 (1493.2–6503.0), $P = 0.002$].

for a large proportion of hospitalized patients with HF in China according to the published data drawn from a Registry Study of 169 participating hospitals. In this study, 31,356 hospitalized patients with HF participated, including 11,034 (35.2%) patients with HFrEF, 6,825 (21.8%) patients with HFmrEF, and 13,497 (43.0%) patients with HFpEF (20). Diagnosis of HFpEF was challenging and required assessment of clinical history, physical examination, natriuretic peptide testing, echocardiography data, and invasive exercise testing (21). Recently, the Heart Failure Association (HFA) of the European Society of Cardiology (ESC) produced an updated consensus recommendation—the HFA–PEFF diagnostic algorithm including clinical assessments (HF symptoms and signs), diagnostic laboratory tests (including NT-proBNP values), and standard echocardiography (22). Notably, a combination of echocardiographic measurements of cardiac structure and function and BNP levels were recommended. Echocardiographic indicators for diagnosing HFpEF included the average septal-lateral E/e' ratio, TR (tricuspid regurgitation peak velocity), and left atrial volume index (22).

Substantial data have demonstrated CKD is an independent risk factor for cardiovascular (CV) events (23–26). The overall rate of CV disease was higher in patients with CKD as compared to those without CKD, in particular, patients with ESKD have an increased incidence of CV death ∼10–20 times that of the general population. HF is more common in CKD patients (23, 27). Among hemodialysis and PD patients, the prevalence of HF is ∼40% (27).

Clinical data have confirmed that HF overlapping with CKD increases the hazard ratio of hospitalization, renal replacement therapy, and death (28). Available data found that patients with ESKD are at increased risk of HF, and CKD is common in HF, especially in HFpEF compared with other forms of HF, in recently published data (11), which might result from renal dysfunction leading to elevated intracardiac filling pressures, fluid retention, and exercise intolerance (16). The available limited data disclosed that HFpEF is highly prevalent in haemodialysis (81%) (29) and PD patients (55%) (12). However, there is still no effective therapy for HFpEF in contrast with HFrEF, where angiotensin converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs), angiotensin receptor neprilysin inhibitors, β-blockers, and mineralocorticoid receptor antagonists (MRAs) can reduce adverse outcomes associated with HFrEF (30).

The pathophysiology of HF in CKD and ESKD is very complex and includes multiple aspects associated with renal impairment: uncontrolled hypertension, left ventricular hypertrophy and fibrosis, excessive preload attributed to salt and water retention, increased afterload attributed to arterial stiffness and high output shunting through arteriovenous fistulae or grafts, neurohormonal activation, impaired iron utilization, anaemia, and bone and mineral disorders (31). Currently, neurohormones are considered to play a key role in the progression of HF in CKD patients except in preload, and left ventricular hypertrophy.

The first-in-class angiotensin receptor neprilysin inhibitor sacubitril-valsartan has been well-recognized to reduce CV and all-cause mortality, as well as the hospitalization rate in patients with HFrEF ($\leq$40%) compared with enalapril (13). In contrast to HFrEF, whether sacubitril-valsartan plays a protective role in patients with HFpEF remains unclear. Interestingly, during the 36 weeks follow-up in 301 patients with HFpEF in the PARAMOUNT-HF trial (15), NYHA class II–III and left ventricular EF $\geq$45%, demonstrated a significantly greater reduction in NT-proBNP from baseline to week 12, and in left atrial size at 36 weeks with sacubitril-valsartan than with valsartan. Moreover, NYHA classification was improved at week 36. Conversely, the PARAGON-HF trial, a recent, promising double blind randomized study in 4,822 patients with HFpEF, the results showed that sacubitril-valsartan did not result in a significantly lower rate of total hospitalizations for HF and death from CV causes among patients with HF and an EF of 45% or higher (16). Nevertheless, subgroup analysis yielded a different conclusion, showing that pharmacological treatments for HFpEF seemed to reduce the risk of HF hospitalization more in women than in men (32). Noticeably, subgroup analyses of the PARADIGM-HF and PARAMOUNT-HF trials all found that sacubitril-valsartan could delay the progression of renal function deterioration in HFrEF or HFpEF patients compared to renin–angiotensin–aldosterone system (RAAS) inhibitors, although there was a modest increase in the urinary albumin/creatinine ratio (UACR) after 8 months. Furthermore, in the subgroup analysis of PARADIGM-HF, sacubitril-valsartan led to greater risk reduction in CV endpoints in patients with CKD compared to enalapril (14, 33). Notably, patients with an estimated GFR (eGFR) <30 ml/min/1.73 m^2 were excluded in the PARAGON-HF trial as well as in the PARAMOUNT-HF trial. Recently, the UK HARP-III trial (United Kingdom Heart and Renal Protection-III) demonstrated that, in a wide range of people with proteinuric CKD, and an estimated GFR 20–60 ml/min/1.73 m^2, sacubitril-valsartan had no extra protective effect on kidney function or albuminuria compared with irbesartan, but it could lower blood pressure and cardiac biomarker levels, including troponin I and NT-proBNP (34). Based on the above inconsistent results, the effects of sacubitril-valsartan treatment in patients with ESKD and HF are unclear, especially for PD patients with HFpEF in whom the data are null.

Recently, the effects of sacubitril-valsartan on advanced CKD patients with HFrEF were investigated in a real-world clinical setting, and the results demonstrated that the

positive role of sacubitril-valsartan was supported by lower incidences of death from any cause, CV death, sudden death, and rehospitalization, including patients with advanced renal impairment (35). Thus, we evaluated, for the first time, the efficacy, safety, and tolerability of sacubitril-valsartan in patients undergoing PD with HFpEF (≥50%). In the present study, we found that the HF phenotype HFpEF seemed more common in PD patients, consistent with a previous study (11). Impressively, we observed a greater treatment effect on reducing heart rate, and cardiac marker NT-proBNP after sacubitril-valsartan use for 3 months or more. In addition, notable improvement of NYHA classification and signs and symptoms of HF, such as dyspnoea, paroxysmal nocturnal dyspnoea and orthopnoea, were found after medication use. Cardiac diastolic function showed a trend of improvement, demonstrated as a lower ratio of E/e' after treatment with ARNI and decreased by 19% compared to before treatment, although we did not find statistically significant improvements in echocardiographic indicators associated with HFpEF (E/e', TR, and LVDd), which might be related to the small sample size and short follow-up time. Available evidence has demonstrated that patients diagnosed with HFpEF are always complicated by concomitant abnormal cardiac diastolic function, which is associated with a poor prognosis due to the lack of effective therapy. In view of the safety of sacubitril/valsartan, renal function was of the greatest concern. Although patients with HFpEF in this study were in advanced renal failure, the majority of PD patients had residual renal function; thus, no obvious reduction in estimated GFR during follow-up and no severe hyperkalaemia or unstable serum creatinine was found during medication.

Some inevitable limitations also need to be considered. First, this was a small-sample, unblinded, non-prospective, single-centre study with shorter follow-up. Second, the included patients all progressed to the ESKD stage and were receiving PD, which means that volume load cannot be completely excluded, although we selected only PD patients with residual renal function demonstrated as average 24 h urine volume was 700 ml, and had already been undergoing CAPD for over 3 months without overt edema. Moreover, patients with inadequate PD and overt hypervolaemia were excluded. Furthermore, in this study, we adopted the self-control method to minimize possible confounding factors, and previous medications continued to be used. In the future, a large-sample, double-blinded, controlled study of PD patients with HFpEF should be performed to verify the effect of sacubitril/valsartan.

CONCLUSIONS

Our study suggested the effectiveness and safety of sacubitril-valsartan in PD patients with HFpEF. This is the first study about ARNI treatment for PD patients with HFpEF, and it may bring hope for these patients due to the lack of other effective methods at present.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Sun Yat Sen Memorial Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JC and YT acquisition, analysis, or interpretation of data. ZX and SF drafted of the manuscript. YC and YT critical revision of the manuscript. BL, JC, QH, YX, and AX statistical analysis. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.657067/full#supplementary-material

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MINI REVIEW
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Optimizing Diet to Slow CKD Progression

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Due to the unique role of the kidney in the metabolism of nutrients, patients with chronic kidney disease (CKD) lose the ability to excrete solutes and maintain homeostasis. Nutrient intake modifications and monitoring of nutritional status in this population becomes critical, since it can affect important health outcomes, including progression to kidney failure, quality of life, morbidity, and mortality. Although there are multiple hemodynamic and metabolic factors involved in the progression and prognosis of CKD, nutritional interventions are a central component of the care of patients with non-dialysis CKD (ND-CKD) and of the prevention of overweight and possible protein energy-wasting. Here, we review the reno-protective effects of diet in adults with ND-CKD stages 3–5, including transplant patients.

Keywords: chronic kidney disease, protein restricted diet, nutrition, salt restriction, renoprotection

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INTRODUCTION

Advanced chronic kidney disease (CKD) is a systemic disorder which is associated with high mortality and poor quality of life. Different treatments and lifestyle modifications are needed to avoid progression to kidney failure, which requires of kidney replacement therapy (maintenance dialysis or transplantation), and exceedingly costly therapy to Society (1–3). Chronic kidney disease progression is largely conditioned by hemodynamic and metabolic factors independent of the primary kidney disease, many of them, such as the high blood pressure (BP), the hyperfiltration, or the proteinuria are highly influenced by diet (4). Moreover, due to the kidney's unique role in nutrient metabolism, patients with advanced CKD are unable to maintain adequate nutrient homeostasis, developing metabolic disorders as sodium and volume overload, hyperkalemia, hyperphosphatemia, metabolic acidosis, altered hormone regulation, and inflammation. Accordingly, nutritional interventions should be a fundamental strategy in the treatment of patients with CKD (5–7).

In recent years, several studies, trials, and meta-analyses have evidenced the effectiveness of protein restriction and others nutritional interventions on kidney outcomes (8–17). This evidence was judged to increase the strength of recommendations for the nutritional management of patients with CKD in the 2020 update of the KDOQI guidelines (18). In this review, we aim to summarize recent studies on the role of diet, focusing on salt and protein restriction, as well as the use of supplements with essential amino acids (AAs) and keto analogs (KAs) to delay the progression of CKD and, at the same time, to preserve the nutritional status of patients with ND-CKD.

DIETARY REQUIREMENTS IN PATIENTS WITH KIDNEY DISEASE

Table 1 shows current recommendations for nutritional requirements in adult patients with non-dialysis CKD (ND-CKD), and kidney transplant recipients (KTR) (18, 19). A caloric intake of 25–35 kcal/kg/day is recommended to counteract the excess resting energy expenditure secondary to inflammation and comorbidities, as well as for preserving a neutral or positive nitrogen balance. However, this recommendation should be individualized according to the patient's profile, including age, lean body mass (which is the primary determinant of energy expenditure), physical activity, and the underlying etiology of kidney disease (20, 21). According to the 2020 KDOQI guidelines, the recommended protein intake for stable patients with ND-CKD 3–5 dialysis is 0.55–0.60 g/kg/day, which can be reduced to 0.28–0.43 g/kg/day if it is supplemented with 7–15 g/day of KAs and essential AAs. In the case of diabetic patients, guidelines suggest a higher protein intake up to 0.6–0.8 g/kg/day to glycemic control. Any intercurrent catabolic episode may require increasing energy and protein intake independently of CKD stage (22). Regarding protein quality, there is no consensus on whether the protein source impacts differently on the risk of CKD progression (18).

Kidney transplant recipients requires a different nutritional management depending on the post transplantation period. During the perioperative period, KTR need to adequate their intake of energy to 35–40 kcal/kg/day and of proteins up to 1.4 g/kg/day for at least 4 weeks (19) to compensate the increase in protein catabolism subsequent to the use of steroid and surgical stress. However, in the maintenance phase, the goal is to optimize the nutritional status with a slight reduction of the caloric intake down to 30 kcal/kg/day. Obese KTR should reduce their caloric consumption to levels lower than their energy expenditure, being values close to 25 kcal/kg/day an adequate approximation (19). Due to the lack of available studies in this population (23), it has been proposed that those with normal kidney function should follow similar recommendations to the general population, whereas for KTR with chronic allograft dysfunction, it is recommended to provide a protein-restricted diet just as in ND-CKD (19).

A modest sodium restriction (<2.3 g/day) is recommended for the management of CKD patients to achieve better volume control, reducing BP, and proteinuria synergistically with available pharmacologic interventions (18). A daily fiber intake of 25–30 g/day or more for CKD patients may be suggested, being this amount similar to recommendations for the general population (7). Potassium restriction in CKD may prevent from complying with this recommendation but in general terms CKD patients do not require aggressive dietary potassium restriction until advanced stages or if hyperkalemia risk is judged high (24–26). Recently, it has been suggested to avoid high potassium foods with poor nutritional value (i.e., bran products, or salt substitutes) and correct other causes of hyperkalemia, such as metabolic acidosis or use of renin-angiotensin-aldosterone system (RAAS) inhibitors, before restricting healthy foods (27). Acidosis is a key risk factor in the progression of CKD, being fruits and vegetables an alternative to oral alkali that may reduce the risk for volume retention and/or hypertension related to bicarbonate supplementation (28). Given the role of calcium balance and the serum phosphate in the development of cardiovascular calcifications, several experts recommend limiting total dietary calcium intake to 800–1,000 mg/day or

TABLE 1 | Nutritional requirements for patients with non-dialysis CKD according to 2020 KDOQI Guidelines (18).

	ND-CKD stage 3–5	Transplantation
Energy (kcal/kg ideal weight/day)[a]	25–35	25–35 in maintenance KTR 25 (obesity) 35–40 for the first 4 weeks after transplantation
Protein (g/kg/day)[a,b]	0.55–0.60 or 0.28–0.43 plus keto/amino acid supplementation	0.8
	0.80–0.90 (diabetes)	0.6–0.8 (CKD stages 3–5 T)
	1.0 (illness)	≥1.4 (for the first 4 weeks after transplantation or if high doses of prednisone is required)
Sodium (g/day)	<2.3	<2.3
Potassium[c]	Adjust dietary potassium intake to maintain serum potassium within the normal range	Adjust dietary potassium intake to maintain serum potassium within the normal range
Calcium (mg/day)	800–1,000[d]	Insufficient data to define optimal dietary calcium intake in KTR (research priority)
Phosphorus[e]	Adjust dietary phosphorus intake to maintain serum phosphate levels in the normal range	Adjust dietary phosphorus intake to maintain serum phosphate levels in the normal range
Fiber (g/day)	25–38	25–38
Vitamin D (IU/day)	600–800	600–800
Vitamin B12 (μg/day)[f]	2.4	2.4
Folic acid (μg/day)[f]	400	400
Vitamin C (mg/day)[f]	90 (M), 75 (W)	90 (M), 75 (W)
Vitamin E (mg/day)[f]	15	15
Vitamin K (μg/day)[f]	120 (M), 90 (W)	120 (M), 90 (W)
Selenium (μg/day)[f]	55	55
Zinc (mg/day)[f]	11 (M), 8 (W)	11 (M), 8 (W)

ND-CKD, non-dialysis chronic kidney disease; KTR, kidney transplant recipients; M, men; W, women.

[a] Energy and protein intake should be adapted to age, gender, level of physical activity, body composition, weight status goals, CKD stage, and concurrent illness or presence of inflammation to maintain normal nutritional status. If present, priority should be given to the correction of protein-energy wasting.

[b] Not enough evidence to make a statement on protein sources.

[c] Guidelines do not suggest specific dietary K range (restriction per se may favor other nutrient deficiencies). Before restricting healthy foods, other causes of hyperkalemia (acidosis, constipation…) should be corrected.

[d] Including dietary calcium, calcium supplementation, and calcium-based phosphate/potassium binders.

[e] When making decisions about phosphorus restriction treatment, consider the bioavailability of phosphorus sources (e.g., animal, vegetable, additives).

[f] No specific recommendations are provided by KDOQI guidelines. In the absence of evidence specific for persons with CKD, recommended Dietary Allowances for Adult General Population should apply.

less (including dietary calcium, calcium supplementation, and calcium-based phosphate binders) in adults with CKD 3–4 not taking active vitamin D analogs. Although phosphate intake to 800–1,000 mg/day (800–1,300 in KTR) was recommended previously (29, 30), new guidelines suggest adjusting dietary phosphorus intake to maintain serum phosphate levels in the normal range (18). Limiting processed foods with phosphorous-based additives and encouraging home-cooked meals from fresh ingredients (preferably plant-based foods) should be the first-line interventions for phosphorus restriction (31). As in the general population, vitamin D intake for CKD patients is recommended at 600–800 IU/day, but the optimal vitamin D levels in serum remain controversial (31, 32).

PRACTICE STRATEGIES: NEED FOR INDIVIDUALIZATED AND NOT TOO RESTRICTIVE DIET

Because a "kidney" diet comes with many restrictions, adherence to such a diet can be difficult and problematic (5). Too many restrictions should be avoided (18), as they can lead to poor intake. Modifications in diet are rarely required for patients with a GFR $\geq$60 ml/min/1.73 m^2. Such patients should be advised to follow the same dietary recommendations as for the general population [low sodium and refined sugar, avoidance of red and processed meats, and high content of fruits, vegetables, legumes, fish, poultry, and whole grains (33)]. However, in the later stages of CKD, diet must be modified across the spectrum of the disease, according to the type of renal replacement therapy if any, and the presence of other comorbidities (5).

ADEQUATE PROTEIN INTAKE TO SLOW CKD PROGRESSION

Biosynthesis and Degradation of Proteins

Protein digestion includes the process of breaking down proteins into their constituent AAs, which can be used either to create proteins or as an energy source, the later especially in times of starvation (34). Unlike carbohydrates and fats, if proteins are consumed in excess, the body has no capacity for their storage. As a result, excess AAs consumed are processed, being the hydrocarbon skeletons stored as fat, while the surplus of nitrogen must be removed, in as much as nitrogenous waste products are harmful, and there are no nitrogenous compounds in energy-transduction pathways (35). This is especially relevant for CKD patients, in whom consuming diets rich in protein leads to the accumulation of nitrogenous waste products and ions, causing the uremic syndrome. Transamination reactions are reversible and can thus be used to synthesize AAs from α-ketoacids (36, 37). However, human beings cannot synthesize all of the AAs (the so-called essential AAs), which must be supplied in the diet. A deficiency of even a single AA leads to a negative nitrogen balance. In this state, more protein is degraded than is synthesized, so more nitrogen is excreted than is ingested (38). The latter is particularly relevant for CKD patients under low protein diet (LPD), who may be at risk for developing protein-energy wasting (PEW) if not adequately monitored.

Effects of Amino Acids and Proteins on Renal Hemodynamics

Excess nutritional load of AAs dilates the "afferent" arteriole, increasing intraglomerular pressure and resulting in "glomerular hyperfiltration" and increased renal plasma flow (39). Glomerular hyperfiltration may contribute to progression of CKD (40–43). Conversely, a lower protein intake leads to greater constriction of the afferent arteriole, resulting in a reduction in GFR (**Figure 1**). In addition to hemodynamic-mediated mechanisms, protein restriction may protect against CKD progression by changes in cytokine expression and matrix synthesis (5).

Metabolic Adaptation to a Reduction in Protein Intake

Most authors agree that, in the absence of intercurrent disease, the protein requirements for patients with CKD are not substantially different from those of healthy subjects (44). In normal healthy adults, the minimum dietary protein intake to prevent negative nitrogen balance is approximately 0.6 g/kg/day. Maintaining this LPD or a very low protein diet (VLPD) of nearly 0.3 g/kg/day supplemented with AAEs and KAs are sufficient to achieve nitrogen balance and normal nutritional parameters (45, 46). As in healthy subjects, CKD patients, can improve AA utilization and nitrogen balance during LPD and VLPD by activating appropriate adaptive responses (47). These include normal anabolic responses to dietary protein restriction (suppression of AA oxidation) and feeding (stimulation of protein synthesis and inhibition of protein degradation), and the recycling of AAs derived from protein breakdown (35, 36). It is important to note that these diets require sufficient caloric intake to effectively use dietary protein (48).

Benefits of Protein Restriction on Kidney Outcomes

The benefits of LPDs include slowing the progression of CKD and reducing uremic symptoms and metabolic disorders (20, 21, 48, 49). Nearly 20 RCTs have assessed the effects of protein restriction on several renal outcomes, including CKD progression, proteinuria, phosphate levels, acidemia, and BP, which have been summarized in several meta-analyses and two Cochrane reviews (8–11, 15, 16, 50–56). Overall, the balance of evidence suggests a benefit of dietary protein restriction. The 2020 KDOQI guidelines recommend, in non-diabetic adults with CKD 3–5 who are metabolically stable, protein restriction for reducing the risk of progression to end-stage renal disease (ESRD) and death (evidence 1A), and improve quality of life (1C). In adults with CKD 3–5 and who have diabetes, the guidelines suggest (with an evidence grade of "opinion") a higher protein intake up to 0.8–0.9 g/kg/day (18). For the study of the effect of LPD on survival, they identified five RCTs. Three studies clearly indicated a beneficial effect of moderate restriction in dietary protein in the development of

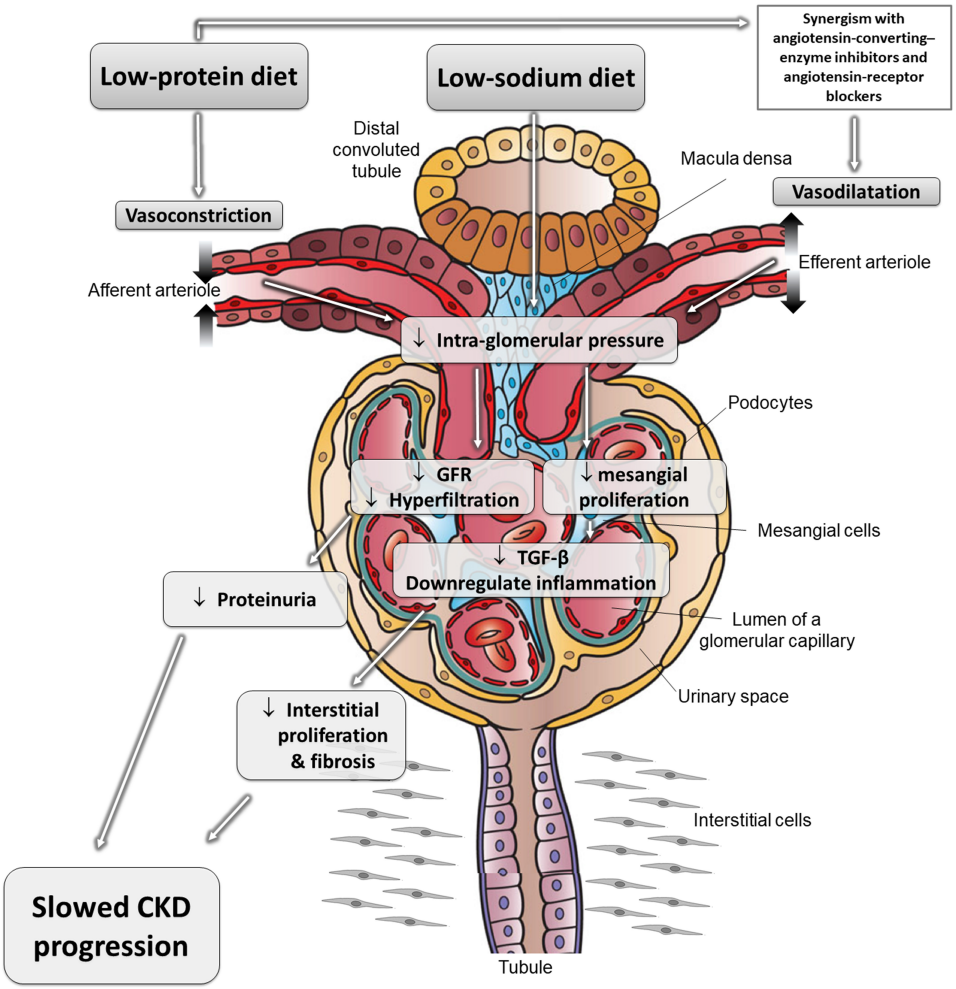

FIGURE 1 | The effects of different nutritional interventions to slow progression of CKD. Schematic representation of reno-protective mechanisms related to protein and diet restriction. These effects can be synergistic with the mechanisms of angiotensin-converting-enzyme inhibitors and angiotensin-receptor blockers, which dilate the efferent arteriole and reduce intraglomerular pressure and glomerular damage. Adapted from Kalantar-Zadeh and Fouque (5). CKD, chronic kidney disease; GFR, glomerular filtration rate; TGF-β, transforming growth factor beta.

ESRD/renal death (57–60), whereas two studies did not (61, 62). The results of the secondary analysis on the number of ESRD/renal death events combined from the three positive studies indicated a beneficial effect of protein restriction (OR 0.621; 95% CI: 0.391–0.985). For the effect of LPD on quality of life, they identified a single RCT that demonstrated how the group with protein restriction presented significantly higher scores for general health and state physical compared to the control group (62). For the study of the effect of VLPD supplemented with AAEs and KAs, they reviewed a total of 13 RCTs and one non-RCT (63–76). The pooled analysis indicated a probable overall benefit of VLCD+KAs supplementation for the development of ESRD/renal death in patients with CKD stages 3–5 (RR 0.65; 95% CI: 0.49–0.85). After the 2020 K-DOQI guidelines, a new systematic review and meta-analysis explored the effectiveness and safety of VLCD supplemented with KAs compared to LPD or a normal protein diet in patients with CKD (15). Seventeen RCTs with a total of 1,459 patients were included. KAs-supplemented VLCD significantly conserved GFR and reduced proteinuria, phosphorus and parathyroid hormone levels, systolic and diastolic BP, as well as serum cholesterol. Additionally, the analysis by subgroups showed how the VLPD supplemented with KAs was superior to the LPD with KA in the rate of decrease in GFR.

Evidence for the benefits of protein restriction in KTR remains elusive (18, 19). Reasons for this limited evidence include the common requirement for a LPD of being metabolically stable, a difficult condition to achieve for patients under immunosuppressive treatment. However, many of KTR have a reduced nephron mass and, therefore, could benefit from protein restriction (77). In a crossover RCT, Rosenberg et al. examined the effects of dietary protein restriction in 14 patients with

chronic kidney rejection. Low protein diet was associated with a significant improvement in plasma renin activity without any change in BP, GFR, or renal plasma flow. Studies are needed to establish the efficacy and the safe level of dietary protein restriction in KTR (78).

Challenges and Risks for Protein Restriction Diets

Major concerns for LPD/VLPD are adherence and safety (79–81). Patients' adherence to these dietary regimens is low, being the knowledge and the satisfaction that the patients obtain from diet compliance the main determinants of adherence (82). Even if the patients are well-informed about the benefits of LPD/VLPD, some of them may find it difficult to adapt their lifestyles to the diet. Accordingly, it is of paramount importance to educate patients about the role of diet therapy with protein restriction for the treatment of CKD, taking into account their eating habits and preferences (81, 83). Regarding nutritional security, it has been definitively demonstrated that PEW is extremely rare in patients with CKD provided that the energy intake is in the normal-high range (30–35 kcal/kg/day), the protein intake is increased in case of acute illness or hospitalization, and provided that a nutritional assessment is periodically conducted (7, 18, 80). Consequently, trained personnel (ideally a registered dietitian nutritionist) is strongly recommended to develop individualized dietary programs and routinely monitor and advise patients (18, 80, 82, 84). However, this approach is time and money consuming (80). An option for those centers in which dedicated personnel (i.e., dietitian) is not available or the expertise of the nephrologist in managing diets is not optimal may be found in simplified and practical approaches to LPD (80, 85, 86). Indeed, even small reductions in protein intake as low as 0.2 g/kg/day may also delay the need for dialysis treatment (10, 56, 80). In these Nephrology Units devoid of dieticians, protein intake may be assessed by urinary urea-N excretion, whereas weight and other anthropometric methods (e.g., skinfold thickness) may be useful for indirectly monitoring caloric intake and body fat (85, 87).

Effects of the Nature of Protein. Is a Vegetarian Diet an Option?

There is insufficient evidence to recommend a particular protein type (plant vs. animal) in terms of the effects on CKD progression or nutritional status (18). However, several observational studies have suggested that plant proteins may have more reno-protective effects than animal proteins. A diet rich in protein from plant sources may slow the progression of CKD (88–92), decrease proteinuria (93, 94), lower the level of uremic toxins (94–99), phosphorus intake, and the endogenous production of acid (89, 90, 100, 101). Moreover, that such diet could potentially improve survival (102). However, the confounding factors inherent in a diet rich in plant-based protein (i.e., higher intakes of vitamins and antioxidants) make it difficult to draw definite conclusions (103, 104). In a RCT, Garneata et al. compared a KA-supplemented vegetarian VLPD with conventional LPD in 207 CKD patients (89). The probability to reach the end point (i.e., KRT or a >50% eGFR reduction) was lower in the supplemented VLPD group than in the LPD group.

DIETARY SALT RESTRICTION

Dietary sodium intake is a modifiable factor that can impact on the risk of CKD progression as well as on cardiovascular disease in CKD patients. Previous reports have demonstrated the effect of sodium intake on fluid overload and hypertension, both predictors of kidney progression and cardiovascular remodeling (105–109). In addition, high sodium intake might have direct toxic effects on blood vessels (109, 110). High salt intake is also a well-established risk factor for hypertension in KTR and can result in decreased graft survival (2, 111).

Conversely, salt restriction RCTs demonstrate a reduction in BP and proteinuria, with potential benefits on CKD progression and survival (5). A Cochrane review summarized the effects of salt restriction in CKD (8). Unfortunately, these studies did not show collectively a beneficial effect of a lower sodium intake on mortality, cardiovascular events, or CKD progression, probably due to their short follow-up and the limited sample size. It is interesting to highlight a significant decrease in proteinuria associated to a low salt diet, that was observed in all the RCTs that reported this outcome (112–115). The 2020 KDOQI guidelines recommend in adults with CKD 3–5 (1B), CKD 5D (1C), or postransplantation (1C), a limitation in the sodium intake to <2.3 g/d (<100 mmol/d) to achieve a BP reduction, an improvement in volume control and a decrease in proteinuria levels (2A) (18). Nevertheless, the lack of long-term RCTs assessing the effectiveness and safety of dietary salt restriction on CKD progression and survival prevents any firm conclusions about these hard outcomes.

REDUCED PHOSPHORUS INTAKE

Phosphate-specific diet therapy provided by a dietitian may reduce phosphate levels in CKD, although overall certainty of evidence is low (116). However, association between hyperphosphatemia and adverse cardiovascular outcomes and CKD progression is robust in this population (117–124), also in KTR (125–127). Altogether, it seems reasonable to recommend adjusting dietary phosphorus intake to maintain serum phosphate levels in the normal range (18, 105).

DIETARY CALORIC RESTRICTION

Obesity constitutes a risk factor for diabetic and non-diabetic kidney disease (128), and there is some evidence suggesting that weight reduction through diet and lifestyle modifications could be considered as a component of the reno-protective regimen of obese patients with ND-CKD (129). Bariatric surgery reduces risk factors implicated in the progression of kidney injury in obesity and type 2 diabetes mellitus (130, 131). Dietary calorie restriction and exercise may reduce oxidative stress and inflammatory in patients with moderate to severe CKD

(132), whereas weight loss may lead to better BP control and reduction of the obesity-related glomerular hyperfiltration and proteinuria (133–136).

FIBER INTAKE AND PROBIOTICS

Evidence is emerging for the effects of fiber intake on uremic toxins generation (104, 137, 138). In a placebo-controlled RCT involving 30 patients with ND-CKD, total plasma p-cresol concentration was reduced by 40% after taking a synbiotic for 4 weeks (139). According to a recent meta-analysis involving eight studies of 261 patients with CKD stages 3–5D, probiotics supplementation may reduce the levels of p-cresol sulfate and elevate the levels of IL-6, thereby protecting the intestinal epithelial barrier of patients with CKD (14). However, it remains uncertain if increasing fiber intake to normalize intestinal microflora could delay CKD progression.

DIETARY PATTERNS AND CKD PROGRESSION

Historically, research recommendations and guidelines have focused primarily on modifying the single intake of micro or macronutrients (57). However, eating habits generally remain little over time for each individual, so that the overall dietary pattern may be more decisive for patients than an excess or deficiency in one specific nutrient (103). Adherence to healthy diet patterns as the Mediterranean and the DASH (The Dietary Approach to Stop Hypertension) diets has been linked to less rapid kidney function decline and favorable effects on cardiovascular morbidity and mortality in ND-CKD patients, including KTR (140, 141). Plant-based diets could also mitigate metabolic acidosis in patients with CKD and potentially slow the progression of kidney disease, but evidence is limited (104). Conversely, a Western diet (rich in saturated fat, red and processed meat, and sweets) has been associated with an increased risk of CKD progression and albuminuria (142). The evidence is not conclusive as not all studies associate healthy dietary patterns and risk of ESRD (143). Evidence from interventional studies is also very limited (144, 145). Altogether, there is a possibility that healthy dietary patterns may prevent the development of ESRD (5, 81, 146).

CONCLUSIONS

Close monitoring to adherence to dietary recommendations and frequent evaluation of nutritional status is fundamental in the management of patients with CKD, since it can affect important health outcomes, including CKD progression, quality of life, morbidity, and mortality. Within these nutritional measures, salt restriction, LPD and VLPD supplemented with AAs and KAs of nitrogen-free AAs, have been shown in recent meta-analyzes of RCTs to be effective in modifying the natural history of CKD, delaying the fall of the GFR, decreasing proteinuria, BP levels, or bone mineral disorder parameters, without increasing the risk of PEW. Patients' preferences and compliance have to be considered when prescribing LPD/VLPD in order to increase the adherence. Additional nutritional measures to delay CKD progression, some of them considered as experimental, may include the limitation of phosphate and calorie intake, the increase of fiber intake, and the promotion of healthy dietary patterns.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Chronic Kidney Allograft Disease: New Concepts and Opportunities

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Chronic kidney disease (CKD) is increasing in most countries and kidney transplantation is the best option for those patients requiring renal replacement therapy. Therefore, there is a significant number of patients living with a functioning kidney allograft. However, progressive kidney allograft functional deterioration remains unchanged despite of major advances in the field. After the first post-transplant year, it has been estimated that this chronic allograft damage may cause a 5% graft loss per year. Most studies focused on mechanisms of kidney graft damage, especially on ischemia-reperfusion injury, alloimmunity, nephrotoxicity, infection and disease recurrence. Thus, therapeutic interventions focus on those modifiable factors associated with chronic kidney allograft disease (CKaD). There are strategies to reduce ischemia-reperfusion injury, to improve the immunologic risk stratification and monitoring, to reduce calcineurin-inhibitor exposure and to identify recurrence of primary renal disease early. On the other hand, control of risk factors for chronic disease progression are particularly relevant as kidney transplantation is inherently associated with renal mass reduction. However, despite progress in pathophysiology and interventions, clinical advances in terms of long-term kidney allograft survival have been subtle. New approaches are needed and probably a holistic view can help. Chronic kidney allograft deterioration is probably the consequence of damage from various etiologies but can be attenuated by kidney repair mechanisms. Thus, besides immunological and other mechanisms of damage, the intrinsic repair kidney graft capacity should be considered to generate new hypothesis and potential therapeutic targets. In this review, the critical risk factors that define CKaD will be discussed but also how the renal mechanisms of regeneration could contribute to a change chronic kidney allograft disease paradigm.

Keywords: review, transplantation, kidney disease, graft survival, regeneration

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INTRODUCTION

CKD is and will be one of the biggest threats for health systems worldwide due to its prevalence and cost, especially when referring to kidney replacement therapy (1). Nowadays, kidney transplantation is the treatment of choice since it has shown its superiority at improving survival and quality of life and reducing costs and comorbidity (2). The incidence of this technique has increased in recent years reaching a median rate of 33 pmp in Europe (3). Therefore, there is a significant number of patients living with a functioning kidney allograft, particularly in high-income countries. On the other side, progressive allograft function loss has become one of the

most important causes of KRT requirement. Looking back, kidney allograft survival has undoubtedly improved. While in the 90's the median survival for kidney grafts was nearly 6.6 years, the current expected lifespan is about 8.8 years (4). However, this gain in allografts expected lifetime is mostly due to the improvement in short time graft survival (4). After the first post-transplant year, it has been estimated that chronic allograft damage may cause a 5% graft loss per year and this rate has seen very little decline in recent years (5). As a result, while short-term graft survival is relatively ensured, the tendency to improve long-term graft survival seems to have slowed down if we compare recent cohorts (3, 5).

From a clinical point of view, there is a wide spectrum of etiologies that explain graft damage (6). Currently, therapeutic interventions target those modifiable factors associated with kidney allograft attrition: reduce ischemia-reperfusion injury, minimize calcineurin-inhibitor exposure, control of classical risk factors or early identification of recurrence of primary renal disease. In addition, a lot of efforts are focused on improving immunologic risk stratification and on the development of new therapeutic strategies against kidney's rejection (7). Separately, all these etiologies can contribute in a major or minor form to the loss of renal mass that will end in chronic graft disease progression (8, 9).

Histologically, chronic graft damage means almost always fibrosis. Fibrosis develops in many patients during the first year after transplantation, especially in the first 3 months (10). Because most of the knowledge we have in this field is based on protocol biopsies, it is difficult to precisely titter the prevalence of fibrosis in allograft, but it varies from 17 to 66% according to the series (11, 12). However, fibrosis will continue to grow in the following years, especially in those patients with more fibrosis in former biopsies (12–14). This progression happens despite the absence or minimization of risk factors (13). Currently, alloimmunological damage is thought to be the main cause of kidney allograft fibrosis (15).

In short, a set of insults from different etiologies are capable of harming the kidney graft and altogether contribute to one common outcome. In this review, we will use the terminology chronic kidney allograft disease (CKaD) to refer to that global damage. We prefer the term CKaD in spite of other formulas (i.e., chronic allograft nephropathy) to emphasize CKaD as a global entity that summarizes the sum of all the damages of each etiology, not only immunological. In addition, we like the similarity with the term CKD as it helps to understand CKaD as a chronic, progressive, multifactorial disease, and above all, as a disease that requires a specific approach with nephroprotective strategies. In fact, despite the progress made in each area, clinical advances in terms of long-term kidney graft survival have been subtle (3) so new approaches are needed and the concept CKaD aims to provide a more holistic point of view. It is important to understand that chronic allograft deterioration is the consequence not only of the damage from various etiologies but also of the imbalance of kidney's own repair mechanisms (16). Based on recent investigations, it can be hypothesized that besides immunological and other mechanisms of damage, the graft's intrinsic capacity to repair could attenuate kidney damage and should be considered in new therapeutic approaches. In this review, the critical risk factors that define CKaD will be discussed but also how the knowledge on kidney mechanisms of repair could contribute to change the CKaD paradigm.

THE HARMS

There are several mechanisms of kidney allograft damage and the most relevant will be described in this part (**Table 1**). Individually, all of them cause nephron loss on a solitary kidney and potentially activate the key mechanism of kidney disease progression by producing glomerular hypertension and hyperfiltration. Renin angiotensin blockade and SGTL-2 inhibitors could alleviate this common mechanism of CKD progression in native and transplanted kidneys. However, this topic is beyond the scope of this revision.

Anti-body Mediated Rejection (ABMR)

Antibody-mediated rejection (ABMR) has been recognized as a major cause of organ-transplant failure during the past two decades (17). Nowadays, it is widely accepted that ABMR is the major risk factor for the development of CKaD and it can explain more than half of graft losses (18). Although there is some lack of knowledge about its natural history yet, understanding about ABMR has drastically increased in last years.

ABMR clinical impact increases poor graft and patient survival outcomes (19). In a large cohort of 885 kidney transplant recipients who underwent biopsies for graft dysfunction, patients with ABMR morphology showed an 8-year graft survival of 53% in C4d-positive and 66% in C4d-negative. This rate was significantly lower than the 81% seen in patients without any rejection features (20). To notice, the presence of complement-fixing antibodies, the extent of graft dysfunction at baseline and the presence of chronic lesions (capillary multilayering, arterial intimal fibrosis, glomerular basement membrane double contours) are associated with the poorest outcomes (21, 22). The main risk factor known for ABMR development is the number of HLA mismatches and poor adherence to medications.

Briefly, its pathogenesis is explained by the existence or *de novo* apparition of donor-specific antibodies (DSA). DSAs recognize some molecules expressed on the recipient's endothelium and this initiates an inflammatory cascade that leads to tissue damage. Among others, the classical pathway of the complement has been identified as an important way for antibody mediated damage (23). This simplistic explanation was the basis for the first definition of ABMR in the Banff classification from 1997 and allowed to stablish three standardized criteria for its diagnosis: First, identification of DSA. Second, evidence of endothelial damage. Third, proof of complement mediated damage through Cd4 staining.

Though, ABMR pathogenesis is more complex and further information has forced to readapt its definition until the actual one (24). Due to its predominant role in ABMR, DSA against HLA antigens are commonly screened in clinical practice, especially if rejection is suspected. However, DSA are not always targeted to HLA antigens but to other endothelial antigens (23). Thus, the current definition of ABMR accepts the diagnostic

TABLE 1 | Harms from different etiology contribute to CKaD.

HARMS		Current therapeutic strategy	Future strategies
ABMR		Plasma exchange + IvIg	Bortezomib (NCT01399593, NCT01567085) C1 esterase inhibitor Tocilizumab Mollecular matching organ allocation
CNI toxicity		CNI minimization strategies	Personalized CNI dosing
GN Recurrence	FSGS	Plasma exchange + Rituximab	Rituximab
	MN	Cyclophosphamide / Rituximab	Rituximab
	IgA Nephropathy	Reduce proteinuria BP control	
	MPGN	Eculizumab/Rituximab	Eculizumab/Rituximab
Diabetic nephropathy recurrence		Insulin Reduce proteinuria	iSGLT2
Infection	CMV	Therapeutic personalized strategy Viremia monitoring Donor/Rcipient serology screening	
	BK virus	Stepwise drug adjust imTOR + tacrolimus	BK virus IVIG Cidofovir, Leflunomide, others
IRI		Minimize cold ischemia time Perfusion machine	C1 esterase inhibitor CNI delayed introduction Others

even without an identified DSA as long as evidence of antibody mediated damage exists (24). In addition, although complement activation is the most deleterious mechanism of allograft injury (25), antibody mediated harm also occurs through other pathways (26). Consequently, ABMR can also be diagnosed in C4d negative cases if DSA are present or in chronic ABMR.

No therapeutic strategy has received FDA approval for the treatment of antibody-mediated rejection. Based on ABMR pathogenesis, plasma exchange plus IVIG to remove circulant DSAs is considered the current standard of care despite low quality evidence (27, 28). Two trials were designed to test the effect of Rituximab plus plasmapheresis and/or IVIG on ABMR in order to decrease the production of DSA. Both trials ended with underpowered results showing no benefit on short graft survival nor on GFR loss prevention (29, 30). Furthermore, targeting plasmatic cells has not shown the desired effect. The BORTEJECT trial was based on the anti-proteosome drug Bortezomib. No differences in GFR attrition were observed but more gastrointestinal and hematological adverse events were observed in the Bortezomib group (31). Two more randomized clinical trials are currently active and will test Bortezomib (ClinicalTrials.gov numbers, NCT01399593 and NCT01567085). The aforementioned impact of the complement cascade in ABMR has been another therapeutic approach. To that end, the anti-C5 antibody Eculizumab, has been used both for ABMR treatment and for ABMR prevention in desensitization protocols. Despite some trends suggesting marginal beneficial effects (32), at this point of time Eculizumab has also failed to prevent graft damage secondary to ABMR (33, 34). Instead of targeting the end of the cascade, another valid strategy could be point to the top through C1 esterase inhibitors. In a phase II study with plasma derived C1 esterase inhibitor, Montgomery et al. (35) showed a secure profile of the treatment and described that no transplant glomerulopathy was found in the treatment group whereas transplant glomerulopathy were present in 3 of 7 patients in the control group. Another therapeutic approach with promising result is the use of the anti-IL-6 antibody Tocilizumab, which in a French cohort of KTR with refractory cABMR showed a graft survival of 80% at 6 years, with a reduction in glomerulitis, peritubular capilaritis and C4d deposition in allograft biopsies after treatment (36). RCTs are currently going on to confirm these therapeutic options. Other new strategies tested in ongoing registered trials are corticotropin or double filtration plasmapheresis (13). However, it should be pointed out that there is a surprising discrepancy between the theoretic high ABMR prevalence and the lack of power of interventional clinical trials on chronic ABMR due to the lack of patients fulfilling inclusion criteria.

Due to the lack of a solid therapeutic approach, prevention of ABMR occurrence may be crucial. Nowadays, to talk about ABMR prevention means improvement of compatibility. Scientific literature is full of evidence showing the correlation with more HLA matches and better graft outcomes (37). However, HLA incompatible transplantations cannot be avoided in current health systems. The study of these HLA miss-matched transplants has identified that some of these miss-matches confers more risk (Unacceptable miss-match) while others are better tolerated (acceptable mismatches) (38, 39). Differences in permissibility between HLA-mismatched combinations may be explained by a different impact of amino-acid polymorphisms on peptide-binding features. Some tools have been developed to calculate this binding-site or epitope miss-match to further compatibility stratification. In a Dutch multi-center study, 2,918 donor–recipient couples were retrospectively stratified using

one of these tools (Predicted Indirectly ReCognizable HLA Epitopes presented by recipient HLA class II: PIRCHE-II) (40). For these donors–recipients couples, PIRCHE-II numbers were related to graft survival in univariate and multivariable analyses. Recently, DQ-antibody verified eplet miss-match has showed to be associated with an increased risk of dnDSA formation, kidney rejection, decline of graft function and graft failure (41). However, no study has reported benefits of these tools in a decision-making prospective protocol.

In short, ABMR is the main cause of long-term graft loss and so far, we have failed to dramatically improve its outcomes. In the absence of effective therapeutic approaches, prevention of ABMR occurrence plays a key role. Probably, molecular matching techniques will be included in organ allocation schemes in the next years. Early treatment and avoiding fibrosis could be essential to prevent long-term allograft attrition.

Calcineurin Inhibitor (CNI) Toxicity

The importance of CNI toxicity on CKaD has drastically decreased over the decades (42). Putting on some perspective, CNI toxicity leading to chronic damage was pointed out after Myers et al. (43) published a case-control study of cardiac transplant recipients under treatment with Cyclosporine for at least 12 months. In this study, 2 patients developed ESKD and the median eGFR of patients treated with CNI presented an important attrition in comparison with controls. Since then, CNI related toxicity leading to ESKD has been observed in other solid organ transplantation cases. Nevertheless, more than three decades have passed and there have been some changes in CNI paradigm: first, the better knowledge of antibody mediated damage and the development of new diagnostic techniques has brought out the important role of alloimmunity in CKaD (see above). Second, CNI levels have been taken to the minimum necessary to ensure safety for both patient and graft survival. Third, Tacrolimus more than Cyclosporine is the current CNI of choice in many cases reducing associated adverse events (44, 45). After all these changes, CNI toxicity has been displaced as a cause of CKaD by some authors. In 2012 Sellarés et al. (18) tried to find a diagnostic explanation beyond "chronic graft nephropathy" for every graft loss in a cohort of 315 patients. In that cohort, no graft failure was attributed to CNI toxicity.

How can CNI toxicity as cause of CKaD have gone from all to nothing? Probably, there are a set of characteristics that make CNI toxicity something like Santa: "you can only see if you believe in it":

a) On one hand, existence of CNI toxicity should be beyond doubt. Its damage mechanisms have been characterized, with an acute reversible damage related to an imbalance between vasodilators and vasoconstrictors and tubular vacuolization; and a chronic damage secondary to reduced glomerular blood flow with ischemic-reperfusion mediated injury (46). In the other hand, when large sparing CNI clinical trials were performed, differences in graft function seemed to be more related to the introduction of IL-2 antibody and MMF optimization than to CNI dosage (7, 47, 48).
b) CNI toxicity is dose-dependent (49, 50). However, the range of tacrolimus levels in those studies were between 5 and 25 ng/ml and the actual recommended target levels are much narrower, conferring less significant differences (51).
c) Histologically, chronic damage due to CNI toxicity shows arteriolar hyalinosis, interstitial fibrosis, tubular atrophy, juxtaglomerular apparatus hyperplasia, glomerular capsular fibrosis and global glomerulosclerosis. Unfortunately, none of these findings are pathognomonic, have not any specific marker and its presence can be explained by other common graft comorbidities.
d) Finally, some factors which are not measured in clinical practice could explain important differences in CNI susceptibility. For example, local concentration of Cyclosporine in kidney is associated with more susceptibility to toxicity and chronic damage (52). However, local concentration does not correlate with systemic blood levels and has a great interindividual variation due to genetic differences and drug interactions. Similarly, while some authors have described the presence of DNA SNPs that confers and increased risk of CNI toxicity (53), personalized CNI dosing according to genetic SNPs exists but is not globally extended.

In conclusion, CNI toxicity is an exclusion diagnosis without any specific marker. From a pragmatic point of view, while in other solid organ transplants chronic kidney damage could reasonably be attributed to CNI toxicity (54); in kidney transplantation is hard to find a situation in which graft loss occurs without any other comorbidity that could contribute to chronic renal damage. Thus, isolated chronic CNI toxicity diagnostic is always controversial and should be performed with caution. However, beyond a categorical diagnostic, CNI effects on renal function and chronic damage through fibrosis are probably always present, contributing to global graft attrition and therefore to CKaD.

Glomerulonephritis (GN) Recurrence

GN recurrence accounts for a large amount of kidney graft recipients evolving to end stage kidney disease. Its exact proportion varies from 3 to 18% of graft losses according to the series (55, 56) but probably, GN recurrence is under diagnosed in transplant recipients (57). There are four main entities that explain the majority of cases of GN recurrence: focal segmental glomerulosclerosis (FSGS), membranous nephropathy (MN), membranoproliferative GN (MPGN), and IgA nephropathy (IgAN). In 2017, Cosio and Cattran (58) published an outstanding review on this topic. So, in this paper we will focused on the key points and actualize the issue with current evidence (**Table 2**).

Recurrent FSGS

FSGS is a common cause of recurrence reaching 30% in 3 years. Of these, nearly 30% will advance to graft loss (58). FSGS recurrence occurs early after transplantation. Genetic forms of FSGS are less probable to appear in the allograft except for podocin mutations (NPHS2) (59). Clinical predictors of GN recurrence are those signs of disease severity in the primary

TABLE 2 | Recurrence profile of the main Glomerulonephritis (GN).

GN		Recurrence	Risk Factors	Graft loss	Treatment
FSGS		30 %	- Aggressive primary disease - Recurrence in former graft - Non-genetic (except NPH2 mutation)	30%	Plasmapheresis Rituximab
MN		30-75%	- Anti-PLA2R + at diagnostic - Elevated Anti-PIA2R titter at transplant or after - HLA-D and PLA2R specific mutations	50% of recurrence when graft loss occurs	Rituximab or Cyclophosphamide
IgA Nephropathy		30% Late recurrence	- Not well-defined - Steroid withdrawal	10%	Avoid steroid withdrawal
MPGN	Polyclonal Ig	30%	- Low C3/C4 blood levels	10%	Rituximab
	Monoclonal Ig	70%		50%	
	C3GN	70%	- alterations in the regulation of the alternative pathway of the complement cascade		Eculizumab (?)
	DDD	80–90%		25%	

FSGS, Focal segmental glomerulosclerosis; MN, Membranous nephropathy; MPGN, Membranoproliferative glomerulonephritis; C3GN, C3 glomerulonephritis; DDD, Dense deposit disease. The symbol (?) pretend to express that evidence is scarce.

episode: evolution to dialysis in <3 years after the diagnosis and high levels of proteinuria after transplantation. Classically, young age is associated with more recurrence though some new evidence contradicts this idea (60). Unfortunately, these factors are imprecise and the most reliable predictor of GN recurrence is the recurrence itself in a former graft.

Although the pathogenesis of primary FSGS remains unknown, the clinical profile after transplantation hardly suggests the presence of a circulating factor (61). In a blind attempt to remove this factor, plasmapheresis is commonly used to treat GN recurrence. The TANGO project aims to create an international collaborative data sharing network about GN recurrence in order to generate stronger evidence on the field (62). As a result of this project, Uffing et al. (60) showed that in current clinical practice the therapeutic response after plasmapheresis was nearly 50%. The second most common pharmacological approach seen in this study was the use of Rituximab. Whether the beneficial effect of Rituximab is through targeting B lymphocyte or directly to podocyte SMPDL-3b protein is currently under discussion (63). Curiously, in the TANGO study there is an almost despicable use of Cyclosporine despite previous data suggested a potentially beneficial effect combined with Plasmapheresis (64).

Recurrent MN

Globally, recurrence of MN is about 50%. However, its range varies from 30 to 75%. This variation is in close relationship with anti-PLA2R titter profile. Thus, primary MN anti-PLA2R mediated has more risk of recurrence than no antibody mediated disease (58, 65, 66). In addition, the presence of persistent elevated titters of antibodies after transplantation carries a higher risk of recurrence. Also, genetic factors have been associated to MN risk of recurrence. For example, the presence of SNP mutations on HLA-D and PLA2R loci confer more risk of recurrence when presented by the donor (67). Most recipients with recurrent MN are under CNI treatment, an effective therapy in native kidneys. Another line of treatment is the use of alkylating agents such as cyclophosphamide, but it has a high risk of medullar toxicity specially if treatment with MMF is not stopped. Current evidence suggest that the use of Rituximab is an effective and safe strategy for these patients, with the additional advantage that do not precise the adjustment of the other immunosuppressors (66, 68) though further studies are needed.

Recurrent IgA Nephropathy

Prevalence of recurrent IgA Nephropathy is 30% according to clinical reports (69). However, two major considerations must be made: first, unlike other primary diseases, IgA recurrence occurs later, even 10 years after transplantation, so some series may underestimate real prevalence due to short follow-up. Second, the histological immunofluorescence pattern characteristic of the disease appears in a very large proportion of patients much before clinical manifestation (70). Though, it should be noticed that some apparently normal donors (living or deceased) may have latent IgA deposits in the kidney (71). Currently, there is no way to know which latent IgA will evolve to clinical recurrence or will otherwise disappear. No robust evidence about other risk factors for IgA recurrence exists, although some authors suggest that primary disease's activity (presence of crescents, rapid evolution to ESKD) could be associated with higher rate of recurrence (72). IgA nephropathy impact on graft survival is mild and survival graft curves just differ from other entities beyond 10 years post transplantation (69). Histological Oxford classification could have prognostic value for allograft failure (73). No specific treatment is recommended for IgA recurrence. According to KDIGO guidelines, treatment should aim to reduce proteinuria, to optimize blood pressure and to reduce inflammation (74). Steroid withdrawal is associated with major incidence of recurrence and poorer graft outcomes (75). Studies from Japan reported favorable outcomes after tonsillectomy, but these results need to be confirmed (76).

Recurrent MPGN

Each type and subtype of MPGN has its particular evolution after transplantation (58). In general, MPGN due to Immunoglobulin

(Ig) deposition has a lower risk of recurrence (30–70%) specially if the Ig are polyclonal. Instead, recurrence of MPGN due to complement deposition rises to 70–90%. Regarding MPGN with Ig deposition, polyclonal Ig MPGN usually appears after some years and presents a slow course (77). Instead, recurrence by monoclonal Ig occurs more often, earlier and is associated with a more aggressive course that often leads to graft failure (77). On the other hand, both DDD and G3GN are associated with a very high risk of recurrence. In addition, C3GN is associated with poor graft survival (78). Treatment of Ig deposition associated MPGN targets Ig production through Rituximab. Though the absence of CT some series has shown promising response rates (79, 80). As C3GN and DDD patients have presented alterations in alternative pathway of the complement cascade, there are several reports on the use of monoclonal antibodies that inhibit activation of the C5 component of complement (eculizumab) with distinct results (81, 82) but so far, no RCT has been done.

Diabetic Nephropathy and Other Classical CKD Risk Factors

Diabetic nephropathy is not only the main cause of ESKD but is also associated with greater morbidity and mortality when it occurs in the kidney graft (83, 84). Post-transplant diabetic nephropathy (PTDN) shares pathophysiological and histological characteristics with primary diabetic nephropathy (85). However, the associated complications seem to develop at an accelerated rate (86). Older age, and obesity have shown to be the main risk factors for the development of PTDN (87). In addition, early low-grade proteinuria (<0.3 g/day) and hypertension (especially systolic blood pressure and elevated pulse pressure) have also been described as risk factors (88). Strategies with reduction or avoidance of steroids tend to decrease the incidence of PTDN (89). CNI are also associated with a high risk of developing PTDN. Among them, Cyclosporine seems to be less hyperglycemic according to the Diabetes Incidence after Renal Transplantation trial (DIRECT) (90). However, Tacrolimus continues to have a safer cardiovascular profile due superior lipid, blood pressure and kidney function effects (91).

The treatment of PTDN does not differ from native diabetic nephropathy. Screening for PTDM should be performed after starting treatment with glucocorticoids, sirolimus, or CNI (74). The choice of oral medication vs. insulin treatment must be done under the exact same rationale than in diabetic nephropathy. To mention, there's little evidence that basal insulin initiation to avoid hyperglycemic status immediately post transplantation can prevent further presentation of PTDN (92). In patients who develop PTDN with overt micro and macroalbuminuria, use of angiotensin inhibitors and statins are strongly recommended. This recommendation is an extrapolation from the effects observed in general population. However, in transplant population with proteinuria of any cause, treatment with angiotensin inhibitor has not demonstrated any benefit in long-term graft survival (93). Lately, iSGLT2 have shown remarkable results in decreasing cardiovascular risk and increasing survival both in general and in chronic kidney disease population (94–96). Given the enthusiasm that they have caused, it was predictable that some papers would appear defending that its use in kidney transplant recipients is safe and effective (97–100). Certainly, randomized trials will attempt this issue soon.

Beyond diabetic nephropathy, there are other risk factors of kidney disease progression but its impact in CKaD are not clearly defined. Briefly, hypertension is a common risk factor of renal disease progression, especially if it is associated with proteinuria. The use of RAAS blockade is one of the main strategies to reduce CKD progression. Hypothetically, the same mechanisms of damage occur on the kidney graft and may lead to CKaD but when it has been studied, results are not clear. While some retrospective cohorts associated the use of ACEI/ARB to better graft and patient survival (101, 102), prospective trials have not confirmed these association with graft survival (93, 103). The effect of obesity in graft outcomes is also controversial. Lafranca et al. (104) recently reviewed its effect, showing better outcomes for graft survival in patients with low BMI (<30). However, patient survival expressed in hazard ratios was in significant favor of high BMI recipients. Hyperlipidemia has also shown important outcomes related to patient survival but not related with CKAD. Though the anti-inflammatory effects of statins, associated with the inhibition of HMG CoA, have reported some good results in other solid organ transplantation (105) its use in kidney transplantation hasn't reported strong evidence in decreasing CKaD (106).

Infections

Infections are one of the most common complications after kidney transplantation due to immunosuppression (107). Besides of its impact in mortality, infections are a well-known risk factor for graft loss (108). Two different scenarios are especially important in relation to CKaD: first, the majority of infections are bacterial urinary tract infections (UTI). They occur more in the elderly due to immunosenescence, frailty, functional impairment and multiple comorbidities (109, 110). Female gender and obesity are also risk factors for the development of urinary infections. They usually occur within the first year after transplantation (111, 112). Despite screening of asymptomatic bacteriuria being common in Kidney Transplant Units (113), its treatment hasn't proved to reduce the incidence of acute pyelonephritis (114). Some series have demonstrated the association between the presence of UTI in the first year after transplant and poorer graft outcomes (108, 115). To explain this, we could extrapolate the effect of acute pyelonephritis on renal scarring and nephron mass loss observed in children (116). However, the association between UTI and loss of kidney functions has been observed also in patients with one or a few episodes of infection in which major renal mass loss is not expected (108). Another explanation is that infections and its clinical context could lead to an immunological imbalance triggering a rejection that would be the culprit for the dysfunction. Interestingly, in one of the few papers that has evaluated this issue, acute pyelonephritis was not independently associated with long term graft survival (117). Altogether, acute pyelonephritis is the most common complication in graft survival and has severe consequences on both patient's and graft's survival.

Second, viral infections play a relevant role in the development of CKaD. Cytomegalovirus (CMV) is the most infectious

pathogen in KT recipients and it has been associated with both poor patient and graft survivals as it has been associated with cardiovascular mortality and an increased risk for acute rejection (118). Luckily, the better knowledge and prevention strategies have led to a drastic reduction of the prevalence of CMV infection from 40–100% to 0–37% (118). On the contrary, human polyomavirus has significantly increased its prevalence and it is associated with an important number of graft losses. BK virus nephropathy (BKVN) is an entity that occurs in up to 10% of renal transplant recipients and can result in graft loss in up to 50% of those affected (119). BK virus is a human polyomavirus of high prevalence and low morbidity with an estimated prevalence in adults of 80– 90% (120). After infection, BK virus may establish itself in a state of non-replicative asymptomatic infection in the renal epithelial and urothelial cells (121). In the host, BK virus can reactive itself in context of both immunosuppression and cellular injury ("two-hit hypothesis"). Three stages of the disease have been described: BKVN starts with viral cytopathic effects (stage A), then leads to an inflammation phase (stage B) and finally tubular atrophy and interstitial fibrosis (stage C) (122). The last stage would explain irreversible graft damage and then CKAD. The lack of strategies to prevent or treat BKVN explain the ominous prognosis of the entity with respect to graft survival. Since treatment options are limited and have poor results, strategies to prevent BKVN are crucial. In kidney transplant units, it is common to perform BK virus surveillance by monitoring BKV viremia post-transplant at different time points (123). This monitoring combined with stepwise drug adjustment is the strategy most commonly used and has become the gold standard. We must assume that lowering immunosuppression implies an increased risk of T-cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR) (124–126). Based on observations from DIRECT (92) and TRANSFORM (127) trials, treatment with ciclosporin and mTOR seemed to be better than Tacrolimus, although the combination of everolimus and tacrolimus was associated with lower incidence of BKVN than MMF with tacrolimus (128, 129). Some other interventions have been attempted with IVIG, cidofovir or Leflunomide among others. Some papers show 90% clearance of BK viremia and sustained graft function after 12 months with IVIG (130–132). Cidofovir has shown to stop polyomavirus replications *in vitro*, as well as good results on achieving BK clearance in a total of 11 cases in the literature (133–135). Conversion from MPA/MMF to Leflunomide has shown therapeutic response in some studies (136) but not in others (137).

Cold Ischemia Time and Ischemia Reperfusion Injury (IRI)

After surgical removal of the organs for transplantation, kidneys are stored in a cold solution to preserve their viability. Cold ischemia time (CIT) is defined as the time that passes from surgical graft removal until the organ is warmed by recipient's blood supply after artery unclamping. CIT is a well-known risk factor for the development of delayed graft function (DGF) (138, 139) and acute rejection (AR) (140). This association implies both poorest graft and patient survival for those transplants with more CIT. Importantly, CIT itself does not seem to determine a decrease in long term graft survival (140–142).

Despite there are many definitions, DGF is the termed used in literature for those grafts that need at least one dialysis session in the first week after kidney transplantation. A longer CIT expanded criteria donors and terminal serum creatinine previous to donation are the main risk factors for DGF (142). The presence of DGF is detrimental for graft performance both in short and long-term. Interestingly, DGF is not only associated with a major rate of acute rejection episodes (143–145) but has also shown to be an independent factor for CKaD (143, 144).

Finally, IRI is a bimodal pathogenic way of tissue damage. Though cold storage and donor hypothermia try to minimize cell metabolism during CIT, some cells like renal tubular epithelial cells remain active in a state of hypoxia. In response to hypoxia, their mitochondria increase the production of reactive oxygen species and tend to develop intracellular acidosis. Thus, prolonged CIT will relentlessly lead to cell death and acute tubular necrosis: the ischemia damage. After reperfusion, the microvascular injury caused by ischemia enhances fluid filtration, with leukocyte plugging in capillaries and damaged endothelial cells secreting factors to favor inflammatory mediators and proteolytic enzymes (146). The global outcome of this ischemia-reperfusion damage is a harmful environment that through DAMPS and PAMPS enhances both innate and cellular immunity (147).

Thus, CIT is associated with a decrease in long term graft survival, but its impact is explained for the risk of developing an AR and DGF. Meanwhile, DGF is independently associated with both decreased long term graft survival and increased AR risk. IRI is a pathogenic phenomenon intimately related long CIT and explains DGF, chronic lesions and rejection risk (148).

Currently, no pharmacological intervention has proved to mitigate DGF neither in terms of duration nor its consequences. In clinical practice some strategies are used based on theoretical rationales without evidence. CNI delayed introduction to avoid toxicity and complement activation is one of these strategies although current evidence does not show significant differences (149). Combined to CNI delayed introduction, the use of Anti-Thymocyte Globulins (ATG) has been used to prevent AR and mitigate damage due to IRI. Even though ATG induction seems to have a better security profile in immunological high-risk patients, it is not clear that this grants any advantages in low-risk recipients compared with Basiliximab, not even in terms of reducing DGF (150). The results of PREDICT-DGF trial (NCT02056938, EudraCT #2014-000332-42) will provide more light about if ATG reduce DGF in comparison with Basiliximab in a selected group of patients at a high risk of developing DGF (151). The role of complement on IRI damage has also been tested as a therapeutic target. The use of C1 esterase inhibitor (C1INH) has shown good results in phase I/II studies, especially in those patients with more risk of DGF (152). Novel therapeutic options are also been tested like siRNA, mesenchymal stem cells or thrombin-targeted per-fluorocarbon nanoparticles (PFC-NP) (153, 154). However, all these strategies are far from being used in clinical practice.

Instead, the only strategy that has been proven effective to reduce CIT related DGF is the use of machine perfusion technology during kidney storage. Specifically, Tingle SJ et al. (155) have recently published a meta-analysis on this topic, showing that in deceased donors storage in hypotermic machine perfusion the incidence of DGF was reduced by 23% in comparison with static cold storage solution.

THE REPARATION MECHANISMS

As reviewed, lots of efforts are currently being put into understanding the whole spectrum of kidney graft damages better. On each field, small strides are being made but with a global vision, CKaD is still worrying. A common limit for all therapeutic strategies is the presence of chronic lesions. These lesions, histologically characterized by fibrosis and extracellular matrix (ECM) deposition, are considered scars which can no longer be repaired. Yet, from another point of view, scars are the result of a wound healing mechanism designed to repair or regenerate kidney damage.

Schematically, when damage of any etiology happens there is a loss of mass of functioning cells and consequently an attrition in organ function. Therefore, the healing process has to (1) stop the damage, (2) refill the gaps left by cell loss, and (3) compensate the loss of organ function (**Figure 1**). Probably, the magnitude and duration of the damage, the age of the organism and the dialogue among the effectors of kidney regeneration will determine the balance between healing and regeneration processes. These are the main regeneration mechanisms for kidney regeneration (**Table 3**).

Macrophages

One of the clearest examples of why the reparations mechanisms are doubled-edged swords is the study of macrophages in CKaD. There is a considerable body of evidence that both circulating and, mainly resident kidney macrophages play a crucial role in kidney inflammation and healing. These hematopoietic cells derived from the yolk sac seem to remain in the kidney during embryogenesis forming niches and they are activated when the harm occurs (156).

After injury, the presence of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) is recognized via Toll-like receptors (TLR) or patterns recognition-receptors (PRR) and activate a subpopulation of macrophages (M1 or activated macrophage). These activated M1 macrophages are proinflammatory cells capable of secreting pro-inflammatory cytokines (IL-6, IL-1, and TNF-α), superoxide anions and oxygen and nitrogen radicals (157). The amplification of the inflammation cascade contributes to fight against the cause of the injury but as a side effect it does also kill host cells increasing tissue damage. These cells will also contribute to kidney fibrosis through secretion of MMP-9, which increases tubular cell ECM transition via the β-catenin pathway (158). In animal models, the depletion of M1 macrophages ameliorated kidney injury (159). These results were also seen in rat models of acute rejection (160).

In addition, there are other populations of macrophages with distinct function in the reparation process. The alternatively activated macrophages (AAM or M2) usually appear later and have an anti-inflammatory function. Up to three types of M2 macrophages can be differentiated: M2a, stimulated by IL-4 and/or IL-3, are capable of secreting ECM components and therefore they also participate in wound healing and tissue remodeling. M2b have an immunoregulatory profile, inducing IL-10 secretion, upregulating antigen presentation through MHC II and downregulating IL-12, IL-6 and TNF. Finally, M2c macrophages are induced by IL-10, TGF-ß and glucocorticoids and produce anti-inflammatory cytokines (161). In a model with depletion of M2 macrophages, a reduction in tubular cell proliferation and repair is observed (159). Altogether, the macrophage system seems to contribute to kidney regeneration in two differentiated phases. First, immediately after kidney injury M1 population contributes to fight the cause of the aggression through inflammation. Second, once the aggression is neutralized, M1 and M2a contribute to restore the damaged tissue by the production of ECM while M2b and M2c populations reduce inflammation to restore kidney homeostasis.

However, an imbalance in this system can contribute to aggravate kidney damage both in animals and humans (159). For example, macrophage depletion is a useful tool to reduce kidney damage in *in vitro* models, yet a selective depletion to manipulate the M1/M2 ratio has different effects. Moreover, the activation of M2 macrophages that usually results in a reduction of the damage can be the main effector of kidney fibrosis when chronic or constitutive damage occurs (162). Interestingly, Wang et al. demonstrated that after kidney rejection, fibrosis is associated with a constitutive activation of macrophages (mostly M2) especially in chronic active forms. In addition, they identified as etiologic factors of fibrosis not only the production of ECM components but also a macrophage to myofibroblasts transition (163).

Cell therapy using the macrophage system has been attempted in animal models to control kidney fibrosis. The phenotype plasticity of this cell type is one of the main limitations. Cao et al. (164) failed to protect kidney function after infusion of bone narrow derived M2 macrophages, mainly due to phenotypic changes of the infused cells. Spleen macrophages, on the contrary, seem to be more stable and have demonstrated a beneficial effect in model of Adriamycin induced nephropathy.

Renal Progenitor Cells (RPCs)

In 2006, a population of progenitor cells surrounding the Bowman's capsule was identified. These cells, which are characterized by the co-expression of CD133 and CD24 markers, display a multipotent capacity of evolving into kidney specific cells terminally differentiated (podocytes and tubular cells) (165). Through differentiation, these RPCs exhibit the capacity to ameliorate acute kidney damage (166). At the same time, under certain conditions, as a sustained injury, these cells could also contribute to crescent formation (167) or glomerulosclerosis (16, 168). Sicking et al. (169) observed how after podocyte damage caused by doxycycline, RPCs from Bowman's capsule tended to leave their position to replace podocytes. After, the remaining

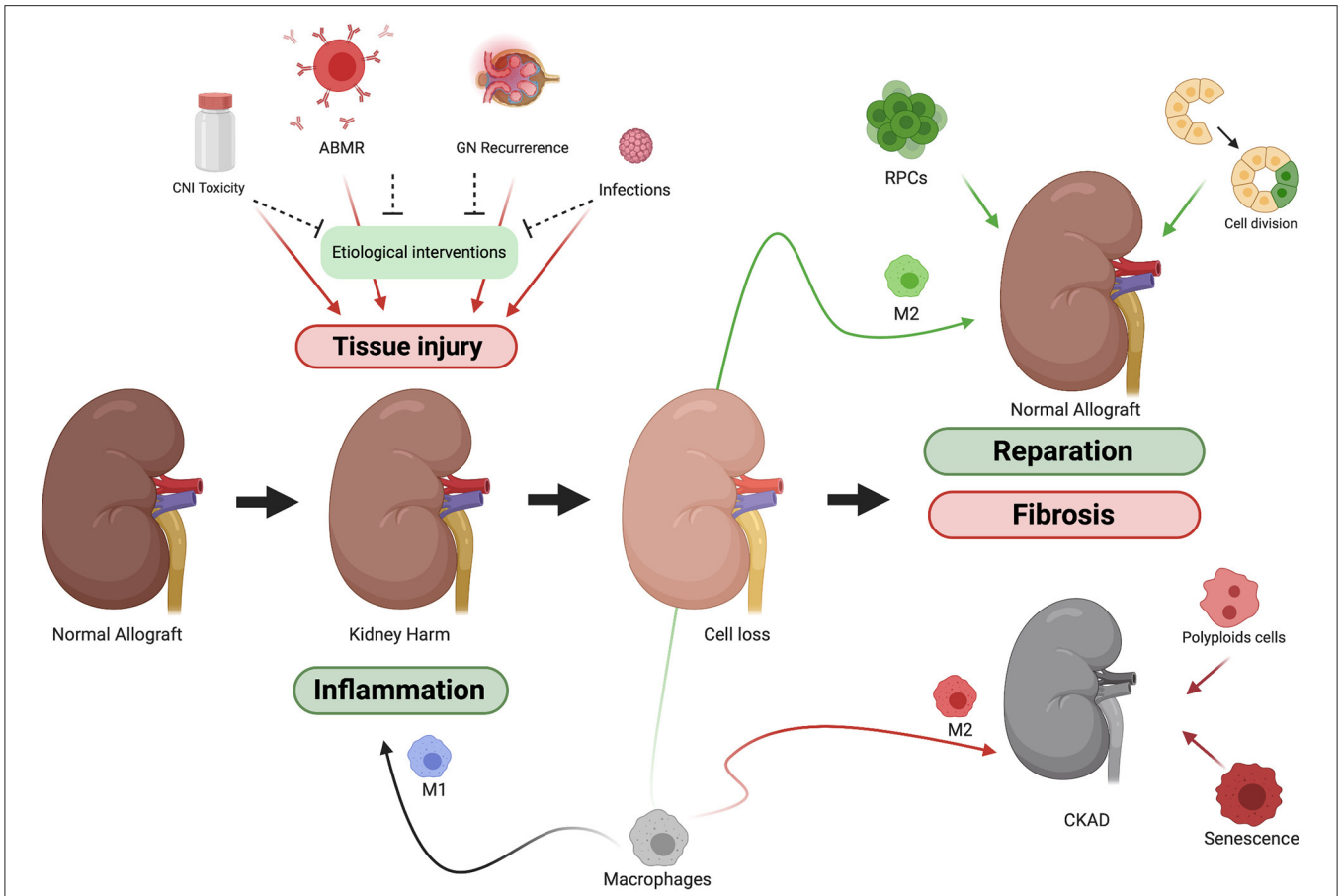

FIGURE 1 | Schematic representation of kidney allograft injury and reparation process. Kidney allograft is exposed to many insults. Although there are mechanisms to protect the graft from each of them, tissue damage is often present at the end. Once the tissue is injured the kidney's owns mechanisms of reparation come into action. To stop the insult, M1 macrophages among others will trigger and inflammatory response. After that, the damage caused (cell loss) must be compensated. Factors like the type and duration of the damage or the age of the donor will determine whether the reparation process tends more toward reparation or scarring (fibrosis).

RPCs formed cellular extensions to cover the denuded Bowman's capsule surface (expressing *de novo* CD44). Throughout the observation period, the induced proliferation of RPCs persisted, resulting in the formation of typical cellular crescents with periglomerular infiltrate. Thus, it can be hypothesized that RPCs act as a physiological renal regenerative mechanism that when overcome tend to scar in order to prevent further damage. Importantly, urinary detection of RPCs is feasible and has been already used to perform functional and genotypic studies without the need of invasive procedures (170, 171).

Recently, Manonelles et al. (172) published the first and only experience of RPCs isolation in kidney transplant recipients. In this study, a cohort of stable kidney transplant recipients with 6 months protocol biopsy was divided into two groups depending on the presence or absence of urinary RPC. A total cohort of 66 patients were then followed for 2 years. Interestingly, at the beginning of the study both groups were identical considering clinical variables, alloimmune response, renal function, albuminuria and graft pathology. However, uRPC+ group showed increased podocyturia and a higher rate of proliferating RPCs along the Bowman's capsule, suggesting that RPCs were proliferating to compensate a podocyte loss. Consequently, 2 years follow up evidenced poorer outcomes in the uRPC+ group with worse renal function, increased albuminuria, wider mesangial expansion and more severe interstitial fibrosis. If these results are confirmed, the detection of urinary RPCs could act as a marker of current injury much before clinical, immunological or histological damage is detected. Whether a therapeutic intervention at this time could prevent function graft attrition must be proved in further studies.

The Tubular Regeneration: Dedifferentiation and Polyploidization

Tubular epithelium is the kidney structure that most commonly suffers damage due to its high metabolic activity and decreased blood supply. Tubular regeneration model has great efficiency and is able to repair after an injury with no or little consequences. One of the important characteristics in this model is that tubular epithelial cells (TECs) are simpler than other kidney cells like podocytes. This feature allows TECs to easily dedifferentiate after an injury in order to do mitosis and repopulate the epithelia (173). This model for regeneration through dedifferentiation

TABLE 3 | Kidney regeneration mechanisms.

Reparation Mechanism	Potential therapeutic target	Current limitations
Machophages	Cell therapy	M1-M2 phenotype plasticity Cell obtention
Renal progenitor Cells	Podocite loss biomarker To control crescent formation	Pre-clinical phase
Tubular regeneration	Senolytic stretagies "AKI to CKD" minimization	Pre-clinical phase
Stem cells	MSC-based therapy	Legal/Administration restrictions Low-grade evidence

has been classically proposed for tubular regeneration but some objections have been done recently.

Lazzeri et al. suggested that the regeneration capacity of the tubule through dedifferentiation had largely been overestimated. Instead, they proposed a model of regeneration highly preserved in other human organs (174). This model is based on two main effectors: first, niches of progenitor cells can be found near the remaining tissue in a non-differentiated state. Second, after injury the remaining differentiated cells increase its content in DNA without undergoing mitosis through a specific cell cycle called endocycle. Thus, these cells can improve their performance reducing the loss of function of the organ while complete regeneration occurs. The same group has already demonstrated the presence of niches of cells that express progenitor markers next to the tubule and also the existence of endocycling cells, in both animals and humans (175).

In this model, the injury would cause a cell loss that implies decrease of organ of function of the organ. After that, progenitor cells would be mobilized from their niches and would finalize their differentiation process to repopulate the epithelia. In the meantime, to preserve organ function (or even survival) the remaining tubular cells would enter endocycle in order to duplicate its DNA content and keep the function of the organ. These cells though, will not be able to perform a normal cell cycle ever again; so, they are doomed to become a hyperfunctioning cell with a hypersecretory state of profibrotic mediators driving fibrogenesis, that is, cell senescence (176). These aberrant profibrotic cells would be responsible for fibrosis after AKI explaining, at least partially, the AKI-to-CKD transition. The balance between progenitors' repopulation and endocycling cells would then be crucial in a complete regeneration of the organ without later damage. Consistently, it has been observed that, the amount of progenitor cells in mammals decrease with aging and endocycling cells take a more important role in reparation processes, which would lead to greater fibrosis (177).

Stem Cells

To involve the kidney's own repair processes as a therapeutic tool is a strategy that has been already tested with promising results through the infusion of stem-cells. In general, based on their therapeutic potential, mesenchymal stem cells (MSCs) from bone marrow or other tissues are considered one the most powerful tools for treating several human diseases. MSC action is based not only on the capacity to differentiate into terminal renal cells but they have also been associated with the release of pro-mitotic, anti-apoptotic, anti-inflammatory and immunomodulatory soluble factors as well as to the mitigation of metabolomic and oxidative stress imbalance (178). A number of clinical trials have been designed to evaluate the safety and efficacy of MSC-based therapy and some good results have been observed in acute kidney injury trials (179). Very recently, the TRITON study (180) has used the infusion of autologous MSCs in 29 kidney transplant recipients to withdraw CNI. After 24 weeks of follow up, no differences were observed in graft function, acute rejection, graft loss, major adverse events or in kidney fibrosis. In a *post-hoc* analysis of this study, a longer follow up (5-year) was performed observing a more preserved renal function in the MSC group. Although there are already many limitations and restrictions to cell therapy, this study shows the feasibility of these treatments and which could be a cornerstone in future kidney transplantation therapeutic regimens.

CONCLUSION

Long-term graft survival is a major concern in the transplant community due to its clinical impact. Until now, lots of efforts have been put into identifying and precisely mitigate the impact of every potential graft damage. Consequently, advances in the treatment of ABMR are expected to report greater outcomes than BK virus or pyelonephritis prevention. However, from a pragmatic point of view, all the aforementioned harmful situations will be present in every kidney graft contributing to the final outcome: Chronic Kidney Allograft Disease. CKaD needs to be addressed by a holistic strategy. A therapeutic approach that considers to abrogate the mechanisms of graft injury and to improve the intrinsic mechanisms of kidney repair could have a transversal impact and lead to a significant improvement in CKaD. Further studies are needed to address this issue in the coming years.

AUTHOR CONTRIBUTIONS

SC reviewed the literature and prepared the manuscript. AM, MT, and AS reviewed the manuscript. JC supervised and reviewed the manuscript. All authors approve this manuscript for publication.

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Non-valvular Atrial Fibrillation in CKD: Role of Vitamin K Antagonists and Direct Oral Anticoagulants. A Narrative Review

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Atrial fibrillation (AF) is the most common arrhythmia in chronic kidney disease (CKD), with a close bidirectional relationship between the two entities. The presence of CKD in AF increases the risk of thromboembolic events, mortality and bleeding. Vitamin K antagonists (VKA) have been the mainstay of treatment for the prevention of thromboembolic events in AF until recently, with confirmed benefits in AF patients with stage 3 CKD. However, the risk-benefit profile of VKA in patients with AF and stages 4–5 CKD is controversial due to the lack of evidence from randomized controlled trials. Treatment with VKA in CKD patients has been associated with conditions such as poorer anticoagulation quality, increased risk of bleeding, faster progression of vascular/valvular calcification and higher risk of calciphylaxis. Direct oral anticoagulants (DOACs) have shown equal or greater efficacy in stroke/systemic embolism prevention, and a better safety profile than VKA in *post-hoc* analysis of the pivotal randomized controlled trials in patients with non-valvular AF and stage 3 CKD, yet evidence of its risk-benefit profile in more advanced stages of CKD is scarce. Observational studies associate DOACs with a good safety/effectiveness profile compared to VKA in non-dialysis CKD patients. Further, DOACs have been associated with a lower risk of acute kidney injury and CKD development/progression than VKA. This narrative review summarizes the evidence of the efficacy and safety of warfarin and DOACs in patients with AF at different CKD stages, as well as their effects on renal function, vascular/valvular calcification and bone health.

Keywords: chronic kidney disease, atrial fibrillation, Direct oral anticoagulants, Vitamin K antagonists, anticoagulant-related nephropathy

INTRODUCTION

Atrial fibrillation (AF) is the most common cardiac arrhythmia and is associated with an increased risk of stroke or systemic embolism (S/SE) (1). Moreover, AF-related stroke is usually more severe, associated with greater disability, increased mortality and longer hospital stays (2).

Chronic kidney disease (CKD) is also common, with increasing prevalence worldwide (3, 4).

Atrial fibrillation is more prevalent in CKD, in both non-dialysis (ND) (16–21% and increasing with higher CKD stages), and particularly end-stage kidney disease (ESKD) patients on dialysis (17–27%) (5). In fact, the relationship between AF and CKD is bidirectional: AF is associated with an increased risk of CKD [reduced glomerular filtration rate (GFR) or albuminuria], while CKD is linked to increased incidence/prevalence of AF (6–9). Atrial fibrillation and CKD share several risks factors (older age, hypertension, diabetes, prevalent cardiovascular disease); while inflammation, frequent in CKD, has been involved in initiation/perpetuation of AF (10). Patients with severe CKD frequently have cardiac abnormalities favoring AF development (left ventricular hypertrophy, myocardial fibrosis or left atrial enlargement) (11). Furthermore, rapid shifts in serum pH and electrolytes during hemodialysis (HD) sessions may be a trigger for AF.

Oral anticoagulation therapy (OAT) with vitamin K antagonists (VKA) and more recently direct oral anticoagulants (DOACs) have been the cornerstone of S/SE prevention in patients with non-valvular AF (NVAF) at risk for stroke. However, the presence of CKD in AF further heightens the risk of S/SE (46–49% in ND-CKD or 83% in ESKD) and death (60–65% higher risk in CKD or ESKD) (5), as well as risk of bleeding in patients under OAT, raising doubts about the risk-benefit profile (5, 9, 12). Atrial fibrillation is associated with a prothrombotic state through numerous pathophysiological pathways, which is further aggravated by CKD, via changes in the left atrium, endothelial dysfunction or activation of coagulation and platelets (9). However, there is no clear evidence of a significant increase in S/SE risk in ESKD patients with AF. Possible explanations for this include: the high prevalence/incidence of non-AF-related stroke in ESKD, uremic platelet dysfunction, use of intradialytic heparin and the paroxysmal and self-limiting nature of hemodialysis-associated AF bursts, which should be associated with a low risk of S/SE, in contrast to the persistent/permanent AF associated with chronic structural atrial changes (13, 14). Thus, if no association between AF and S/SE in hemodialysis patients was proven, there would not be a need for OAT in this population (13).

VITAMIN K ANTAGONISTS IN PATIENTS WITH AF AND CKD

Vitamin K antagonists reduce S/SE incidence in patients with NVAF in the general population (15). However, VKA have several drawbacks: slow onset of action and cessation of effects, a narrow therapeutic range requiring frequent monitoring and dose adjustments, and significant drug-drug and food-drug interactions (16), which may be aggravated by dietary restrictions in CKD (17).

Evidence of the efficacy and safety of VKA in CKD patients with AF from randomized controlled trials (RCT) is scarce. Warfarin targeted at an international normalized ratio (INR) of 2–3 vs. aspirin plus fixed low-dose of warfarin, reduced the risk of S/SE by 76% without increasing the risk of major bleeding in the AF patient subset with stage 3 CKD (18). However, there is no RCT evidence of the risk/benefit ratio of VKA in patients with AF and stage $\geq$ stage 4 CKD.

In a meta-analysis of observational studies, warfarin reduced the risk of S/SE and mortality (30 and 35%, respectively) without increasing the risk of major bleeding in the ND-CKD group; in ESKD patients, however, warfarin did not decrease the risk of S/SE or mortality, yet increased the risk of major bleeding by 30%, suggesting that VKAs have a favorable risk/benefit profile in ND-CKD patients, but not in ESKD (19). This agrees with an updated meta-analysis of 15 observational studies in ESKD patients with AF, in which those receiving warfarin, had a 49% increased rate of hemorrhagic stroke with no benefits on ischemic stroke (IS), major bleeding or mortality vs. those not receiving warfarin (20), and also with the results of two recent studies; an observational study (21) and a retrospective study of patients with advanced CKD and incident AF before initiating dialysis (22). Despite the limitations of these studies (observational, results subject to confounding, moderate to high heterogeneity, no data on quality of anticoagulation), they suggest that VKA may not be effective for S/SE prevention in patients with AF and ESKD, and indeed may be harmful (20, 23). In addition, the time in therapeutic range (TTR) is lower in CKD patients; the worse the renal function, the lower the TTR; while poor anticoagulation quality is associated with increased risk of thromboembolic and bleeding events (24–26). INR variability has also been associated with bleeding and mortality in ESKD patients (27). Further, patients with moderate-severe CKD usually require lower doses of VKA, and show a higher bleeding risk with supra-therapeutic INR (28, 29). Further information on the net clinical benefit of VKA in this population is forthcoming from two ongoing RCTs comparing the hemorrhagic and thrombotic risk of VKA vs. no anticoagulation in hemodialysis patients with AF: the Oral Anticoagulation in Hemodialysis Patients (AVKDIAL) (NCT02886962), and the Danish Warfarin-Dialysis Study - Safety and Efficacy of Warfarin in Patients With Atrial Fibrillation on Dialysis (DANWARD) (NCT03862859).

DIRECT ORAL ANTICOAGULANTS IN PATIENTS WITH AF AND CKD

The efficacy of DOACs (dabigatran, rivaroxaban, apixaban and edoxaban) in S/SE prevention and their safety profile vs. VKA have been demonstrated in pivotal trials (30–33). A meta-analysis of these trials found that DOACs have greater efficacy in reducing S/SE, mainly driven by reducing hemorrhagic stroke, lowering the risk of mortality, and conferring a non-significant lower risk of major bleeding (significant for intracranial hemorrhage) (34).

However, DOAC rely on the kidney, to variable extent, for drug elimination. Renal clearance is about 80% with dabigatran, 50% with edoxaban, 33% with rivaroxaban and 27% with apixaban (35), and these may require dose adjustments in moderate-severe CKD (**Table 1**). In this regard, it could be useful

TABLE 1 | DOAC pharmacological properties and dose adjustments by renal function.

	Dabigatran	Apixaban	Rivaroxaban	Edoxaban
Bioavailability	3–7%	50%	66–100 %	62%
Protein binding	35%	87%	>90%	55%
Half-life	12–14 h	∼12 h	5–9 h (young) 11–13 h (elderly)	10–14 h
Renal elimination	>80%	27%	33%	50%
Dializability	Yes (50–60%)	Small	No	No
Reversal agent	Idarucizumab	Andexanet alfa	Andexanet alfa	Andexanet alfa
Usual dose	150 mg bid or 110 mg bid (Europe)	5 mg bid	20 mg/d	60 mg/d
Dose adjustments by renal function				
Europe	CrCl 30–50 110 mg bid if high bleeding risk CrCl < 30 Contraindicated	CrCl> 30: Sr Cr ≥ 1.5 mg/dl and 1 of 2 (age ≥ 80 yrs or weight ≤ 60 kg) 2.5 mg bid, otherwise 5 mg bid CrCl ≤ 30 2.5 mg bid CrCl < 15 Contraindicated	CrCl 15–50 15 mg/d CrCl< 15 Contraindicated	CrCl 15–50 30 mg/d CrCl < 15 Contraindicated
United States	CrCl > 30 150 mg bid CrCl 15–30 75 mg bid CrCl< 15 Contraindicated	At least 2/3, Sr Cr ≥ 1.5 mg/dl, age ≥ 80 yrs, weight ≤ 60 kg 2.5 mg bid, otherwise 5 mg bid ESKD 5 mg bid (age < 80 yrs or weight > 60 kg)	CrCl 15–50 15 mg/d CrCl < 15 or dialysis 15 mg/d (limited data)	CrCl > 95 Contraindicated CrCl 15–50 30 mg/d CrCl < 15 Contraindicated

bid, twice daily; ESKD, End-stage kidney disease; CrCl, Creatinine clearance; Sr Cr, Serum creatinine.

to measure the anticoagulant effect of DOACs in advanced CKD or in patients with deteriorating renal function (36).

Among patients with moderate CKD *post-hoc* analysis of pivotal RCTs of DOACs vs. warfarin in patients with NVAF (37–41), showed a favorable risk-benefit profile for DOACs, which has been confirmed in several meta-analyses (42–44). In the Cochrane meta-analysis including 12,545 AF patients with CKD (390 patients in stage 4), DOACs reduced the incidence of S/SE (RR 0.81, 95% CI 0.65–1.00) and non-significantly reduced the incidence of major bleeding (RR 0.79, 95% CI 0.59–1.04) vs. warfarin (42). However, these studies included patients with creatinine clearances (CrCl) > 30 ml/min [the Apixaban for the Prevention of Stroke in Subjects with Atrial Fibrillation (ARISTOTLE) trial included patients with CrCl > 25 ml/min]. Therefore, the risk/benefit profile of DOACs in patients with AF and stages 4–5D CKD is uncertain. A *post-hoc* analysis of the ARISTOTLE trial confirmed the safety profile of apixaban with a 66% lower risk of major bleeding among patients with CrCl 25–30 ml/min (45).

Several observational studies have evaluated the effectiveness and safety of DOACs vs. warfarin, covering a range of different CKD stages (26, 46–62) (see **Table 2**). Despite the low quality of the evidence, due to heterogeneity or retrospective study designs, they suggest that DOACs are a viable alternative to VKA in patients with ND-CKD, with a net clinical benefit observed in a meta-analysis (69).

In ESKD patients observational studies comparing DOACs vs. VKA show high heterogenicity (63–68, 70), with initial studies showing safety concerns with dabigatran or rivaroxaban vs. warfarin (63). In contrast, a retrospective study comparing apixaban vs. warfarin showed similar event rates for IS/SE but, significantly lower rates of major bleeding events. Furthermore, 5 mg/12 h apixaban was associated with lower risk for IS/SE and mortality vs. warfarin (65). However, recent studies have not confirmed these benefits (67), or the benefit of DOACs vs. non-OAT in this population (66).

A RCT in hemodialysis patients with NVAF compared the effect of VKA, rivaroxaban at 10 mg/d and rivaroxaban at 10 mg/d plus vitamin K2 at 2,000 μg/thrice weekly for 18 months on the progression of cardio-aortic calcium deposits (primary endpoint). After the initial follow-up, only severe bleeding events were lower in rivaroxaban-treated patients (71). However, after a follow-up extension of the trial for at least 18 additional months, a reduction was observed in the composite outcome of fatal and non-fatal cardiovascular events and of life-threatening or major bleeding complications compared with VKA, although the risk of stroke or death were not different between groups (72).

In a recent meta-analysis in ESKD patients with AF, DOACs showed comparable effectiveness and safety to VKA, while OAT vs. no anticoagulation was associated with a higher risk of IS/SE and a similar risk of major bleeding (67). Thus, there is no evidence of a net clinical benefit of DOACs in ESKD patients with AF, despite an urgent need. Several ongoing RCT are assessing OAT use and comparing the safety and efficacy of DOACs vs. VKAs in this population [Compare Apixaban and vitamin-K antagonists in patients with AF and ESKD

TABLE 2 | Observational studies comparing DOACs vs. VKA or no anticoagulation in CKD patients.

References	Study design	Patients	DOACs type	Comparator	Efficacy outcomes (HR, 95% CI)*	Safety outcomes (HR, 95% CI)*
Lee et al. (46)	Retrospective	eGFR < 60 ml/min per 1.73 m^2	Dabigatran Rivaroxaban ($n = 59$)	VKA ($n = 174$)	Stroke (HR 0.78, 0.21–3.00) Composite of death, hospitalization and stroke (HR 0.3, 0.11–0.77) No differences in mortality	Major bleeding (HR 0.23, 0.07–0.69)
Harel et al. (47)	Population-based Nested case-control study	Stages 3–4 CKD, aged ≥ 66 yrs	Dabigatran ($n = 200$) Rivaroxaban ($n = 33$)	VKA ($n = 8,176$)		Major bleeding Dabigatran [adjusted odds ratio (aOR), 0.9, 0.64–1.25] Rivaroxaban (aOR, 0.97, 0.44–2.11) vs. warfarin
Stanton et al. (48)	Retrospective Matched cohort	CrCl < 25 ml/min or ESKD (AF 72.6%)	Apixaban ($n = 73$)	VKA ($n = 73$)	Stroke (7.5 vs. 7.5%)	Major bleeding (9.6 vs. 17.8%, $p = 0.149$)
Weir et al. (49)	Retrospective IPTW	CKD (CrCl < 50 ml/min)	Rivaroxaban ($n = 229$)	VKA ($n = 647$)	IS (HR 0.09, 0.01–0.72) Composite event (VTE, MI, IS) (HR 0.56; 0.26–1.18)	Major bleeding (HR 1.20, 0.66–2.20)
Shin et al. (55)	Retrospective Propensity- score matched cohort	eGFR < 60 ml/min per 1.73 m^2	Dabigatran Rivaroxaban Apixaban ($n = 1,122$)	VKA ($n = 1,122$)	IS (HR 1.02, 0.76–1.37)	Bleeding (HR 1.23, 1.02–1.48)
Di Lullo et al. (56)	Retrospective	Stages 3b-4 CKD	Rivaroxaban ($n = 247$)	VKA ($n = 100$)	All strokes (warfarin 25 in 24 patients vs. 0 with rivaroxaban $p \leq 0.002$)	GI bleeding (8 warfarin vs 2 rivaroxaban, $p < 0.001$)
Becattini et al. (50)	Prospective	Stages 1–4 CKD ($n = 449$)	Dabigatran Rivaroxaban Apixaban	Stable vs. worsening renal function (WRF)		Major bleeding (HR 1.02, 1.01–1.04) for every 1 mL min^{-1} decrease in eGFR (HR 2.43, 1.33–4.45) for WRF with change in eGFR staging
Kumar et al. (51)	Population-based Retrospective Propensity score	Incident AF, CKD (eGFR < 50 ml/min per 1.73 m^2) and ≥ 65 yrs, (patients on dialysis excluded)	OAT ($n = 2424$) DOACs or VKA	No OAT ($n = 2424$)	IS (HR 2.60, 2.00–3.38) Mortality (HR 0.82, 0.74–0.91)	Bleeding (HR 2.42, 1.44–4.05)
Schafer et al. (52)	Retrospective	Stages 4–5 CKD or dialysis (NVAF or VTE)	Apixaban ($n = 302$)	VKA ($n = 302$)	S/SE similar for both groups in each of the time periods	Bleeding 0–3 months (OR 0.58; CI 0.31–1.11) 6–12 months (OR 0.16; 0.05–0.5)
Loo et al. (57)	Population-based Retrospective Propensity score	Stages 3–5 CKD, dialysis and KTR and incident AF	Rivaroxaban Apixaban Dabigatran Edoxaban ($n = 2,596$)	VKA ($n = 2,596$)	IS/SE (HR 0.79, 0.40–1.58)	Major bleeding (HR 0.88, 0.47–1.62) GI bleeding (HR 0.99; 0.63–1.55)
Bonnemeier et al. (54)	Retrospective database Propensity score	New users of OAT and CKD	Rivaroxaban ($n = 4,164$)	VKA ($n = 7,002$)	IS (HR 0.72, 0.55–0.94)	ICH (HR 0.66, 0.38–1.14)
Coleman et al. (58)	Retrospective	Stages 4–5 CKD (88% stage 5 or hemodialysis)	Rivaroxaban ($n = 1,986$)	VKA ($n = 4,848$)	S/SE (HR 0.55, 0.27–1.10) IS (HR 0.67, 0.30–1.50)	Major bleeding: (HR 0.68, 0.47–0.99)

(Continued)

TABLE 2 | Continued

References	Study design	Patients	DOACs type	Comparator	Efficacy outcomes (HR, 95% CI)*	Safety outcomes (HR, 95% CI)*
Wetmore et al. (53)	Retrospective IPTW	Stages 3–5 CKD (not on dialysis) and incident AF	Apixaban (n = 6,738) Rivaroxaban (n = 3,904) Dabigatran (n = 1,588)	VKA (n = 10,529)	S/SE (HR 0.70, 0.51–0.96 for apixaban) (HR 0.80, 0.54–1.17 for rivaroxaban) (HR 1.15, 0.69–1.94 for dabigatran) No differences in mortality	Major bleeding (HR 0.47, 0.37–0.59 for apixaban) (HR 1.05, 0.85–1.30 for rivaroxaban) (HR 0.95, 0.70–1.31 for dabigatran)
Chan et al. (60)	Retrospective	Stages 4–5 CKD (25% on dialysis)	DOACs (n = 280) VKA (n = 520)	No OAT (n = 2,971)	IS/SE Warfarin [adjusted hazard ratio (aHR) 3.1, 2.1–4.6] vs. no OAT DOAC (aHR 1.1; 0.3–3.4) vs. no OAT	Major bleeding Warfarin (aHR 2.8; 2.0–3.8) DOACs (aHR 3.1, 1.9–5.2).
Weir et al. (26)	Retrospective Propensity score	Stages 4–5 CKD and dialysis	Rivaroxaban (n = 781)	VKA (n = 1536)	IS/SE (HR 0.93, 0.46–1.9)	Major bleeding (HR 0.91, 0.65–1.28)
Yao et al. (61)	Retrospective US adiministrative claims database Stabilized IPTW	New users of OAT, eGFR $\geq$ 15 ml/min per 1.73 m^2	Apixaban (n = 10,880) Dabigatran (n = 3,007) Rivaroxaban (n = 8,269)	VKA (n = 10,680)	Apixaban: Stroke (HR 0.57, 0.43–0.75), Mortality (HR 0.68, 0.56–0.83); Dabigatran: Stroke, (HR, 0.94, 0.65–1.35) Mortality (HR, 0.68, 0.48–0.98); Rivaroxaban: Stroke (HR, 0.69, 0.51–0.94), Mortality (HR, 0.73, 0.58–0.91). No interaction between treatment and eGFR for any outcome	Major bleeding Apixaban (HR 0.51, 0.44–0.61) Dabigatran (HR 0.57, 0.43–0.75) Rivaroxaban (HR 0.84, 0.72–0.99)
Ashley et al. (62)	Retrospective Population-level High-dimensional propensity core	Incident patients, $\geq$ 66 years, starting OAT of all stages of CKD	DOACs (n = 27552) (dabigatran 32%, rivaroxaban 41%, apixaban 27%)	VKA (n = 27552)	Cardiovascular event or mortality (HR 0.82, 0.75, 0.90) Interaction eGFR and primary outcome by OAT class (p = 0.02) eGFR $\geq$ 60 (HR 1.01, 0.92–1.12), eGFR 30–59 (HR 0.83, 0.75–0.93), eGFR < 30 (HR 0.75, 0.51, 1.10)	Bleeding (HR 0.73, 0.58, 0.91) No interaction
Chan et al. (63)	Retrospective	Hemodialysis patients Fresenius Medical Care North America ESKD database (n = 29,977)	Rivaroxaban (n = 244) Dabigatran (n = 281)	VKA (n = 8,064) Aspirin (n = 6,018)		Hospitalization or death from bleeding vs. warfarin Dabigatran (RR 1.48; 1.21–1.81) Rivaroxaban (RR 1.38, 1.03–1.83) Hemorrhagic death: Dabigatran (RR, 1.78; 1.18–2.68) Rivaroxaban (RR, 1.71; 0.94–3.12)
Sarrat et al. (64)	Retrospective In center	Hemodialysis patients	Apixaban (n = 40)	VKA (n = 120)		Major bleeding (0 vs. 5.8%, p = NS)
Siontis et al. (65)	Retrospective cohort, Propensity score	Dialysis patients of Medicare	Apixaban (n = 2,351)	VKA (n = 7,053)	S/SE (HR 0.88, 0.69–1.12) Mortality (HR, 0.85; 0.71–1.01)	Major bleeding (HR 0.72, 0.59–0.87) Intracraneal bleeding (HR, 0.79; 0.49–1.26)
Mavrakanas et al. (66)	Retrospective US Renal Data System cohort Propensity score	Dialysis patients with incident AF	Apixaban (n = 521)	No treatment (n = 1561)	Stroke, TIA, SE (HR 1.24, 0.69–2.23) Mortality (HR, 0.58; 0.43–0.78)	Fatal /IC bleeding (HR 2.74, 1.37–5.47)

(Continued)

TABLE 2 | Continued

References	Study design	Patients	DOACs type	Comparator	Efficacy outcomes (HR, 95% CI)*	Safety outcomes (HR, 95% CI)*
Makani et al. (59)	Retrospective	Stages 3–5 CKD or dialysis patients	Dabigatran Rivaroxaban Apixaban Edoxaban (n = 4,748)	VKA (n = 5,895)	Embolic stroke Stage 3 CKD (HR 0.87, 0.73–1.04) Stage 4–5 CKD (HR 0.60, 0.34–1.09) Mortality Stage 3 CKD (HR 0.74, 0.68–0.81) Stage 4–5 CKD (HR 0.76, 0.63–0.92)	Bleeding Stage 3 CKD (HR 0.83, 0.74–0.94) Stage 4–5 CKD (HR 0.69, 0.50–0.93)
See et al. (67)	Retrospective Population-based cohort Propensity score	Dialysis patients with NVAF	DOACS (n = 448)	Warfarin (n = 448)	IS/SE (HR 1.21, 0.76–1.92)	ICH (HR 0.78, 0.29–2.1) Major bleeding (HR 0.98; (0.64–1.51))
See et al. (67)	Retrospective Population-based cohort Propensity score	Dialysis patients with NVAF	OAT (n = 2,977)	No OAT (n = 2,977)	IS/SE (HR 1.54, 1.29–1.84)	Major bleeding (HR 1.14, 0.97–1.34) ICH (HR 1.41, 0.99–2.02)
Miao et al. (68)	Retrospective IPTW	ESKD or on dialysis patients	Rivaroxaban (n = 787)	Apixaban (n = 1,836)	S/SE (HR 1.18, 0.53–2.63) IS (HR 1.12, 0.45–2.76)	Major bleeding (HR 1.00, 0.63–1.58)

**Unless otherwise stated. CKD, Chronic kidney disease; CrCl, Creatinine clearance; DOACs, Direct oral anticoagulants; ESKD, End-stage kidney disease; GI, Gastrointestinal; HR, Hazard ratio; ICH, Intracerebral hemorrhage; IPTW, Inverse-probability-of-treatment weighting approach; IS, Ischemic stroke; KTR, Kidney transplant recipient; MI, Myocardial infarction; NVAF, Non-valvular atrial fibrillation; OAT, Oral anticoagulation therapy; SE, Systemic embolism; USRDS, US Renal Data System; VKA, Vitamin K antagonists; VTE, Venous thromboembolism.*

(AXADIA); NCT02933697] [Strategies for the Management of Atrial Fibrillation in patiEnts Receiving Dialysis (SAFE-D); NCT03987711] which will likely yield useful data.

CONTROL OF RENAL FUNCTION DURING ORAL ANTICOAGULATION

Worsening of kidney function (WRF) is common among AF patients (73, 74) and is associated with an increased risk of cardiovascular events, all-cause mortality and bleeding (41, 73–75). This worsening is also common in observational studies (50, 76, 77), especially among CKD patients, and is a better predictor of IS/SE and bleeding than renal dysfunction *per se* (50, 78). Together with changes in renal function over time because of concomitant treatments (e.g., RAAS blockade and diuretics) or acute events, WRF during follow-up requires monitoring to adjust DOAC dosage. Discordance in DOAC prescription with drug labeling is common in clinical practice, and may result in overdosing or underdosing. Overdosing of DOAC due to inadequate adjustment increases the risk of bleeding, while underdosing is associated with an increased risk of stroke, especially with apixaban (79, 80).

Dose adjustment of DOAC according to drug labeling is based on estimated CrCl using the Cockroft-Gault (CG) formula, while laboratories usually report eGFR (MDRD or CKD-EPI equations), which provide a better estimation of measured GFR, especially for bed-ridden patients whose weight is difficult to estimate (81, 82). Discrepancies in results between different equations may result in inappropriate dosing and patient misclassification (e.g., as eligible for DOACs when they are contraindicated) (83–86). This issue will likely be addressed in the near future, but until more data are available it is reasonable to use the CG-eCrCl formula to adjust DOAC dose.

Guidelines recommend measuring renal function at DOAC treatment initiation and annually thereafter, but suggest more frequent monitoring in CKD patients > 75 years-old, with frailty, and those treated with DOAC with higher renal clearance (87). In CKD patients the interval for monitoring kidney function (in months) can be calculated by dividing eGFR/10. Monitoring renal function in patients under DOAC treatment should also be considered when WRF is suspected, in contexts such as acute events or when prescribing drugs that affect renal hemodynamics (87).

COMPLICATIONS DURING OAT: DOACS VS. VKA

Effects on Kidney Function

Acute Kidney Injury/Anticoagulant-Related Nephropathy

Warfarin-related nephropathy (WRN) is an episode of acute kidney injury (AKI) associated with warfarin and was first reported in 2009 (88). It is characterized by an increase in serum creatinine, with micro or macrohematuria, in the absence of other causes of AKI, often following supra-therapeutic INR values. Subsequent studies have reported further cases of WRN,

most associated with an INR > 3 (89). Kidney biopsies showed signs of acute tubular injury and glomerular hemorrhage with red blood cells in Bowman's space and renal tubular obstruction by red blood cell casts. Although all cases had underlying renal disease in the initial study (88), later studies reported WRN in both CKD and non-CKD patients, and it was also associated with increased mortality risk (89) and faster CKD progression (90).

Several cases have also been reported of AKI in patients treated with DOACs, particularly with dabigatran. Pathological findings were similar to those found in WRN, and this entity is now known as anticoagulant-related nephropathy (ARN). In a recent histologic study of ARN most cases had underlying renal disease (91), suggesting that excessive anticoagulation aggravates an underlying glomerular disease and increases glomerular hematuria. Other possible mechanisms of AKI during OAT include oxidative stress due to free iron release and hemoglobin tubular toxicity.

The risk of AKI in patients with AF is high. In an observational study, one in seven had an AKI episode within 2 years (76). The risk of this injury was higher among patients with CKD and those receiving VKA vs. DOAC-treated patients in several observational studies (76, 92–95).

CKD Development and Progression

Post-hoc analysis of *the Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation* (ROCKET) (73) and *Randomized Evaluation of Long-Term Anticoagulant Therapy with Dabigatran etexilate* (RE-LY) (96) trials suggest that DOACs have a more beneficial effect on renal function decline than warfarin, which has been confirmed in multiple observational studies (76, 94, 95, 97–99). The renal benefit of DOACs was more apparent with rivaroxaban and dabigatran, and was notably better than in VKA-treated patients with supra-therapeutic INR levels.

The renal benefit of DOACs vs. VKA may be owing to its more predictable dose-response effect and lower risk of hemorrhages; the lower risk of AKI which could influence progressive deterioration of renal function, and its potential renal anti-inflammatory effect, as observed in experimental models (100). In contrast, VKA can promote vascular calcification (as discussed below), increasing arterial stiffness, favoring systolic hypertension and a pulsatile flow at the microvasculature, which may be deleterious for the kidney (101).

Lastly, the *Factor XA Inhibition in REnal Patients with Non-valvular Atrial Fibrillation-Observational Registry* (XARENO) (NCT02663076) is an observational prospective study that will compare CKD progression with rivaroxaban, VKA or no anticoagulation in patients with CKD stages 3–4 and NVAF.

Vascular and Valvular Calcification and Risk of Calciphylaxis

A common complication in CKD patients, vascular calcification (VC) is inversely associated with renal function and positively associated with worse prognosis in this population (102). Further, vascular medial calcification favors arterial stiffness, which is associated with systolic hypertension, left ventricular hypertrophy and reduced coronary flow reserve, and is a risk factor for cardiovascular events, and CKD progression (101). Vitamin K antagonists impede the -carboxylation of coagulation factors, as well as other vitamin K-dependent proteins which inhibit VC, such as matrix Gla protein, Gla-rich protein, and growth arrest–specific protein 6, preventing their activation (103).

In fact, VKA have been associated with increased progression of VC (104), and arterial stiffness (105), which may be especially relevant in CKD patients, and supporting that DOACs are safer than VKA as regards these two conditions, although the differential effect was not demonstrated in a recent RCT (71). Although switching from warfarin to rivaroxaban improved arterial stiffness in a prospective study (106), this finding was not substantiated in the above mentioned RCT (71). Further, rivaroxaban was also found to reduce the progression of aortic or mitral valve calcification vs. VKA in an observational, retrospective study in stage 3b-4 CKD patients (107).

Calciphylaxis is a rare but severe complication in dialysis patients, characterized by skin necrosis and ulceration, with panniculitis, calcification and luminal occlusion of small arterioles and subcutaneous capillaries (108, 109). Calciphylaxis is associated with high morbidity and mortality. Treatment with VKA increases several fold the risk of calciphylaxis in both uremic and non-uremic patients (110), suggesting a beneficial effect for DOACs in preventing this complication.

OAT and Bone Health

Patients with ND-CKD and ESKD are prone to bone fractures and osteoporosis due to factors such as bone mineral disease (111). Vitamin K antagonists inhibit the γ-carboxylation of osteocalcin which plays an important role in bone matrix formation (17). Nonetheless, the role of VKA on bone fractures and bone density is controversial. In a recent meta-analysis, VKA for >1 year was not associated with increased risk of fracture vs. controls or compared to DOACs, although the risk was increased in females and elderly patients vs. controls (112).

The available evidence suggests that DOACs are associated with a lower impact on bone metabolism and potentially with lower risk of fractures than VKA (113–120), although this warrants corroboration in properly designed studies. Interestingly, switching from warfarin to rivaroxaban was associated with an improvement in bone metabolism markers (106).

LEFT ATRIAL APPENDAGE OCCLUSION

Given the increased risk of bleeding among patients with severe CKD, left atrial appendage occlusion (LAAO) may be an attractive alternative to reduce the risk of S/SE events while avoiding the need for OAT. All the RCTs showing LAAO as non-inferior to OAT in preventing stroke and bleeding events had excluded patients with severe CKD; however a recent prospective study showed that, at 2 years' follow-up, LAAO in dialysis patients was associated with no thromboembolic events and lower bleeding incidence compared to a cohort treated

with oral anticoagulants (121), similar results were found in a small study (122). Besides this study, a prospective single arm study of the Watchman device in dialysis patients is currently ongoing (NCT03446794).

KEY CONCEPTS

1. Atrial fibrillation prevalence and incidence is higher in CKD and rises with the degree of kidney dysfunction. The presence of CKD in AF confers an increased risk of stroke/systemic embolism, mortality, and bleeding.
2. Oral anticoagulation with VKA in mild-moderate CKD patients with AF results in a net clinical benefit, although there are no conclusive data on their risk/benefit ratio in more advanced stages of CKD, especially among ESKD patients. Vitamin K antagonist treatment in CKD is associated with poorer anticoagulation quality and increased risk of bleeding.
3. Pivotal RCTs have demonstrated a net clinical benefit for DOACs vs. warfarin in NVAF with mild-moderate CKD, but there is little evidence in patients with AF and stages ≥ 4 CKD. Observational studies suggest that the benefit remains in ND-CKD patients, but is controversial in ESKD.
4. Vitamin K antagonists are associated with a higher risk of anticoagulant-related nephropathy, acute kidney injury and faster CKD progression than DOACs, especially among CKD patients.
5. In patients with NVAF, worsening renal function is common and associated with poorer outcomes. Renal function should be periodically monitored and DOAC dose adjusted to avoid the risk of over or underdosing. Renal function should be measured using the CG-eCrCl, rather than eGFR.
6. Vitamin K antagonists have been associated with an increased risk of vascular/valvular calcification in CKD and likely with higher risk of osteoporosis/fractures, which may be particularly relevant in CKD.

AUTHOR CONTRIBUTIONS

AC and PG conceived and drafted the manuscript. AC, PG, JB, and EP carried out the literature search. JA, JP, and JG provided a critical revision of the manuscript. All authors contributed to the article and approved the final version of the manuscript.

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