HIGHLIGHTS

- Oxidative stress in chronic kidney disease
- Impaired autophagy in Fabry disease
- Residential greenness and clinical outcomes of patients with CKD
- Alcohol use on the risk of end-stage kidney disease
- Gd–IgA1 for recurrent IgAN in kidney transplant
Aims and Scope

Kidney Research and Clinical Practice (KRCP; formerly The Korean Journal of Nephrology; ISSN 1975-9460, launched in 1982), the official journal of the Korean Society of Nephrology, is an international, peer-reviewed journal published in English. Its ISO abbreviation is Kidney Res Clin Pract.

The journal considers articles on all aspects of nephrology and hypertension as well as molecular genetics, anatomy, pathology, physiology, pharmacology, and immunology related to kidney disease. In particular, the journal focuses on translational renal research that helps bridging laboratory discovery with the diagnosis and treatment of human kidney disease. The journal publishes the topics covered basic science with possible clinical applicability and the papers on the pathophysiological basis of the kidney disease. Original studies from areas of diagnostic and interventional nephrology or dialysis access are also welcomed. Major article types considered for publication include original research and reviews on current topics of interest.

To provide an efficient venue for dissemination of knowledge and discussion of topics related to basic research, translational study and clinical practice in nephrology, the journal offers online only open access, in which all published articles are free for everyone to read and download.

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The image on the front cover: Chung et al showed the role of impaired autophagy in the renal fibrosis in Fabry disease. On electron micrographic findings, enhanced autophagosomes and autophagic vesicles in proximal tubular cells were observed in Fabry mice treated with UUO. Please see the text for more details (pp. 208-219).
Acute kidney injury (AKI) is one of the most frequent complications in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection during the coronavirus disease 2019 (COVID-19) pandemic. Although COVID-19 is characterized by atypical pneumonia and subsequent severe respiratory failure, approximately 10% of inpatients with COVID-19 reportedly suffer from AKI [1], which is significantly associated with poor outcomes [2]. Successive recent publications have reported an even higher global incidence of AKI. Patients with COVID-19 who are being treated in intensive care units (ICUs) are the most susceptible to severe AKI, which requires renal replacement therapy. However, the pathophysiology of COVID-19-associated AKI remains to be determined; systemic inflammation, hypovolemia, nephrotoxic drugs, various lung-kidney interactions (such as possible renal congestion presumably caused by high levels of positive end expiratory pressure), and direct viral cytopathic effects on the renal epithelium and podocytes are all possible mechanisms currently in discussion [3]. With no specific treatment for COVID-19-associated AKI available, early recognition of patients at risk and early diagnosis of AKI are key to confronting this emerging health burden on clinical nephrology.

In this issue of *Kidney Research and Clinical Practice*, Alfano et al. [4] reported that absolute change in serum creatinine level (ΔsCr) within 24 hours of admission was significantly associated with 30-day in-hospital mortality in patients with COVID-19. The authors conducted a retrospective observational study on 224 non-ICU inpatients at a single center in Italy and stratified them into three groups: 1) stable kidney function group with ΔsCr within the range of −0.05 mg/dL to +0.05 mg/dL, 2) decreased kidney function group with ΔsCr higher than +0.05 mg/dL, and 3) improved kidney function group with ΔsCr lower than −0.05 mg/dL. They reported the highest survival rate in the stable kidney function group, while the decreased kidney function group demonstrated the highest rates of both AKI and mortality during hospitalization.

The study by Alfano et al. [4] offers evidence that even modest renal dysfunction during the early stage of admission is associated with poor prognosis in patients with COVID-19 who are treated in general wards. The timing of AKI development in SARS-CoV-2 infection must be considered. In
previous investigations of COVID-19, two phenotypes of AKI were reported: 1) AKI that was already present at the time of admission and 2) AKI that newly developed about one week after admission [2,5]. According to Hirsch and colleagues’ report on 5,449 patients hospitalized with COVID-19 in New York [2], 37.3% of patients with AKI either arrived with AKI or developed it within 24 hours of admission. The current report by Alfano et al. [4] calls attention to the former group that had evident renal dysfunction during the early stage of admission. Further evaluation is necessary to clarify the impact of newly developed AKI during hospitalization on patient outcomes; Chan et al. [6] reported that about 35% of COVID-19 patients who experienced an AKI episode during hospitalization had residual renal impairment even after discharge.

Another point highlighted by Alfano et al. [4] is that the threshold for deteriorating or improving renal function was much smaller in their patients than described in previous reports (0.05 mg/dL in serum creatinine). This very slight change in serum creatinine is likely to be recognized as nonsignificant in clinical practice. However, the risk stratification associated with this threshold showed a significant difference in survival of patients with COVID-19. The Acute Kidney Injury Network first incorporated an absolute increase of 0.3 mg/dL in serum creatinine into the AKI diagnostic criteria, which is valid in its present form as revised by the Kidney Disease Improving Global Outcomes. The rationale behind these international guidelines included several preceding studies on patients after cardiac surgery [7] and in general inpatient settings [8]. In the present study by Alfano et al. [4], even in the decreased kidney function group, only 30% of the COVID-19 patients met the current AKI diagnostic criteria. The current threshold of serum creatinine change of 0.3 mg/dL might need to be reconsidered. In addition, it should be kept in mind that the average laboratory measurement error in serum creatinine among hospitals is approximately 0.20 ± 0.09 mg/dL [9].

It is also noteworthy that a poor prognosis was reported not only in the decreased kidney function group but also in the improved kidney function group with serum creatinine decline of >0.05 mg/dL; Kaplan-Meier survival analysis revealed that the stable kidney function group with ΔsCr between -0.05 mg/dL and +0.05 mg/dL had the highest survival rate [4]. In addition to kidney function, changes in a patient’s fluid balance and intravascular volume status might affect short-term (within 24 hours) changes in creatinine level. The improved kidney function group was comprised of patients who had reversible renal impairment at the time of admission, patients who were dehydrated upon admission, and patients who required fluid resuscitation due to severe septic conditions. Moreover, serum creatinine fluctuations as a result of impaired renal autoregulation can explain the survival difference to some extent. Previous clinical studies have reported a higher mortality in patients with greater kidney function variability in both outpatient [10] and inpatient [11] settings. Kao et al. [11] investigated the intraindividual variability of inpatient serum creatinine in 6,011 participants and found that creatinine change during hospitalization was significantly associated with in-hospital mortality. They proposed a threshold of 25 µmol/L (0.28 mg/dL) for the creatinine change to predict a significantly higher mortality compared with those patients who had stable kidney function.

Several limitations should be considered when interpreting the results of Alfano et al. [4]: 1) their investigation was a retrospective study that selected patients with multiple creatinine measurements obtained within 24 hours; 2) the time between measurements varied within the 24-hour range; and 3) it is unclear whether the results are specific to non-ICU patients with COVID-19 or are relevant to a broader population, such as all critically ill patients with COVID-19 or patients with renal dysfunction associated with other diseases.

In conclusion, this timely report by Alfano et al. [4] sheds light on a small, potentially overlooked change in serum creatinine during the early stage of admission in patients with COVID-19. Future studies must validate the threshold that indicates significant creatinine change and the optimal measurement interval and also provide novel insights on the characteristics of COVID-19-associated AKI.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

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Biomarker for recurrent immunoglobulin A nephropathy in kidney allografts: promising but still a long way to go

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Immunoglobulin A (IgA) nephropathy (IgAN), the most common primary glomerulonephritis worldwide, frequently leads to end-stage renal disease, which requires renal replacement therapy, including kidney transplantation (KT). Recurrence of IgAN after KT was reported to range from 35% to 60%, with a graft loss rate of 7%–10% [1]. Diagnosis of recurrent IgAN is dependent upon a pathologic diagnosis by an invasive allograft biopsy, and no effective noninvasive methods have been successfully proposed. In this regard, the KDIGO guidelines for care of KT recipients recommend screening for IgAN recurrence using urinalysis and kidney function tests [2]. KT recipients await serological biomarkers for a noninvasive determination of recurrence or severity of IgAN in kidney allograft. To discuss the possibility of biomarkers for recurrent IgAN in kidney allograft, we need to understand the proposed mechanism of development of IgAN. IgAN is characterized by IgA deposits in the kidney, resulting in mesangial cell proliferation, extracellular matrix expansion, and inflammation, finally progressing to fibrosis. Although the origin of the disease is under investigation, aberrantly glycosylated IgA1 might be a key element in the pathogenesis of the disease [3]. Defective galactosylation can lead to self-polymerization of IgA1 and facilitate its deposition in the kidney. An autoimmune response against the galactose-deficient (Gd) IgA1 molecule is initiated, with production of glycan-specific immunoglobulin G (IgG) or IgA autoantibodies. Therefore, IgAN can be caused by immune complex deposition resulting from generation of IgG and IgA antibodies to aberrant nonglycosylated IgA1 [3]. Based on this information, serum levels of “Gd-IgA1,” “Gd-IgA1-specific IgG and IgA,” and “its related immune complexes” have been proposed as biomarkers for progression of IgAN in the native kidney [4]. A study by Berthoux et al. [5] analyzed serum samples from 97 patients with IgAN, 30 healthy volunteers, and 30 patients with non-IgAN disease. The study found that the level of the putative antigen anti-glycosylated IgA1 correlated with the disease process in the native kidney. It has been suggested that this hypothesis for pathogenesis of IgAN can be adapted to an allograft [6]. Therefore, these serological biomarkers might be suitable for characterization of the stage of recurrent IgAN in kidney allografts. To date, four reports (including the current study [7]) investigated the role of serum Gd-IgA1 or its related autoantibody/immune complexes for prediction of recurrent IgAN in a kidney allograft (Table 1). Berthelot et al. [8] reported the predictive
value of Gd-IgA1 for IgAN recurrence in allograft kidneys for the first time. They analyzed three markers, Gd-IgA1, IgG anti-IgA autoantibodies, and IgA-soluble CD89 complexes, using serum obtained at pretransplant in 38 KT recipients, and their results showed that all three markers significantly predicted disease recurrence. In another study by Berthoux et al. the prognostic significance of the levels of Gd-IgA1 autoantigen and Gd-IgA1–specific IgG and IgA autoantibodies in serum obtained at the time of transplant or native-kidney IgAN diagnosis was assessed for clinicopathologic recurrence, allograft failure, and patient death over 10 years. Compared to healthy controls, the patients had significantly elevated serum Gd-IgA1 level at diagnosis and transplant, but the level was not associated with any outcomes, including IgAN recurrence. In contrast, the level of serum Gd-IgA1–specific IgG autoantibodies at transplant was associated with a higher risk of recurrence. In contrast to previous studies that used samples at pretransplant or at transplant, a more recent study measured serum Gd-IgA1 level at a mean time of 51 ± 29 months after KT. As a result, the level of Gd-IgA1 in recurrent IgAN patients was significantly higher than those in nonrecurrent IgAN patients or healthy controls. In a current study, Park et al. enrolled 27 KT recipients who underwent allograft biopsy and measured the serum Gd-IgA1 level using serum collected at the allograft biopsy. The mean serum Gd-IgA1 level was significantly higher in the recurrent IgAN group than in the nonrecurrent IgAN group, and serum Gd-IgA1 level was an independent factor predicting IgAN recurrence. They concluded that serum Gd-IgA1 could be used as a diagnostic biomarker for recurrent IgAN in KT.

All the above studies found that serum Gd-IgA1 or its related autoantibody level showed significance in predicting recurrent IgAN in kidney allograft. However, many obstacles must be overcome or clarified before these biomarkers can be applied in clinical practice. First, the previous studies used Gd-IgA1 level at specific time points including pretransplant, at KT, or at allograft biopsy. Thus, the dynamics of Gd-IgA1 level during the posttransplant period and their association to IgAN recurrence after KT were not shown. Therefore, it is unclear whether Gd-IgA1 level remained high and induced IgAN recurrence, or whether it was low but became high at the time of recurrence. To clarify this issue, longitudinal assessment in a prospective cohort should determine the prototypical course of Gd-IgA1 level and its association with clinicopathologic recurrence of IgAN in allograft. Second, most studies did not show an association between Gd-IgA1 level and clinical outcomes, such as al-

### Table 1. Published reports of serologic biomarkers for recurrent IgAN after KT

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Patient group</th>
<th>Measured biomarker</th>
<th>Sampling time</th>
<th>Observation period (yr)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berthelot et al. [8]</td>
<td>2015</td>
<td>38 KTRs (recurrence)</td>
<td>Serum Gd-IgA1</td>
<td>At transplant</td>
<td>8.7 ± 2.5</td>
<td>All three markers predicted recurrence of IgAN</td>
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<tr>
<td></td>
<td></td>
<td>22 KTRs (non-recurrence)</td>
<td>IgG anti-IgA</td>
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<td></td>
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<td>autoantibodies</td>
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<td></td>
<td></td>
<td>17 HCs</td>
<td>IgA-soluble CD89</td>
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<td></td>
<td></td>
<td></td>
<td>complexes</td>
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<tr>
<td>Berthoux et al. [9]</td>
<td>2017</td>
<td>96 KTRs</td>
<td>Serum Gd-IgA1</td>
<td>At diagnosis of IgAN</td>
<td>12.4 ± 6.1</td>
<td>Only IgG predicted clinic-pathologic recurrence of IgAN</td>
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<td></td>
<td></td>
<td></td>
<td>(pretransplant)</td>
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<td></td>
<td></td>
<td>30 HCs</td>
<td>IgA1-specific IgG</td>
<td>At transplant</td>
<td>7.0 ± 3.0</td>
<td>Serum Gd-IgA1 predicted recurrence of IgAN</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>and IgA autoantibody</td>
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<tr>
<td>Temurhan et al. [10]</td>
<td>2017</td>
<td>18 KTRs (recurrence)</td>
<td>Serum Gd-IgA1</td>
<td>Posttransplant</td>
<td>12.8 ± 7.0</td>
<td>Serum Gd-IgA1 predicted recurrence of IgAN</td>
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<td></td>
<td></td>
<td>23 KTRs (non-recurrence)</td>
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<td>44 non-KT IgAN patients</td>
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<tr>
<td></td>
<td></td>
<td>11 HCs</td>
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<tr>
<td>Park et al. [7]</td>
<td>2021</td>
<td>14 KTRs (recurrence)</td>
<td>Serum Gd-IgA1</td>
<td>Posttransplant</td>
<td>12.8 ± 7.0</td>
<td>Serum Gd-IgA1 predicted recurrence of IgAN</td>
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<td>13 KTRs (non-recurrence)</td>
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Gd-IgA1, galactose-deficient immunoglobulin A1; HC, healthy controls; IgAN, immunoglobulin A nephropathy; IgG, immunoglobulin G; KT, kidney transplant; KTR, KT recipient.
lograft failure, even though it was useful in predicting IgAN recurrence. Perhaps an association was not demonstrated because previous studies were performed in a relatively small-sized patient group. If there is no correlation with allograft survival, the need for biomarkers will be halved. Furthermore, an effective therapeutic strategy for recurrent IgAN has not been established. Hence, strategies to improve allograft outcomes when recurrent IgAN is suspected based on high Gd-IgA1 level remain unclear. All KT patients receive strong maintenance immunosuppression, so few other treatments can be added in recurrent IgAN. Hence, new therapeutic agents to change the clinical outcomes need to be developed to maximize the effectiveness of biomarkers for recurrent IgAN. Lastly, the assay method needs to be standardized. In earlier studies, serum Gd-IgA1 level was measured by lectin enzyme-linked immunosorbent assay using *Helix aspersa* agglutinin, a lectin that binds to terminal galactosyl-N-acetylamine residues [7,8]. In contrast, the current study [7] and the study by Temurhan et al. [10] used a lectin-independent Gd-IgA1 assay (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan). In addition, the significant level associated with IgAN recurrence was different between the studies. Therefore, standardization of measurement methods to overcome inter- or intralaboratory disparity should be achieved.

In summary, theoretically, serum Gd-IgA level has potential as a biomarker for recurrent IgA nephropathy. Areas that require further investigation, however, include an understanding of IgAN recurrence, especially the dynamics during the posttransplant period, and the effectiveness of serum Gd-IgA level for predicting allograft survival. A prospective multicenter study including serial protocol biopsies and measurement of serum Gd-IgA1 level by nephrologists involved in KT should further clarify these issues.

**Conflicts of interest**

The author has no conflicts of interest to declare.

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Implications of oxidative stress in chronic kidney disease: a review on current concepts and therapies

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Moderate levels of endogenous reactive oxygen species (ROS) are important for various cellular activities, but high levels lead to toxicity and are associated with various diseases. Levels of ROS are maintained as a balance between oxidants and antioxidants. Accumulating data suggest that oxidative stress is a major factor in deterioration of renal function. In this review, we highlight the possible mechanism by which oxidative stress can lead to chronic kidney disease (CKD). This review also describes therapies that counter the effect of oxidative stress in CKD patients. Numerous factors such as upregulation of genes involved in oxidative phosphorylation and ROS generation, chronic inflammation, vitamin D deficiency, and a compromised antioxidant defense mechanism cause progressive detrimental effects on renal function that eventually lead to loss of kidney function. Patients with renal dysfunction are highly susceptible to oxidative stress, as risk factors such as diabetes, renal hypertension, dietary restrictions, hemodialysis, and old age predispose them to increased levels of ROS. Biomolecular adducts (DNA, proteins, and lipids) formed due to reaction with ROS can be used to determine oxidative stress levels. Based on the strong correlation between oxidative stress and CKD, reversal of oxidative stress is being explored as a major therapeutic option. Xanthine oxidase inhibitors, dietary antioxidants, and other agents that scavenge free radicals are gaining interest as treatment modalities in CKD patients.

Keywords: Antioxidants, Chronic kidney disease, Oxidative stress, Renal dialysis

Background

Living organisms require oxygen to sustain their existence, and oxidative compounds such as reactive oxygen species (ROS) and reactive nitrogen species in cells are produced from molecular oxygen as a consequence of aerobic metabo-
lism. ROS can be classified as either free radicals or non-radicals; free radicals include superoxide anion radical (O$_2^-$), peroxyl (ROO$^•$), alkoxyl (RO$^•$), nitric oxide (NO$^•$), and hydroxyl radical (OH$^•$). Non-radical species include peroxynitrite (ONOO$^-$), hydrogen peroxide (H$_2$O$_2$), and hypochlorous acid (HOCl) [1]. ROS exhibit both beneficial and harmful effects on the cell. Oxidative compounds aid in physiological cell processes when produced in low to moderate concentration, but higher concentration causes detrimental effects including damage to molecular components such as DNA, proteins, and lipids; production of pro-and anti-inflammatory cytokines; and activation of several stress-induced transcription factors [2]. Endogenous sources of ROS include several cellular enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox), xanthine oxidase (XO), mitochondrial oxidases, cyclooxygenase, myeloperoxidase, amino acid oxidase, lipoxigenase, and peroxisomes. Exogenous sources of oxidants include cigarette smoke, ozone exposure, hyperoxia, ionizing radiation, and heavy metal ions.

To counterbalance the effects of oxidants, the human body is equipped with enzymatic and nonenzymatic antioxidant defense mechanisms. Antioxidant enzyme defenses include superoxide dismutase (SOD), catalase, glutathione peroxidase, thioredoxin and peroxiredoxin, and glutathione transferase. Nonenzymatic antioxidants include vitamin C, vitamin E, glutathione, and carotenoids.

When the balance between oxidants and antioxidants shifts in favor of oxidants, oxidative stress is produced. Oxidative stress is known to trigger several pathological conditions including neurological disorders [3], cardiovascular diseases (CVDs) [4], diabetes [5], cancer, and asthma [6] and has been associated with kidney dysfunction [7]. In pyelonephritis, renal dysfunction is caused by ROS-mediated lipid peroxidation and DNA damage, leading to structural and functional aberrations in the kidney [8]. Administration of free radical scavengers such as catalase and dimethyl-sulfoxide neutralizes ROS production, resulting in reversal of oxidative damage and histopathological changes in a chronic pyelonephritis mouse model [9]. Over the last few decades, a large number of clinical, experimental, and theoretical investigations have been conducted for detection of signs of oxidative stress in renal failure patients [10–12]. Oxidative stress is widely considered a biochemical hallmark of chronic kidney disease (CKD) influencing progression of renal function deterioration [13] and onset of major systemic comorbidities including CVD.

**Understanding the pathogenesis and mediators of oxidative stress in CKD**

Kidneys are responsible for homeostasis of extracellular fluids. Progressive decline in kidney function causes CKD, which leads to accumulation of toxic waste (uremia). CKD has become a global health concern, with more than one million annual deaths from end-stage renal disease (ESRD) [14]. CKD is diagnosed by either a reduction in glomerular filtration rate (GFR) and/or the presence of albumin, red blood cells, or white blood cells in the urine. The normal GFR in a healthy individual is ≥90 mL/min/1.73m$^2$, whereas GFR <60 mL/min/1.73m$^2$ for three months or more is indicative of decreased kidney function or presence of CKD [15]. Onset and progression of CKD are associated with various components of metabolic syndrome (MetS) including hypertension, diabetes, obesity, and dyslipidemia. The relationship between MetS and CKD is complex and bidirectional. However, it is difficult to define the etiological role of MetS in CKD as the individual components of MetS are sensitive to lifestyle modifications, medications, and other factors. Some of the additional risk factors for CKD include exposure to nephrotoxins, acute kidney disease, smoking, and aging [16]. All these risk factors significantly disturb the redox balance in the body. Increased oxidative species and decreased antioxidant capacity have been documented in various renal insufficiencies including CKD (Fig. 1).

In kidney diseases, cellular oxidative stress induces apoptosis and senescence, reduced regenerative capability of cells, and fibrosis in the kidney cells. Oxidative stress leads to accumulation of extracellular matrix proteins, podocyte damage, mesangial expansion, renal hypertrophy, endothelial dysfunction, tubulointerstitial fibrosis, and glomerulosclerosis [1]. Thus, oxidative stress further contributes to deterioration of renal function and disease progression.

The mitochondrial electron transport complex is major source of ROS production via oxidative phosphorylation system (OXPHOS) in the cell. In CKD patients, mitochondrial deregulation causes overproduction of ROS and enhances oxidative stress. Several genes involved in OXPHOS have been found to be upregulated in CKD patients [17]. Other enzymes including Nox, XO, and lipooxygenases, which initiate ROS production, are upregulated in CKD [18]. Several isoforms
of Nox have been implicated in renal diseases including nephrolithiasis, hypertension, membranous nephropathy, renal transplantation, and acute kidney injury [19]. Nox4, the predominant Nox isoform in kidney, acts as a major source of ROS and plays a central role in chronic renal diseases such as diabetic nephropathy [20]. Increased Nox-dependent superoxide generation has been reported in patients at an early stage of chronic renal failure [21] and has been shown to contribute to microvascular dysfunction in CKD [22].

XO is the oxidative radical-producing isoform of xanthine oxidoreductase (XOR), also known as urate-producing enzyme. The XO enzyme catalyzes oxidation of hypoxanthine to xanthine and then xanthine to uric acid together with ROS release. XO activity is higher in plasma of CKD patients and has been suggested to be an independent predictor of cardiovascular events in CKD patients [23]. Recently, Terawaki et al. [24] reported relationships between estimated GFR (eGFR) and both XO and XOR activity. Further, these researchers showed higher XOR redox, the ratio of XO to total XOR, in the plasma of advanced CKD patients, indicating its role in elevated ROS production.

Nitric oxide (NO) influences kidney function and aids in maintaining normal blood pressure by promoting natriuresis and diuresis, aiding in adaptation to variations in dietary salt intake. NO also acts as a powerful anti-oxidative agent that minimizes the adverse effects of $O_2^–$. Studies have reported reduced NO production in CKD patients [25]. Multiple factors are responsible for the diminished levels of NO including decreased availability of L-arginine, the substrate for NO synthesis, and increased levels of NO synthase inhibitors such as asymmetric dimethylarginine [26]. The decreased NO activity and further deactivation by superoxide anion radical increase vascular resistance in renal arteries and manifest as hypertensive nephropathy and CVD [27].

**Figure 1. Factors influencing oxidative stress in chronic kidney disease (CKD).** Disturbance in the balance of antioxidants (pink box) and oxidants (light blue box) causes oxidative stress to can lead to CKD. Additional factors that contribute to CKD by enhancing oxidative stress are shown in the green box. Comorbid conditions associated with CKD are listed in orange.

NADPH, nicotinamide adenine dinucleotide phosphate; PMN, polymorphonuclear neutrophil.
CKD patients have severe vitamin D deficiency that is further decreased by reduced activity of the enzyme 1-α hydroxylase (CYP27B1), which converts 25-hydroxyvitamin D to its more active form, 1,25-dihydroxyvitamin D. Deficiency of vitamin D causes oxidative stress, inflammation, hypertension, and hypocalcemia, which lead to progression of CKD and CVD [28].

Elevated levels of lipid-associated oxidation markers such as F2-isoprostanes and malondialdehyde (MDA); protein-associated oxidation markers including oxidized low-density lipoproteins, carbonyls, and glycations; and DNA-associated oxidation markers such as 8-oxo-2′-deoxyguanosine reflect the status of oxidative stress in CKD and can be correlated to disease severity (Fig. 2). The free radicals generated due to oxidative stress have high reactivity and short half-lives (seconds) and are difficult to quantitate in clinical settings. Therefore, biomolecular adducts having longer half-lives (hours to weeks) have become an important tool to measure the levels of oxidative stress.

Oxidative stress is an important contributor to chronic inflammation in CKD. Long-term low-grade inflammation has been implicated in the pathophysiology of CKD. Damage to the kidney causes inflammation and recruits macrophages and leucocytes to result in an “oxidative burst” that causes overproduction of ROS. Accumulation of ROS triggers an inflammatory chain reaction by recruiting macrophages and secreting cytokines, chemokines, and eicosanoids. Cytokines and inflammatory mediators such as tumor necrosis factor (TNF)-α, transforming growth factor β, and interleukins (ILs) have been shown to modulate GFR, renal blood flow, and sodium excretion [29]. In addition, oxidative stress activates nuclear factor (NF)-κB, a transcription factor responsible for expression of inflammatory mediator genes [30]. Oxidative stress affects the phosphorylation and degradation of I-κB, an inhibitory protein that maintains NF-κB in an inactivated state, and leads to activation of NF-κB. The presence of antioxidants inhibits activation of NF-κB by ROS [31]. Patients with advanced-stage CKD have high levels of inflammation markers such as C-reactive protein, TNF-α, and IL-6 as well as oxidative stress markers such as plasma protein carbonyls and F2-isoprostanes, supporting the link between inflammation and oxidative stress in disease pathogenesis [32,33].

Antioxidant defense mechanisms have been shown to be compromised in patients with renal dysfunction. The free radical scavenger SOD is down-regulated in renal patients [34]. Genetic polymorphism in glutathione-S transferase, another antioxidant enzyme, contributes to elevated oxidative stress in ESRD patients [35]. In addition, reduced plasma levels of antioxidant enzymes including catalase, glutathione peroxidase, intracellular glutathione, and thiol have been reported in patients with CKD [36].

**ROS increase in renal patients**

Increased susceptibility to oxidative stress in patients with renal dysfunction can be attributed to various mechanisms (Fig. 1). Risk factors such as diabetes, renal hypertension, and old age predispose these patients to increased levels of oxidative stress compared to the normal population. Another possible reason is nutritional limitation of fresh vegetables and fruits to avoid hyperkalemia and low level/intake of vitamin C. Normal potassium level is critical to maintain normal heart function; in hyperkalemia, the reduced ability...
of the kidney to excrete potassium from blood can disrupt potassium hemostasis and lead to abnormal heart rhythms. Severe hyperkalemic condition results in mortality.

Renal dysfunction leads to accumulation of several uremic toxins such as indoxyl sulfate, p-cresol, and p-cresyl sulfate, which trigger progression of CKD and increase the risk of CVD [37–39]. Indoxyl sulfate stimulates oxidative stress to contribute to atherosclerotic vascular disease, arrhythmia, and chronic heart failure, indicating its role in the high prevalence of CVD that accelerates progression of CKD [40]. In addition, accumulation of uremic toxins in CKD causes uremic sarcopenia and uremic osteoporosis [41,42]. Similarly, p-cresyl sulfate increases oxidative stress and is involved in various mechanisms associated with cardiovascular and renal dysfunction [43].

Hemodialysis (HD), which is currently one of the major renal replacement therapies, is associated with increased oxidative stress [44]. HD is a nonselective procedure that removes solutes and results in loss of antioxidant molecules including water-soluble vitamins [45–48] and trace elements [49]. Furthermore, the bioincompatible dialyzer membranes used in HD cause ROS production via activation of polymorphonuclear neutrophils (PMNs). Activated PMNs generate myeloperoxidase, which is a key trigger for ROS activation and inactivation of nitrogen oxide. Studies have shown that increased serum myeloperoxidase is associated with markers of both inflammation and mortality in HD patients [50]. The presence of bacterial endotoxins such as lipopolysaccharide or anticoagulants in the dialysate triggers formation of oxidative species [51–53].

Therapies to counter oxidative stress in CKD

A growing body of evidence clearly suggests a role of oxidative stress in the pathogenesis of CKD and has prompted researchers worldwide to explore the possibility of reversing oxidative stress. Oxidative stress is enhanced in patients undergoing HD. Studies indicate that supplementation of antioxidants is beneficial in treating and preventing progression of CKD in predialysis as well as dialysis patients [54]. Several clinical trials have been performed to examine the therapeutic potential of various antioxidants in slowing progression of CKD.

XO inhibitors (XOIs) have been the primary choice for treatment of hyperuremia associated with various diseases including CKD. The first-generation XOIs allopurinol has been shown to exert a moderate nephroprotective effect by reducing ROS generation and inflammation and improving endothelial function [55,56]. Recently, new randomized clinical trials with second-generation XOIs (febuxostat and topiroxostat) have been initiated [57]. N-acetylcysteine (NAC), a precursor of glutathione, has also emerged as a potential molecule for slowing CKD progression to ESRD by attenuating systemic oxidative stress [58]. NAC has been shown to improve endothelial dysfunction in CKD patients on HD [59].

Nxes are a major source of ROS in the kidney; therefore, Nxes inhibitors are emerging as potential therapeutics for CKD [60]. Preclinical studies have shown that GKT137831 (setanaxib), a dual inhibitor of Nox1 and Nox4, exhibits renoprotective effects by attenuating glomerular structural changes, podocyte loss, extracellular matrix accumulation, and albuminuria in a mouse model of diabetic nephropathy [61,62]. Owing to the crucial role of setanaxib in attenuating renal pathology, it has now been enrolled in a Phase 2 clinical trial for type I diabetes and kidney disease. Recently, Cha et al. [63] showed that APX-115, a novel pan-Nox inhibitor, provides better protection than setanaxib. APX-115 decreased oxidative stress and improved insulin resistance in diabetic db/db mice. Moreover, APX-115 decreased albuminuria and preserved creatinine level [63]. Another study showed that APX-115 effectively prevented kidney injury such as oxidative stress, inflammation, and fibrosis in diabetic mice [64]. Moreover, APX-115 treatment effectively inhibited mitochondrial and peroxisomal dysfunction, suggesting pan-Nox inhibition as an effective therapy.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is another emerging treatment target to counteract oxidative stress and inflammation in CKD [65]. Nrf2 is a transcription factor responsible for regulation of various antioxidant genes. Enhancing Nrf2 activity in renal tubules decreases oxidative stress and prevents kidney disease progression [66]. Bardoxolone methyl (BARDO), a semisynthetic triterpenoid, is one of the most potent activators of Nrf2. BARDO has been shown to increase estimated GFR and preserve kidney function in stage 4 CKD patients. This strongly suggests that restoring Nrf2 activity could potentially retard CKD progression [67].

The protective effect of dietary antioxidant micronutrients in various diseases associated with oxidative stress has been well documented. Antioxidant therapy reduces serum creatinine level and improves kidney function which contributes
to reduced risk of progression to ESRD. Vitamins E and C are strong and powerful antioxidants that have been considered for CKD therapy [68-70].

Alpha-tocopherol, the biologically active form of vitamin E, counteracts oxidative stress by protecting against peroxidation of lipids and increasing low-density lipoprotein resistance [71]. Vitamin E is a strong scavenger of peroxyl radical and also regulates the expression of inflammatory genes. Supplementation with vitamin E in HD patients causes significant decrease in serum MDA and induces SOD1 and catalase activity [68]. In addition, vitamin E-modified cellulose membrane use in HD has been shown to suppress oxidative stress and inflammation and improves endothelial function. Furthermore, hemolipodialysis has been shown to reduce oxidative stress caused during HD using vitamin C and liposomes containing vitamin E in the dialysate [72]. However, daily administration of vitamin E in ESRD patients significantly reduces cardiovascular complications but does not affect mortality [73]. Reduced vitamin C level has been observed in CKD patients undergoing HD. Vitamin C prevents oxidative damage by directly scavenging superoxide anion and hydroxyl radical. A moderate dose of vitamin C has been suggested as a corrective measure in CKD. Co-administration of vitamin C and vitamin E decreases the formation of carbonyl compounds and MDA concentration and increases total antioxidant capacity levels in peritoneal dialysis patients [74]. CKD patients are also supplemented with 1, 25-vitamin D, the active form of vitamin D, as these patients have very a high rate of vitamin D deficiency [28]. Numerous studies have reported a beneficial effect of vitamin D supplementation, including reduced proteinuria, improvement in endothelial cardiovascular markers, increased serum level of 1, 25-vitamin D, and decreased inflammation markers and serum parathyroid hormone levels in CKD and dialysis patients [75–79]. A recent meta-analysis demonstrated that vitamin D supplementation modulates various parameters of oxidative stress including total antioxidant capacity, glutathione, and MDA [80]. Experimental evidence suggests that vitamin D supplementation reduces oxidative stress and inflammation by increasing Nrf2 and up-regulating antioxidant enzymes [81].

Along with lifestyle changes such as exercise, smoking cessation, and dietary measures, treatment of CKD is focused on controlling albuminuria, blood pressure, blood glucose, and lipids. Agents such as beta blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), and direct renin inhibitors (DRI) suppress the renin-angiotensin-aldosterone-system (RAAS), a regulator of blood pressure. These agents have been demonstrated to attenuate oxidative stress and therefore play a protective role in early as well as end stages of kidney disease [82,83]. A meta-analysis of randomized trials showed that ACE inhibitors were effective in decreasing blood pressure and excretion of urinary protein and in slowing progression of renal disease [84]. Monotherapy with ARBs or ACE inhibitors has been shown to reduce proteinuria. Similarly, a combination of ACE inhibitors and ARBs maximizes RAAS inhibition and normalizes proteinuria and GFR. Based on experimental evidence, a combination of RAAS inhibitors was suggested to be more effective than monotherapy in attenuating the progression of renal dysfunction. However, this regimen was linked with higher occurrence of adverse events such as hypotension and hyperkalemia [85]. The effect of triple RAAS blockade therapy through administration of aldosterone antagonists in combination with ACE inhibitors and/or ARBs for treatment of patients with kidney disease is undetermined [86]. Aliskiren, the first orally bioactive DRI, has been predicted to have greater potential for suppression of RAAS than any other class of drug. Additionally, aliskiren attenuates oxidative stress and provides protection of renal tubules in patients with CKD [83,87].

Natural compounds that target mitochondria, alone or in combination with conventional therapies and lifestyle modifications, are gaining worldwide interest as treatment modalities in CKD patients undergoing both conservative and dialysis treatment because of the low prevalence of adverse effects associated with their use. Although these antioxidant therapies seem promising, their use is controversial. Most studies demonstrating a benefit are either in vivo, isolated, or non-holistic studies. Large-scale randomized controlled trials (RCTs) are lacking for most of these compounds. Currently, there are ongoing trials for various antioxidants including resveratrol, NAC, coenzyme Q10, tocopherols, and curcumin. Table 1 describes various therapies that have shown promise and/or are under investigation [61–64,67–70,88–100].

**Conclusion**

There is an abundance of crosstalk between pathways of inflammation and oxidative stress. Both inflammation and
Oxidative stress have been implicated in various pathological systems that are prevalent in CKD, leading to progressive patient deterioration. Due to the complex nature of oxidative stress and the numerous molecular pathways involved, poly-pharmacotherapy with antioxidants might be effective in CKD patients. Many compounds have shown a beneficial role in reducing oxidative stress due to their free radical scavenging properties, indirect antioxidant properties, or anti-inflammatory actions. However, the most significant limitations of most of the relevant studies are small sample size and short-term follow-up. Hence, none of these molecules are routinely used in clinical practice. Thus, well-organized RCTs and comparative studies with long-term follow-up are warranted.

Table 1. Potential antioxidant therapeutics in CKD

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Rationale for use</th>
<th>Role in CKD</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAC</td>
<td>Essential precursor for reduced glutathione, which further detoxifies reactive species by enzymatic or nonenzymatic reactions</td>
<td>Reduces the levels of serum oxidative stress biomarkers such as MDA and anti-MDA</td>
<td>[88]</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Superoxide anion and hydroxyl radical scavenger, vitamin E regeneration</td>
<td>Inhibits lipid peroxidation and reduces endothelial dysfunction</td>
<td>[69,70]</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>A lipid-soluble antioxidant that scavenges peroxyl radical</td>
<td>Increases level of serum NO and activities of erythrocytic SOD and catalase, decreases serum MDA</td>
<td>[68]</td>
</tr>
<tr>
<td>Vitamin B</td>
<td>Inhibits formation of AGEs</td>
<td>Decreases oxidative stress and albuminuria</td>
<td>[89]</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Causes hypercalcemia and hyperphosphatemia in CKD</td>
<td>Slows disease progression and albuminuria</td>
<td>[90]</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>Xanthine oxidase inhibitor</td>
<td>Decreases uric acid level and cardiovascular risk and slows progression of renal disease</td>
<td>[91]</td>
</tr>
<tr>
<td>GKT137831</td>
<td>Dual inhibitor of Nox1 and Nox4</td>
<td>Attenuates glomerular structural changes, podocyte loss, ECM accumulation, and albuminuria</td>
<td>[61,62]</td>
</tr>
<tr>
<td>APX-115</td>
<td>Pan-Nox inhibitor</td>
<td>Decreases oxidative stress and albuminuria and preserves creatinine level. Also inhibits mitochondrial and peroxisomal dysfunction</td>
<td>[63,64]</td>
</tr>
<tr>
<td>BARD</td>
<td>Activator of Nrf2</td>
<td>Increases estimated glomerular filtration rate and preserves kidney function</td>
<td>[67]</td>
</tr>
<tr>
<td>L-arginine</td>
<td>Precursor to NO and maintains endothelial function</td>
<td>Plays a protective role in ischemic acute renal failure</td>
<td>[92]</td>
</tr>
<tr>
<td>L-carnitine</td>
<td>Transporter of long-chain fatty acid chains across the mitochondrial inner membrane, protects membrane structures</td>
<td>Increases glutathione level and glutathione peroxidase activity and decreases MDA level</td>
<td>[93,94]</td>
</tr>
<tr>
<td>Coenzyme Q10</td>
<td>Highly lipophilic molecule localized in mitochondria that prevents membrane lipid peroxidation</td>
<td>Improves mitochondrial function and decreases oxidative stress</td>
<td>[95,96]</td>
</tr>
<tr>
<td>Omega-3 PUFA</td>
<td>Include DHA and EPA and possess anti-inflammatory properties</td>
<td>Reduces the inflammatory markers IL-6, IL-1β, TNF-α, and CRP</td>
<td>[97]</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Used as antioxidant, anti-inflammatory, antibacterial, and antimicrobial reagent</td>
<td>Attenuates proteinuria, TGF-β, and IL-8</td>
<td>[98,99]</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Directly scavenges ROS and modulates the expression and activity of antioxidant enzymes</td>
<td>Strong anti-inflammatory and antioxidant effects</td>
<td>[100]</td>
</tr>
</tbody>
</table>

AGE, advanced glycation end products; BARD, bardoxolone methyl; CKD, chronic kidney disease; CRP, C-reactive protein; DHA, docosahexanoic acid; ECM, extracellular matrix; EPA, eicosapentaenoic acid; ESRD, end-stage renal disease; IL, interleukin; NAC, N-acetyl cysteine; NO, nitric oxide; Nox, nicotinamide adenine dinucleotide phosphate oxidase; Nrf2, nuclear factor erythroid 2-related factor 2; MDA, malondialdehyde; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; SOD, superoxide dismutase; TGF-β, transforming growth factor-β; TNF, tumor necrosis factor.

Conflicts of interest
All authors have no conflicts of interest to declare.

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Investigation: SV, PS, SK
Writing–original draft: SV, PS, SK, VT, VB
Writing–review & editing: All authors
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**References**


Extracellular vesicles (EVs) refer to all endogenously produced membrane-bound vesicles that are released from cells into the extracellular space [1]. EVs contain various molecules, such as DNA, messenger RNA (mRNA), proteins, lipids, and microRNAs (miRNAs). Different subtypes of EVs have been described including exosomes, microvesicles or microparticles, apoptotic bodies, ectosomes, and oncosomes based on their origin, size, contents, and biogenesis (Table 1). In the narrow sense, EVs usually refer to exosomes or microvesicles. In this review, we will use the compre-
hensive term ‘EVs’ to focus on exosomes and microvesicles because of overlapping characteristics among the different subtypes. EVs were initially discovered over 30 years ago, but it was thought that their physiological role was limited to the excretion of intracellular or membrane components of cells. However, recent advancements have shown that EVs are involved in multiple biological processes such as immune modulation, hemostasis, and tissue proliferation/regeneration, which can affect the development and regeneration of organs [1–3]. EVs are also widely found in pathologic conditions. Their presence in any body fluid expands the diagnostic role of EVs as biomarkers in various types of diseases. EVs also contribute to disease progression by affecting the level of inflammation, thrombosis, and tumorigenesis [4,5]. On the other hand, EVs have received a lot of medical attention as a potential therapeutic vehicle based on their capacity of shuttling proteins and genetic materials. In this review, we will focus on the role of EVs in renal physiology and disease processes as well as their potential applicability as diagnostics and therapeutics in multiple renal diseases.

**Physiologic role of extracellular vesicles in kidneys**

In physiologic conditions, the major biological functions of EVs are to get rid of unwanted substances from host cells. EVs can also transfer important biological information to their recipient cells by delivering genetic material, proteins, lipids, and receptors. The conveyance of RNAs or miRNAs can have a substantial effect on the recipient cells by reprogramming their genetic characteristics. Below, we will discuss in detail the biological function of EVs in renal physiology (Fig. 1A [6]).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exosome</th>
<th>Microvesicle</th>
<th>Apoptotic body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formation</td>
<td>Endosomal pathway, exocytosis</td>
<td>Outward blebbing of the plasma membrane</td>
<td>Cell shrinkage and fragmentation</td>
</tr>
<tr>
<td>Size</td>
<td>30–100 nm</td>
<td>100–1,000 nm</td>
<td>1–5 µm</td>
</tr>
<tr>
<td>Content</td>
<td>Proteins, lipids, mRNA, miRNA, and cytosol</td>
<td>Proteins, lipids, mRNA, miRNA, and cytosol</td>
<td>Proteins, lipids, nuclear fractions, DNA, rRNA, organelles, and cytosol</td>
</tr>
<tr>
<td>Main protein markers</td>
<td>Tetraspanins (CD63, CD9), Alix, and TSG101</td>
<td>Integrins, selectins, and CD40 ligand</td>
<td>Histones</td>
</tr>
<tr>
<td>Appearance by electron microscopy</td>
<td>Cup shape</td>
<td>Irregular shape</td>
<td>Heterogenous</td>
</tr>
</tbody>
</table>

mRNA, messenger RNA; miRNA, microRNA; rRNA, ribosomal RNA; TSG101, tumor susceptibility gene 101.

**Homeostasis and cell survival**

One of the most important biological roles of EVs is in the removal of intracellular toxic materials to extracellular spaces. As cells go through multiple biological processes, they build up damaged organelles and cellular waste, which can induce cellular stress leading to cell death or inflammation. Eukaryotic cells have developed a self-defense mechanism for the removal of intracellular waste—the secretion of EVs into the extracellular space [7]. In this way, cells can effectively eliminate potentially harmful chromosomal DNA fragments through exosomes. The inhibition of this process can induce an innate immune response, DNA damage, and apoptosis in normal human cells. Kidney cells are relatively susceptible to biological stress. Under hypoxic conditions, renal tubular cells proactively augment the amount of exosome secretion and alter the composition of exosomes to meet specific biological needs [8]. EV uptake into recipient cells is also a very important biological process in intercellular communication. The most common mechanism of cellular uptake is endocytosis whereby the EVs are engulfed by recipient cells [9]. The balance between EV release and uptake depends on the physiologic condition of the parent/recipient cells, the type of parent/recipient cells, and the recognition of ligands/receptors on the EVs and recipient cells [10].

**Inflammation/immunomodulation**

EVs are a miniature version of their parent cells, so their immunologic function significantly depends on their origin and the microenvironment their parent cells are exposed to. For example, EVs from dendritic cells can function as
antigen-presenting vesicles through the direct presentation of peptide-major histocompatibility complex (MHC) complexes to T cells [11]. These peptide-MHC complexes can be transferred to other recipient cells, indirectly presenting antigens, and subsequently leading to immune cell stimulation. On the other hand, EVs secreted by tumor cells can mediate an immuno-suppressive response by switching monocytes and T cells to tolerogenic subtypes, inhibiting the differentiation of monocytes, or inducing the apoptosis of bystander T cells, so-called activation-induced cell death [12]. The physiological state of the parent cells significantly affects the content of the EVs. For example, exosomes derived from hypoxic tumor cells could induce higher levels of differentiation and the activation of myeloid-derived suppressor cells compared to exosomes derived from cells under normoxic conditions [13].

**Antimicrobial effect**

The urinary tract system is constantly exposed to microorganisms from the exterior environment, putting it at high risk of urinary tract infection. However, most of the urinary tract, except the urethra, generally remains sterile. This resistance to infection is mediated by several factors. Traditionally, anatomical barriers, such as the glycoprotein plaque and a layer of hydrated mucus, as well as immunologic barriers from various resident immune cells and the epithelial cell lining, were considered to be major host defense mechanisms [14]. Recently, EVs were found to serve a significant role against infection within the urinary tract. A proteomic study showed that enriched innate immune proteins including calprotectin and lysozyme C in urinary EVs could mediate an antimicrobial effect [15]. Even though autophagy is a well-developed cellular defense mechanism to clear foreign pathogens, some
bacteria have evolved defense mechanisms to overcome autophagy by neutralizing lysosomal pH. A study by Miao et al. [16] shows that bladder epithelial cells can overcome this phenomenon through exosomes—by expulsing exosome-encased bacteria from the intracellular space.

**Kidney regeneration/repair**

Mesenchymal stem cells (MSCs) are well-known for their regenerative and reparative potential in different organs including the kidneys [17]. However, the reparative potential of MSCs is mediated through EVs rather than the differentiation potential of the MSCs themselves. MSC-derived EVs contain different types of antioxidants and growth factors to stimulate the differentiation of resident progenitor cells. Delivery of genetic materials like DNA, miRNAs, or mRNAs through EVs can also introduce the reparative potential of parental MSCs to recipient cells. In a study by Ranghino et al. [18], glomerular MSC-derived EVs mediated tubular epithelial cell regeneration and alleviated ischemic acute kidney injury (AKI) by transferring mRNAs and miRNAs with potential proregenerative effects.

**Hemostasis and platelet aggregation**

An ample number of publications have reported the coagulant properties of plasma EVs in humans [19,20]. EVs originating from platelets, endothelial cells, or leukocytes were found to be involved in the course of hemostasis. These EVs can initiate the coagulation process by exposing phosphatidylserine (PS) on their surfaces to allow binding sites for coagulation factors [21]. Another mechanism is through the expression of tissue factors on their surfaces, initiating the extrinsic pathway of coagulation and leading to thrombin burst and platelet clot formation [22]. A study by Yu et al. [23] showed that patients with diabetic kidney disease had a significantly higher level of PS-expressing microvesicles compared to control groups. They also found that higher levels of PS-positive microvesicles were associated with hypercoagulable status as well as worse renal function. A similar finding was shown in patients with immunoglobulin A nephropathy [24].

Recently, several studies have revisited the procoagulation property of EVs because of technical improvements in blood collection, plasma preparation, and EV isolation [25]. Interestingly, plasma EVs isolated from healthy individuals promote fibrinolysis more dominantly than coagulation. This discrepancy suggests an ambivalent role of EVs in hemostasis processes, which depends on the microenvironment. In addition, proper EV sample preparation is important for a better understanding of their role in the human body.

**Vessel integrity and revascularization**

Endothelial cell-derived EVs help to maintain vessel integrity and promote vascular endothelial cell survival by removing cell-death signals and releasing excess complements from vascular endothelial cells [26]. In damaged vessels, platelet-derived EVs induce the adhesion of endothelial cells to the extracellular matrix, which helps the regeneration of endothelium and attenuates vascular permeability [27].

Tumor-derived EVs are well-known for their angiogenic properties, but EVs can also induce angiogenesis under physiologic conditions. Endothelial cell-derived EVs modulate angiogenesis by promoting endothelial cell invasion and capillary-like structure formation [28]. Recently, the transfer of certain miRNAs, including miR-126 or miR-124, using endothelial cell-derived EVs and recipient endothelial cells has been found to have a significant role in angiogenesis [29,30]. MSC-induced EVs also induce angiogenesis by transferring proteins like phosphorylated signal transducer and activator of transcription-3 or nuclear factor-kB pathway-associated proteins, leading to the transcription of proangiogenic proteins [31,32]. MSC-derived EVs can also transfer several growth factors including epidermal growth factor and vascular endothelial growth factor to endothelial cells promoting angiogenesis. More detailed information about the angiogenic mechanism of EVs from multiple cellular origins can be found in a review article by Todorova et al. [2].

**Electrolyte and water balance**

Sodium/water reabsorption is one of the major functions of renal tubular cells. Two-thirds of filtrated sodium is reabsorbed in proximal tubular epithelial cells and fine-tuning for sodium reabsorption occurs in the distal tubule and collecting ducts. Therefore, interactive communication between proximal and distal/collection tubular cells are important for proper maintenance of electrolyte balance and volume control. A study by Jella et al. [33] showed that exosomes from proximal tubular cells can regulate epithelial sodium channel
activity in the distal tubule and collecting ducts through the exosomal delivery of glyceraldehyde 3-phosphate dehydrogenase. Upon exposure to vasopressin, cortical collecting duct tubular cells increase the production of aquaporin water channels within themselves as well as within their exosomes [34]. These exosomes could transfer functional aquaporin channels to other tubular cells, increasing their water transport capacity. By mediating inter and intracellular communication of renal epithelial cells among the different nephron segments, exosomes provide a significant role in electrolyte and fluid balance in the human body.

**Extracellular vesicles as mediators of kidney disease**

In pathologic conditions, the production of EVs significantly increases, contributing to the initiation and propagation of disease through immunomodulation, thrombogenesis, and oncogenesis. The detailed role of renal EVs in different disease courses are discussed below (Fig. 1B).

**Glomerular disease**

In advanced glomerular disease, tubular damage and fibrosis are the leading mechanisms of renal dysfunction. A recent study by Jeon et al. [35] showed that miRNAs in EVs released by injured podocytes can induce tubular epithelial cell apoptosis, suggesting possible glomerulotubular crosstalk. This study also showed that the miRNA profile in EVs from injured podocytes are different from those of non-injured podocytes, and miRNA mimics of injured podocytes could reproduce a proapoptotic effect on renal tubular epithelial cells. In diabetic nephropathy, exposure to high glucose levels can induce EV production from podocytes. Tubular epithelial cells uptake these podocyte-derived EVs and generate tubular fibrosis through p38 phosphorylation [36].

**Acute kidney injury**

A recent miRNA sequencing study showed that urinary exosomal miRNAs have characteristic profiles and functional properties that depend on the AKI stage [37]. Several exo-miRNAs (miR-16, miR-24, and miR-200c) were increased during the early injury state, and the expression of their target mRNAs was significantly affected in the renal medulla. During the early recovery phase, exo-miRNAs that share Zeb1/2 as a common target were significantly upregulated, implying their role in transforming growth factor (TGF)-β1-associated renal fibrosis. In ischemia-reperfusion injury, hypoxic damage induces the increased production of EVs in tubular epithelial cells that contains TGF-β1 mRNA [8]. Exosomal delivery of these TGF-β1 mRNAs into fibroblasts ultimately leads to fibroblast activation and proliferation. On the other hand, Ranghino et al. [18] recently showed that MSCs within the glomeruli can contribute to postischemic renal recovery primarily through the release of EVs.

In sepsis-related AKI, platelet-derived and neutrophil-derived EVs demonstrate proinflammatory and procoagulant effects, which can prevent the growth of bacteria and its dissemination [38]. However, these EVs can systematically induce oxidative stress and disseminate inflammatory responses, leading to poorer clinical outcomes.

**Tubulointerstitial inflammation**

Tubulointerstitial inflammation is a common characteristic of both acute and chronic kidney disease. Lv et al. [39] found a significant increase in tubular epithelial cell-derived exosomal miRNA-19b-3p in both a lipopolysaccharide-induced AKI model and an adriamycin-induced chronic proteinuric kidney disease model. Tubular epithelial cell-derived exosomal miRNA-19b-3p promoted M1 macrophage activation and tubulointerstitial inflammation. This group also showed that exosomal delivery of CCL2 mRNA from tubular epithelial cells to macrophages could mediate albumin-induced tubulointerstitial inflammation [40]. Hypoxia is another well-known stimulator of tubulointerstitial inflammation in the kidney. Hypoxic stimulation induces the expression of hypoxia-inducible factor-1α in tubular epithelial cells. This stimulus leads to exosomal miRNA-23a transfer from tubular epithelial cells to macrophages, which triggers macrophage activation and promotes tubulointerstitial inflammation [41]. Thus, modulation of macrophage activation by blocking exosomal miRNA or mRNA delivery from tubular epithelial cells can be a novel therapeutic approach.

**Fibrosis**

Fibrosis in glomeruli and tubules is an ultimate pathologic
process of chronic kidney diseases and end-stage renal disease, and the pathophysiological role of EVs in fibrosis has been explored in several studies [5,8,42,43]. Various stimuli on renal cells, such as hypoxia, oxidative stress, or high glucose, can lead to EV secretion or changes in the EV composition [8,42]. EVs secreted by damaged kidney cells can be transferred to other normal kidney cells, changing their phenotype and activating fibroblasts. In hypoxic conditions, damaged tubular epithelial cells transfer TGF-β1 mRNA to fibroblasts through exosome secretion, ultimately leading to the proliferation/activation of fibroblasts and progression of renal tubular fibrosis [8]. An in vitro study by Wu et al. [42] showed that EVs secreted from high glucose-treated glomerular endothelial cells can trigger podocyte epithelial-mesenchymal transition by transferring TGF-β1 mRNA into glomerular mesangial cells. This process induces cellular proliferation and overproduction of extracellular matrix within glomeruli, which is a key pathological mechanism of diabetic nephropathy. Several miRNAs in EVs, such as miR-21, miR-192, and miR-34a, also affect the biological processes of tubular fibrosis [43,44] and glomerular fibrosis [45], respectively.

Nephrolithiasis

Given the significant role of EVs as mediators of various renal diseases, several studies have speculated that EVs have a role in stone formation. For instance, an in vitro study by He et al. [46] showed that exposure of renal tubular cells to different concentrations of oxalates could affect the size and composition of the exosomes from tubular cells. This result suggests that the biological role of exosomes may depend on the level of oxalate exposure in a given microenvironment. Other in vitro studies by Singhto et al. [47,48] showed that exosomes derived from human macrophages exposed to calcium oxalate crystals could induce the migration/activation of monocytes/T-cell/neutrophils and enhance the production of proinflammatory cytokine interleukin-8. These exosomes had a greater capacity to bind calcium oxalate crystals and subsequently enhanced crystal invasion through the extracellular matrix, suggesting the possible role of macrophage-derived exosomes in the crystal invasion of the renal interstitium.

Renal cancer

EVs mediate intercellular communication between tumor cells. EVs from cancer cells contain proangiogenic mRNAs and various miRNAs with tumor invasive properties [49]. Clear renal cell carcinoma-derived EVs can negatively affect adjacent immune cells by the activation of the TGF-β/SMAD signaling pathway [50]. EVs are also known to mediate chemoresistance in renal cancer cells through the delivery of long noncoding/competing endogenous RNA, ultimately leading to higher expression of prometastatic receptors [51]. Further information about the role of EVs in urological malignancies is well described in a review article by Linxweiler and Junker [4].

Extracellular vesicles as biomarkers in various kidney diseases

Most of the EV studies in kidney diseases have been focused on biomarker discoveries. EVs can be found in multiple types of biological fluids including serum, urine, breast milk, cerebrospinal fluid, saliva, ascites, and bronchoalveolar lavage fluid. Under physiologic conditions, almost all urinary EVs originate from kidney cells because circulating EVs do not generally go through the glomerular basement membrane. Therefore, the organ-specific origin of urinary EVs comprises the major advantage of using urinary EVs as biomarkers in kidney diseases. Also, urinary EVs can be obtained noninvasively and repeatedly, providing important information regarding diagnosis, prognosis, and response to treatment. Even though circulating EVs can originate from different organs, serum, and plasma, they can still provide significant information about the extent and prognosis of renal disease because the clinical outcome is determined not only from the kidney damage itself but also from the dysfunction of distant organs including the lungs, heart, brain, liver, and immune system [52]. Easier clinical accessibility is also a huge benefit of using circulating EVs as biomarkers in kidney disease.

Recent advancements in ‘omic’ studies have expedited the discovery of novel EV biomarkers enabling the detection of pathologic processes even earlier than traditional biomarkers. Salih et al. [53] compared urinary EVs from autosomal dominant polycystic kidney disease (ADPKD) patients and
healthy controls using quantitative proteomics. Among the proteins with higher abundancy, five proteins including complements and plakins were selected for further analysis based on their significance in pathway analysis. Especially, a higher level of complement in urinary EVs was found in younger ADPKD patients with preserved kidney function, raising its potential as a biomarker. Due to the vast amount of information from various studies and limited space, the role of EVs as biomarkers is summarized in Table 2 [53–78].

**Extracellular vesicles as therapeutics in various kidney diseases**

**Extracellular vesicles as a cargo of therapeutic material**

The role of EVs as biocarriers has received a lot of attention.
Table 2. Continued

<table>
<thead>
<tr>
<th>Disease</th>
<th>Source/method</th>
<th>Main finding</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKD</td>
<td>Human urine/proteomics</td>
<td>Higher levels of complement C3 and C9 are found in urinary EVs derived from ADPKD patients.</td>
<td>[53]</td>
</tr>
<tr>
<td></td>
<td>Human and rat urine/Western blotting</td>
<td>Increased urinary exosomal AGS3 expression in PKD patients/rats.</td>
<td>[70]</td>
</tr>
<tr>
<td>LN</td>
<td>Human urine/RT-PCR</td>
<td>Urinary exosomal miR-29c correlates with the degree of renal chronicity but not with renal function in patients with LN.</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td>Human urine/RT-PCR</td>
<td>Decreased level of urinary exosomal let-7a and miR-21 in patients with active LN compared to inactive LN.</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td>Human urine/miRNA sequencing</td>
<td>Urinary exosomes from LN patients complicated with cellular crescent have a unique miRNA expression profile including miR-3135b, miR-654-5p, and miR-146a-5p.</td>
<td>[73]</td>
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<tr>
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<td>Human urine/RT-qPCR</td>
<td>Down-regulation of urinary exosomal miR-29c level with worsening renal function and progression of tubulointerstitial fibrosis in CKD patients.</td>
<td>[74]</td>
</tr>
<tr>
<td></td>
<td>Human and mouse urine/RT-qPCR</td>
<td>Upregulation of urinary exosomal miR-21 in CKD patients as well as in nephrotoxic serum-treated mice.</td>
<td>[75]</td>
</tr>
<tr>
<td></td>
<td>Human urine/RNA-sequencing</td>
<td>Urinary exosomal miRNA-181a significantly decreased in CKD patients compared to healthy controls.</td>
<td>[76]</td>
</tr>
<tr>
<td>RCC</td>
<td>Human plasma/RT-qPCR</td>
<td>Increased hsa-miR-301a-3p and decreased hsa-miR-1293 levels were found in plasma EVs in patients with metastatic clear cell RCC compared to those with localized disease.</td>
<td>[77]</td>
</tr>
<tr>
<td></td>
<td>Human plasma/RT-qPCR</td>
<td>Higher levels of plasma EV-derived TIMP-1 mRNA were found in patients with metastatic RCC or huge tumor burden. Also, patients with a high level of EV-derived TIMP-1 mRNA had poorer survival compared to those with a low level.</td>
<td>[78]</td>
</tr>
</tbody>
</table>

ADPKD, autosomal dominant polycystic kidney disease; AGS3, activator of G-protein signaling 3; AKI, acute kidney injury; ATF3, activating transcription factor 3; CKD, chronic kidney disease; DN, diabetic nephropathy; DM, diabetes mellitus; EV, extracellular vesicle; FSGS, focal segmental glomerulosclerosis; IgA, immunoglobulin A; LN, lupus nephritis; IncRNA, long noncoding RNA; MCD, minimal change disease; miRNA and miR, microRNA; mRNA, messenger RNA; NGAL, neutrophil gelatinase-associated lipocalin; PKD, polycystic kidney disease; RCC, renal cell carcinoma; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; SH2D1B, SH2 domain-containing protein 1B; TIMP, tissue inhibitor of metalloproteinase; TNF-α, tumor necrosis factor alpha; WT-1, Wilms tumor 1.

recently. EVs carry several biological benefits as a vector over other methods, including stability, small size, and low immunogenicity. Specific cell surface molecules on EVs increase their targeting capacity with fewer off-target effects. Nanosized EVs with certain surface molecules can even penetrate natural barriers such as the blood-brain barrier [79].

Also, EVs use the native mechanisms of recipient cells in the course of internalization and intracellular trafficking [80]. Delivery of biomolecular cargos such as mRNA, miRNA, and proteins can modify the genetic profile and biological response of recipient cells, leading to the modulation of disease processes [81].

EV loading of traditional drugs with anti-inflammatory properties has been studied for therapeutic applicability [82,83]. Dexamethasone, for example, is widely used to suppress inflammation in various renal diseases, but its clinical use is limited due to significant adverse effects. By incubating macrophages with dexamethasone, macrophage-derived dexamethasone containing EVs were successfully produced and delivered to the inflamed kidneys of mice, which showed significant suppression of renal inflammation and fibrosis [82]. Notably, dexamethasone containing EVs substantially decreased the adverse effects from systemic steroid treatment such as hyperglycemia and suppression of the hypothalamic-pituitary-adrenal axis.

During the past decade, there has been an explosive advancement in EV research. Interdisciplinary cooperation between medicine and biomedical engineering has harnessed EVs as potent drug delivery tools through the application of nanotechnology. Several studies have successfully loaded genetic material into EVs and shown effects on modifying disease courses. Yim et al. [84] recently developed a novel technique for the effective intracellular transfer of soluble proteins through exosomes by using optogenetic engineering.
This technology was adopted in an experimental sepsis model, showing the protective effect of exosomal super-repressor inhibitor of kappa B (IkB) delivery in a murine septic AKI model [85]. Recently, our group has also demonstrated the beneficial effect of exosomal super-repressor IkB delivery in an ischemic AKI model (under review).

**Extracellular vesicles for kidney repair**

Stem cell therapy has great benefits in multiple experimental kidney injury models, not through their differentiation potential but the paracrine effects of their EVs [17]. EVs secreted from MSCs or endothelial progenitor cells have been shown to play a significant role in the amelioration of tubular cell apoptosis/fibrosis and proliferation of tubular and endothelial cells in various AKI models [86, 87]. This beneficial effect was also found in experimental chronic kidney disease models, ameliorating epithelial-to-mesenchymal transition and preventing inflammatory cell infiltration [88, 89]. MSCs that were engineered to overexpress miRNA-let7c could selectively deliver miRNA-let7c to damaged kidney cells using exosomes, which led to protection from renal injury and decreased fibrosis in a unilateral ureteral obstruction model [90]. Nagaishi et al. [91] showed that MSC-derived renal trophic factors including exosomes could alleviate albuminuria and immune cell infiltration into the kidneys in a diabetic nephropathy model. MSC-derived exosomes showed an antiapoptotic effect and protected tight junction structure in tubular epithelial cells. Intravenous injections of urinary stem cell-derived exosomes could also reduce urinary microalbumin excretion and prevent podocyte/tubular epithelial cell apoptosis in diabetic rats [92].

Apart from MSCs, EVs from other origins have shown a renoprotective effect in animal kidney injury models. Exosomes derived from sera of animals after remote ischemic preconditioning could attenuate sepsis-induced renal damage, and this was mediated by the upregulation of exosomal miRNA-21 [93]. Dominguez et al. [94] showed that intravenous delivery of EVs from rat renal tubular cells could markedly reduce renal damage in recipients after ischemic insult. This group also showed that human renal exosomes can prevent ischemic renal injury in nude rats [95].

However, the clinical evidence of the renoprotective effect of EV therapy in humans is relatively scarce. Several problems limit the therapeutic application of EVs in humans; the low yield of EVs from cultured cells and premature EV isolation/purification with a risk of contamination from other molecules with similar density [96, 97]. Although several studies have shown promising advances in EV isolation and purification, which is reviewed elsewhere [98], further efforts are needed to improve isolation efficiency and purity. Also, large-scale production is required for the therapeutic application of EVs in humans. Yang et al. [99] recently used a cellular nanoporation method to produce large-scale exosomes containing therapeutic mRNAs and targeting peptides. By using this strategy, they could produce a significantly higher number of exosomes and exosomal mRNA transcripts compared with bulk electroporation and other exosome production strategies. Lastly, advanced techniques in the fine modulation of EV content using transfection or cell engineering is needed to deliver specific transcripts or protein composition into target cells. This will lead to a better understanding of the biological role of certain EVs in specific renal disease models and will broaden the therapeutic applicability of EVs in various renal diseases.

**Extracellular vesicles as potential therapeutic targets**

As we have mentioned, EVs can mediate the propagation of renal damage in septic, inflammatory, or thrombogenic conditions. Therefore, temporarily blocking the release and uptake of EVs can possibly alleviate tissue damage. Various pharmacologic agents including antiplatelet agents, statins, calcium channel blockers, and abciximab were found to negatively affect the process of EV release and uptake, which is reviewed elsewhere [1]. However, whether blocking the release and uptake of EVs can directly affect the disease course has not been fully investigated. A study by Mossberg et al. [100] showed that C1-inhibitor can significantly reduce the release of chemotactic kinin B1-receptor-positive endothelial microvesicles, suggesting it has therapeutic potential in inflammatory diseases. Further studies are required to understand the clinical applicability of controlling EV interaction with recipient cells in systemic inflammatory conditions.

**Conclusions and perspectives**

EVs are involved in cell-to-cell communications within kidneys, mediating podocyte-tubulointerstitial/proximal-distal tubular/glomerulus-tubular crosstalk. EVs can either pro-
vide physiological roles in renal repair and immunomodulation or mediate pathological processes inducing thrombosis and inflammation. Recent studies have significantly improved our understanding of the roles of EVs in kidney physiology and pathology. However, most of the results are limited to descriptive transcriptomic/proteomic analysis and lack consistency as well as reliability. Stringent validation through clinical trials and large-scale cohort studies is necessary before entering into clinical application. Further optimization of EV isolation techniques and meticulous manipulation of the genetic materials or protein compositions of EVs are also necessary for clinical consideration. These efforts will extend our knowledge on the role of EVs in the development/progression of kidney diseases and will broaden the clinical applicability of EVs as novel diagnostics and therapeutics of renal diseases.

Conflicts of interest

Tae-Hyun Yoo is a Scientific Advisory Board member at ILIAS Biologics Inc. and the Editor-in-Chief of Kidney Research and Clinical Practice. Chulhee Choi is the founder and a shareholder of ILIAS Biologics Inc. The authors have no additional financial interests.

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Writing—original draft: SAL
Writing—review & editing: CC, THY
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Fabry disease exacerbates renal interstitial fibrosis after unilateral ureteral obstruction via impaired autophagy and enhanced apoptosis

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Background: Fabry disease is a rare X-linked genetic lysosomal disorder caused by mutations in the GLA gene encoding alpha-galactosidase A. Despite some data showing that profibrotic and proinflammatory cytokines and oxidative stress could be involved in Fabry disease-related renal injury, the pathogenic link between metabolic derangement within cells and renal injury remains unclear.

Methods: Renal fibrosis was triggered by unilateral ureteral obstruction (UUO) in mice with Fabry disease to investigate the pathogenic mechanism leading to fibrosis in diseased kidneys.

Results: Compared to kidneys of wild-type mice, lamellar inclusion bodies were recognized in proximal tubules of mice with Fabry disease. Sirius red and trichrome staining revealed significantly increased fibrosis in all UUO kidneys, though it was more prominent in obstructed Fabry kidneys. Renal messenger RNA levels of inflammatory cytokines and profibrotic factors were increased in all UUO kidneys compared to sham-operated kidneys but were not significantly different between UUO control and UUO Fabry mice. Protein levels of Nox2, Nox4, NQO1, catalase, SOD1, SOD2, and Nrf2 were not significantly different between UUO control and UUO Fabry kidneys, while the protein contents of LC3-II and LC3-I and expression of Beclin1 were significantly decreased in UUO kidneys of Fabry disease mouse models compared with wild-type mice. Notably, TUNEL-positive cells were elevated in obstructed kidneys of Fabry disease mice compared to wild-type control and UUO mice.

Conclusion: These findings suggest that impaired autophagy and enhanced apoptosis are probable mechanisms involved in enhanced renal fibrosis under the stimulus of UUO in Fabry disease.

Keywords: alpha-Galactosidase, Autophagy, Fabry disease, Fibrosis

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Introduction

Fabry disease (OMIM #301500) is a rare X-linked lysosomal storage disorder resulting from an error in glycosphingolipid metabolism caused by a lack of alpha-galactosidase A (α-Gal A) [1,2]. Deficiency of α-Gal A can cause progressive accumulation of glycosphingolipids such as globotriaosylceramide (Gb3, also known as CD77 or GL3) in lysosomes. Clinical manifestations of Fabry disease are consequences of such Gb3 accumulation in various tissues and organs, including the heart, liver, spleen, and kidney [1,3]. Renal involvement is observed in approximately 55% of patients with Fabry disease [4]. In males with the classical form of Fabry disease, renal manifestation can start with microalbuminuria in infancy. Disease manifestation becomes evident with higher albuminuria and overt proteinuria in childhood and early adulthood [3,5]. In later adulthood, around the third or fourth decade of life, the glomerular function begins to be affected, leading to chronic kidney disease and ultimately end-stage kidney disease within the fifth decade [3]. It is difficult to recognize prodromal Fabry disease due to its highly variable and nonspecific phenotype, and low prevalence rate. Many patients are diagnosed late or never. This remains an unsolved problem [6]. Commercially approved enzyme replacement therapy (ERT) and pharmacological chaperones are available to treat Fabry disease.

Despite marked advances in patient care and improved overall outlook on Fabry disease [5], current therapeutic strategies are insufficient to stop or reverse most processes of Fabry disease. There is a significant need to develop other therapeutic approaches based on an increased understanding of Fabry disease beyond the primary enzyme deficiency [7]. The exact mechanism through which the disease can cause multisystemic damage is poorly understood. High levels of glycolipids in cells and plasma affected by Fabry disease are insufficient to explain the pathophysiology of this disease and the inter- or infrafamilial phenotype variability in patients with the same GLA mutations [8]. Researchers have suggested that abnormal accumulation of Gb3 or globotriaosylphingosine (lyso-Gb3) due to α-Gal A deficiency could trigger different cellular mechanisms and affect phenotypic expression of Fabry disease [8,9]. Lysosomal deposits can act in a damage-associated molecular pattern (DAMP), known to represent endogenous molecules released from injured or dying cells [8]. In addition, intracellular accumulation of substrates could cause DAMP production by injured cells with subsequent proinflammatory activity such as increased recruitment of cytokines, proinflammatory factors, and leukocyte activity [8,10]. Indeed, most studies have emphasized the role of inflammation in tissue damage in Fabry disease [1,7,11]. Previous studies have shown that increased oxidative stress and altered antioxidant defenses could be involved in the vasculopathy of Fabry disease [7,12]. Altered nitric oxide generation and increased levels of reactive oxygen species (ROS) and nitrotyrosine have been observed in patients and animal models of Fabry disease [13–15]. Another study has shown that decreased level of tetrahydrobiopterin, an essential cofactor for the normal enzymatic function of all isoforms of nitric oxide synthase and aromatic amino acid hydroxylases, in Fabry mouse tissues contributes to disease pathogenesis through oxidative stress, associated with antioxidant capacity of cells and uncoupling of nitric oxide synthase [13]. Expectedly, transforming growth factor-β1 (TGF-β1), a regulatory cytokine with key functions under inflammatory and oxidative stress conditions, could promote fibrosis by enhancing the synthesis of extracellular matrix in cells and tissues of patients with Fabry disease via epithelial-to-mesenchymal transition (EMT) [8,16,17].

The autophagy-lysosome pathway could be another important signaling pathway contributing to the onset and progression of Fabry disease in involved cells and tissues [18]. A few investigations have suggested that dysregulation of autophagy resulting from Gb3 deposition could contribute to tissue damage [7,8,18]. A previous study has revealed that expression of microtubule-associated protein light chain 3 (LC3), a marker of autophagic vacuoles, is increased substantially in brains with α-Gal A deficiency in a mouse model of Fabry disease [18]. However, a connection between altered autophagy and renal involvement has yet to be established in Fabry nephropathy. The objective of this study was to examine how kidneys would respond and react to proinflammatory and profibrotic stimuli triggered by unilateral ureteral obstruction (UUO) in a mouse model of Fabry disease and to determine which pathophysiological signaling pathways are most altered in such kidneys.
Methods

Experimental animals

Fabry disease mice, a rodent model with α-Gal A gene-disruption, were bred to produce sufficient numbers. Heterozygous female mice were bred with control males to maintain the mouse colony. Mating between mutant males and females was performed to generate littermates with α-Gal A deficiency [18]. All mice were fed a rodent diet and water ad libitum. For this study, male Fabry disease mice or wild-type mice weighing 20 to 25 g underwent left ureteral ligation with 4-0 silk thread under isoflurane anesthesia. Sham operations were similarly performed for wild-type mice without ligation as described previously [19]. Mice were divided into three groups (n = 4 or 5 mice per group): sham-operated wild-type mice (Sham), UUO-operated wild-type control mice (UUO Cont), and UUO-operated Fabry disease mice (UUO Fabry). At 7 days after sham operation or UUO, mice were euthanized by exsanguination under isoflurane anesthesia, followed by trans-cardiac perfusion with phosphate-buffered saline (PBS). Sham-operated or obstructed kidneys were harvested for histological evaluation and molecular analysis. All experiments were performed following protocols approved by the Institutional Animal Care and Use Committee of The Catholic University of Korea Yeouido St. Mary’s Hospital (No. YEO-2020-009FA).

Genotyping

To genotype each mouse, polymerase chain reaction (PCR) was performed as described previously [16] using the following primers: Exon3-forward: 5’-GCAACTGTTCATGCAGATGG-3’; Exon3-reverse: 5’-CTGCAGATCAATGTCATAGG-3’; Neo-forward: 5’-GAAGGGACTGGCTGCTATTG-3’; Neo-reverse: 5’-AATATCACGGGTAGCCAACG-3’. Results were used to indicate which mice were wild-type or α-Gal A deficient (Supplementary Fig. 1, available online).

Histology

To evaluate the severity of tubulointerstitial fibrosis, 4% phosphate-buffered paraformaldehyde-fixed kidney sections were stained with Masson’s trichrome or with sirius red following the manufacturer’s protocols (Merck KGaA, Darmstadt, Germany) [20]. More than 20 fields were randomly selected for quantitative evaluation and analyzed in a blinded manner using ImageJ 1.53a software (National Institutes of Health, Bethesda, MD, USA).

Quantitative reverse transcription polymerase chain reaction

According to the manufacturer’s manual, total renal RNA was isolated using TRIzol reagents (Thermo Fisher Scientific, Waltham, MA, USA). Quantitative reverse transcription (qRT) PCR assays were performed using SYBR Premix (TaKaRa Bio Inc., Otsu, Japan). Primer sequences for each gene used in PCR are listed in Supplementary Table 1 (available online). The specificity of PCR was confirmed by analyzing the melting curve. All PCR assays were performed in duplicate. Results were normalized to messenger RNA (mRNA) expression in the sham-operated kidney tissues of wild-type mice.

Immunoblotting

Total proteins from harvested kidneys were extracted using a PRO-PREP Protein Extraction Kit (iNtRON Biotechnology, Seongnam, Korea). Protein concentrations were determined using a protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). After electrophoresis, proteins in gels were transferred into nitrocellulose membranes and incubated with primary antibodies against the following proteins were used: NAD(P)H:quinone oxidoreductase 1 (NQO1; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), catalase (Abcam, Cambridge, UK), superoxide dismutase 1 (SOD1; Enzo Life Science, Inc., Farmingdale, NY, USA), SOD2 (Abcam), heme oxygenase-1 (HO-1; Thermo Fisher Scientific), nuclear factor-erythroid-2-related factor 2 (Nrf2; Santa Cruz Biotechnology Inc.), Nox2 (BD Bioscience, San Jose, CA, USA), Nox4 (Cell Signaling Technology, Danvers, MA, USA), LC3B (Novus Biologicals, Littleton, CO, USA), Beclin1 (Novus), β-actin (Sigma-Aldrich, St. Louis, MO, USA), β-tubulin (Sigma-Aldrich), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Sigma-Aldrich). After washing with PBS, all blots were incubated with a secondary antibody conjugated with horseradish peroxidase. Immunoreactive proteins were detected by enhanced chemiluminescence reagents. Densities of protein bands were...
measured and normalized to those of respective housekeeping proteins in the same sample.

**Transmission electron microscopy**

For electron microscopic examination, small sections of kidney tissues were fixed in 2.5% glutaraldehyde in phosphate buffer. After washing and dehydrating in serial alcohol and propylene oxide, tissues were infiltrated with a series of graded ethyl alcohol solutions and embedded in Epon substitute. Thin sections were prepared and stained with uranyl acetate and lead citrate. Samples were observed under a transmission microscope to identify representative lesions in glomeruli and proximal tubules.

**Terminal deoxynucleotidyl transferase dUTP nick end labeling assay**

To examine DNA fragmentation indicative of apoptosis, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (Millipore, Billerica, MA, USA) was performed according to the manufacturer’s protocol. TUNEL-positive cells were evaluated in 20 randomly selected fields for each section using ImageJ 1.53a software.

**Statistical analysis**

Quantitative data are expressed as mean ± standard error of mean. All statistical analyses were performed using GraphPad Prism (GraphPad Software LLC, La Jolla, CA, USA). In all analyses, p-values less than 0.05 were considered to indicate a statistically significant difference.

**Results**

**Fabry disease aggravates renal tubulointerstitial fibrosis triggered by unilateral ureteral obstruction**

Compared with sham-operated kidneys, obstructed kidneys induced by UUO showed renal tubulointerstitial fibrosis, as indicated by Masson’s trichrome and sirius red staining (Fig. 1). Besides, UUO-induced renal fibrotic areas were larger in UUO kidneys of Fabry disease mice than in sham-operated kidneys.

**Fabry disease has no further effect on inflammation or epithelial-mesenchymal transition triggered by unilateral ureteral obstruction**

Results of qRT-PCR revealed that renal mRNA levels of interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNFa) were significantly increased on day 7 post-UUO compared to those of sham-operated kidneys (Fig. 2A–C). However, there was no additional increase in the mRNA levels of inflammatory cytokines in obstructed kidneys of Fabry disease mice as indicated by sirius red staining (*p = 0.009 vs. Sham, **p < 0.001 vs. Sham, p = 0.02 vs. UUO Cont). (D) More enhanced renal fibrotic areas in obstructed kidneys of Fabry disease mice as indicated by sirius red staining (*p = 0.009 vs. Sham, **p < 0.001 vs. Sham, p = 0.02 vs. UUO Cont). Cont, control; Sham, sham-operated wild-type mice.
Figure 2. Renal messenger RNA (mRNA) expression levels of genes involved in inflammation and epithelial-mesenchymal transition. (A) Quantitative reverse transcription polymerase chain reaction analysis showing the increased expression level of renal interleukin (IL)-1β on day 7 after unilateral ureteral obstruction (UUO), without significant difference between UUO control (Cont) and UUO Fabry (*p = 0.05 vs. Sham, **p = 0.02 vs. Sham). (B) Increased IL-6 mRNA level in UUO kidneys (*p = 0.02 vs. Sham, **p = 0.04 vs. Sham). (C) Similarly increased tumor necrosis factor-α (TNFα) mRNA level in both UUO Cont and UUO Fabry (*p = 0.048 vs. Sham, **p = 0.046 vs. Sham). (D) Significantly enhanced matrix metalloproteinase (MMP)2 mRNA level in the obstructed kidneys of all UUO groups (*p = 0.03 vs. Sham, **p = 0.04 vs. Sham). (E) Increased renal MMP9 mRNA level observed in all UUO groups (*p = 0.01 vs. Sham, **p = 0.02 vs. Sham). (F) No significant increase in epithelial cadherin (E-cadherin) mRNA level in all UUO groups compared to Sham. (G) Significantly increased renal mRNA level of vascular E-cadherin (VE-cadherin) in UUO Cont (*p = 0.03 vs. Sham). (H) Significantly enhanced renal α-smooth muscle actin (α-SMA) mRNA levelin UUO Cont (*p = 0.009 vs. Sham). (I) Increased fibronectin mRNA level in all UUO groups (*p = 0.006 vs. Sham, **p = 0.05 vs. Sham). (J) Increased renal vimentin mRNA levelin all UUO groups (*p = 0.002 vs. Sham, **p = 0.004 vs. Sham). (K) Similarly enhanced mRNA level of renal transforming growth factor (TGF)-β in all UUO groups (*p = 0.01 vs. Sham,**p = 0.007 vs. Sham). (L) Increased collagen type IV α1 chain (COL4A1) mRNA level on day 7 after UUO in obstructed kidneys of all UUO groups (*p = 0.004 vs. Sham, **p = 0.04 vs. Sham). Sham, sham-operated wild-type mice.
vascular endothelial cadherin (VE-cadherin) and α-smooth muscle actin (α-SMA) were significantly enhanced in UUO control mice compared to sham-operated mice and tended to be increased in UUO Fabry mice, without statistical significance (Fig. 2G, H). Fibronectin, vimentin, TGF-β1, and collagen type IV α1 chain (COL4A1) mRNA levels were increased at day 7 after UUO in both UUO control and UUO Fabry without a significant difference between the two groups (Fig. 2I-L). Collectively, the expression of inflammation or EMT-related genes was significantly increased by a similar amount in obstructed kidneys of both wild-type and Fabry mice, suggesting that Fabry disease had no additional effect on renal inflammation or EMT stimulated by UUO surgery.

Figure 3. Effect of Fabry disease on antioxidant enzymes in unilateral ureteral obstruction (UUO) kidneys. (A) Representative western blot results for NAD(P)H:quinone oxidoreductase 1 (NQO1), catalase, superoxide dismutase (SOD)-1, heme oxygenase-1 (HO-1), total nuclear factor-erythroid-2-related factor 2 (Nrf2), and SOD2. (B) Increased renal protein expression of NQO1 after UUO in both wild-type and Fabry disease mice, without significant difference between the two UUO groups (*p < 0.001 vs. Sham, **p < 0.001 vs. Sham). (C) Similarly increased catalase expression after UUO in UUO control (Cont) and UUO Fabry (*p < 0.001 vs. Sham, **p < 0.001 vs. Sham). (D) Decreased expression of SOD1 in both UUO Cont and UUO Fabry groups, with UUO Fabry having a slightly higher level than UUO Cont (*p < 0.001 vs. Sham, **p < 0.001 vs. Sham, p = 0.02 vs. UUO Cont). (E) Renal HO-1 expression significantly increased in all UUO groups (*p = 0.003 vs. Sham, **p = 0.02 vs. Sham). (F) Significantly enhanced total Nrf2 expression in all UUO groups (*p < 0.001 vs. Sham, **p = 0.002 vs. Sham). (G) Lower renal SOD2 expression in both UUO Cont and UUO Fabry than in Sham (*p < 0.001 vs. Sham, **p < 0.001 vs. Sham). Sham, sham-operated wild-type mice.
Obstructed kidneys of Fabry disease mice have similar renal antioxidant enzyme and oxidative stress status

On day 7 post-UUO, obstructed kidneys showed suppressed expression of NQO1, catalase, SOD1, and SOD2 proteins (Fig. 3A–D, G). However, Fabry disease did not confer further suppression of those antioxidant enzymes. Rather, renal SOD1 expression in UUO Fabry was slightly higher than that of UUO Cont but still significantly lower than that of Sham Cont (Fig. 3A, D). By contrast, protein expression levels of HO-1 and Nrf2, a transcription factor involved in the induction of cytoprotective antioxidants, were similarly increased by UUO in both wild-type and Fabry disease mice (Fig. 3A, E, F).

Results of Western blot analysis revealed that protein level of Nox2, one of the NADPH oxidases in the Nox family, was significantly increased in UUO control and tended to be increased in UUO Fabry without a statistically significant difference from that of the sham group (Fig. 4A, B). Renal Nox2 expression level was significantly increased in all UUO groups compared to that in the sham group (Fig. 4A, C). These results demonstrate that Fabry disease mice experience no further aggravation of antioxidant enzymes or oxidative stress in obstructive nephropathy compared to wild-type.

Obstructed kidneys of Fabry disease mice have altered autophagy in unilateral ureteral obstruction kidneys

It has been reported that autophagy is involved in the development and progression of renal fibrosis induced by UUO [21,22]. The ratio of LC3-II to LC3-I, a reliable marker of autophagy, was significantly increased in UUO control

**Figure 4.** Effect of Fabry disease on oxidative stress in unilateral ureteral obstruction (UUO) kidneys. (A) Representative western blot results for Nox2 and Nox4. The β-tubulin image was reused because the membrane for immunoblot analyses of superoxide dismutase-1, heme oxygenase-1, and total nuclear factor-erythroid-2-related factor 2 in Fig. 3 was reprobed with anti-Nox2 and anti-Nox4. (B) Renal Nox2 expression was significantly increased in UUO control (Cont) compared to Sham. Nox2 level in UUO Fabry was not significantly different from that in Sham or UUO Cont (*p = 0.01 vs. Sham). (C) Both UUO Cont and UUO Fabry had a significant increase in Nox4 level in obstructed kidneys (**p < 0.0046 vs. Sham, **p = 0.008 vs. Sham). Sham, sham-operated wild-type mice.

**Figure 5.** Effect of Fabry disease on autophagy in unilateral ureteral obstruction (UUO) kidneys. (A) Western blot results representing microtubule-associated protein light chain 3 (LC3) and Beclin1. The β-actin image was reused because the membrane for immunoblot analysis of NAD(P)H:quinone oxidoreductase 1 and catalase in Fig. 3 was reprobed with anti-LC3 and anti-Beclin1. (B) LC3-II/LC3-I level was increased in UUO control (Cont) but decreased in UUO Fabry (*p = 0.01 vs. Sham, **p < 0.001 vs. UUO Cont). (C) Renal expression of Beclin1 was lower in UUO Fabry than in Sham or UUO Cont (*p < 0.001 vs. Sham, **p < 0.001 vs. Sham, p = 0.03 vs. UUO Cont). Sham, sham-operated wild-type mice.
but decreased in UUO Fabry (Fig. 5A, B). In addition, renal expression of Beclin1, a protein involved in the initiation of autophagosome formation by forming a multiprotein complex [23], was significantly decreased in UUO control but further decreased in UUO Fabry (Fig. 5A, C). On electron microscopy, there were more autophagosomes and autophagic vesicles in proximal tubular cells of the UUO Cont group than in those of the Sham group (Fig. 6). However, definite autophagosomes were rarely recognized in obstructed kidneys of Fabry disease mice except prominent electron-dense inclusions. Taken together, these results indicated that autophagy was induced in UUO kidneys of wild-type mice but attenuated in those of Fabry disease mice.

**Fabry disease mice have a larger number of apoptotic tubular cells after unilateral ureteral obstruction**

In TUNEL assay analyzing renal apoptosis, while a few TUNEL-positive cells were detected in sham-operated kidneys, there was a significant increase in the number of

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**Figure 6. Impaired autophagy in obstructed kidneys of Fabry disease mice.** Representative electron micrographs of glomeruli (A, B) and proximal tubules (C, D). (Sham A–D) Sham-operated alpha-galactosidase A wild-type mice had normal glomerulus and proximal tubule appearance. (Unilateral ureteral obstruction [UUO] control [Cont] A–D) Following UUO, disruption of glomerular and tubular barriers, irregular cytoplasmic processes, and autophagic vacuoles were shown. (UUO Fabry A–D) Inclusion bodies containing globotriaosylceramide were observed in renal proximal tubular cells but not in podocytes of UUO-operated Fabry disease mice. Scale bars: 2 μm for A and C 0.2 μm for B and D.

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TUNEL-positive cells in UUO control (Fig. 7). Interestingly, the number of these apoptotic cells was increased further in UUO kidneys of Fabry disease mice, suggesting that Fabry disease is associated with enhanced tubular apoptosis in obstructed kidneys after UUO.

Discussion

This study demonstrated that UUO kidneys could induce renal tubulointerstitial fibrosis, inflammation, EMT, oxidative stress, autophagy, and apoptosis in similar patterns as described in previous reports [19,22,24]. In the setting of Fabry disease, a further increase in fibrosis without any change in levels of inflammation, EMT, antioxidant enzymes, or oxidative stress was observed in obstructed kidneys after UUO. These findings might be explained by altered autophagy and enhanced apoptosis in kidneys affected by Fabry disease.

Chronic immune system stimulation in Fabry disease has been reported [3]. Increase of inflammatory markers such as TNF, IL-1β, IL-6, TNF receptor (TNFR) 1, TNFR2, monocyte chemoattractant protein-1, intercellular adhesion molecule-1, and soluble vascular adhesion molecule in patients with Fabry disease implicates chronic inflammation in Fabry disease as a major driver of Fabry disease pathogenesis [25,26]. It has been suggested that glycolipids such as lyso-Gb3 bind to toll-like receptor 4, nuclear factor κB, and T lymphocytes, leading to chronic inflammation and associated vasculopathy [8]. However, another study has shown that treatment with only Gb3 could not induce an increased level of IL-1β, IL-6, or TNFα in an in vitro experiment using monocyte-derived dendritic cells and macrophages purified from peripheral blood mononuclear cells [10]. Although there is limited research on Fabry disease-related inflammation in kidneys, TGF-β1, one of the key regulatory cytokines in many diseases, has been reported to play a role in renal pathogenesis in Fabry disease [16]. Upregulated TGF-β1 level appears to be associated with dysfunction of endothelial cells and podocytes affected by Fabry disease [16,27]. However, under renal injury induced by UUO in our experiment, Fabry disease mice did not show additional changes in proinflammatory cytokines such as IL-1β, IL-6, TNFα, or TGF-β1 compared to wild-type mice. UUO in wild-type mice exerted the fibrotic response associated with EMT, as indicated by increased levels of MMP2, MMP9, VE-cadherin, α-SMA, fibronectin, vimentin, and COL4A1, consistent with previous reports [19,21]. However, the levels of these fibrotic factors were not higher in the obstructed kidneys of Fabry disease mice. Since TGF-β1 plays a role in the formation and proliferation of myofibroblasts [3], profibrotic cytokines and EMTs might have followed a similar pattern to the expression of TGF-β1.

Oxidative stress has been implicated as one of the major underlying mechanisms behind the pathogenesis of obstructive nephropathy [19,23]. Since mitochondria are the major source of ROS, mitochondria-rich proximal tubules appear to be the most susceptible to obstructive injury [23]. Increased ROS can also result from impaired antioxidant capacity [19]. Our study showed that the kidneys of UUO-operated mice had decreased levels of antioxidant enzymes, including NQO1, catalase, SOD1, and SOD2. Meanwhile, increased levels of renal HO-1 and Nrf2 proteins were noted on day 7 after UUO, suggesting that levels of these antioxidant proteins might not be enough to reverse the extensive renal injury induced by UUO. In Fabry disease, accumulated Gb3 has been suggested to be associated with oxidative stress, 

Figure 7. Increased apoptosis in obstructed kidneys of Fabry disease mice. (A) Representative image of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (×200). (B) Quantification of renal TUNEL-positive cells showing increased apoptosis in wild-type mice with unilateral ureteral obstruction (UUO) and more highly enhanced apoptosis in Fabry disease mice with UUO (*p = 0.003 vs. Sham, **p < 0.001 vs. Sham, p = 0.002 vs. UUO control [Cont]). Sham, sham-operated wild-type mice.
However, our experiment’s results rejected the initial hypothesis that Fabry disease would have more highly augmented oxidant stress and more impaired antioxidants under the stimulus of UUO. One representative finding is that the expression of Nox4, the major NADPH oxidase isoform expressed in mitochondrial fractions of the kidney, is increased in obstructed kidneys [23,28] but not further increased in obstructed kidneys affected by Fabry disease.

Since homeostatic processes are particularly active in renal proximal tubular cells, in which reabsorption and transport properties are the most active in the kidney, autophagy-mediated turnover of damaged mitochondria would be required for protecting proximal tubules from renal injury [29,30]. Considering that the lysosomal system captures and degrades worn-out intracellular constituents through autophagy, it is expected that altered autophagy could damage cells through defective mitochondrial clearance [30,31]. Like our study, numerous previous studies have reported that UUO can induce autophagy, as evidenced by increased LC3-II/LC3-I [32–34]. Autophagy has controversial roles in numerous diseases, including cancer, infection, neurodegeneration, aging, cardiovascular disease, and various kinds of kidney diseases [33,35]. Although autophagy could upregulate or downregulate the fibrotic process in different cell lines [32], pharmacological inhibition of autophagy or genetic attenuation of its activity can worsen renal fibrosis [32,33]. In the brain of α-Gal A-deficient mice, LC3 itself is substantially increased, while the expression of autophagosomes is relatively lacking, indicating induction of aberrant autophagy in Fabry disease [18]. Autophagic activity or flux is generally low under basal conditions but can be induced by various stimuli [33]. Our study results suggest that kidneys of Fabry disease mice could not respond appropriately to the need for autophagic activity.

Autophagy can interact with the apoptotic machinery by acting upstream of apoptosis, converging with the apoptotic pathway, or mediating steps downstream of apoptosis. Autophagy can prevent apoptosis by selectively removing damaged mitochondria that might otherwise accumulate under stress conditions [36]. A previous study has indicated that Beclin1 can regulate the crosstalk between autophagy and apoptosis. In addition, Beclin1 can protect renal tubular epithelial cells from apoptosis after UUO [24]. Indeed, autophagy can degrade unnecessary or dysfunctional components and prevent cell apoptosis [37], and increased autophagy is observed alongside decreased apoptosis [38]. As some reports have demonstrated that inhibition of apoptosis could delay or reverse renal tubulointerstitial fibrosis [39], apoptosis might be an early event that precedes the onset of fibrosis [40]. According to our results, Fabry disease mice showed more severe renal fibrosis after UUO, but they did not show greater enhancement in inflammation, EMT, or oxidative stress in this rapidly progressive renal tubulointerstitial fibrosis mouse model. We speculate that the defective autophagic machinery in Fabry disease failed to upregulate autophagic activity in response to UUO stimuli, leading to renal apoptosis and subsequent fibrosis.

Although it has been suggested that ERT could slow the progression of renal involvement in Fabry disease, its limited effect in specific situations has been observed [5]. Considering that altered autophagy, a unique attribute of Fabry disease, might serve as one of the main mechanisms of renal tubulointerstitial fibrosis, findings from the current study indicate that autophagy is another therapeutic target in Fabry disease.

**Conflicts of interest**

Sungjin Chung is a deputy editor of *Kidney Research and Clinical Practice* and was not involved in the review process of this article. All authors have no other conflicts of interest to declare.

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**Authors’ contributions**

Conceptualization: SC, HSK
Data curation: SC, MS, YC, SO, ESK
Formal analysis: SC, YKK, SJS, SWP, SCJ, HSK
Funding acquisition: SC
Investigation: SC, MS, YC, SO, ESK  
Writing—original draft: SC, HSK  
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Effect of estimating equations for glomerular filtration rate on novel surrogate markers for renal outcome

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Backgrounds: Recently, alternative surrogate endpoints such as a 30% or 40% decline in estimated glomerular filtration rate (eGFR) or eGFR slope over 2 to 3 years have been proposed for predicting renal outcomes. However, the impact of GFR estimation methods on the accuracy and effectiveness of surrogate markers is unknown.

Methods: We retrospectively enrolled participants in health screening programs at three hospitals from 1995 to 2009. We defined two different participant groups as YR1 and YR3, which had available 1-year or 3-year eGFR values along with their baseline eGFR levels. We compared the effectiveness of eGFR percentage change or slope to estimate end-stage renal disease (ESRD) risk according to two estimating equations (modified Modification of Diet in Renal Disease equation [eGFRm] and Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation [eGFRc]) for GFR.

Results: In the YR1 and YR3 groups, 9,971 and 10,171 candidates were enrolled and ESRD incidence during follow-up was 0.26% and 0.19%, respectively. The eGFR percentage change was more effective than eGFR slope in estimating ESRD risk, regardless of the method of estimation. A 40% of decline in eGFR was better than 30%, and a 3-year baseline period was better than a 1-year period for prediction accuracy. Although some diagnostic indices from the CKD-EPI equation were better, we found no significant differences in the discriminative ability and hazard ratios for incident ESRD between eGFRc and eGFRm in either eGFR percentage change or eGFR slope.

Conclusion: There were no significant differences in the prediction accuracy of GFR percentage change or eGFR slope between eGFRc and eGFRm in the general population.

Keywords: Chronic kidney disease, End-stage renal disease, Glomerular filtration rate, Renal endpoint, Surrogate endpoint
**Introduction**

Chronic kidney disease (CKD) has been recognized as a leading cause of morbidity and mortality [1,2]. Nevertheless, current therapies for preventing CKD progression are still lacking [3], which is largely attributable to the absence of well-validated surrogate endpoints for renal outcomes [4]. Established endpoints for CKD progression, such as doubling of serum creatinine or end-stage renal disease (ESRD), are late events indicating substantial kidney damage. Furthermore, such studies require long-term follow-up and a large number of participants to produce reliable data. Considering these challenges, several alternative surrogate markers for CKD progression have been proposed [5]. In the 2012 scientific workshop sponsored by the National Kidney Foundation and the US Food and Drug Administration, a decline of 30% or 40% in the estimated glomerular filtration rate (eGFR) over 2 to 3 years was proposed as an acceptable alternative surrogate endpoint for clinical trials involving CKD [6]. While these newly-suggested endpoints have been utilized in some studies [7,8], experts recommend their careful application in clinical trials [6]. In particular, surrogate endpoints based on percentage changes in eGFR have been mainly validated in the CKD population and are less applicable in patients with a high baseline GFR [9]. Surrogate markers could prove more useful if they are validated in patients with early-stage CKD who have high GFRs. Therefore, different candidate surrogate endpoints such as GFR slope or changes in albuminuria have been evaluated in recent studies [9–12]. However, the efficacy of these endpoints needs to be further validated. Moreover, differences in the efficacy of novel surrogate endpoints based on the GFR-estimation equations are not well-studied.

Currently, the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equations are widely used to estimate GFR [13,14]. The later-developed CKD-EPI equation is increasingly used due to better accuracy at GFRs of >60 mL/min/1.73 m². However, none of these equations are optimally applicable across all GFR ranges and in different populations [15]. The eGFR decline was noninferior to the measured GFR decline in predicting renal outcomes [16]; however, the specific GFR estimation method which may improve predictive accuracy when utilizing novel surrogate endpoints is unknown. Application of different equations could lead to misclassification of CKD [17] or to variability in the calculation of GFR decline. Therefore, we aimed to evaluate the impact of GFR estimation methods on the efficacy of GFR decline or GFR slope for predicting renal outcomes in the general population.

**Methods**

**Study design**

We retrospectively enrolled 143,890 participants aged ≥18 years with eGFR of ≥15 mL/min/1.73 m² who underwent routine health screenings between May 1995 and April 2009 at one of three university-affiliated hospitals (Seoul National University Hospital, Seoul National University Bundang Hospital, and Seoul National University Boramae Medical Center) in Korea. Clinical characteristics including age, sex, medical history, and laboratory values, were collected from the electronic medical records of each participant at each hospital. The eGFR was calculated using the modified MDRD equation (eGFRm) or the CKD-EPI 2009 equation (eGFRc) using isotope dilution mass spectrometry-traceable creatinine values [13,14]. To determine eGFR decline and slope, we defined two different baseline period groups, YRI and YR3, which consisted of participants with available 1-year or 3-year eGFR values along with their baseline eGFR levels. The development of ESRD by December 2017 was determined using the ESRD registry of the Korean Society of Nephrology. The study was approved by the Institutional Review Board (IRB) of Seoul National University Hospital (No. B-1801/442-003). The requirement for written informed consent was waived by the IRB, and all data were fully anonymized before analysis.

**Statistical analyses**

Data were presented as percentages for categorical variables and as mean ± standard deviation for continuous variables. Each variable was compared using the t-test for continuous variables and the chi-square or Fisher exact test for categorical variables. Receiver operating characteristic (ROC) curve analyses were performed to examine the discriminant ability of each surrogate marker for predicting ESRD development. The Bland-Altman plot was also used to represent the agreement of eGFR percentage change and eGFR slope according
to the equations used for GFR estimation. The areas under the ROC curves (AUCs) of different surrogate markers were compared using the DeLong test.

The performance of surrogate markers was also presented in a manner similar to that of diagnostic tests [18]. To evaluate overall performance, diagnostic accuracy was shown by using paired indicators such as sensitivity and specificity, positive predictive value (PPV) and negative predictive value, and positive likelihood ratio and negative likelihood ratio. In particular, likelihood ratios are useful measures from a clinical perspective and are directly linked to posttest probabilities of event occurrence [19]. Overall diagnostic accuracy and diagnostic odds ratios (DOR) were used as global measures. DORs are not dependent on disease prevalence [20] and are therefore more useful for comparing accuracy between surrogate markers for rare events. Comparisons of sensitivity and specificity achieved with different threshold values were performed using the McNemar test, and positive and negative predictive values were compared using a weighted generalized score statistic [21].

Multivariable Cox proportional hazards analysis with a restricted cubic spline of three knots was performed to examine the association of GFR decline and GFR slope with renal survival. Hazard ratios (HRs) were adjusted by age, sex, and factors related to incident ESRD including systolic blood pressure, diabetes mellitus, eGFR, uric acid, albumin, alkaline phosphatase, glucose, hemoglobin, and urine dipstick protein results. The completeness of these variables is shown in Supplementary Table 1 (available online), and listwise deletion was used in the multivariable analyses. The p-values of <0.05 were considered statistically significant. All analyses were performed using IBM SPSS version 23 (IBM Corp., Armonk, NY, USA) and R software version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics

Of 143,890 subjects aged ≥18 years with eGFRc of ≥15 mL/min/1.73 m², we finally included 9,972 and 10,171 individuals in the YR1 and YR3 groups, respectively, after excluding patients with either missing eGFRc values or inadequate follow-up data (Fig. 1). The YR1 and YR3 groups were followed for 180.1 ± 38.6 months and 178.8 ± 33.4 months, respectively. The mean age of the YR1 group was 54 years, 60.0% were male, the mean eGFRc was 95.8 mL/min/1.73 m², and 10.6% were diabetic (Table 1). The mean age of the YR3 group was 53 years, 59.0% were male, the mean eGFRc was 95.9 mL/min/1.73 m², and 10.4% were diabetic. In total, 1.8% and 2.3% of YR1 participants and 1.7% and 2.3% of YR3 patients had an eGFRc of <60 mL/min/1.73 m² and an eGFRm of <60 mL/min/1.73 m², respectively. The YR1 and YR3 groups did not differ with respect to sex, presence of diabetes, blood pressure, or laboratory values.

At baseline, eGFRc showed a greater mean value compared to eGFRm in both the YRI (95.8 mL/min/1.73 m² vs. 95.4 mL/min/1.73 m²) and YRII (95.9 mL/min/1.73 m² vs. 95.0 mL/min/1.73 m²) groups. The standard deviation and intersubject coefficient of variation (CV) were greater for eGFRm, as these values were more wildly distributed across the range of eGFR than eGFRc values were (Supplementary Table 2, available online). The Bland-Altman plot showed how differences between eGFRc and eGFR in both the YRI and YRII groups were prominent at high GFR levels (≥90 mL/min/1.73 m²) (Supplementary Fig. 1, available online).

Figure 1. Selection of study participants. 1-year period, 6–18 months after the first examination; 3-year period, 30–42 months after the first examination. eGFR, estimated glomerular filtration rate; eGFRc, eGFR by the 2009 Chronic Kidney Disease-Epidemiology Collaboration creatinine equation; eGFRm, eGFR by the modified Modification of Diet in Renal Disease equation.
Table 1. Baseline characteristics of the study participants

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<td>AST (U/L)</td>
<td>26 ± 15</td>
<td>26 ± 18</td>
<td>0.59</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>29 ± 28</td>
<td>29 ± 27</td>
<td>0.86</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>69 ± 21</td>
<td>69 ± 20</td>
<td>0.50</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.5 ± 1.4</td>
<td>5.5 ± 1.5</td>
<td>0.69</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.1 ± 0.5</td>
<td>9.1 ± 0.5</td>
<td>0.21</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>3.7 ± 0.8</td>
<td>3.7 ± 0.8</td>
<td>0.98</td>
</tr>
</tbody>
</table>

| eGFRc (mL/min/1.73 m²)    | 95.8 ± 15.4          | 95.9 ± 15.4          | 0.39     |
| ≥90                      | 6,773 (67.9)         | 6,949 (68.3)         |          |
| <90, ≥60                 | 3,024 (30.3)         | 3,049 (30.0)         |          |
| <60, ≥30                 | 162 (1.6)            | 161 (1.6)            |          |
| <30                      | 13 (0.1)             | 12 (0.1)             |          |

| eGFRm (mL/min/1.73 m²)    | 95.4 ± 27.3          | 95.0 ± 25.9          | 0.31     |
| ≥90                      | 5,148 (51.6)         | 5,233 (51.5)         |          |
| <90, ≥60                 | 4,592 (46.0)         | 4,702 (46.2)         |          |
| <60, ≥30                 | 219 (2.2)            | 224 (2.2)            |          |
| <30                      | 13 (0.1)             | 12 (0.1)             |          |


Data are expressed as number only, mean ± standard deviation, or number (%).

ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; eGFRc, eGFR by the 2009 Chronic Kidney Disease-Epidemiology Collaboration creatinine equation; eGFRm, eGFR by the modified Modification of Diet in Renal Disease equation; HDL-C, high density lipoprotein cholesterol; SBP, systolic blood pressure; YR1, 1-year eGFR group; YR3, 3-year eGFR group.

*Comparison between YR1 and YR3.

During the baseline period, percentage changes in eGFR differed between eGFRc and eGFRm in YR1, but not in YR3 (Supplementary Table 2). Likewise, while the eGFRc slope was steeper than the eGFRm slope in YR1, this difference in inclination was not observed in YR3. The CV for percentage change in eGFR and for the eGFR slope was much greater for eGFRm compared to eGFRc in both the YR1 and YR3 groups. The Bland-Altman plot showed that the larger the absolute value of eGFR percentage change and eGFR slope, the larger the difference between eGFRc and eGFRm. In particular, subjects with high eGFRs showed greater differences in eGFR percentage change and eGFR slope between the CKD-EPI and MDRD equations.

Prediction accuracy for ESRD according to surrogate markers

During the follow-up period, the rate of ESRD incidence was 0.26% and 0.19% in the YR1 and YR3 groups, respectively. Surrogate markers showed better discrimination for ESRD development in the YR3 group compared to the YR1 group (Table 2). ROC analysis showed that ESRD prediction accuracy was significantly greater when using percentage change in eGFR compared to eGFR slope (Fig. 2, Table 2). Among the studied surrogate markers, the percentage change in eGFRc in YR3 showed the highest AUC (0.837), while eGFRc slope in YR1 showed the lowest AUC (0.632). AUCs for percentage changes in eGFRc and eGFRm, and those for yearly slopes for eGFRc and eGFRm values, did not differ in either the YR1 or YR3 groups (Table 2). When diagnostic indicators were evaluated based on percentage changes in eGFR and the estimating equations, changes of ≥30% in eGFRc and eGFRm showed an acceptable specificity of >90% and a high DOR (Table 3). Although the PPV of each endpoint was relatively small due to the low incidence of ESRD, application of a higher threshold yielded a greater PPV. Overall, the diagnostic indices according to the two equations revealed similar patterns. However, the eGFRc criteria had higher DOR, specificity, and PPV than the eGFRm criteria at all eGFR thresholds in both the YR1 and YR3 groups (Table 3). In particular, the PPV and specificity of a 30% or 40% decline in eGFR were statistically different based on the GFR-estimating equation used (Supplementary Table 3, available online).

The PPV associated with a 40% decline in eGFR was not statistically different compared to conventional endpoints (57% eGFRc or 55% eGFRm decline) in YR3, but not for a 30% decline or less. Subgroup analyses showed that 30% and 40% declines in eGFR showed higher DORs in patients with age...
of <65 years and proteinuria grade >1+. The eGFRc values demonstrated a better DOR than eGFRm values in patients aged <65 years and in those with eGFR of ≥60 mL/min/1.73 m$^2$ (Supplementary Table 4, 5; available online).

### Associations between changes in eGFR and ESRD risk

In the restricted cubic spline model, percentage decline in eGFR was not significantly associated with the risk for ESRD in YR1 (Fig. 3). However, in YR3, a greater percentage decline in eGFR was significantly associated with a higher risk for developing ESRD at all ranges. These associations between changes in eGFR and HR were comparable using eGFRc and eGFRm. A negative eGFR slope was associated with a modest increase in the adjusted HR for ESRD in YR1 (Fig. 4), while this correlation with eGFR slope was stronger in YR3. While eGFRm slope had a higher estimated HR than eGFRc, the former demonstrated a wider confidence interval.

In subgroup analyses stratified by age, sex, presence of diabetes, eGFR, and presence of proteinuria, adjusted HRs for percentage change in eGFR for each criterion were not

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### Table 2. Comparison of AUCs of ROC curves for eGFR changes to estimate incident ESRD

<table>
<thead>
<tr>
<th>Period</th>
<th>Variable</th>
<th>Percent of eGFRc change (AUC, 0.71)</th>
<th>Slope of eGFRc per year</th>
<th>Percent of eGFRm change (AUC, 0.68)</th>
<th>Slope of eGFRm per year</th>
<th>Percent of eGFRc change (AUC, 0.84)</th>
<th>Slope of eGFRc per year</th>
</tr>
</thead>
<tbody>
<tr>
<td>YR1</td>
<td>Percent of eGFRc change</td>
<td>0.20</td>
<td>0.004</td>
<td>&lt;0.001</td>
<td>0.03</td>
<td>0.08</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Slope of eGFRc per year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>YR3</td>
<td>Percent of eGFRc change</td>
<td>0.08</td>
<td>0.004</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.04</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Slope of eGFRc per year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

AUC, area under the ROC curve; eGFR, estimated glomerular filtration rate; eGFRc, eGFR by the 2009 Chronic Kidney Disease-Epidemiology Collaboration creatinine equation; eGFRm, eGFR by the modified Modification of Diet in Renal Disease equation; ESRD, end-stage renal disease; ROC, receiver operating characteristic; YR1, 1-year eGFR group; YR3, 3-year eGFR group.

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### Figure 2. Receiver operating characteristic curves for changes in eGFR

During 1-year (A) and 3-year (B) periods for ESRD estimation. The eGFR was estimated based on isotope dilution mass spectrometry-traceable creatinine. eGFR, estimated glomerular filtration rate; eGFRc, eGFR by the 2009 Chronic Kidney Disease-Epidemiology Collaboration creatinine equation; eGFRm, eGFR by the modified Modification of Diet in Renal Disease equation; ESRD, end-stage renal disease.

---

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<table>
<thead>
<tr>
<th>Variable</th>
<th>Criteria for eGFRc change (No. of subjects at risk)</th>
<th>Criteria for eGFRm change (No. of subjects at risk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YR1 group (n = 9,972)</td>
<td>YR3 group (n = 10,171)</td>
</tr>
<tr>
<td></td>
<td>30.0% (n = 139)</td>
<td>30.0% (n = 186)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.115 (0.024–0.302)</td>
<td>0.526 (0.289–0.756)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.986 (0.984–0.989)</td>
<td>0.983 (0.980–0.985)</td>
</tr>
<tr>
<td>PPV</td>
<td>0.022 (0.004–0.062)</td>
<td>0.054 (0.026–0.097)</td>
</tr>
<tr>
<td>NPV</td>
<td>0.998 (0.996–0.999)</td>
<td>0.999 (0.998–1.000)</td>
</tr>
<tr>
<td>LR (+)</td>
<td>8.438 (2.873–24.782)</td>
<td>9.409 (2.792–31.709)</td>
</tr>
<tr>
<td>LR (-)</td>
<td>0.897 (0.781–1.030)</td>
<td>0.907 (0.810–1.023)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.984 (0.981–0.986)</td>
<td>0.998 (0.998–1.000)</td>
</tr>
<tr>
<td></td>
<td>40.0% (n = 39)</td>
<td>50.0% (n = 49)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.077 (0.009–0.251)</td>
<td>0.368 (0.163–0.616)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.996 (0.995–0.997)</td>
<td>0.996 (0.994–0.997)</td>
</tr>
<tr>
<td>PPV</td>
<td>0.051 (0.006–0.173)</td>
<td>0.143 (0.059–0.272)</td>
</tr>
<tr>
<td>NPV</td>
<td>0.996 (0.996–0.998)</td>
<td>0.998 (0.998–0.999)</td>
</tr>
<tr>
<td>LR (+)</td>
<td>20.678 (5.255–81.360)</td>
<td>22.318 (5.090–97.861)</td>
</tr>
<tr>
<td>LR (-)</td>
<td>0.927 (0.829–1.035)</td>
<td>0.962 (0.891–1.039)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.994 (0.981–0.999)</td>
<td>0.999 (0.998–1.000)</td>
</tr>
<tr>
<td></td>
<td>57.0% (n = 5)</td>
<td>50.0% (n = 6)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.038 (0.001–0.196)</td>
<td>0.105 (0.013–0.331)</td>
</tr>
<tr>
<td>Specificity</td>
<td>1.000 (0.999–1.000)</td>
<td>1.000 (0.999–1.000)</td>
</tr>
<tr>
<td>PPV</td>
<td>0.200 (0.005–0.716)</td>
<td>0.333 (0.043–0.777)</td>
</tr>
<tr>
<td>NPV</td>
<td>0.996 (0.996–0.998)</td>
<td>0.996 (0.996–0.999)</td>
</tr>
<tr>
<td>LR (+)</td>
<td>2.329 (0.943–5.754)</td>
<td>2.329 (0.943–5.754)</td>
</tr>
<tr>
<td>LR (-)</td>
<td>0.906 (0.769–1.067)</td>
<td>0.906 (0.769–1.067)</td>
</tr>
<tr>
<td>DOR</td>
<td>4.448 (1.524–12.982)</td>
<td>4.448 (1.524–12.982)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.998 (0.996–0.999)</td>
<td>0.998 (0.996–0.999)</td>
</tr>
<tr>
<td></td>
<td>30.0% (n = 661)</td>
<td>30.0% (n = 820)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.115 (0.024–0.302)</td>
<td>0.526 (0.289–0.756)</td>
</tr>
<tr>
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<td>0.986 (0.984–0.989)</td>
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<tr>
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</tr>
<tr>
<td>DOR</td>
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</tr>
<tr>
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<td>0.998 (0.996–0.999)</td>
<td>0.998 (0.996–0.999)</td>
</tr>
<tr>
<td></td>
<td>40.0% (n = 261)</td>
<td>40.0% (n = 245)</td>
</tr>
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<td>Sensitivity</td>
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</tr>
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<td>0.998 (0.996–0.999)</td>
<td>0.998 (0.996–0.999)</td>
</tr>
</tbody>
</table>

Data are expressed as eGFR change (%) or index (95% confidence interval). DOR, diagnostic odds ratio; eGFR, estimated glomerular filtration rate; eGFRc, eGFR by the 2009 Chronic Kidney Disease-Epidemiology Collaboration creatinine equation; eGFRm, eGFR by the modified Modification of Diet in Renal Disease equation; ESRD, end-stage renal disease; LR (-), negative likelihood ratio; LR (+), positive likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; YR1, 1-year eGFR group; YR3, 3-year eGFR group.
significant in YR1, except for eGFR of ≥60 mL/min/1.73 m² (Supplementary Table 6, available online). Meanwhile, percentage changes in eGFR in YR3 were associated with significantly higher HRs for each subgroup, especially in young patients and those with proteinuria. Overall, estimated HRs based on eGFRc or eGFRm were similar, though HRs estimated using eGFRc were 2 to 3 times greater than those derived using eGFRm in patients with eGFR of ≥60 mL/min/1.73 m².

**Discussion**

In this study, we evaluated the predictive accuracy of several novel surrogate markers for ESRD, including eGFR percentage change and eGFR slope over 1- and 3-year baseline periods in the general population, using different estimating equations. Our findings showed that a 30% or 40% decline in eGFR may be a more accurate surrogate endpoint than the eGFR slope when predicting renal outcome. We also showed that these values are more predictive over a baseline period of 3 years compared to 1 year. Percentage changes in eGFRc...
showed higher specificity and PPV compared to those in eGFRm and were more likely to be associated with the development of ESRD in patients with eGFR of ≥60 mL/min/1.73 m². Nevertheless, there were no significant differences in discriminative ability (AUC) or adjusted HRs between eGFRc and eGFRm for predicting ESRD risk.

Recent studies have provided evidence in favor of using alternative surrogate endpoints such as eGFR percentage decline and eGFR slope for predicting renal outcomes [10–12,22,23]. Indeed, an ongoing randomized trial to evaluate the effect of sodium-glucose cotransporter-2 inhibitors on renal outcomes set eGFR decline of ≥40% as one of the primary endpoints [24]. Nevertheless, novel surrogate endpoints have not been well validated in the general population. Overall, our findings are similar to those of previous studies in the CKD population showing that eGFR changes over 1 year were insufficient to predict renal outcomes. Researchers have recommended the use of surrogate markers over 2 to 3 years [6]. Furthermore, a 40% decline in eGFR is more acceptable than a 30% decline [25], which was also consistent with our results. We found that a 40% decline in eGFR over 3 years was the most reliable candidate surrogate endpoint in this study. Since our study was based on the Korean population, our findings highlight the significance of using eGFRc and eGFRm for predicting renal outcomes.
of these early surrogate markers in Asian populations. While the eGFR slope showed moderate predictive accuracy as well, it was lower than that achieved with percentage change in eGFR and was a comparatively weaker surrogate marker. These findings may be related to characteristics of eGFR slope such as greater variability in eGFR at higher levels, nonlinear trajectories, and nonuniform distributions [26].

These early-identifiable markers are important, as conventional endpoints such as creatinine doubling or ESRD require much longer follow-up periods in the general population. The application of these surrogate endpoints can enable early detection and management of CKD progression. However, there are some considerations regarding surrogate markers of eGFR change in patients with high baseline GFR levels. The MDRD and CKD-EPI equations are most commonly used to assess kidney function in medical research and clinical practice. Clinically meaningful differences between the two equations are mainly seen when GFR levels are high [27]. The CKD-EPI equation shows greater accuracy than the MDRD equation when estimating GFR in individuals with normal kidney function [14]. Thus, using the CKD-EPI equation can decrease the chances of misclassifying patients at low risk of ESRD [28]. Since misclassification of at-risk patients affects the efficacy of surrogate endpoints, it is clinically important to assess their accuracy based on the equation used for GFR estimation. Our findings demonstrated that both the CKD-EPI and MDRD equations were acceptable at estimating the risk for ESRD in the general population based on the results of the ROC and Cox proportional hazards analyses. These results support the application of both equations for predicting renal outcome and alleviate concerns about the use of the MDRD equation in utilizing novel surrogate endpoints.

However, we found that the CKD-EPI equation was better with respect to some diagnostic indices such as specificity, DOR, and PPV, representing more accurate prediction for true endpoint. Our findings can be attributed to (1) less bias in estimating GFR and (2) lower within-subject variability of CKD-EPI equation in patients with high GFR [14,29]. As shown in our Bland-Altman plot analysis (Supplementary Fig. 1), the differences between eGFRc and eGFRm increased at higher GFRs, and greater differences in both eGFR percentage change and slope were observed mainly in patients with higher GFRs. These findings suggest that the greater bias associated with the MDRD equation could affect its predictive accuracy. In addition, our results may be indicative of the high within-subject variability of the MDRD equation. Within-subject variability was usually higher at higher GFRs and was significantly higher in the application of the MDRD equation compared to the CKD-EPI equation [29]. Changes in eGFR between two time points may be partly affected by this within-subject variability.

There are certain limitations in this study. The number of enrolled patients was relatively small, and the rate of incidence of ESRD was low due to the characteristics of the studied population. Surrogate markers based on changes in eGFR were assessed using serum creatinine measurements merely at the beginning and the end of the defined baseline period. Therefore, GFR variability and acute treatment effects during the baseline period were not considered. In addition, several unmeasured confounders could be present, considering the observational nature of the study. Nevertheless, our study rigorously analyzed the differences in different novel surrogate endpoints with respect to the equations used for estimating eGFR, which allowed us to provide detailed implications. To the best of our knowledge, the impact of GFR-estimating equations on the efficacy of novel surrogate markers for predicting renal outcomes has not been evaluated previously.

In conclusion, there were no significant differences in estimated ESRD risk using the GFR percentage change or eGFR slope between the CKD-EPI equation and the modified MDRD equation in the general population. Surrogate markers using the CKD-EPI equation may be slightly more accurate in patients with high GFRs.

Conflict of interest

All authors have no conflicts of interest to declare.

Authors’ contributions

Conceptualization: HJC
Data curation: KK, EB, SG, HES, JYR, YY, JCJ, SK
Formal analysis: KK, YY, HJC
Investigation: EB, SG, HES, JYR
Writing–original draft: KK
Writing–review & editing: All authors
All authors read and approved the final manuscript.
References


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Background: The prognostic value of within-day sCr variation serum creatinine variation is unknown in the setting of the novel coronavirus disease 2019 (COVID-19). We evaluated the prognostic significance of 24-hour serum creatinine variation in COVID-19 patients.

Methods: A monocentric retrospective analysis was conducted in COVID-19 patients not admitted to the intensive care unit. Three groups were subdivided based on 24 hours serum creatinine variation from admission. In the stable kidney function group, 24-hour serum creatinine variation ranged from +0.05 to –0.05 mg/dL; in the decreased kidney function group, 24-hour serum creatinine variation was >0.05 mg/dL; in the improved kidney function group, 24-hour serum creatinine variation was <–0.05 mg/dL.

Results: The study population included 224 patients with a median age of 66.5 years and a predominance of males (72.3%). Within 24 hours of admission, renal function remained stable in 37.1% of the subjects, whereas it displayed improved and deteriorated patterns in 45.5% and 17.4%, respectively. Patients with decreased kidney function were older and had more severe COVID-19 symptoms than patients with stable or improved kidney function. About half of patients with decreased kidney function developed an episode of acute kidney injury (AKI) during hospitalization. Decreased kidney function was significantly associated with AKI during hospitalization (hazard ratio [HR], 4.6; 95% confidence interval [CI], 1.9–10.8; p < 0.001) and was an independent risk factor for 30-day in-hospital mortality (HR, 5.5; 95% CI, 1.1–28; p = 0.037).
**Introduction**

The novel coronavirus disease 2019 (COVID-19) is a complex disease presenting principally with pneumonia and multiorgan disease in aged and highly comorbid patients [1]. Acute decline of kidney function is a relatively frequent complication of COVID-19, especially in patients with severe lung involvement [2]. The etiological mechanisms causing acute kidney injury (AKI) are still unknown. The interplay between hyperactive immune response, cytopathic effects of the virus, and homeostatic reactions to balance pulmonary and hemodynamic responses have been postulated to be the main causes of kidney damage [3]. The rate of this complication ranges from 0.5% to 36.6% [2,4-9]. In intensive care unit (ICU), the prevalence of this disease was higher, accounting for about 80% of patients [10].

The diagnosis of AKI relies on the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines and is defined as the combination of a peak increase in serum creatinine (sCr) level and abrupt reduction of urinary output [11]. In the real world, small sCr variations are frequent and often overlooked. The prognostic value of sCr within-day variation has been poorly investigated because fluctuations of sCr can be linked to nonpathological issues including analytical, biological, and dietary factors [12,13]. A study of 14,912 adults who underwent two sCr measurements within 24 hours showed that monitoring 24-hour sCr variation was useful to identify AKI earlier than with the conventional criteria. A 24-hour sCr variation greater than 0.1 mg/dL (or an increase of 5%) was associated with increased all-cause mortality, especially in inpatient settings [14].

As a predictor of poor outcomes, change in sCr can be a useful risk-stratification tool to quickly identify high-risk COVID-19 patients requiring intensive management. Comprehensive risk assessment assumes paramount importance in the management of these vulnerable subjects. Identification of high-risk patients soon after admission allows timely supportive treatment and limits further insult to the kidneys.

Based on this background, we evaluated 24-hour sCr differences (ΔsCr) after admission in a cohort of hospitalized patients with COVID-19 to assess the prognostic effect of early sCr variation.

**Methods**

We retrospectively reviewed the electronic charts of all non-ICU hospitalized patients with COVID-19 at the University Hospital of Modena, Italy from March 4 to June 20, 2020. The study was approved by the regional ethical committee of Emilia Romagna (No. 0013376/20). Written informed consent was waived due to containment restrictions between COVID-19 patients and healthcare workers.

**Population**

Demographic, clinical, and laboratory data of 432 consecutive adult patients (≥18 years) admitted with COVID-19 were collected. According to the international guidelines, diagnosis of COVID-19 was defined as a positive real-time reverse transcriptase-polymerase chain reaction assay of nasopharyngeal swabs or lower respiratory tract specimens [15]. Study inclusion criteria comprised patients older than 18 years with a second measurement of sCr within 24 hours from admission. Patients on renal replacement therapy were excluded from the analysis.

The study population comprised 224 patients. The ΔsCr was calculated by subtracting the first sCr value from the second value collected within 24 hours of admission (ΔsCr = sCr<sub>24h</sub> – sCr<sub>baseline</sub>). The entire cohort was subdivided according to 24-hour ΔsCr. In the stable kidney function group, the 24-hour ΔsCr ranged from +0.05 to -0.05 mg/dL; in the decreased kidney function group, the 24-hour ΔsCr was >0.05 mg/dL; in the improved kidney function group, the 24-hour ΔsCr was <-0.05 mg/dL.

**Conclusion:** COVID-19 patients with decreased kidney function within 24 hours of admission were at high risk of AKI and 30-day in-hospital mortality.

**Keywords:** Acute kidney injury, Biomarkers, Coronavirus, Kidney, Creatinine, Mortality
Baseline clinical characteristics

All measurable comorbidities were quantified in our study population at admission. Chronic kidney disease (CKD) was defined as a chronic (>3 months) reduction of glomerular filtration rate (GFR) < 60 mL/min; cardiovascular disease (CVD) included a wide array of diseases affecting the cardiac muscle and the vascular system supplying the heart, brain, and other vital organs; chronic obstructive pulmonary disease was defined as a previous diagnosis of airflow obstruction with reduction of forced expiratory volume in 1 second; hypertension referred to high blood pressure (≥140/90 mmHg) requiring at least one antihypertensive medication; diabetes included altered glucose metabolism requiring treatment; AKI has been defined according to 2012 KDIGO guidelines without urine output criteria [11].

All patients were treated in agreement with the Regional Guidelines of Emilia Romagna [12] regarding the treatment of COVID-19; these were continuously updated during the period of the study. The treatments consisted of (1) hydroxychloroquine (400 mg twice a day [BID] on day 1 followed by 200 mg BID on days 2 to 5, eventually adjusted for creatinine clearance estimated by a CKD algorithm); (2) azithromycin (500 mg once a day [QD] for 5 days) at physician discretion when suspecting a bacterial respiratory superinfection; (3) low molecular weight heparin for prophylaxis of deep vein thrombosis according to body weight and kidney function; (4) darunavir/cobicistat (800/150 mg QD) or lopinavir/ritonavir (400/100 mg BID) for 14 days were used until March 18, when a clinical trial on the former did not show any benefit of protease inhibitors against the standard of care. Other antiviral agents showing promising results, such as remdesivir, were not used in our patients due to lack of availability in the market.

Serum creatinine measurement

The sCr level was measured in a single laboratory at the University Hospital of Modena using the Jaffe (CREJ2, Roche/Hitachi Cobas Systems; Roche Diagnostics GmbH, Mannheim, Germany) or the enzymatic method (Ortho Clinical Diagnostics, Rochester, NY, USA). The latter has been used to measure sCr in urgent blood tests. Precision in sCr measurement ranged from 1.2% to 5.0% for the Jaffe method and from 1.3% to 2.6% for the enzymatic method. The estimated GFR was calculated using the Chronic Kidney Disease-Epidemiology Collaboration equation [16]. Peak sCr value was selected among patients with multiple sCr measurements (>2 times) performed on the same day. A cut-off of ±0.05 mg/dL was chosen to overcome interlaboratory variability [17].

Outcome

The primary outcome measure was 30-day in-hospital mortality among patients stratified according to 24-hour ΔsCr.

Statistical analysis

Comparisons of population characteristics were performed using the paired Student t-test or Wilcoxon signed-rank test, as appropriate, and the chi-square test was used for categorical variables. One-way analysis of variance evaluated differences in continuous variables between groups, and Tukey test was used for post hoc analysis. Kaplan-Meier analysis assessed the survival of patients. Cox regression was used to estimate time-to-event data; the hazard ratio (HR) for death was adjusted for age, sex, PaO₂/FiO₂ ratio, CKD, AKI, CVD, hypertension, diabetes, C-reactive protein, and GFR. The stable kidney function group was considered as a reference. All independent variables satisfied the proportional hazard assumption. A two-sided p-value less than 0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS version 27 (IBM Corp., Armonk, NY, USA).

Results

The median patient age was 66.5 years (interquartile range, 55.5–77.7 years), with a predominance of males (72.3%). Clinical and laboratory examinations of all enrolled patients are reported in Table 1.

Within 24 hours from admission, kidney function decreased in 39 subjects (17.4%), improved in 102 (45.5%), and remained stable in 83 (37.1%). Patients with decreased kidney function were older and presented with more severe impairment of respiratory function than patients with stable kidney function or improved kidney function. No differences were observed in the administration of fluid or potentially nephrotoxic agents across groups. The fluctuations in sCr detect-
Table 1. Demographic, laboratory, and clinical characteristics of COVID-19 patients stratified according to 24-hour ΔsCr

<table>
<thead>
<tr>
<th>Variable</th>
<th>All patients (n = 224)</th>
<th>Decreased kidney function (ΔsCr &gt; 0.05 mg/dL) (n = 39)</th>
<th>Stable kidney function (0.05 mg/dL ≤ ΔsCr ≤ –0.05 mg/dL) (n = 83)</th>
<th>Improved kidney function (ΔsCr &lt; 0.05 mg/dL) (n = 102)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basal characteristic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>66.5 (55.5–77.7)</td>
<td>77.6 (71.4–83.7)</td>
<td>61.9 (53.0–72.1)</td>
<td>66.2 (57.8–76.9)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Range of age (yr)</td>
<td>28.8–97.3</td>
<td>39.9–94.5</td>
<td>29.1–94.4</td>
<td>28.9–97.3</td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>162 (72.3)</td>
<td>26 (66.7)</td>
<td>61 (73.5)</td>
<td>75 (73.5)</td>
<td>0.22</td>
</tr>
<tr>
<td>White blood cell (/mm$^3$)</td>
<td>6,525 (4,867–9,085)</td>
<td>7,310 (5,675–8,935)</td>
<td>6,805 (4,707–7,957)</td>
<td>6,380 (4,720–8,990)</td>
<td>0.43</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>12.4 (10.9–13.5)</td>
<td>11.6 (10.1–13.2)</td>
<td>12.3 (11.3–13.5)</td>
<td>12.8 (10.9–13.6)</td>
<td>0.09</td>
</tr>
<tr>
<td>Platelet (×10$^9$/L)</td>
<td>213.0 (82.5–300.0)</td>
<td>192.0 (81.7–256.5)</td>
<td>230.5 (163.5–317.5)</td>
<td>206.0 (156.0–294.0)</td>
<td>0.12</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>102.5 (82.5–132.7)</td>
<td>102.0 (81.7–133.2)</td>
<td>101.0 (86.0–128.5)</td>
<td>81.0 (52.2–149.7)</td>
<td>0.009*</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>137.0 (135.0–140.0)</td>
<td>143.0 (110.0–199.0)</td>
<td>137.0 (135.7–140.0)</td>
<td>137.0 (135.0–139.7)</td>
<td>0.08</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>45.0 (31.0–64.8)</td>
<td>58.0 (47.7–105.2)</td>
<td>38.0 (30.2–56.5)</td>
<td>42.0 (26.7–55.5)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>626.0 (449.0–694.0)</td>
<td>545.0 (432.0–646.0)</td>
<td>656.0 (506.0–751.7)</td>
<td>616.0 (470.0–684.0)</td>
<td>0.42</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.8 (2.6–3.3)</td>
<td>2.6 (2.4–3.0)</td>
<td>3.0 (2.6–3.3)</td>
<td>2.9 (2.6–3.3)</td>
<td>0.11</td>
</tr>
<tr>
<td>D-dimer (mg/L)</td>
<td>1,490.0 (820.0–2,680.0)</td>
<td>1,725.0 (807.5–2,982.5)</td>
<td>1,533.0 (800.0–2,795.0)</td>
<td>2,380.0 (1,245.0–4,770.0)</td>
<td>0.63</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.59 (0.47–0.83)</td>
<td>0.70 (0.49–1.10)</td>
<td>0.57 (0.42–0.83)</td>
<td>0.56 (0.41–0.68)</td>
<td>0.05*</td>
</tr>
<tr>
<td>aPTT</td>
<td>1.15 (1.02–1.30)</td>
<td>1.20 (1.14–1.60)</td>
<td>1.08 (1.03–1.20)</td>
<td>1.20 (1.10–1.90)</td>
<td>0.08</td>
</tr>
<tr>
<td>PT</td>
<td>1.07 (1.02–1.14)</td>
<td>1.10 (1.06–1.26)</td>
<td>1.08 (1.03–1.20)</td>
<td>1.06 (1.02–1.10)</td>
<td>0.45</td>
</tr>
<tr>
<td>Alamine amino-transferase (U/L)</td>
<td>35.5 (23.0–58.0)</td>
<td>31.0 (20.0–48.0)</td>
<td>36.0 (23.0–61.0)</td>
<td>38.0 (23.0–59.0)</td>
<td>0.11</td>
</tr>
<tr>
<td>CPK (U/L)</td>
<td>111.5 (54.0–265.0)</td>
<td>107.5 (35.0–341.0)</td>
<td>111.0 (47.5–271.7)</td>
<td>83.0 (37.0–185.0)</td>
<td>0.07</td>
</tr>
<tr>
<td>Lactic dehydrogenase (U/L)</td>
<td>622.0 (475.2–812.0)</td>
<td>660.0 (432.0–823.0)</td>
<td>630.0 (486.0–781.0)</td>
<td>595.0 (451.0–848.0)</td>
<td>0.09</td>
</tr>
<tr>
<td>PCR (mg/dL)</td>
<td>6.2 (3.2–16.9)</td>
<td>5.9 (3.5–19.8)</td>
<td>5.9 (2.7–17.5)</td>
<td>6.7 (3.4–16.4)</td>
<td>0.68</td>
</tr>
<tr>
<td>Admission sCr (mg/dL)</td>
<td>0.86 (0.68–1.12)</td>
<td>0.90 (0.71–1.39)</td>
<td>0.76 (0.62–0.92)</td>
<td>0.92 (0.79–1.21)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td>83.5 (60.2–99.4)</td>
<td>77.9 (45.9–91.0)</td>
<td>96.1 (76.3–111.9)</td>
<td>76.6 (58.7–93.9)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>24-hour sCr (mg/dL)</td>
<td>0.81 (0.66–1.08)</td>
<td>1.23 (0.90–1.90)</td>
<td>0.75 (0.60–0.97)</td>
<td>0.79 (0.66–1.04)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>ΔsCr (mg/dL)</td>
<td>–0.04 (–0.13 to 0.03)</td>
<td>0.17 (0.11–0.38)</td>
<td>–0.01 (–0.02 to 0.02)</td>
<td>–0.13 (–0.21 to –0.09)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>ΔsCr range (mg/dL)</td>
<td>1.24–2.78</td>
<td>0.05–2.78</td>
<td>–0.04 to 0.04</td>
<td>–1.24 to –0.05</td>
<td></td>
</tr>
<tr>
<td>Time elapsed from first sCr (hr)</td>
<td>13 (13–19)</td>
<td>13 (10–13)</td>
<td>16 (13–19)</td>
<td>13 (13–19)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>120 (110–133)</td>
<td>110 (100–130)</td>
<td>120 (110–130)</td>
<td>120 (110–140)</td>
<td>0.32</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>70 (61–80)</td>
<td>70 (60–75)</td>
<td>70 (70–80)</td>
<td>70 (60–80)</td>
<td>0.33</td>
</tr>
<tr>
<td>PaO$_2$ /FiO$_2$</td>
<td>245.0 (147.0–291.0)</td>
<td>167.0 (91.7–239.0)</td>
<td>264.0 (216.0–298.0)</td>
<td>232.2 (149.0–303.0)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.2 ± 1.0</td>
<td>37.6 ± 1.04</td>
<td>37.1 ± 1.0</td>
<td>37.1 ± 1.06</td>
<td>0.13</td>
</tr>
<tr>
<td>Dyspnea$^c$</td>
<td>96 (52.1)</td>
<td>16 (66.7)</td>
<td>34 (49.3)</td>
<td>46 (50.5)</td>
<td>0.31</td>
</tr>
<tr>
<td>SOFA</td>
<td>2.88 ± 1.98</td>
<td>4.00 ± 2.53</td>
<td>2.36 ± 1.30</td>
<td>2.96 ± 2.00</td>
<td>0.009*</td>
</tr>
</tbody>
</table>

(Continued to the next page)
<table>
<thead>
<tr>
<th>Variable</th>
<th>All patients (n = 224)</th>
<th>Decreased kidney function (ΔsCr &gt; 0.05 mg/dL) (n = 39)</th>
<th>Stable kidney function (0.05 mg/dL ≤ ΔsCr ≥ –0.05 mg/dL) (n = 83)</th>
<th>Improved kidney function (ΔsCr &lt; 0.05 mg/dL) (n = 102)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKD (&lt;60 mL/min)</td>
<td>12 (5.4)</td>
<td>4 (10.3)</td>
<td>2 (2.4)</td>
<td>6 (5.9)</td>
<td>0.18</td>
</tr>
<tr>
<td>CVD</td>
<td>123 (54.9)</td>
<td>16 (41.0)</td>
<td>51 (61.4)</td>
<td>56 (54.9)</td>
<td>0.11</td>
</tr>
<tr>
<td>COPD</td>
<td>20 (8.9)</td>
<td>4 (10.3)</td>
<td>6 (7.2)</td>
<td>10 (9.8)</td>
<td>0.79</td>
</tr>
<tr>
<td>High blood pressure</td>
<td>136 (60.7)</td>
<td>20 (51.3)</td>
<td>55 (66.3)</td>
<td>61 (59.8)</td>
<td>0.28</td>
</tr>
<tr>
<td>Diabetes</td>
<td>41 (18.3)</td>
<td>10 (25.6)</td>
<td>14 (16.9)</td>
<td>17 (16.7)</td>
<td>0.45</td>
</tr>
<tr>
<td>First 24-hr treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl 0.9% infusion</td>
<td>71 (31.7)</td>
<td>14 (35.9)</td>
<td>25 (30.1)</td>
<td>32 (31.4)</td>
<td>0.81</td>
</tr>
<tr>
<td>NaCl 0.9% infusion (L)</td>
<td>62.0 (0.6)</td>
<td>9.3 (0.5)</td>
<td>21.8 (0.6)</td>
<td>31 (0.6)</td>
<td>0.73</td>
</tr>
<tr>
<td>Dextrose 5% infusion</td>
<td>5 (2.2)</td>
<td>2 (5.1)</td>
<td>0 (0)</td>
<td>3 (2.9)</td>
<td>0.16</td>
</tr>
<tr>
<td>Dextrose 5% infusion (L)</td>
<td>5 (0.8)</td>
<td>1 (0.5)</td>
<td>0 (0)</td>
<td>4 (1.0)</td>
<td>0.40</td>
</tr>
<tr>
<td>Danuravir/cobicistat</td>
<td>56 (25.0)</td>
<td>9 (23.1)</td>
<td>27 (32.5)</td>
<td>20 (19.6)</td>
<td>0.12</td>
</tr>
<tr>
<td>Lopinavir/ritonavir</td>
<td>1 (0.4)</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.09</td>
</tr>
<tr>
<td>RAAS-blocker</td>
<td>14 (6.2)</td>
<td>2 (5.1)</td>
<td>5 (6.0)</td>
<td>7 (6.9)</td>
<td>0.92</td>
</tr>
<tr>
<td>NSAID</td>
<td>2 (0.9)</td>
<td>0 (0)</td>
<td>2 (2.4)</td>
<td>0 (0)</td>
<td>0.18</td>
</tr>
<tr>
<td>Difference in outcomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKI</td>
<td>41 (18.3)</td>
<td>17 (43.6)</td>
<td>8 (9.6)</td>
<td>16 (15.7)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Hospitalization (day)</td>
<td>11.1 (6.5–17.8)</td>
<td>15.9 (8.3–21.1)</td>
<td>13.9 (9.1–27.7)</td>
<td>14.9 (7.8–23.4)</td>
<td>0.997</td>
</tr>
<tr>
<td>Death</td>
<td>44 (19.6)</td>
<td>17 (43.6)</td>
<td>6 (7.2)</td>
<td>21 (20.6)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Still admitted</td>
<td>8 (3.6)</td>
<td>1 (2.6)</td>
<td>1 (1.2)</td>
<td>6 (5.9)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Values are presented as median (interquartile range), number (%), or mean ± standard deviation unless otherwise specified.

AKI, acute kidney injury; aPTT, activated partial thromboplastin time; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; COVID-19, the novel coronavirus disease 2019; CVD, cardiovascular disease; CPK, creatine phosphokinase; GFR, glomerular filtration rate; INR, international normalized ratio; NSAID, nonsteroidal anti-inflammatory drugs; PCR, polymerase chain reaction; RAAS, renin-angiotensin-aldosterone system; sCr, serum creatinine; SOFA, sequential organ failure assessment score; ΔsCr, variation in serum creatinine.

*Statistically significant difference between the decreased kidney function group and the stable kidney function group; †statistically significant difference between the decreased kidney function group and the improved kidney function group. Data missing. Total liter of intravenous infusion. *p < 0.05.
ed in the decreased kidney function group are detailed in Fig. 1. Notably, 69.2% of ΔsCr results were <0.3 mg/dL, a cut-off currently used to diagnose AKI [11].

AKI was detected in 43.6%, 9.6%, and 15.7% of patients with decreased kidney function, stable kidney function, and improved kidney function, respectively. As expected, patients with decreased kidney function experienced more episodes of AKI than did the other patients (p < 0.001) (Table 1). Positive correlation was found between the continuous variable ΔsCr and AKI (r = 0.31; p = 0.001) and in-hospital mortality (r = 0.21; p = 0.002); univariate Cox regression analysis showed that ΔsCr was a predictor of AKI (HR, 7.9; 95% CI, 4.2–14.7; p < 0.001) and in-hospital mortality (HR, 4.0; 95% CI, 2.2–7.2; p < 0.001).

Cox regression analysis was performed to assess the association between groups and outcomes. Subjects with decreased kidney function had a 4.6-fold greater risk of developing episodes of AKI (95% CI, 1.9–10.8; p < 0.001); conversely, improvement of kidney function, was not associated with AKI (HR, 1.7; 95% CI, 0.6–3.7; p = 0.273).

Kaplan-Meier estimates revealed statistically significant differences in survival between the groups (log-rank test, p < 0.001). The chi-square test estimated that patients with decreased kidney function (p < 0.001) and improved kidney function (p = 0.02) had higher mortality compared to subjects with stable kidney function (Fig. 2). Cox hazards regression analysis showed that decreased kidney function was an independent risk factor for 30-day in-hospital mortality, with an adjusted HR of 5.5 (95% CI, 1.1–28.0; p = 0.04) (Table 2).

**Discussion**

In clinical practice, sCr is the most common endogenous marker for assessing kidney function. Small increases in sCr have been recognized as a sign of impaired kidney function. According to the current guidelines, an increase in sCr
≥ 0.3 mg/day within 48 hours is widely recognized as the minimum criterion for the diagnosis of AKI [11]. In hospitalized patients, sCr variation is a frequent event, principally related to hemodynamic instability, systemic inflammatory response, dehydration, and use of nephrotoxic agents. Fluctuations in sCr generally occur in aged patients, especially if they are affected by systemic disease [18].

This study confirms that sCr elevation portends adverse prognostic significance in hospitalized patients with severe symptoms of COVID-19. We found that the decrease of kid-

![Kaplan-Meier curves describing the survival of patients with COVID-19 stratified according to the value of 24-hour ΔsCr. Thirty-day survival of patients with stable kidney function (+0.05 ≤ ΔsCr ≤ +0.05 mg/dL), decreased kidney function (ΔsCr > 0.05 mg/dL) and improved kidney function (ΔsCr < −0.05 mg/dL).](image)

COVID-19, the novel coronavirus disease 2019; ΔsCr, variation in serum creatinine.

Table 2. Unadjusted and adjusted Cox regression analyses to predict 30-day in-hospital mortality

<table>
<thead>
<tr>
<th>Kidney function</th>
<th>Unadjusted analysis</th>
<th>Adjusted analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR 95% CI p-value</td>
<td>Model 1 HR 95% CI p-value</td>
</tr>
<tr>
<td>Stable (Reference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased</td>
<td>5.8 2.3–14.9 &lt; 0.001*</td>
<td>6.2 2.3–16.9 &lt; 0.001*</td>
</tr>
<tr>
<td>Improved</td>
<td>2.8 1.1–6.9 0.03*</td>
<td>2.8 1.1–7.1 0.23</td>
</tr>
</tbody>
</table>

HR has been adjusted for sex and age (model 1) and for PaO_{2}/FiO_{2}, acute kidney injury, diabetes, chronic kidney disease, cardiovascular disease, blood pressure, C-reactive protein, and glomerular filtration rate at admission (model 2). CI, confidence interval; HR, hazard ratio.

*p < 0.05.
ney function within 24 hours from admission is an independent factor for poor outcomes in a group of non-ICU-admitted patients with symptoms of COVID-19.

In this study, the majority of patients (>80.0%) had stable (37.1%) or improved (45.5%) kidney function within 24 hours from admission. A small subset of the population (17.4%) experienced an increase in sCr over baseline. As the rates of supportive therapies and nephrotoxic agents were similar among the three groups, it is unclear why sCr changed in only some patients. Theoretically, supportive therapy including fluid administration, oxygen delivery, modulation of antihypertensive therapy, and withdrawal of offensive agents is expected to stabilize or even improve kidney function in ill and potentially dehydrated patients. Conversely, an increase in sCr, despite the best supportive care, is likely associated with a serious medical condition. Indeed, kidney function decline was documented in a subset of the population with different demographic and clinical characteristics compared to patients with stable or improved kidney function. Twenty-four-hour sCr elevation occurred in older subjects presenting with the higher sequential organ failure assessment score (SOFA score), including a lower mean PO2/FiO2 and a higher baseline sCr, compared to patients in the stable kidney function group. We suppose that baseline kidney function and severity of COVID-19 as key drivers of change in sCr within 24 hours from admission. As expected, the continuous variable ΔsCr was independently associated with AKI (HR, 7.9; 95% CI, 4.2–14.7; p < 0.001) and in-hospital mortality (HR, 4.0; 95% CI, 2.2–7.2; p < 0.001). Subsequent analysis showed that an increase in sCr > 0.05 mg/dL within 24 hours from admission occurred in a high-risk cohort of patients who experienced poorer outcomes (Fig. 2). This subset of the population was associated with a 4.6-fold risk of developing AKI during hospitalization and with a 5.5-fold risk of 30-day in-hospital mortality. Similar results have been found in a previous study aimed to clarify the clinical meaning of within-day ΔsCr in adult hospitalized subjects. The results of that study reported that each 5% or 0.1 mg/dL elevation in ΔsCr was associated with increased 30-day all-cause mortality (adjusted HR, 1.08; 95% CI, 1.06–1.10) [14].

As previously mentioned, modest sCr variations are often overlooked in the real world because these changes are often linked to analytical (e.g., interindividual variability or analytic error) or biological (e.g., muscle mass, renal tubular secretion, or protein-rich intake) variations. The cut-off of 0.05 mg/dL, indicating an increase of small magnitude, is theoretically greater than the “desirable” biologic variations of the laboratory methods used to measure sCr. The intralaboratory precision of sCr measurement is currently set at <4% to 5% below 1.13 mg/dL and at <2% above 1.13 mg/dL [17, 19].

Based on these results, decrease in kidney function might be clinically relevant because it can affect patient survival. The change in sCr can be a useful prognostic marker to stratify COVID-19 patients at high risk of AKI or poor outcomes. In the context of the emerging and rapidly evolving situation of the COVID-19 pandemic, timely identification of patients at high risk of AKI allows hospitals to prioritize supportive treatment in the most vulnerable subjects. In attempt to prevent and limit kidney injury, adequate care bundles for subjects with AKI should include adequate hydration, avoidance of nephrotoxic drugs, and withdrawal of potentially deleterious drugs such as renin-angiotensin system blockers and metformin [20].

In the absence of more detailed data about the pathogenicity of severe acute respiratory syndrome coronavirus (SARS-CoV-2) in the renal parenchyma [21], we suppose that the results of this study are not exclusively related to COVID-19 and might be translated to other general systemic diseases including bloodstream infection or sepsis of other origins. In particular, sepsis is a unique milieu to evaluate the clinical significance of sCr fluctuations. In this setting, sCr elevation is associated with dramatic increases in morbidity and mortality because reduced creatinine production due to the proinflammatory state tends to magnify even small variations in sCr [22].

The retrospective nature of our study, the small sample size and the lack of intra-group stratified analysis are the main drawbacks and limit the generalizability of our results. Although all patients received the same treatment for COVID-19 at admission, we cannot estimate the effects of other medications administered before admission nor can we control for other potential confounding variables able to interact with the most relevant outcomes. Given the general interest in identification of an early marker of morbidity and mortality in the pathogenesis of COVID-19, confirmatory studies are needed before drawing firm conclusions. Furthermore, further investigations should confirm these findings in the general population.

In conclusion, sCr elevation (greater than 0.05 mg/dL)
within 24 hours from admission selected a group of aged patients with severe manifestations of COVID-19 at high risk of 30-day in-hospital mortality.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Authors’ contributions

Conceptualization: GA, AF
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Intellectual contributions: FF, JM, G Cappelli, GG
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Supplementary material

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References


Acute kidney injury and mortality in coronavirus disease 2019: results from a cohort study of 1,280 patients

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Background: The development of acute kidney injury (AKI) in patients with coronavirus disease 2019 (COVID-19) is associated with a high risk of death. Published data demonstrate the possibility of severe kidney injury in patients suffering from COVID-19. However, these data are still controversial.

Methods: A total of 1,280 patients with a proven diagnosis of COVID-19 were included in our study. COVID-19 was confirmed in all patients using reverse transcriptase polymerase chain reaction test of a nasopharyngeal swab, and based on the typical computed tomography findings. Demographic data, underlying comorbidities, and laboratory blood tests were assessed. We assessed the incidence of AKI and its associated mortality defined by survival status at discharge.

Results: Proteinuria was identified with 648 patients (50.6%) with COVID-19. AKI was identified in 371 patients (29.0%). Ten of these patients (2.7%) required dialysis. The risk factors for AKI included age of > 65 years, augmentation of C-reactive protein, ferritin and an increase in values of activated partial thromboplastin time. Overall, 162 of the 1,280 hospitalized patients (12.7%) and 111 of the 371 patients (29.9%) with AKI did not survive. The hazard ratio (HR) for mortality was 3.96 (95% confidence interval, 2.83–5.54) for patients with AKI vs. no AKI.

Conclusion: AKI was a relatively common finding among patients with COVID-19. The risk factors for AKI in COVID-19 included old age, the inflammatory response, the severity of lung involvement, and disseminated intravascular coagulation. These same factors, in addition to arterial hypertension, were found to increase the risk of mortality.

Keywords: Acute kidney injury, COVID-19, Hematuria, Mortality, Proteinuria

Introduction

The recently detected zoonotic coronavirus disease 2019 (COVID-19) is a novel virus that is closely related to severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV).
This coronavirus was initially detected in China in December 2019, after which it spread all over the world. COVID-19 is a severe acute respiratory syndrome that can involve alveolar and interstitial pneumonia. However, other organs may also be involved, including the kidneys, heart, gastrointestinal tract, and nervous system [1].

Acute kidney injury (AKI) is a serious complication of COVID-19, which also determines the prognosis of the disease. Various studies have suggested that the frequency of AKI with COVID-19 varies from 0.5% to 28% [1–3]. Wang et al. [4] observed no obvious renal function abnormality or AKI in more than 100 patients with COVID-19 during their admission to the hospital. However, despite their descriptions, many other studies have demonstrated that the development of AKI is associated with a high risk of death in patients with COVID-19 [1,3,5].

Previous coronavirus infections, such as SARS-CoV and MERS-CoV, have a high degree of sequence homology to SARS-CoV-2, and can also cause AKI that is associated with adverse outcomes. AKI, which affected 26.7% of MERS-CoV patients, was a risk factor for mortality [6]. In contrast, 17% of patients with SARS were diagnosed with AKI. The development of AKI during the course of the SARS infection was associated with catastrophic outcomes, such as multiple organ failure (MOF) and death [7]. Multiple studies similarly have suggested that COVID-19 can be complicated by AKI; however, these data are still controversial. Therefore, there is a need for additional studies.

Methods

Study population

Between April and June 2020, COVID-19 patients who were admitted to the City Clinical Hospitals of Moscow Healthcare Department were selected for enrollment in this study. The study was approved by the local Ethics Committee of the Moscow State Budgetary Healthcare Institution (No. 07-20) and the written informed consent from the patients was not required.

The included patients had a laboratory-settled SARS-CoV-2 infection and viral pneumonia. The confirmed cases of COVID-19 were defined by a positive reverse transcriptase polymerase chain reaction (RT-PCR) test that was obtained by direct analysis of a nasopharyngeal swab. All of these patients also had typical images on computed tomography (CT) scan of the lungs. The typical radiographic features of coronavirus include a white mist or “ground glass” pattern in both lungs. In patients with a negative RT-PCR result, SARS-CoV-2 pneumonia was defined by severe acute respiratory infection with typical CT scan findings [8] and no other obvious etiology.

Demographic and clinical data, underlying comorbidities, and laboratory blood tests (including chemistry blood analysis, coagulation tests, assessment of renal function, C-reactive protein [CRP], lactate dehydrogenase [LDH], ferritin, and routine urine analysis) were collected. The international normalized ratio (INR), activated partial thromboplastin time (aPTT), ferritin, LDH, D-dimer, and CRP levels were evaluated at admission, and from baseline to maximum laboratory values.

Thoracic CT scan results and patient treatments (i.e., retroviral therapy, glucocorticoid administration, breathing and renal support) were obtained from electronic medical records. These data were transcribed into data compilation tables.

Proteinuria levels were measured in spot urine in g/g creatinine (Cr) by the colorimetric method using pyrogallol red (cutoff value, 0.15 g/g Cr). Proteinuria >0.15–1.0 g/g Cr was defined as mild, >1.0–3.0 g/g Cr as moderate, more than 3.0 g/g Cr as severe. Changes in proteinuria levels were assessed in 279 patients during their hospitalization.

The dynamics of fibrinogen and platelet levels were evaluated in 906 patients during their hospital stay. Increasing D-dimer levels were associated with an increase in aPTT and a decrease in platelet count according to the criteria of the International Society on Thrombosis and Haemostasis. These changes were attributed to signs of disseminated intravascular coagulation (DIC). We identified and focused on preexisting comorbidities such as diabetes mellitus and systemic arterial hypertension, CT involvement score, and percentage of lung involvement.

Outcome definitions

The primary endpoint was AKI, which was defined according to the Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline for AKI [9]. The standard definition of AKI in adults was designated as follows; an increase in serum creatinine (SCr) by ≥0.3 mg/dL within 48 hours, an
increase in SCr to >1.5 times baseline within the previous 7 days, or urine volume < 0.5 mL/kg/hr for >6 hours. The severity of AKI was staged according to the following criteria: stage 1, SCr 1.5–1.9 times baseline or ≥0.3 mg/dL increase; stage 2, SCr 2.0–2.9 times baseline; stage 3, SCr 3.0 times baseline, an increase in SCr to ≥4.0 mg/dL, or initiation of renal replacement therapy [9]. Recovery from AKI was identified by comparing the last creatinine level with the baseline creatinine level and was divided into stages 1, 2, or 3.

According to the percentage of lung tissue involvement, the severity of pneumonia was determined as mild, moderate, severe, or extremely severe, as follows: mild involvement (CT1) was defined by involvement of up to 25% of the lung parenchyma; moderate (CT2) involvement involved 25% to 50% of the lung parenchyma; severe (CT3), 50% to 75%; and extremely severe (CT4), more than 75% of the lung parenchyma. Acute respiratory distress syndrome (ARDS) was diagnosed in cases in which the O₂ saturation decreased to <92% and there was a need for respiratory support. Septic shock was diagnosed based on MOF and the need for vasopressor support.

We finally assessed mortality during hospitalization, which was defined by survival status on the day of discharge.

### Statistical analysis

The results are presented as medians and interquartile ranges (IQR) for continuous variables with a non-normal distribution. Differences in the proportions and continuous data were tested using the Pearson chi-square test and Mann-Whitney U test, respectively. The cumulative incidence of AKI was estimated using Kaplan-Meier analysis.

The principal components analysis and rotation method varimax with Kaiser normalization were used to choose the number of components to extract. Linear regression models with the following covariates were used to estimate the risk factors for AKI: age, body mass index, changes in INR, aPTT, CRP, ferritin, fibrinogen, D-dimer, LDH levels over time (from baseline to maximum laboratory values), maximum proteinuria, CT stage, presence and absence of SARS, diabetes mellitus, and arterial hypertension. HRs with 95% confidence intervals (CIs) for mortality were calculated. A p-value of <0.05 was considered statistically significant. The statistical analysis was performed using the IBM SPSS version 23 (IBM Corp., Armonk, NY, USA).

### Results

#### Demographic characteristics

A total of 1,280 patients with a proven diagnosis of COVID-19 were included in our study. The average age of the hospitalized patients was 63 years (IQR, 52–75 years); 644 were males and 636 were females. The demographic characteristics are shown in Table 1.

In 648 (50.6%) of the hospitalized patients with COVID-19, proteinuria was identified. Severe proteinuria was observed in 38 patients (3.0%); mild proteinuria was identified in 610 (94.1%). In 34 patients with COVID-19 infection, proteinuria was newly developed. Proteinuria increased in 22 patients. In contrast, proteinuria disappeared or dramatically decreased during the hospitalization in 223 patients. Hema- turia was detected in 77 patients (6.0%). Leukocyturia was detected in 282 hospitalized patients (22.0%).

Potassium levels of <3.5 mmol/L were observed in 113 patients (8.8%). In 90 patients with hypokalemia, mild proteinuria (>0.15 g/g Cr) was detected, manifested by hematuria in 15 patients and by leukocyturia in 41 patients. In 23 patients, there were no changes in the urine tests. In 51 patients, hypokalemia was associated with AKI (32 patients, stage 1; 12 patients, stage 2; seven patients, stage 3).

#### Incidence and severity of acute kidney injuries

The cumulative frequency of AKI was 371 of 1,280 patients (29.0%). Ten of these patients (2.7%) required dialysis (Supplementary Fig. 1, available online). The proportions of patients with AKI stages 1, 2, and 3 among the hospitalized patients were 256 (69.0%), 68 (18.3%), and 47 (12.7%), respectively.

AKI was diagnosed in 63 of 134 patients (47.0%) admitted to the intensive care unit (ICU). The numbers of AKI patients
with stages 2 and 3 were higher than those in the common group of hospitalized patients (17% and 11%, respectively). In 96 patients of 371 patients (25.9%), the Cr levels had already increased at the time of admission 1.62 mg/dL (IQR, 1.33–1.80 mg/dL).

According to a factorial analysis and the rotation method of varimax with Kaiser normalization, we identified three groups of AKI risk factors as follows: 1) inflammation (an augmentation of CRP and ferritin levels), 2) an increase in aPTT values, and 3) age of >65 years. The linear regression model showed the significance of AKI development with the following parameters; older age, glomerular filtration rate (GFR) on hospital admission, degree of CRP increase, ferritin levels, the lengthening of the aPTT in dynamics from baseline to maximum laboratory values, the maximum values of D-dimer during hospitalization and the development of ARDS (Table 2).

### Outcomes

Overall, 162 of the 1,280 hospitalized patients (12.7%) and 111 of the 371 patients (29.9%) with AKI did not survive. In total, 860 of 907 patients (94.8%) without AKI survived, while 258 patients in the AKI group (23.1%) survived (Supplementary Table 1, available online). According to the Kaplan-Meier analysis, the survival probability gradually decreased among patients with AKI 1, 2, and 3 in the general group (Fig. 1A). Fifty-two patients (73.2%) without AKI and 34 patients (39.5%) with AKI survived in the ICU (Supplementary Table 2, available online).

In the ICU, the survival probability was significantly lower in patients with AKI 3 than it was in those without AKI, or with AKI 1 or 2. There was a significant difference between the AKI 3, AKI 2 and no AKI groups. However, there was no difference in the survival probability between the no AKI, AKI 1, and AKI 2 groups (Fig. 1B).

The only difference between the AKI 2 and 3 groups was

### Table 1. Characteristics of the study subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (n = 1,280)</th>
<th>Without AKI (n = 909)</th>
<th>AKI (n = 371)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>62.8 (52–75)</td>
<td>62.0 (52–74)</td>
<td>65.0 (53–78)</td>
<td>0.007</td>
</tr>
<tr>
<td>Male sex</td>
<td>645 (50.4)</td>
<td>455 (50.1)</td>
<td>190 (51.2)</td>
<td>0.70</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>373 (29.1)</td>
<td>179 (19.7)</td>
<td>194 (52.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>222 (17.3)</td>
<td>127 (14)</td>
<td>95 (25.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>Obesity</td>
<td>495 (38.7)</td>
<td>349 (38.4)</td>
<td>146 (39.3)</td>
<td>0.75</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>29.5 (24.8–32.4)</td>
<td>29.2 (24.8–32.4)</td>
<td>30.1 (24.7–32.6)</td>
<td>0.16</td>
</tr>
<tr>
<td>Intensive care unit</td>
<td>134 (10.5)</td>
<td>70 (7.7)</td>
<td>64 (17.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>ARDS</td>
<td>31 (2.4)</td>
<td>0 (0)</td>
<td>31 (8.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Invasive ventilation</td>
<td>26 (2.0)</td>
<td>0 (0)</td>
<td>26 (7.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Shock</td>
<td>10 (0.8)</td>
<td>0 (0)</td>
<td>10 (2.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Length of hospital stay (day)</td>
<td>10 (8–14)</td>
<td>10 (7–13)</td>
<td>12 (8–16)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Laboratory parameter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteinuria</td>
<td>648 (50.6)</td>
<td>407 (44.8)</td>
<td>241 (65.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GFR (mL/min/1.73 m^2)</td>
<td>70 (45–93)</td>
<td>80 (58–100)</td>
<td>41 (27–47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>INR (n = 1,179)</td>
<td>1.11 (1.02–1.23)</td>
<td>1.08 (0.99–1.16)</td>
<td>1.17 (1.05–1.35)</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>aPTT (sec) (n = 906)</td>
<td>26.2 (24.7–31.0)</td>
<td>25.2 (22.0–29.7)</td>
<td>29.3 (24.8–41.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ferritin (µg/L) (n = 1,087)</td>
<td>420.0 (266–580)</td>
<td>357.0 (178–521)</td>
<td>533.5 (360–649)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDH (U/L) (n = 1,217)</td>
<td>607 (458–856)</td>
<td>543 (432.5–687.5)</td>
<td>742 (518–1,105)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/L) (n = 1,280)</td>
<td>81.0 (34–135)</td>
<td>64.2 (28.9–116.9)</td>
<td>122.4 (70.2–197.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D-dimer (mg/L) (n = 760)</td>
<td>0.3 (0.03–0.60)</td>
<td>0.1 (0.02–0.48)</td>
<td>0.4 (0.04–0.94)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range) or number (%). AKI, acute kidney injury; aPTT, activated partial thromboplastin time; ARDS, acute respiratory distress syndrome; BMI, body mass index; CRP, C-reactive protein; GFR, glomerular filtration rate; INR, international normalized ratio; LDH, lactate dehydrogenase.

* Differences in proportions and continuous data were tested using the Pearson chi-square test and Mann-Whitney U test, respectively. Obesity is defined as having a BMI ≥ 30.0 kg/m^2. ARDS was diagnosed in cases of O_2 saturation of < 92% and the need for respiratory support.
the D-dimer level. The D-dimer level was significantly higher in patients with AKI 3 (4.6 [0.87–6] mg/L) than it was in patients with AKI 2 (0.75 [0.34–1.91] mg/L, p < 0.05) and in patients without AKI (0.27 [0.08–1.67] mg/L, p < 0.001). Cox regression univariate analysis was performed on data from 1,280 COVID-19 patients. The incidence rate of death was higher in the COVID-19 patients with AKI than it was in those without AKI. The HR for AKI patients compared with the non-AKI patients was 3.96 (95% CI, 2.83–5.54, p < 0.0001; AKI stage 1: 1.40 [0.99–1.97], p = 0.06; AKI stage 2: 1.99 [1.24–3.17], p < 0.004; AKI stage 3: 6.01 [4.14–8.71], p < 0.0001) which indicates that the mortality of patients with AKI was higher than that of non-AKI patients.

In multivariate Cox proportional hazards analysis, the following clinical features were predictors of unfavorable outcomes in COVID-19 patients; AKI 3 stage, lower GFR on admission, DIC with elongation of aPTT, and arterial hypertension (Fig. 2).

**Discussion**

Renal damage in COVID-19 results from the interaction of many different pathological processes. This renal damage may be due to the direct cytotoxic effect of the virus on kid-
ney cells, given that viral particles have been identified in podocytes, tubular epithelium, and endothelial cells [10,11]. Tubular damage may also be associated with a direct toxic effect of high cytokine concentrations, which lead to tubular dysfunction or tubular necrosis. The SARS-CoV-2 virus recruits CD68+ macrophages into the tubulointerstitium. This suggests that proinflammatory cytokines derived from macrophages induce tubular damage [11].

The effect of the virus on the podocyte explains a high percentage of the proteinuria levels in patients affected by COVID-19. However, proteinuria is often transient and rarely exceeds 3 g in this setting. In our study, proteinuria was detected in half of the COVID-19 patients and was a common manifestation of kidney damage.

Rare cases of collapsing nephropathy with nephrotic syndrome have been described in African American patients. A relationship between the APOL1 genotype with the mutation of the APOL1 gene has been advocated [12,13]. The majority of patients in our study had transient proteinuria, which may indicate its association with COVID-19 infection. Other observed changes in the urinary sediment analysis included hematuria and leukocyturia. It should be noted that the frequency of proteinuria was comparable with other similar studies; nevertheless, we evidenced that hematuria was present less often [3,14]. Leukocyturia was detected more frequently than was hematuria in our results. However, it is difficult to prove that there is a direct connection between the urinary sediment and viral action.

Hypokalemia was observed in 8.8% of patients. Our data do not match the data of a prior study, which showed that hypokalemia occurs in 93% of patients at the time of admission. The reasons for hypokalemia in COVID-19 patients are discussed. Chen et al. [15] identified a connection between hypokalemia and kaliuresis, which were identified in some patients with COVID-19. Gaetano et al. [16] suggested that hypokalemia may be related to kaliuresis due to increased angiotensin II levels, and uptake of diuretics and other nephrotoxic drugs. However, there is insufficient evidence to interpret kaliuresis as a result of tubular damage caused by COVID-19 infection.

The second most common renal lesion with COVID-19 was AKI, with a cumulative incidence of 29%. However, the sum of the incidences of stage 2 and stage 3 was 9%. According to previous studies, the incidence of AKI varies widely.
The well-known risk factors for AKI are underlying diseases such as diabetes mellitus, systemic arterial hypertension, elderly age, and/or chronic kidney disease [19]. We noticed a direct, proportional relationship between inflammatory marker levels (CRP, ferritin, LDH) and AKI development. The independent prognostic elements of AKI were older age, increased CRP, ferritin, and aPTT lengthening. The findings suggest that in addition to the severity of inflammation in COVID-19, there are other important factors in kidney damage that activate the clotting chain. We also identified a significant increase in D-dimer levels along with aPTT extension and ARDS development in the AKI group. Previous studies found that COVID-19 can induce a hypercoagulable state that is associated with progression of respiratory failure and poor prognosis [10,11]. Endothelial-induced injury caused by the virus results in activated complement, which contributes to the augmentation of thrombotic microangiopathy. SARS-CoV-2 is known to activate the complement lectin pathway [19]. The complement activation reaction in patients with COVID-19 was confirmed by the detection of deposits of C5b-9 (membrane attack complex), C4d, and mannose binding lectin-associated serine protease 2 in the vessels of the skin, lungs, and kidney tissues [20].

In a previous series of autopsies of patients with COVID-19, there was pronounced infiltration of lung and kidney tissues by macrophages and neutrophils [11]. Activation of innate immunity by the virus (macrophages and neutrophils) promotes the release of tissue factors and activation of the extrinsic coagulation pathway. This activation leads to the release of the protease network by neutrophils, which is the so-called neutrophil extracellular trap process (NETosis). This process promotes the activation of the intrinsic coagulation pathway [21,22].

Drawing analogies with macrophage activation syndrome, the association of immune inflammation with local or systemic blood coagulation in COVID-19 has been defined as an immunothrombotic process. This process initially starts in the lung (pulmonary intravascular coagulation), but by wide decompensation, can progress to DIC [23,24].

We identified an association between extended aPTT and high D-dimer levels without a tendency to decrease in patients with AKI. Similar results were obtained by Cheng et al. [14]. Patients with AKI were found to have coagulation abnormalities, including prolonged aPTT, higher D-dimer levels, and lower platelet counts. An additional adverse factor for developing AKI is hypoxia. The evidence that suggests that hypoxia contributes to AKI development comes from a comparison of the percentage of lung tissue involved and the decrease in oxygen saturation rates in patients with AKI [25]. In our study, ARDS was found to be an independent risk factor for AKI.

A lower GFR on admission and the presence of AKI significantly increased the risk of death in patients with COVID-19; these findings were comparable to published data [3,5,11,14]. However, factors other than AKI also influence the mortality rate of critically ill patients, as evidenced by the absence of differences between AKI stages 1, 2 and no AKI. In addition to the severity of the COVID-19 infection itself, the presence of arterial hypertension and DIC can also increase mortality.

Our study has several limitations. In many patients, the Cr levels were missed before hospital admission. Therefore, we were not able to estimate the incidence of chronic kidney disease. We also did not evaluate the levels of sodium, magnesium, chloride, or calcium in the serum. These measurements may have allowed for more reliable conclusions about the degree of tubular disorders in our patients. We did not evaluate the degree of hypovolemia or the administration of nephrotoxic drugs in our cohort. However, because the patients were admitted to the hospital, these additional AKI factors were quickly corrected and should not have affected our results. The dynamics of proteinuria can only be traced in some patients with COVID-19 infection, which did not allow us to accurately judge the direct relationship of proteinuria with COVID-19 infection.

In conclusion, acute kidney damage is a serious complication of COVID-19, because it is a predictor of increased mortality. Two factors that influence AKI development are old age and hyperactivation of the inflammatory response. In addition, DIC and the severity of lung involvement were associated with severe AKI development. An increased risk of mortality in patients with COVID-19 was connected to the same factors and the presence of arterial hypertension.

**Conflicts of interest**

All authors have no conflicts of interest to declare.
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Authors’ contributions

Conceptualization: NC
Data curation: NC
Formal analysis: NC, SB
Investigation: SB, AB, TA, ML
Writing–original draft: NC, ML
Writing–review & editing: SM
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References


Changes in metabolic parameters and adverse kidney and cardiovascular events during glomerulonephritis and renal vasculitis treatment in patients with and without diabetes mellitus

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**Background:** Cardiovascular disease causes significant morbidity and mortality in patients with glomerulonephritis, which is increasingly diagnosed in older individuals who may have diabetes mellitus (DM). We evaluated the impact of DM on metabolic profile, renal and cardiovascular outcomes during treatment and follow-up of individuals with glomerulonephritis.

**Methods:** We performed a retrospective cohort study of 601 consecutive adults with biopsy-proven glomerulonephritis for factors associated with kidney failure, hospitalization for cardiovascular events, and death. Biopsies with isolated diabetic nephropathy were excluded.

**Results:** The median patient age was 49.8 years (36.7–60.9 years) with estimated glomerular filtration rate of 56.7 mL/min/1.73 m² (27.7–93.2 mL/min/1.73 m²). DM was present in 25.4%. The most frequent diagnoses were minimal change disease (MCD) or focal segmental glomerulosclerosis (FSGS) (29.5%), lupus nephritis (21.3%), immunoglobulin A (IgA) nephropathy (19.1%), and membranous nephropathy (12.1%). The median follow-up was 38.8 months (interquartile range [IQR], 26.8–55.8 months). Among 511 individuals with lupus nephritis, anti-neutrophil cytoplasmic antibody-associated vasculitis, MCD/FSGS, membranous nephropathy, and IgA nephropathy, 52 (10.2%) developed kidney failure at a median 16.4 months (IQR, 2.3–32.2 months), while 29 (5.7%) had cardiovascular-related hospitalizations at 12.9 months (IQR, 4.8–31.8 months) and 31 (6.1%) died at 13.5 months (IQR, 2.5–42.9 months) after diagnosis. Cox regression analysis found that baseline DM was independently associated with kidney failure (adjusted hazard ratio [HR], 2.07; 95% confidence interval [CI], 1.06–4.05, p = 0.03) and cardiovascular-related hospitalization (adjusted HR, 2.69; 95% CI, 1.21–5.98, p = 0.02) but not with mortality.

**Conclusion:** DM was strongly associated with kidney failure and hospitalization for cardiovascular events in patients with biopsy-proven glomerulonephritis.

**Keywords:** Cardiovascular diseases, Diabetes mellitus, Glomerulonephritis, Renal insufficiency
Introduction

Glomerulonephritis and renal vasculitis continue to be frequent causes of end-stage kidney disease (ESKD) worldwide [1–3], and mortality from acute and chronic glomerulonephritis rose 25% over the past 10 years [4]. Individuals with glomerulonephritis are increasingly older [5–9] and are more likely to have metabolic diseases that are associated with older age, such as diabetes mellitus (DM) and hypertension [7,9]. In addition, treatment with immunosuppressants such as glucocorticosteroids may cause or exacerbate DM, hypertension, and obesity [10–12]. These conditions are established cardiovascular risk factors [13,14], while certain systemic diseases that cause glomerulonephritis, such as anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, may be intrinsically associated with increased cardiovascular risk [15,16]. Cardiovascular disease is a major contributor to morbidity and mortality in patients with glomerulonephritis [16–20]. Given the propensity for metabolic and cardiovascular disease among individuals with glomerulonephritis and renal vasculitis, we aimed to evaluate the impact of DM on the metabolic profile, as well as adverse renal and cardiovascular outcomes during treatment and follow-up of individuals with glomerulonephritis.

Methods

This was a retrospective cohort study of consecutive adults aged ≥21 years with biopsy-proven glomerulonephritis and renal vasculitis diagnosed between January 2011 and July 2015 at the Singapore General Hospital, an academic medical center and tertiary referral center. Subjects were identified from the kidney biopsy procedural log, which recorded all native kidney biopsies performed. Biopsies with isolated diabetic nephropathy (n = 64) were excluded. This study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the local Institutional Review Board (No. 2015/2882). The requirement for informed consent was waived because of the retrospective nature of the study.

Demographic, comorbid disease, laboratory data, and medication data were retrieved from electronic medical records. Presence of DM at presentation was defined according to physician diagnosis of DM, fasting glucose ≥ 7 mmol/L, glycated hemoglobin (HbA1c) ≥ 6.5%, or when glucose-lowering medications were required. Baseline hypertension, hyperlipidemia, and ischemic heart disease were identified from medical records. Laboratory data collected included serum creatinine and urine protein-to-creatinine ratio (UPCR) within 1 month before kidney biopsy; fasting triglyceride (TG), high-density lipoprotein cholesterol and low-density lipoprotein cholesterol (LDL-C) within 24 months preceding biopsy and within 6 months after immunosuppressant; and HbA1c and fasting glucose within 6 months before and after immunosuppressant. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [21]. Nephrotic-range proteinuria was defined if UPCR was greater than 3 g/g. All laboratory investigations were conducted at our center, the laboratory of which is accredited by the College of American Pathologists. Pharmacotherapy data retrieved included angiotensin converting enzyme (ACE) inhibitor, angiotensin II receptor blocker (ARB), antidiabetic, antilipid, and immunosuppressant medication (glucocorticosteroid, calcineurin inhibitor, mycophenolate mofetil or sodium, cyclophosphamide, and azathioprine) prior to and after kidney biopsy, as well as peak daily dose of each immunosuppressant after diagnosis of biopsy-confirmed glomerulonephritis.

We assessed (1) changes in glycemic and lipid indices and need for antidiabetic therapy during therapy for glomerulonephritis and renal vasculitis; (2) occurrence and time to ESKD, defined by need for dialysis or transplant or if serum creatinine was >500 µmol/L; (3) hospitalization for cardiovascular events including acute myocardial infarct, congestive cardiac failure or cardiac catheterization showing >50% coronary artery stenosis; and (4) death. Data were retrieved until last hospital visit or death.

Statistical analyses were performed using IBM SPSS version 26 (IBM Corp., Armonk, NY, USA). Categorical variables were presented as proportions and continuous variables were summarized as medians with interquartile ranges (IQR [25th percentile–75th percentile]). The baseline clinical characteristics, metabolic profiles, and medications were compared for individuals with and without DM using the Pearson chi-square test for categorical variables and the Mann-Whitney U test for non-normally distributed continuous variables. When analyzing factors associated with clinical outcomes, we included the most frequent diagnoses (minimal change disease [MCD] or focal segmental glomerulosclerosis [FSGS], lupus nephritis, immunoglobulin A (IgA)
nephropathy, membranous nephropathy, and ANCA-associated vasculitis) and excluded patients with “other diagnoses” in order to reduce the heterogeneity of the study cohort. Kaplan-Meier survival curves for the clinical outcomes were compared using log-rank tests. Cox regression (stepwise method) was performed to obtain hazard ratios (HRs) and 95% confidence intervals (CIs) for factors that were independently associated with the clinical outcomes.

Covariates (age, sex, glomerulonephritis diagnosis, DM, hypertension, hyperlipidemia, ischemic heart disease, eGFR, UPCR, use of ACE inhibitor or ARB, and immunosuppressant treatment after biopsy) were chosen a priori. Multicollinearity was checked by examining the correlation matrix for coefficient values of ≥0.80. Stratified analyses to test for associations between DM and clinical outcomes were performed according to age (<50 years vs. ≥50 years), sex (female vs. male), ischemic heart disease (no vs. yes), eGFR category (≥60 mL/min/1.73 m² vs. <60 mL/min/1.73 m²), UPCR category (<3 g/g vs. ≥3 g/g), use of ACE inhibitor or ARB (no vs. yes), and immunosuppressant use (no vs. yes). The p-values of interactions for effect modification were obtained by including the interaction term (DM*variable) in the regression model. Subgroup analyses were performed for those with and without DM. All tests were two-tailed and statistical significance was defined as p < 0.05.

Results

Cohort clinical characteristics

We evaluated 601 individuals with biopsy-proven glomerulonephritis and renal vasculitis. Our multiethnic Southeast Asian cohort included Chinese (464 patients, 77.2%), Malay (77, 12.8%), Indian (22, 3.7%), and other ethnicities (38, 6.3%). The median age was 49.8 years (36.7–60.9 years) with eGFR 56.7 mL/min/1.73 m² (27.7–93.2 mL/min/1.73 m²). DM was present in 153 (25.5%) at biopsy. The most frequent diagnoses were MCD or FSGS (177 patients, 29.5%), lupus nephritis (128, 21.3%), IgA nephropathy (115, 19.1%), and membranous nephropathy (73, 12.1%). Eighteen patients (3.0%) had ANCA-associated vasculitis while other diagnoses (90 patients, 15.0%) included thin membrane disease, Alport disease, ANCA-negative pauci-immune vasculitis, undifferentiated immune-complex mediated glomerulonephritis, antiglomerular basement membrane disease, and tubulointerstitial nephritis. Histological features of diabetic nephropathy were present concurrently with glomerulonephritis in 34 biopsies (5.7%); 22 minimal changes or FSGS, three IgA nephropathies, two membranous nephropathies, one each of lupus nephritis and ANCA-associated vasculitis, and five others.

Table 1 compares the clinical characteristics and pharmacotherapy of patients with and without DM at presentation. Those with DM were older, more likely to be male, and to have hypertension, hyperlipidemia, and ischemic heart disease. They also had lower eGFR and greater proteinuria. Individuals with DM were more likely to have received blood pressure-lowering medications, including ACE inhibitor and ARB, before biopsy. After diagnosing biopsy-proven glomerulonephritis, treatment with ACE inhibitor or ARB did not differ between groups. Individuals with DM were less likely to receive immunosuppressive therapy such as glucocorticosteroid, but the peak daily dose and cumulative treatment duration of prednisolone and the use and treatment duration of calcineurin inhibitor were not different between groups.

Effect of baseline diabetes mellitus on metabolic profile after immunosuppressive therapy

Table 2 describes the metabolic profiles of 408 individuals who received immunosuppressant treatment. During the 6-month follow-up after starting immunosuppressant treatment, fasting glucose, TG, and LDL-C results were available for 294 individuals, while HbA1c was available for 92 individuals. Patients with DM were more likely to have increased TG and LDL-C after immunosuppressant, compared to individuals without DM. Antidiabetic and antilipid medications at baseline and after immunosuppressive therapy were also more frequently prescribed among those with DM. Fig. 1 shows that the need for oral antidiabetic therapy nearly doubled and the need for insulin therapy increased four-fold among individuals with DM treated with immunosuppressants. The need for antidiabetic medications decreased over time in both DM (Fig. 1A) and non-DM (Fig. 1B) patients, possibly due to tapering doses of induction immunosuppressant.

Effect of baseline diabetes mellitus on clinical outcomes

Among 511 individuals with lupus nephritis, ANCA-associated vasculitis, MCD/FSGS, membranous nephropathy, and IgA nephropathy, 52 (10.2%) developed ESKD at a median of 16.4
months (IQR, 2.3–32.2 months), while 29 (5.7%) had cardio-
vascular-related hospitalizations at a median of 12.9 months
(4.8–31.8 months), and 31 (6.1%) died at a median 13.5 months
(2.5–42.9 months) after diagnosis.

The follow-up duration was comparable between those
with and without DM (median of 36.5 months [IQR, 24.4–
53.8 months] vs. 40.0 months [IQR, 27.3–57.2 months], re-
spectively; p = 0.31). In the DM group (n = 120), there were
21 ESKD, 18 cardiovascular-related hospitalizations, and 10
deaths. In the no-DM group (n = 391), there were 31 ESKD,
11 cardiovascular-related hospitalizations, and 21 deaths.
Individuals with DM at baseline were more likely to develop
ESKD (17.5% vs. 7.9%, p = 0.002) and to experience a car-
diovascular event requiring hospitalization (15.0% vs. 2.8%,
with increased cardiovascular-related hospitalization in both female (HR, 6.92; 95% CI, 1.95–24.57) and male patients (HR, 3.97; 95% CI, 1.54–10.26), but by varying degrees.

Other factors associated with end-stage kidney disease, cardiovascular-related hospitalization, and mortality

Multivariate analyses for ESKD, cardiovascular-related hospitalization, and death are shown in Tables 3 and 4. Older age was independently associated with increased risk of mortality but reduced risk of ESKD. Male sex increased the risks of cardiovascular-related hospitalization and death. Compared to IgA nephropathy, both lupus nephritis and ANCA-associated vasculitis independently increased the risks of cardiovascular-related hospitalization and death, but while patients with ANCA-associated vasculitis had greater

<table>
<thead>
<tr>
<th>Variable</th>
<th>Biopsy-proven glomerulonephritis and renal vasculitis treated with immunosuppressants (n = 408)</th>
<th>Without DM (n = 323)</th>
<th>With DM (n = 85)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antidiabetic medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>0 (0)</td>
<td>40 (47.1)</td>
<td>40 (47.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>After immunosuppressant</td>
<td>19 (5.9)</td>
<td>62 (72.9)</td>
<td>62 (72.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antilipid medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>111 (34.4)</td>
<td>53 (62.4)</td>
<td>53 (62.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>After immunosuppressant</td>
<td>213 (65.9)</td>
<td>70 (83.4)</td>
<td>70 (83.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline (mmol/L)</td>
<td>5.1 (4.8–5.7)</td>
<td>7.0 (5.8–8.2)</td>
<td>7.0 (5.8–8.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak value after immunosuppressant (mmol/L)</td>
<td>5.6 (4.9–6.8)</td>
<td>7.8 (6.6–9.5)</td>
<td>7.8 (6.6–9.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change from baseline (%)</td>
<td>9.6 (–4.2 to 33.2)</td>
<td>10.0 (–15.2 to 45.5)</td>
<td>10.0 (–15.2 to 45.5)</td>
<td>0.76</td>
</tr>
<tr>
<td>HbA1c</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline (%)</td>
<td>5.4 (5.1–5.7)</td>
<td>6.4 (5.8–7.2)</td>
<td>6.4 (5.8–7.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak value within 6 mo after immunosuppressant (%)</td>
<td>5.7 (5.3–6.2)</td>
<td>6.7 (6.0–8.7)</td>
<td>6.7 (6.0–8.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change from baseline (%)</td>
<td>2.6 (0–13.6)</td>
<td>8.0 (–3.1 to 33.6)</td>
<td>8.0 (–3.1 to 33.6)</td>
<td>0.40</td>
</tr>
<tr>
<td>Fasting triglyceride</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline (mmol/L)</td>
<td>1.69 (1.20–2.30)</td>
<td>1.73 (1.19–2.50)</td>
<td>1.73 (1.19–2.50)</td>
<td>0.79</td>
</tr>
<tr>
<td>Peak value after immunosuppressant (mmol/L)</td>
<td>1.43 (1.03–2.05)</td>
<td>1.93 (1.22–2.45)</td>
<td>1.93 (1.22–2.45)</td>
<td>0.004</td>
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<tr>
<td>Change from baseline (%)</td>
<td>–11.2 (–42.9 to 21.4)</td>
<td>5.0 (–23.9 to 42.3)</td>
<td>5.0 (–23.9 to 42.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Fasting LDL-cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline (mmol/L)</td>
<td>3.93 (2.91–6.10)</td>
<td>3.10 (2.24–4.21)</td>
<td>3.10 (2.24–4.21)</td>
<td>0.001</td>
</tr>
<tr>
<td>Peak value after immunosuppressant (mmol/L)</td>
<td>3.36 (2.67–4.16)</td>
<td>3.21 (2.27–4.23)</td>
<td>3.21 (2.27–4.23)</td>
<td>0.29</td>
</tr>
<tr>
<td>Change from baseline (%)</td>
<td>–14.3 (–52.2 to 15.4)</td>
<td>4.0 (–35.6 to 51.4)</td>
<td>4.0 (–35.6 to 51.4)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Data are expressed as number (%) or median (interquartile range). DM, diabetes mellitus; HbA1c, glycated hemoglobin; LDL, low-density lipoprotein.

Fasting glucose, triglyceride, and LDL-cholesterol results were available for 294 individuals during the initial 6 months after immunosuppressants were started. HbA1c was available for 92 individuals during the initial 6 months after immunosuppressants were started.

p < 0.001). Mortality was not significantly different between the groups (8.3% vs. 5.4%, p = 0.24). Survival analyses (Fig. 2) showed that the cumulative probabilities of renal survival and survival without cardiovascular-related hospitalization were significantly lower in those with DM at baseline. Multivariate analyses shown in Tables 3 and 4 confirmed that DM was independently associated with ESKD (adjusted HR, 2.07; 95% CI, 1.06–4.05; p = 0.03) and cardiovascular-related hospitalization (adjusted HR, 2.69; 95% CI, 1.21–5.98; p = 0.02) but not with mortality. Interactions were observed between DM and proteinuria for ESKD, and for DM and sex for cardiovascular-related hospitalizations (Supplementary Table 1, available online): DM was associated with increased ESKD in patients with nephrotic-range proteinuria (HR, 2.33; 95% CI, 1.18–4.63) but not in those without nephrotic-range proteinuria (HR, 2.86; 95% CI, 0.86–9.54); DM was associated with increased cardiovascular-related hospitalization in both female (HR, 6.92; 95% CI, 1.95–24.57) and male patients (HR, 3.97; 95% CI, 1.54–10.26), but by varying degrees.
risk of cardiovascular-related hospitalization than lupus nephritis, the inverse was true for mortality risk. Baseline ischemic heart disease increased the risk of cardiovascular-related hospitalization. A higher eGFR reduced the risks of all three adverse clinical events. While greater proteinuria increased the risk of ESKD, treatment with immunosuppressant reduced it. None of the covariates were significantly correlated with any other.

**Subgroup analyses by diabetes mellitus status at baseline**

In the DM group, older age (adjusted HR, 0.91; 95% CI, 0.86–0.96; p = 0.001), higher eGFR (adjusted HR, 0.92; 95% CI, 0.88–0.96; p < 0.001 for per 1 mL/min/1.73 m²), and treatment with ACE inhibitor or ARB (adjusted HR, 0.80; 95% CI, 0.02–0.32; p < 0.001) were independently associated with reduced risk of ESKD. Higher eGFR (adjusted HR, 0.98; 95% CI, 0.96–0.99; p = 0.02 for per mL/min/1.73 m²) and ACE inhibitor or ARB therapy (adjusted HR, 0.22; 95% CI, 0.05–0.86; p = 0.03) were associated with reduced risk of cardiovascular-related hospitalization, while baseline ischemic heart disease was associated with increased risk (adjusted HR, 6.94; 95% CI, 2.64–18.25; p < 0.001). Concurrent histological diabetic nephropathy was not a significant predictor...
of ESKD or cardiovascular hospitalization when added as a variable in the multivariable models. Multivariate analysis was not performed for mortality in DM due to the small number of events.

In the no-DM group, eGFR was the only independent factor associated with ESKD (adjusted HR, 0.95; 95% CI, 0.93–0.97; p < 0.001). Multivariate analysis was not performed for cardiovascular-related hospitalization due to the small number of events. Older age (adjusted HR, 1.09; 95% CI, 1.05–1.14; p < 0.001), male sex (adjusted HR, 3.46; 95% CI, 1.39–8.66; p = 0.008), and lupus nephritis (adjusted HR, 18.64; 95% CI, 2.37–146.54; p = 0.005) were independently associated with increased mortality risk.

Discussion

DM was frequent among our multiethnic cohort of 601 individuals with biopsy-proven glomerulonephritis and renal vasculitis and was associated with worsening of the metabolic profile after immunosuppressant treatment. After accounting for age, sex, glomerulonephritis diagnosis, hypertension, hyperlipidemia, baseline ischemic heart disease, eGFR, proteinuria, and treatment with ACE inhibitor or ARB and immunosuppressants, DM was also found to be associated with a one-fold greater risk of ESKD and 1.7-fold greater risk of cardiovascular-related hospitalization in glomerulonephritis and renal vasculitis.

While DM is an established risk factor for kidney failure and cardiovascular disease in the general population and in individuals with chronic kidney disease [13,22–24], there has been little focus on the additive risks of DM in glomerulonephritis and renal vasculitis, which may in themselves result in inherently increased risks of ESKD and cardiovascular disease [25,26]. We confirmed that DM doubled the risk of cardiovascular-related hospitalization independent of other cardiovascular risk factors and underlying glomerulonephritis diagnosis. The few prior studies that evaluated the cardiovascular risks of DM in glomerulonephritis generally limited their cohorts to individuals with a single glomerulonephritis diagnosis and reported conflicting results. DM was associated with increased risk of cardiovascular events (age-adjusted HR, 7.07; 95% CI, 1.88–26.54) in a medical records review of 58 incident ANCA-associated vasculitis [27], while a larger cohort of 504 Chinese patients with ANCA-associated vasculitis did not find DM to be associated

Figure 2. Kaplan-Meier survival curves. They show that cumulative probabilities of survival without the event were significantly different for (A) end-stage kidney disease (ESKD; log rank p = 0.001) and (B) cardiovascular-related hospitalization (log rank p < 0.001), but not for (C) mortality (log rank p = 0.22).
with cardiovascular events [16]. Notably, a meta-analysis of observational studies on cardiovascular events in ANCA-associated vasculitis stressed that most early studies published before 2016 did not provide adequate information on traditional cardiovascular risk factors [28]. In a prospective cohort of 299 systemic lupus erythematosus (SLE) patients in North America [29], DM was associated with increased odds of coronary artery disease (univariate odds ratio [OR], 4.63; 95% CI, 2.75–7.82; p = 0.02). In contrast, DM did not predict cardiovascular events among 277 SLE patients in Sweden who were followed up over 7 years [30]. A systematic review that evaluated 28 studies from the PubMed database from inception to 2012 highlighted that many cohort studies did not examine DM as a risk factor, while the few that did had small numbers of individuals with DM and did not find DM to be an independent risk factor for cardiovascular disease [31]. Among 221 patients with IgA nephropathy, DM was correlated with coronary heart disease in univariate analysis (OR, 5.87; 95% CI, 1.61–21.5; p < 0.05), but not after adjusting for other vascular risk factors.

DM causes endothelial dysfunction and inflammation and thus vascular disease [32]. We also considered the plausibility that pre-existing DM exacerbated the metabolic derangements associated with immunosuppressant therapy in glomerulonephritis, thereby increasing cardiovascular risk. In particular, glucocorticosteroid was administered to 95.8% of patients treated with immunosuppressive therapy as monotherapy or in combination with other immunosuppressant and is associated with adverse changes in blood pressure, body weight, insulin resistance, and DM [10,12,33], which are traditional risk factors for cardiovascular disease [13,14,33]. While baseline DM was associated with significantly worse lipid indices after treatment with immunosuppressants, we did not identify immunosuppressant therapy as an independent risk factor for adverse cardiovascular events. However, prior studies of individuals with inflammatory and autoimmune diseases treated with moderate to high-dose glucocorticosteroid found increased risk of myocardial infarction and symptomatic coronary artery disease [10,34,35]. The association between low-dose glucocorticosteroid and cardiovascular events was less clear [36,37]. Interestingly, steroid-sparing or steroid-minimization regimens have been gaining traction in recent years [38,39]. In a randomized controlled trial of 176 individuals with IgA nephropathy, mycophenolate mofetil combined with reduced prednisolone dose was associated with lower risk of new-onset DM than

### Table 3. Factors associated with ESKD during treatment and follow-up of 511 individuals with lupus nephritis, ANCA-associated vasculitis, MCD or FSGS, membranous nephropathy, and IgA nephropathy

<table>
<thead>
<tr>
<th>Variable</th>
<th>ESKD (n = 52)</th>
<th>Univariate HR (95% CI)</th>
<th>p-value</th>
<th>Multivariate HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (/year increase)</td>
<td></td>
<td>0.97 (0.94–0.99)</td>
<td>0.02</td>
<td>0.97 (0.95–0.99)</td>
<td>0.02</td>
</tr>
<tr>
<td>Male sex</td>
<td></td>
<td>1.42 (0.73–2.74)</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td></td>
<td>0.56 (0.20–1.59)</td>
<td>0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANCA-associated vasculitis</td>
<td></td>
<td>0.65 (0.12–3.46)</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCD/FSGS</td>
<td></td>
<td>0.62 (0.29–1.33)</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membranous nephropathy</td>
<td></td>
<td>0.70 (0.18–2.69)</td>
<td>0.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM at baseline</td>
<td></td>
<td>2.21 (1.08–4.53)</td>
<td>0.03</td>
<td>2.07 (1.06–4.05)</td>
<td>0.03</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td>2.41 (0.99–5.91)</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td></td>
<td>0.71 (0.34–1.45)</td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td></td>
<td>0.99 (0.34–2.95)</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR (/mL/min/1.73 m² increase)</td>
<td></td>
<td>0.95 (0.93–0.97)</td>
<td>&lt;0.001</td>
<td>0.95 (0.93–0.97)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>UPCR (/g/g increase)</td>
<td></td>
<td>1.07 (1.01–1.14)</td>
<td>0.02</td>
<td>1.07 (1.02–1.13)</td>
<td>0.01</td>
</tr>
<tr>
<td>ACEi or ARB</td>
<td></td>
<td>0.52 (0.22–1.27)</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunosuppressant</td>
<td></td>
<td>0.41 (0.20–0.85)</td>
<td>0.02</td>
<td>0.36 (0.19–0.68)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

ACEi, angiotensin converting enzyme inhibitor; ANCA, anti-neutrophil cytoplasmic antibody; ARB, angiotensin II receptor blocker; CI, confidence interval; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; FSGS, focal segmental glomerulosclerosis; HR, hazard ratio; IgA, immunoglobulin A; MCD, minimal change disease; UPCR, urine protein-to-creatinine ratio.

Lim, et al. Metabolic indices, ESKD, and CVD in glomerulonephritis
<table>
<thead>
<tr>
<th>Variable</th>
<th>Cardiovascular hospitalization (n = 29)</th>
<th></th>
<th></th>
<th>Death (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariate HR (95% CI)</td>
<td>p-value</td>
<td>Multivariate HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (/year increase)</td>
<td>0.99 (0.96–1.03)</td>
<td>0.77</td>
<td>-</td>
<td>1.09 (1.05–1.13)</td>
</tr>
<tr>
<td>Male sex</td>
<td>2.32 (0.96–5.59)</td>
<td>0.06</td>
<td>2.12 (0.94–5.23)</td>
<td>0.07</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>10.54 (1.12–99.25)</td>
<td>0.04</td>
<td>9.55 (1.07–84.93)</td>
<td>0.04</td>
</tr>
<tr>
<td>ANCA-associated vasculitis</td>
<td>14.23 (1.37–147.55)</td>
<td>0.03</td>
<td>13.76 (1.25–129.21)</td>
<td>0.02</td>
</tr>
<tr>
<td>MCD/FSGS</td>
<td>4.86 (0.61–38.56)</td>
<td>0.13</td>
<td>4.98 (0.64–38.96)</td>
<td>0.13</td>
</tr>
<tr>
<td>Membranous nephropathy</td>
<td>4.66 (0.43–50.35)</td>
<td>0.21</td>
<td>4.17 (0.44–39.65)</td>
<td>0.21</td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>1.00 (reference)</td>
<td>1.00</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>DM at baseline</td>
<td>2.39 (1.01–5.68)</td>
<td>0.05</td>
<td>2.69 (1.21–5.98)</td>
<td>0.02</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.31 (0.58–9.14)</td>
<td>0.24</td>
<td>-</td>
<td>1.18 (0.39–3.51)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>1.54 (0.64–3.71)</td>
<td>0.34</td>
<td>-</td>
<td>0.87 (0.38–1.99)</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>7.67 (3.23–19.66)</td>
<td>&lt;0.001</td>
<td>8.51 (3.58–20.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR (/mL/min/1.73 m² increase)</td>
<td>0.98 (0.96–0.99)</td>
<td>0.01</td>
<td>0.98 (0.96–0.99)</td>
<td>0.006</td>
</tr>
<tr>
<td>UPCR (/g/g increase)</td>
<td>0.98 (0.90–1.07)</td>
<td>0.69</td>
<td>-</td>
<td>0.98 (0.91–1.06)</td>
</tr>
<tr>
<td>ACEi or ARB</td>
<td>0.42 (0.13–1.32)</td>
<td>0.14</td>
<td>-</td>
<td>0.60 (0.21–1.74)</td>
</tr>
<tr>
<td>Immunosuppressant</td>
<td>1.11 (0.42–2.97)</td>
<td>0.83</td>
<td>-</td>
<td>1.44 (0.46–4.45)</td>
</tr>
</tbody>
</table>

ACEi, angiotensin converting enzyme inhibitor; ANCA, anti-neutrophil cytoplasmic antibody; ARB, angiotensin II receptor blocker; CI, confidence interval; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; FSGS, focal segmental glomerulosclerosis; HR, hazard ratio; IgA, immunoglobulin A; MCD, minimal change disease; UPCR, urine protein-to-creatinine ratio.
full-dose prednisolone (1% vs. 14% respectively, p = 0.002). Rapid prednisolone taper in the Plasma Exchange and Gluco-
corticoids in Severe ANCA-Associated Vasculitis (PEXIVAS) trial was comparable in efficacy to standard, higher-dose prednisi-
 lone but did not reduce the risk of endocrine adverse events [40]. While there are no long-term data on the cardiovascular effects of steroid-minimization therapies in glomerulonephritis, steroid avoidance and withdrawal therapies in renal transplantation were associated with significant cardiovascular risk reduction [41].

In this study, we also found that cardiovascular risk was increased inherently by ANCA-associated vasculitis and lupus nephritis, consistent with previous evidence [15,25,26,28,31,42]. The Joint European League Against Rheumatism and European Renal Association-European Di-
alysis and Transplant Association (EULAR/ERA-EDTA) not-
ed in 2017 that while hypertension and DM were prevalent among patients with ANCA-associated vasculitis, the cardio-
vascular risk appeared to be greater than that conferred by traditional risk factors alone [26]. A subsequent meta-analy-
thesis of observational studies that compared ANCA-associated vasculitis with the general population or controls with chron-
ic kidney disease found that ANCA-associated vasculitis had relative risks of 1.65 (95% CI, 1.23–2.22) for all cardiovascular events and 1.60 (95% CI, 1.39–1.84) for ischemic heart disease [28]. In SLE, the increased risk of cardiovascular disease has been attributed to accelerated atherosclerosis and presence of antiphospholipid antibodies [31,43]. A nested case-control study of 52,676 patients with SLE and 758,034 matched pa-
tients without SLE found that SLE was associated with greater odds of atherosclerotic cardiovascular disease (adjusted OR, 1.46; 95% CI, 1.41–1.51) [42]. The aforementioned systematic review summarized epidemiologic data that SLE had at least 2- to 3-fold elevated risks of myocardial infarction, congestive heart failure, and overall cardiovascular mortality compared to the general population [31].

While DM increased the risk of ESKD in this study, evidence from the available literature is conflicting. An early retrospective cohort of 536 French patients with FSGS, membranous nephropathy, and IgA nephropathy followed up for a mean of 7 years found that DM was strongly asso-
ciated with ESKD (adjusted HR, 2.6; 95% CI, 1.2–5.8) [44]. However, a subsequent retrospective cohort of 580 Taiwanese patients diagnosed with membranous nephropathy, MCD, FSGS, and IgA nephropathy and followed up over a median of 5.9 years did not find DM to be a risk factor for ESKD [45]. Unfortunately, two large retrospective cohorts of primary glomerulonephritis, one of 1,943 membranous nephropathy, MCD, FSGS, IgA nephropathy, and membranoproliferative glomerulonephritis patients in Korea and another of 2,350 with membranous nephropathy, MCD, FSGS, IgA nephropa-
thy, and lupus nephritis patients in South California, did not analyze DM as a risk factor for ESKD [46,47]. A Taiwanese Na-
tional Health Insurance Research Database study compared 1,317 SLE patients with DM and propensity score-matched controls without DM who were followed up for a mean of 5 years and found that ESKD was higher in the DM group (inci-
dence rate ratio, 2.71; 95% CI, 1.70–4.32) [48].

Interestingly, treatment with ACE inhibitor or ARB was associated with reduced risk of cardiovascular-related hos-
pitalizations and ESKD among our patients with glomerulo-
nephritis and renal vasculitis with DM. Previous systematic reviews of cardiovascular risk in ANCA vasculitis and SLE did not evaluate these medications as risk factors [28,31]. Simi-
larly, these medications were not analyzed as risk factors for ESKD in a Taiwanese cohort of primary glomerulonephritis patients [45], while baseline ACE inhibitor or ARB was not associated with ESKD in a French cohort of primary glomer-
ulonephritis patients (adjusted HR, 0.9; 95% CI, 0.5–1.6) [44]. However, both ACE inhibitor and ARB reduced major car-
diovascular events (OR, 0.82; 95% credible interval, 0.71–0.92 and OR 0.76; 95% credible interval, 0.62–0.89, respectively) and kidney failure (OR, 0.61; 95% credible interval, 0.47–0.79 and OR, 0.70; 95% credible interval, 0.52–0.89, respectively) compared to placebo in a meta-analysis of 119 randomized controlled trials of 64,768 participants with chronic kidney disease [49,50].

Our study had several limitations. We did not retroactively adjudicate the diagnosis of primary FSGS. However, the glo-
merulonephritis diagnosis was corroborated with the phy-
sician-documented clinical diagnosis, which took into con-
sideration clinical and histologic features that supported the diagnosis [31], including the degree of podocyte foot efface-
ment routinely reported by our pathologists, and that were used to differentiate primary from secondary FSGS [52]. The impacts of ACE inhibitor, ARB, and immunosuppressants on clinical outcomes may be confounded by indications, while the effect of DM may be confounded by other cardiovascular risk modifiers such as smoking, use of antplatelet or antic-
agulation drugs, and statins, which were not included in this
observational study. Thus, causality cannot be confirmed, and the results of this study are only intended for hypothesis-generation. The small number of cardiovascular events may limit the power to detect significant associations and likely contributed to imprecise risk estimates with large CIs. Similarly, the absence of significant interactions may also be due to the lack of power to detect heterogeneity in the effects measured [53]. While we found qualitative heterogeneity in the risk effect as nephrotic-range proteinuria was an effect modifier for the time to ESKD analysis [53], we acknowledge that multiple analyses may be prone to Type 1 errors. Lastly, the results of our study, obtained in a relatively old population with high prevalence of traditional cardiovascular risk factors, may not be generalizable to other cohorts with different demographic and clinical profiles.

Despite its limitations, this study has several strengths such as a long observation period sufficient for development of the outcomes of interest and the use of multivariate regression analysis to adjust for imbalances in risk factors between exposure groups. Importantly, we identified risk factors for long-term renal and cardiovascular outcomes among individuals with biopsy-proven glomerulonephritis and renal vasculitis, including modifiable factors amenable to interventions to reduce adverse events. Considering our findings and the current evidence, we propose that individuals with DM and biopsy-proven glomerulonephritis should be carefully and regularly assessed for cardiovascular risk and have their modifiable risk factors optimized. Patients with DM or at high cardiovascular risk may benefit from ACE inhibitor or ARB therapy and consideration for steroid-minimization immunosuppressive regimens, but further research will be required to evaluate the long-term cardiovascular benefits of such strategies.

Conflicts of interest

Jason C. J. Choo has served on Advisory Boards for Novartis and Pfizer and has donated honoraria to Singapore General Hospital to support research and education.

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Authors’ contributions

Conceptualization, Data curation, Methodology, Project administration: CCL, JCJC
Formal analysis: CCL
Investigation: THZ, IM, CYM, CCM, WKT
Writing-original draft: CCL
Writing-review & editing: All authors
All authors read and approved the final manuscript.

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References


Manifestation of rs1888747 polymorphisms in the FRMD3 gene in diabetic kidney disease and diabetic retinopathy in type 2 diabetes patients

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Background: FRMD3 polymorphisms has suggested that they could be an alternative test to differentiate diabetic nephropathy (DN) from nondiabetic renal disease (NDRD) in type 2 diabetes mellitus (DM) patients. This study was performed to investigate the relationship between the FRMD3 gene and clinical characteristics of DN.

Methods: Patients who already had renal pathologic results were tested for FRMD3 polymorphisms. The subjects were classified into three groups; DN with diabetic retinopathy (DR), DN without DR, and DM with NDRD. FRMD3 polymorphisms were analyzed in each group.

Results: The prevalence of GG, CG, and CC was 44.4%, 42.2%, and 13.3% respectively. There was no significant difference in clinical parameters, which consisted of disease duration, proteinuria, and complications in DN with or without DR and DM with NDRD. The G allele was mainly found in DN with DR patients (50.8%) whereas the C allele was found in DM with NDRD patients (43.5%) (p = 0.02). There was a significant association between the CC genotype in NDRD when compared to GG (p = 0.001). In addition, the C allele was 2.10-fold more often associated with NDRD than the G allele (p = 0.03). The CC genotype was correlated with risk for NDRD than the GG and GC genotypes, with odds ratios of 6.89 and 4.91, respectively (p = 0.02).

Conclusion: C allele presentation, especially homozygous CC, was associated with NDRD pathology in patients with overt proteinuria. Hence, kidney biopsy is suggested in those with the C allele or homozygous CC genotype, regardless of retinopathy manifestations.

Keywords: Diabetic kidney disease, Diabetic nephropathies, Diabetic retinopathy, FRMD3 gene

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**Introduction**

Diabetic kidney disease or diabetic nephropathy (DN) are two of the most common complications in diabetic patients. Approximately 20% to 40% of diabetic patients develop diabetic kidney disease, which can progress to chronic kidney disease [1–3]. Its pathophysiology is similar to the progression of diabetic retinopathy (DR). Recent studies showed that both metabolic and hemodynamic stimuli could activate key intracellular signaling pathways along with transcription factors that contribute to microvascular damage in both glomeruli and the retina [1,4,5].

The diagnosis of DN can be made when patients manifest persistent proteinuria (exceed 500 mg within 24 hours) and hypertension along with a progressive decline in renal function [3,6]. This condition is usually preceded by the stage of microalbuminuria [7]. Without intervention, diabetic patients with microalbuminuria progress to proteinuria and then overt DN [4,8]. However, there are reports of non-diabetic renal disease (NDRD) that are isolated or superimposed on DN, such as immunoglobulin (Ig) A nephropathy, minimal change disease, and idiopathic focal segmental glomerulosclerosis.

The prevalence of NDRD in diabetic patients confirmed by tissue pathology reports ranges from 10% to 85% [9–12]. This wide prevalence range may result from selection criteria, biopsy threshold, or sample size [13]. Due to its indolent nature, chronic kidney disease diagnosed after DR is typically presumed to be DN [5]. However, diabetic patients with high amounts of proteinuria without DR are recommended for a kidney biopsy to rule out other glomerular diseases [13–16]. Because frequent complications from kidney biopsy often occur, many patients may refuse to receive one. Many nephrologists are also reluctant to perform renal biopsy on diabetic patients due to its potential complications, such as hematoma, arterial embolization, and even necessity of nephrectomy. Additionally, many primary hospitals lack the facilities necessary for kidney biopsy. Therefore, nephrologists must provide a diagnosis based on the patients’ clinical manifestations and laboratory results [3,8,10].

Novel studies have observed that the FRMD3 genotype in the rs1888747 position most likely correlates with diabetic kidney disease. The prominent gene expression can be narrowed down to three alleles. First, the CC allele was observed to be a protective gene for diabetic kidney disease, suggesting other glomerular pathology. Second, GC and GG were both progressive alleles which were predisposing factors favoring diabetic kidney disease. Hence, identifying single nucleotide polymorphisms (SNPs) of rs1888747 in the FRMD3 gene from blood cells could potentially serve as a less invasive procedure compared to kidney biopsy [17–23].

The objective of this research was to study the association between SNP expression in the FRMD3 gene and diabetic kidney disease and DR. Furthermore, we attempted to create a diagnostic guideline for the necessity of kidney biopsy to confirm the presence of diabetic kidney disease.

**Methods**

Clinical data collection

This was a correlation study between gene polymorphisms and renal pathology in patients with type 2 diabetes mellitus (DM) who had greater than 500 mg/day of urine protein at Her Royal Highness Princess Maha Chakri Sirindhorn Medical Center (MSMC), Thailand between March 13, 2019 and March 12, 2020. All patients voluntarily signed informed consent before participating in the study. The protocol was approved by the Human Research Ethics Board of Srinakharinwirot University (No. SWUEC/E-406/2561) in compliance with Declaration of Helsinki in 1964 and its amendments. Data was collected from type 2 DM patients who were older than 18 years of age. We collected details about their history and diagnosis of diabetes as well as its duration, DR and other complications such as cardio/cerebrovascular disease, peripheral vascular disease, DN, and diabetic neuropathy from medical records. Blood pressure, serum creatinine, serum glucose, hemoglobin A1c (HbA1C), and proteinuria were examined on the day of the hospital visit. The FRMD3 genotype was also tested at the same visit. Four weeks later, a renal biopsy was performed to determine the cause of proteinuria. We excluded patients with uncertain biopsy results, those with mixed pathological results, and inadequate biopsy tissue.

Patients who were diagnosed with DN had to have class II pathological results according to the Pathologic Classification of Diabetic Nephropathy 2010 [24]. Class II in this classification was defined as diffuse diabetic glomerulosclerosis.
mixed with an increase in extracellular material in the mesangium such that the width of the interspace exceeded two mesangial cell nuclei in at least two glomerular lobules [24]. Meanwhile, DR was evaluated and diagnosed according to the Early Treatment Diabetic Retinopathy Study (ETDRS) report number 7, by showing at least one microaneurysm with soft exudates, cotton-wool spots with venous beading, or definitely-present intraretinal microvascular abnormalities [25].

Clinical and laboratory evaluation

Serum glucose was analyzed by hexokinase/glucose-6-phosphate dehydrogenase. Serum creatinine and HbA1C levels were measured by the enzymatic method. Proteinuria levels were analyzed with the urine protein-creatinine ratio (g/g), which was analyzed by the benzethonium chloride and enzymatic method with the ARCHITERT C8000 (Abbott, Abbott Park, IL, USA).

DNA isolation and genotyping

Blood samples were tested for the *FRMD3* genotype during routine diabetic follow-up. DNA was extracted from patients’ blood leukocytes and was analyzed for genotyping by real-time polymerase chain reaction (RT-PCR). We studied the rs1888747 SNPs which were associated with DN [18]. DNA was extracted from peripheral blood leukocytes with a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s recommendations. SNPs (rs1888747) were genotyped using primer probes contained in the human TaqMan SNP Genotyping Assays (Applied Biosystems, Waltham, MA, USA). RT-PCR reactions were conducted in 96-well plates, in 20-µL total reaction volume using 20 ng of genomic DNA, TaqMan GTXpress Master Mix (2×; Applied Biosystems), and TaqMan SNP Genotyping Assays for rs1888747 (20×). Plates were positioned in an RT-PCR thermal cycler (QuantiStudio 5 RT-PCR System; Applied Biosystems) and enzyme activation was performed for 20 seconds at 95°C, followed by 45 cycles at 95°C for 3 seconds and 60°C for 30 seconds.

Patient allocation

*FRMD3* gene polymorphisms were analyzed as homozygous GG, heterozygous GC, and homozygous CC in accordance to DN with DR, DN without DR, and DM with NDRD groups, respectively.

Statistical analysis

The researchers employed the IBM SPSS version 23.0 (IBM Corp., Armonk, NY, USA). Through descriptive statistics, data collection was arranged into groups in terms of both frequency and percentage. The p-value and standard deviation were compared for continuous data by using the Student t-test. The chi-square test was used to reveal the relationship between continuous and categorical variables. The Spearman correlation coefficient was used to assess the correlation of selection algorithms with continuous and ordinal variables. The p-values of ≤0.05 were considered statistically significant.

Sample size calculation was based on the hypothesis that the ratio of DN to NDRD was 1:1. Appropriate sample size was calculated by setting the statistical power (1-β) level at 0.80 with a error probability at 0.05 [26]. Including the consideration of DN and NDRD incidence in type 2 DM patients, the calculated applicable sample size was 82 (in this study, n = 90).

Results

Data were gathered from 90 type 2 DM patients, and 37.8% of these had end-stage renal disease, requiring 2 to 3 hemodialysis sessions per week. Each patient also underwent DR examination with an ophthalmologist and diabetic neuropathy examination with monofilament testing within 4 weeks after the genotype was confirmed. This allowed accuracy when correlating genotype detection and microvascular complications of type 2 DM.

Demographic data

Of the 90 subjects, 40 (44.4%) were female. The mean age was 61.7 ± 13.1 years old, and the mean duration of DM was 13.9 ± 9.4 years. As for type 2 DM complications, 45 patients (50.0%) had diabetes retinopathy, 52 patients (57.8%) had diabetic neuropathy, and 29 patients (32.2%) had experienced macrovascular complications (cardio/cerebrovascular accident or peripheral vascular disease). As for renal complica-
tions, the mean urine protein was 3.43 g/g (range, 0.53–23.80 g/g), and the results from renal biopsies confirmed that 64 cases (71.1%) were DN and the remaining 26 (28.9%) were NDRD, including minimal change disease, focal segmental glomerulosclerosis, and IgA nephropathy. In addition, we also found that patients with retinopathy (n = 45) manifested renal pathology consistent with DN in 40 of 45 patients (88.9%, p < 0.001). However, 22 of 45 patients (48.9%) without retinopathy also showed renal pathology consistent with DN.

Patients were then categorized into GG, GC, and CC groups according to the rs1888747 genotypic characteristic, and the number of patients in each category was 40 (44.4%), 38 (42.2%), and 12 (13.3%), respectively. With respect to clinical manifestations, as shown in Table 1, there were fewer male subjects in the homozygous CC group (p = 0.04). However, no other significant clinical parameters differed significantly, including years of disease, amount of proteinuria, blood pressure, or rate and severity of microvascular and macrovascular complications. In addition, there was no difference in the amount of proteinuria in patients with DN with or without DR and DM with NDRD (Fig. 1). Therefore, only polymorphism variables were analyzed further in multivariable regression models (Table 1, 2).

Table 1. The correlation between clinical demographic data and genotypic polymorphism

| Demographic variable                  | GG (n = 40) | GC (n = 38) | CC (n = 12) | p-value  
|--------------------------------------|------------|------------|------------|----------
| Sex, male:female                     | 26:14      | 21:17      | 3:9        | 0.04*    
| Age (yr)                             | 60.0 ± 14.3| 64.0 ± 9.8 | 59.8 ± 17.3| 0.18     
| Duration of type 2 DM (yr)           | 13.9 ± 10.0| 13.3 ± 8.9 | 15.3 ± 9.3 | 0.79     
| Proteinuria at biopsy (g/g)          | 3.04 (0.44–10.65) | 3.71 (0.53–23.80) | 3.76 (0.57–15.04) | 0.52     
| Diabetic retinopathy                 | 22 (55.0)  | 17 (44.7)  | 5 (41.7)   | 0.57     
| Peripheral neuropathy                | 23 (57.5)  | 21 (55.3)  | 8 (66.7)   | 0.78     
| Macrovascular complicationa          | 15 (37.5)  | 11 (28.9)  | 3 (25.0)   | 0.61     
| SBP (mmHg)                           | 138.9 ± 14.8| 138.3 ± 17.5| 143.1 ± 18.6| 0.90     
| DBP (mmHg)                           | 77.5 ± 9.8 | 75.4 ± 11.2| 78.5 ± 21.0| 0.49     
| HbA1C                                | 7.4 ± 1.7 | 6.9 ± 1.4 | 8.9 ± 3.2 | 0.49     
| Cr at biopsyb (mg/dL)                | 1.38 (0.60–2.71) | 1.59 (0.69–2.80) | 1.46 (0.53–2.25) | 0.20     
| eGFR (CKD-EPI) at biopsyb (mL/min)   | 60.3 (24.1–111.2) | 49.6 (16.0–99.0) | 55.1 (19.9–124.3) | 0.17     
| ESRD (diagnosis dependent)           | 18 (45.0)  | 12 (31.6)  | 4 (33.3)   | 0.45     

Data are expressed as number only, mean ± standard deviation, median (range), or number (%). CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration equation 1; Cr, serum creatinine; DBP, diastolic blood pressure; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HbA1C, hemoglobin A1c; SBP, systolic blood pressure.

aCerebrovascular (ischemic or hemorrhagic stroke) or ischemic heart disease or peripheral vascular disease. bExclude patients with ESRD.

*p < 0.05.
The correlation between the rs1888747 single nucleotide polymorphism of the \textit{FRMD3} gene with overt proteinuria and diabetic retinopathy

The distribution pattern of alleles and genotypes are reported in Fig. 2 and 3. The G allele was found in DN with DR, DN without DR, and DM with NDRD 50.8%, 24.6%, and 24.6% of the time, respectively. Meanwhile, the C allele was found in 32.3%, 24.2%, and 43.5% of cases, respectively. The results indicated that the G allele was found mainly in patients with DN whereas the C allele was found in patients with NDRD \((p = 0.02)\) (Fig. 2). Moreover, the CC polymorphism was found significantly more often in patients with NDRD when compared to the GG polymorphism \((p = 0.04)\) (Fig. 3).

The risk of nondiabetic renal disease in diabetic patients based on the rs1888747 single nucleotide polymorphisms polymorphism in the \textit{FRMD3} gene

The association between genotype and renal pathology in diabetic patients with overt proteinuria and DR is mentioned in Fig. 2 and 3. These results indicate that patients with the C allele and genotype CC were presumed to have NDRD. Hence, subgroup analysis and logistic regression of patients with and without DR were performed to identify the predicted risk of developing NDRD in diabetic patients, as shown in Table 2. The C allele was 2.10 times more likely to be associated with NDRD, compared to the G allele \((p = 0.03)\). Furthermore, the CC genotype increased the risk of NDRD compared to other genotypes \((\text{omnibus test of model coefficients } p = 0.02)\); this genotype carried a 6.89-fold risk compared to the genotype GG by multivariate analysis \((p = 0.02)\). However, patients with the GC genotype were 4.91-fold more likely to have NDRD when compared to those with the GG genotype, although without statistical significance by multivariate analysis.

In the subgroup analysis of patients with and without DR, each group contained 45 participants. Using the power of the test \((Z_\beta)\) to find the reliability of the subgroup analysis \((\text{effect size})\), it was found that the power was more than 80% in both groups \((94\% \text{ in both groups})\), which indicated high reliability of results from subgroup analysis. We found that patients with DR who had the C allele had more than a 7-fold risk of NDRD in addition to DN when compared to those carrying the G allele \((p = 0.01)\). This could be interpreted as a 33-fold risk when comparing CC to GG \((p = 0.01)\). Using multivariate analysis, we found that once patients with DR as their only diabetic complication developed proteinuria, it could not promptly be concluded that DN was the pathology responsible for the protein leakage, especially in patients with the CC polymorphism. In contrast, in type 2 DM patients who had proteinuria but did not have DR, there was no statistical difference among polymorphisms that increased the risk of NDRD.

**Discussion**

The diagnosis of DN predominantly relies on clinical judgment. Important findings contributing to the diagnosis of DN in type 2 DM patients with abnormal proteinuria usually include 1) a diagnosis of type 2 DM for longer than 10 years; 2) the presence of DR; 3) the absence of urine sediment;
and lastly, 4) negative viral serologies and immunologic tests for systemic lupus erythematosus [15]. If clinical clues suggesting a different diagnosis from DN are present, renal biopsy should be considered to avoid missing a diagnosis of NDRD. Literature indicates that diabetic patients with NDRD showed significant improvements in proteinuria and renal function following treatment with immunosuppressive agents [15, 27]. Patients with NDRD that has similar clinical features to DN, such as primary focal segmental glomerulosclerosis, membranous nephropathy, or IgM nephropathy, may not receive a renal biopsy. This may result in a misdiagnosis, leading to a loss of opportunity for treatment. Thus, the development of a tool to distinguish between NDRD and DN in type 2 DM patients with proteinuria is of vital clinical importance, especially in regards to disease management and prognosis.

This study demonstrated that clinical parameters such as glucose level, urine protein level, and the presence of diabetic complications such as retinopathy in diabetic patients cannot distinguish DN from other glomerular diseases. Furthermore, nearly half of diabetic patients without retinopathy also manifested renal pathology consistent with DN.

In previous studies, diabetic patients who had over 10 years of diabetes with overt proteinuria and no DR were often perceived as having other glomerular pathology other than DN. Data showed that in type 2 DM patients, DN tended to develop with DR [5, 13, 14, 16, 28, 29], suggesting that patients diagnosed with overt proteinuria alone likely had other glomerular diseases rather than DN since there was no evidence of DR. This subset of patients was recommended to undergo kidney biopsy to further evaluate the correct clinicopathologic cause [3, 4, 7, 8, 10, 15, 16].

We have shown that the most frequent SNP polymorphism of the FRMD3 gene at rs1888747 location was GG, then CG, and then CC. Moreover, the G allele and homozygous GG specifically appeared to be more dominant in DN, while C allele and homozygous CC were less common. This observation was similar to previous studies [17–23] which found that expression of the C allele and CC genotype resulted in the prevention of DN. In our report, however, expression of the C allele and CC genotype in patients without retinopathy did not lead to a significant increase in DN when compared to the GG and CG genotypes. On the other hand, patients with DR who expressed the CC polymorphism might have

Figure 2. Distribution of G and C alleles in diabetic nephropathy (DN) with diabetic retinopathy (DR), DN without DR, and type 2 diabetes mellitus (DM) with nondiabetic renal disease (NDRD). The proportion of the G allele was 50.8%, 24.6%, and 24.6%, respectively, in each group. Meanwhile, the proportion of the C allele was 32.3%, 24.2%, and 43.5%, respectively (p = 0.02). The error bars mean 95% confidence intervals.
a 33-fold increased risk for NDRD compared to those with the GG polymorphism. These results demonstrated that the occurrence of the C allele and homozygous CC genotype increases the risk of diabetic patients acquiring NDRD as compared to DN.

Based on the results mentioned above, the clinical management of diabetic patients should correspond with the given evidence. A close observation approach is reasonable in patients diagnosed with overt proteinuria without retinopathy (assuming that the renal pathology is DN). However, this approach requires acknowledgment that this group of patients exhibits SNP polymorphisms of the FRMD3 gene with a homozygous GG phenotype to further support the diagnosis. However, if the homozygous CC genotype is present despite having DR, a kidney biopsy is advised in order to identify the differential diagnosis of other glomerular diseases.

Although the CC genotype was associated with NDRD, there were no statistically significant differences between the GG, GC, and CC genotypes in other diabetic complications such as retinopathy, neuropathy, and macrovascular complications. We hypothesized that this was because of bio-differences. The bio-differences are the differences between genotype and phenotype; even though each target tissue expressed the same gene (rs1888747), they create diabetic complications differently due to differences in the hemodynamic system and the exposure to metabolic triggers. For example, the kidney receives an overwhelming portion of the total cardiac output compared to other organs and also has the ability to concentrate solutes. Therefore, the kidneys accumulate metabolites, increasing their effect on the kidney. Therefore, there can be varied outcomes even though different tissue may express the same genes [30–32]. This is a compelling field that has not been thoroughly studied and is an excellent objective for future research. Another limitation of this study is its sample size. Hence, we cannot convincing-ly show that NDRD can be diagnosed without a renal biopsy based on the predominant pattern of the C allele, as the reason C allele is predominantly found in NDRD may be due to the small proportion of the G allele as a risk factor for DN.
Nonetheless, there are a handful of patients who refuse to undergo kidney biopsy since it is an invasive procedure that may lead to life-threatening complications including intraabdominal hemorrhage, kidney failure, and death. This study supports close monitoring under the assumption that DN develops in patients with the homozygous GG polymorphism in the FRMD3 gene. However, a kidney biopsy is suggested in patients who test positive for the CC or CG phenotypes in order to diagnose other glomerular diseases. The limitation of this study was the absence of qualitative measurements of gene expression levels from each phenotype. Further research should identify the predictive power of these genes and the occurrence of the disease via a receiver operating characteristic curve analysis.

In conclusion, this study demonstrated that in patients diagnosed with type 2 DM, parameters such as glomerular filtration rate, level of proteinuria, and presence of DR were unable to distinguish DN from other glomerular diseases. Meanwhile, the C allele may manifest a certain phenotype that has a renoprotective effect against DN. Therefore, we can imply that there may be underlying glomerular diseases in patients with overt proteinuria who manifest the C allele, especially the homozygous CC phenotype.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Authors’ contributions

Conceptualization, Funding acquisition: CK
Data curation, Project administration: PP
Formal analysis: CK, SP, PP
Investigation: CK, PP, RY
Methodology: CK, PP
Visualization: CK, PP, TD
Writing—original draft: CK, PP, TP, KW, TD
Writing—review & editing: CK, TP, KW, TD
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References

6. Gheith O, Farouk N, Namphoony N, Halim MA, Al-Otaibi T. Diabetic kidney disease: world wide difference of prevalence and
Effects of residential greennis on clinical outcomes of patients with chronic kidney disease: a large-scale observation study

Jae Yoon Park¹,², Jiyun Jung³, Yong Chul Kim⁴, Hyewon Lee⁵, Ejin Kim⁶, Yon Su Kim⁴, Ho Kim⁶,⁷, Jung Pyo Lee⁴,⁸

Background: As industrialization and urbanization are accelerating, the distribution of green areas is decreasing, particularly in developing countries. Since the 2000s, the effects of surrounding greenness on self-perceived health, including physical and mental health, longevity, and obesity have been reported. However, the effects of surrounding green space on chronic kidney disease are not well understood. Therefore, we investigated the impact of residential greenness on the mortality of chronic kidney disease patients and progression from chronic kidney disease to end-stage renal disease (ESRD).

Methods: Using a large-scale observational study, we recruited chronic kidney disease patients (n = 64,565; mean age, 54.0 years; 49.0% of male) who visited three Korean medical centers between January 2001 and December 2016. We investigated the hazard ratios of clinical outcomes per 0.1-point increment of exposure to greenness using various models.

Results: During the mean follow-up of 6.8 ± 4.6 years, 5,512 chronic kidney disease patients developed ESRD (8.5%) and 8,543 died (13.2%). In addition, a 0.1-point increase in greenness reduced all-cause mortality risk in chronic kidney disease and ESRD patients and progression of chronic kidney disease to ESRD in a fully adjusted model. The association between mortality in ESRD patients and the normalized difference vegetation index was negatively correlated in people aged >65 years, who had normal weight, were nonsmokers, and lived in a nonmetropolitan area.
Introduction

According to the World Health Organization’s report in 2016, 23% of all deaths worldwide (around 12.6 million) were attributable to environmental factors (e.g., air pollutants), and these factors accounted for 22% of the global burden of disease [1]. As industrialization and urbanization are accelerating, the distribution of green areas is decreasing, particularly in developing countries. Since the 2000s, the effects of surrounding greenness on self-perceived health, including physical (e.g., fatigue) and mental health (e.g., mood), longevity, and obesity have been reported [2]. Greenness has been hypothesized to benefit health by reducing exposure to air pollution, extreme heat, and noise, providing opportunities for physical activity and social engagement, and decreasing psychological stress and depression through direct contact with nature [3]. In recent years, it has been reported that a decrease in green areas adversely affects specific clinical outcomes, such as mortality due to cardiovascular (CV) disease, respiratory disease, and kidney disease, as well as birth outcomes and mental and physical health.

Chronic kidney disease (CKD) has become a critical public health issue worldwide, with a steadily increasing prevalence and poor clinical outcomes, and it imposes a high medical cost burden. Approximately 26 million adults in the United States and 4.6 million adults in Korea have CKD [4], and the annual per-person medical cost attributable to CKD was $1,700 for stage 2, $3,500 for stage 3, and $12,700 for stage 4 [5]. Individuals with CKD are 8- to 10-fold more likely to have CV mortality than those without renal dysfunction [6]. The traditional risk factors for CKD are diabetes mellitus (DM), hypertension, dyslipidemia, smoking, old age, and male sex [7]. Although the management of these factors has advanced considerably, the risk of CKD and its complications remain significant.

Accordingly, it is important to investigate and control new threats as well as traditional risk factors in order to establish a new perspective on CKD management. Recent reports have provided evidence that environmental factors, such as air pollution and sunlight exposure, also play important roles in kidney disease [8]. However, there is limited evidence for the impact of greenness on CKD patients.

Therefore, we have investigated the impact of residential greenness on mortality in CKD and end-stage renal disease (ESRD) patients and on disease progression from CKD to ESRD using a large-scale observational cohort from multiple metropolitan hospitals in Korea.

Methods

Study population

A cohort of 66,492 patients who visited three medical centers (Seoul National University Hospital, Seoul National University Bundang Hospital, and Seoul National University Boramae Medical Center) in Korea were followed from January 2001 to December 2016. We defined CKD patients according to the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline for the Evaluation and Management of CKD report [9]. The guideline defined CKD as abnormalities of kidney structure or function which were present for >3 months with implications for health. We excluded those whose residences were unclear (n = 7) and who were followed up for less than 3 months (n = 1,920). Therefore, 64,565 CKD patients were eventually enrolled in the study. We investigated the effect of greenness on three types of clinical outcomes; all-cause mortality in CKD patients, ESRD development in CKD patients, and all-cause mortality in ESRD patients. The date of death was confirmed from Statistics Korea. During follow-up, the date of ESRD outcome is selected as the earliest of the dates when the diagnostic code was entered, the dialysis prescription was first ordered, or when surgery for dialysis access (arteriovenous fistula or catheter insertion for peritoneal dialysis) was performed.
Ethical aspects

The study complied with the Declaration of Helsinki and received full approval from the Institutional Review Board of Seoul National University Hospital (No. J-1704-121-848), Seoul National University Bundang Hospital (No. B-1706/401-402), and Seoul National University Boramae Medical Center (No. 20170414/16-2017-65/051). The written informed consent waiver was also approved.

Normalized difference vegetation index

Green space was defined using the normalized difference vegetation index (NDVI) derived from the Moderate-Resolution Image Spectroradiometer (MODIS) Terra satellite images, which are generated every 16 days at 250-m spatial resolution by NASA [10]. Previous studies have used NDVI as a representative indicator of green space [11]. The NDVI is calculated as the ratio of the sum and the difference between red and near-infrared (NIR) light (NDVI = (NIR – Red)/(NIR + Red)), based on the fact that the mesophyll leaf structure reflects NIR, whereas chlorophyll in plants absorbs the red frequencies of visible light [12]. It ranges from –1 to 1, with higher positive values indicating dense vegetation areas and higher negative values representing bare soil, water, and snow [13]. Patients’ exposure to vegetation was estimated using the average NDVI in summer (June–August) within a radius of 250 m and 1,250 m around participants’ residences. We assigned the summer NDVI with high vegetation at 1 year before cohort enrollment as an estimate of maximum exposure [14]. NDVI within 250 m and 1,250 m around the participants’ residences in the year before their cohort entry was used to investigate the long-term effects, as NDVI measured from MODIS images was available from 2000. Measurement within a radius of 250 m indicated the directly accessible greenness, whereas vegetation within a radius of 1,250 m around each patient’s residence referred to greenness accessible within a 10- to 15-minute walkable distance [15].

Covariates

The following covariates were included: age at baseline, sex (one, male; two, female), hemoglobin, hypertension, DM, estimated glomerular filtration rate (eGFR), average concentration of particulate matter less than 10 μm (PM$_{10}$), population density, financial independence rate, and number of hospital beds. We calculated eGFR as follows [16]:

$$eGFR = 175 \times \text{Creatinine}^{-1.154} \times \text{Age}^{-0.203} \times 0.742 \text{ (if female)}$$

Subjects were defined as having hypertension if they were prescribed antihypertensive medications, were registered with diagnostic codes for hypertension on the electronic medical chart, and met the following hypertension criteria at the time of blood pressure measurement; systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg. In addition, subjects were defined as having DM if they were being prescribed DM medication, or were registered with diagnostic codes for DM on the electronic medical chart at the time of enrollment. The hourly PM$_{10}$ was obtained from 274 monitoring stations nationally through the Urban Atmospheric Monitoring Network between 2000 and 2016. Average PM$_{10}$ values of monitoring stations within 3 km, 5 km, and 10 km radii around participants’ residences were used, since the monitoring stations are not distributed equally throughout the country. Therefore, PM$_{10}$ was not assigned if there was no observatory within 3 km (n = 15,798), 5 km (n = 7,365), and 10 km (n = 4,172) from the residence. In addition, the mean concentration of PM$_{10}$ was updated every year, from 1 year before entry into the cohort, to reflect the time variance in this parameter. We included geographic characteristics by examining the population density, financial independence rate, and the number of hospital beds in the districts of each participant’s residence.

Statistical analyses

We applied Cox proportional hazard models to estimate the hazard ratios (HRs) and their 95% confidence intervals (CIs) for the associations between 0.1-point increases in residential greenness (NDVI) and clinical outcomes in four types of models. (1) Model 1 was a crude model that analyzed the effects of NDVI. (2) Model 2 was additionally stratified by 5-year age groups from 20 to 90 years of age and was adjusted for sex, eGFR, hemoglobin, hypertension, and DM. (3) Model 3 was additionally modified by PM$_{10}$ within 3 km, 5 km, and 10 km around patients’ residences. (4) Model 4 (fully adjusted model) was additionally adjusted for geographical variables, such as population density, financial indepen-
Results

Supplementary Fig. 1 (available online) shows the flow chart of 64,565 participants in the cohort. Table 1 presents the baseline characteristics of the study population with several exclusions in Supplementary Fig. 1. The average age was 54.0 years, and 49.0% of patients were male. Those who were living in the highest quartile of the 250-m NDVI tended to be slightly older, had lower hemoglobin levels, air pollutants, and financial independence rates, and more likely to live in nonmetropolitan areas compared to those living in the lowest 250-m NDVI quartile. During a mean follow-up period of 6.8 years, 8,546 deaths occurred in the cohort (13.2%) and 5,512 CKD patients developed ESRD (8.5%). The mortality rate of ESRD patients was 23.5% (n = 1,918). The mean eGFR of participants was 62.6 mL/min/1.73 m². In addition, more than half of the CKD patients (55.0%) lived in metropolitan areas. The mean concentration of PM_{10} was measured within 3 km, 5 km, and 10 km. Fig. 1 shows the NDVI distribution in Korea, in June, from 2001 to 2015. The green environment had improved substantially by 2015 as compared to 2001. In the south.

We investigated the HRs of clinical outcomes per 0.1-point increment of exposure to greenness using various models (Supplementary Table 2, available online). In model 1, every 0.1-point increase in NDVI within both the 250 m and 1,250 m radii was associated with an increased risk of all-cause mortality and occurrence of ESRD in CKD patients. However, we found protective effects of green space on the mortality risk of ESRD patients in the crude model. After adjustment for individual-level covariates (model 2), the increased risk effects of NDVI were reduced. HRs by 0.1-point NDVI increase in green space within the 1,250 m and 250 m were 0.98 (95% CI, 0.96–1.01) for mortality and 1.00 (95% CI, 0.98–1.02) for progression to ESRD, respectively. The impact of a green environment on reducing mortality risk and progression to ESRD was stronger when the model was additionally adjusted for the mean concentration of PM_{10} regardless of radius.

In the fully adjusted model, we found a consistent association between both continuous values and quartiles of greenness and clinical outcomes (Table 2). Patients living in the highest quartile of greenness according to the 1,250-m radius CKD and ESRD patients had a 1.1% (0%–2.3%) and 2.3% (0.4%–4.1%) lower mortality risk, respectively than those living in the lowest greenness quartile. In addition, CKD patients living in the highest quartile of NDVI, according to the 250-m radius, showed less frequent progression to ESRD than those living in the lowest NDVI quartile (HR, 0.99; 95% CI, 0.99–1.01). We confirmed the linear trend of association between mortality and NDVI (p-values of 0.006–0.046). Greenness within 1,250 m had significant protective effects against mortality in CKD patients (HR, 0.96; 95% CI, 0.93–0.996) and ESRD patients (HR, 0.91; 95% CI, 0.87–0.97). The HR for each 0.1-point increase in NDVI in the 250-m radius on progression to ESRD was 0.98 (95% CI, 0.95–1.01).

Table 3 shows the results of stratified analyses by model 4, which was adjusted for potential confounders such as urbanity, smoking, alcohol, age, and BMI. Strong protective effects of both a 250-m and 1,250-m buffer of greenness in terms of reducing mortality risk were seen in CKD patients.
older than 65 years. In the association between greenness and progression from CKD to ESRD, we observed inverse HRs in nonmetropolitan residents as compared to metropolitan residents, but the differences between groups were not significant (p for interaction = 0.44). A green environment within a 1,250-m radius reduced the mortality risk of ESRD patients in an urbanity-, smoking-, age-, and BMI-specific association. We found stronger protective effects of greenness in patients with normal weight who lived in metropolitan areas than in individuals with BMI over 25.0 kg/m² living in nonmetropolitan areas, although there was no significant difference between these groups (p for interaction = 0.43–0.46). When stratified by age, exposure to greenness had positive effects in people older than 65 years (HR, 0.89;

### Table 1. Baseline characteristics of the study population (n = 64,565) according to the quartile of the NDVI within a 250-m radius

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDVI 250 m</td>
<td>0.38 ± 0.15</td>
<td>0.22 ± 0.03</td>
<td>0.31 ± 0.02</td>
<td>0.41 ± 0.04</td>
<td>0.60 ± 0.09</td>
</tr>
<tr>
<td>NDVI 1,250 m</td>
<td>0.41 ± 0.14</td>
<td>0.25 ± 0.04</td>
<td>0.34 ± 0.03</td>
<td>0.45 ± 0.03</td>
<td>0.61 ± 0.07</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>54.0 ± 17.0</td>
<td>53.3 ± 16.7</td>
<td>54.0 ± 17.2</td>
<td>54.4 ± 17.2</td>
<td>54.4 ± 16.9</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31,613 (49.0)</td>
<td>7,580 (47.0)</td>
<td>7,933 (49.2)</td>
<td>7,889 (48.9)</td>
<td>8,206 (50.8)</td>
</tr>
<tr>
<td>Female</td>
<td>32,952 (51.0)</td>
<td>8,559 (53.0)</td>
<td>8,206 (50.8)</td>
<td>8,250 (51.1)</td>
<td>7,934 (49.2)</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td></td>
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<tr>
<td>&lt;25</td>
<td>28,079 (43.5)</td>
<td>6,350 (39.3)</td>
<td>7,183 (44.5)</td>
<td>7,332 (45.4)</td>
<td>7,211 (44.7)</td>
</tr>
<tr>
<td>≥25</td>
<td>13,676 (21.2)</td>
<td>2,983 (18.5)</td>
<td>3,398 (21.1)</td>
<td>3,594 (22.3)</td>
<td>3,699 (22.9)</td>
</tr>
<tr>
<td>Missing</td>
<td>22,810 (35.3)</td>
<td>6,806 (42.2)</td>
<td>5,558 (34.4)</td>
<td>5,213 (32.3)</td>
<td>5,230 (32.4)</td>
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<td>Observation period (yr)</td>
<td>6.8 ± 4.5</td>
<td>8.1 ± 4.7</td>
<td>6.5 ± 4.4</td>
<td>6.1 ± 4.2</td>
<td>6.2 ± 4.2</td>
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<td>Hypertension</td>
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<td>Yes</td>
<td>20,634 (32.0)</td>
<td>4,971 (30.8)</td>
<td>5,250 (32.5)</td>
<td>5,292 (32.8)</td>
<td>5,127 (31.8)</td>
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<tr>
<td>No</td>
<td>43,931 (68.0)</td>
<td>11,168 (69.2)</td>
<td>10,889 (67.5)</td>
<td>10,847 (67.2)</td>
<td>11,013 (68.2)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14,239 (22.1)</td>
<td>3,466 (21.5)</td>
<td>3,411 (21.1)</td>
<td>3,599 (22.3)</td>
<td>3,761 (23.3)</td>
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<tr>
<td>No</td>
<td>50,326 (77.9)</td>
<td>12,673 (78.5)</td>
<td>12,728 (78.9)</td>
<td>12,540 (77.7)</td>
<td>12,379 (76.7)</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>62.6 ± 31.9</td>
<td>64.0 ± 29.1</td>
<td>63.4 ± 33.4</td>
<td>62.0 ± 32.0</td>
<td>61.2 ± 32.6</td>
</tr>
<tr>
<td>≥25</td>
<td>12.9 ± 2.2</td>
<td>13.1 ± 2.2</td>
<td>12.9 ± 2.2</td>
<td>12.9 ± 2.2</td>
<td>12.8 ± 2.2</td>
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<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3,321 (5.1)</td>
<td>869 (5.4)</td>
<td>926 (5.7)</td>
<td>781 (4.8)</td>
<td>744 (4.6)</td>
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<tr>
<td>No</td>
<td>27,427 (42.5)</td>
<td>8,622 (53.4)</td>
<td>7,792 (48.3)</td>
<td>6,067 (37.6)</td>
<td>4,944 (30.6)</td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4,774 (7.4)</td>
<td>1,244 (7.7)</td>
<td>1,389 (8.6)</td>
<td>1,130 (7.0)</td>
<td>1,009 (6.3)</td>
</tr>
<tr>
<td>No</td>
<td>25,963 (40.2)</td>
<td>8,245 (51.1)</td>
<td>7,325 (45.4)</td>
<td>5,715 (35.4)</td>
<td>4,677 (29.0)</td>
</tr>
<tr>
<td>PM₁₀ (µg/m³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 km</td>
<td>56.5 ± 12.7</td>
<td>59.4 ± 14.6</td>
<td>55.7 ± 12.2</td>
<td>54.7 ± 11.1</td>
<td>55.13 ± 11.12</td>
</tr>
<tr>
<td>5 km</td>
<td>56.7 ± 11.8</td>
<td>59.6 ± 12.9</td>
<td>55.9 ± 11.5</td>
<td>55.3 ± 10.8</td>
<td>55.7 ± 10.9</td>
</tr>
<tr>
<td>10 km</td>
<td>57.2 ± 11.2</td>
<td>60.2 ± 12.6</td>
<td>56.2 ± 10.9</td>
<td>55.7 ± 9.9</td>
<td>56.4 ± 10.3</td>
</tr>
<tr>
<td>Metropolitan resident</td>
<td>35,528 (55.0)</td>
<td>14,548 (90.1)</td>
<td>10,926 (67.7)</td>
<td>8,624 (42.3)</td>
<td>3,230 (20.0)</td>
</tr>
<tr>
<td>Population density (person/km²)</td>
<td>11,064 ± 8,265</td>
<td>16,481 ± 6,599</td>
<td>13,125 ± 7,692</td>
<td>9,380 ± 7,847</td>
<td>5,124,64 ± 6,389</td>
</tr>
<tr>
<td>Financial independence rate (%)</td>
<td>48.5 ± 18.6</td>
<td>48.2 ± 18.2</td>
<td>46.6 ± 17.9</td>
<td>47.4 ± 17.8</td>
<td>45.3 ± 19.1</td>
</tr>
<tr>
<td>The number of hospital beds</td>
<td>2,596 ± 1,273</td>
<td>2,773 ± 1,460</td>
<td>2,665 ± 1,209</td>
<td>2,514 ± 1,123</td>
<td>2,198 ± 1,096</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or number (%). eGFR, estimated glomerular filtration rate; NDVI, normalized difference vegetation index; PM₁₀, particulate matter less than 10 µm.
95% CI, 0.82–0.96), although the difference between groups was not significant (p = 0.24). We also confirmed that non-smokers had a greater protective effect of greenness against progression to ESRD compared to smokers.

**Discussion**

In this study, we evaluated the long-term effects of NDVI on mortality and progression to ESRD in patients with CKD in Korea between 2001 and 2016. Participants with CKD and ESRD exposed to a green space within a walkable distance of 10 to 15 minutes had markedly reduced mortality risks, while the risk of progression from CKD to ESRD was also significantly decreased by living within an area with directly accessible green space. We found that the benefits of residential greenness on mortality were greater in metropolitan residents who were 65 years or older with a healthy lifestyle (as indicated by nonsmoking and normal weight).

Previously, several studies reported a lower mortality risk among people living in greener areas. An 11-year study using a large cohort of individuals living in Canada reported that a 0.15-point increase in NDVI around participants’ residences reduced non-accidental, CV disease-, and respiratory disease-related deaths by 8.5%, 8.9%, and 10.1%, respectively [17]. In addition, US women living in the highest quintile of greenness within a 250-m radius had 12%, 13%, and 34% lower risk of all-cause non-accidental, cancer-related, and respiratory disease-related mortality, respectively, than those living in the lowest quintile [15]. Several studies have also reported a protective effect of greenness against infant mortality [18], anxiety and depression, and impaired fetal growth [19]. However, a meta-analysis of six studies conducted in 2016, which estimated the association between all-cause mortality and greenness showed nonsignificant...
results when greenness increased by 0.1 units (relative risk, 0.992; 95% CI, 0.98–1.01) [20]. However, the results of the study were not generalizable, as this meta-analysis was performed using a relatively small number of studies.

In recent years, studies that explored the relationship between residential greenness and major causes of CKD, such as DM, hypertension, obesity, and dyslipidemia, have been reported. In 2014, the United Kingdom researchers reported that the risk of DM, the most common cause of CKD, was inversely associated with green space [21]. More recently, studies from China also reported that the risk of DM was reduced by greenness, which was measured using the NDVI [22]. The risk of hypertension, the second most important cause of CKD, has been consistently reported to be decreased by a green environment since the 2000s. The risk of metabolic syndrome, which involves obesity and dyslipidemia, has also been reported to be decreased in green spaces [23]. The specific mechanisms of the relationship between greenness and the major risk factors of CKD are not fully understood, but the reports commonly mentioned that green space would reduce ambient air pollution [24]. Ambient air pollutants, such as nitric oxide, PM$_{10}$, and PM$_{2.5}$, have been reported as risk factors for DM in several studies [25]. Air pollution also has been reported as a risk factor for hypertension, obesity, and dyslipidemia [26]. Other general positive effects of greenness included increased physical activity, social cohesion, and microbial exposure and decreased psychological stress, air temperature levels, exposure to volatile organic compounds, and noise [27].

In subgroup analysis, our results demonstrated that green space could reduce mortality in CKD and ESRD patients over 65 years of age. While previous reports did not reveal a significant association between age and greenness [28], at the beginning of 2020, studies from China reported more

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### Table 3. Stratified hazard ratio (95% confidence interval) for clinical outcomes by the NDVI$^a$

<table>
<thead>
<tr>
<th>Variable</th>
<th>CKD patient</th>
<th>ESRD</th>
<th>ESRD patient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDVI 250 m</td>
<td>NDVI 1,250 m</td>
<td>NDVI 250 m</td>
</tr>
<tr>
<td>Urbanicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.05 (0.99–1.10)</td>
<td>1.06 (1.00–1.13)</td>
<td>1.00 (0.93–1.08)</td>
</tr>
<tr>
<td>Yes</td>
<td>0.97 (0.92–1.01)</td>
<td>0.97 (0.92–1.03)</td>
<td>0.98 (0.94–1.03)</td>
</tr>
<tr>
<td>$p_{interact}$</td>
<td>0.35</td>
<td>0.97</td>
<td>0.44</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0.98 (0.94–1.03)</td>
<td>0.98 (0.93–1.04)</td>
<td>0.99 (0.96–1.03)</td>
</tr>
<tr>
<td>Yes</td>
<td>1.12 (0.99–1.26)</td>
<td>1.09 (0.95–1.25)</td>
<td>1.00 (0.90–1.11)</td>
</tr>
<tr>
<td>$p_{interact}$</td>
<td>0.05</td>
<td>0.04</td>
<td>0.86</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>1.00 (0.96–1.05)</td>
<td>1.00 (0.95–1.05)</td>
<td>1.00 (0.96–1.04)</td>
</tr>
<tr>
<td>≥65</td>
<td>1.03 (0.91–1.16)</td>
<td>1.04 (0.94–1.22)</td>
<td>0.92 (0.82–1.03)</td>
</tr>
<tr>
<td>$p_{interact}$</td>
<td>0.23</td>
<td>0.04</td>
<td>0.56</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>0.97 (0.93–1.02)</td>
<td>0.96 (0.92–1.01)</td>
<td>0.98 (0.94–1.01)</td>
</tr>
<tr>
<td>≥25</td>
<td>0.99 (0.91–1.07)</td>
<td>1.01 (0.92–1.10)</td>
<td>0.98 (0.95–1.01)</td>
</tr>
<tr>
<td>$p_{interact}$</td>
<td>0.19</td>
<td>0.08</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Multivariable fully adjusted model included covariates of age (5-year age groups from 20 to 90 years), sex, hypertension, diabetes, hemoglobin, estimated glomerular filtration ratio, average concentration of PM$_{10}$ monitoring stations within 5 km around the residence, population density, financial independence rate, and the number of hospital beds.

CKD, chronic kidney disease; ESRD, end-stage renal disease; NDVI, normalized difference vegetation index; $p_{interact}$, $p$ for interaction between stratification variable and NDVI.

$^a$NDVI 0.1 unit increase
significant protective effects of greenness against metabolic syndrome in participants aged under 65 years [29]. The most problematic issue in the elderly population is frailty, which is a condition in which various physiological functions have deteriorated due to age. These individuals are vulnerable to sudden health problems caused by even minor stress events [30]. Our findings are consistent with frailty in elderly people. We also observed significant effects of greenness on sub-populations with BMI less than 25.0 kg/m² who do not consume alcohol or smoke. These results indicate that the effect of greenness was more significant in CKD patients with well-controlled modifiable risk factors. Consequently, the results could suggest that deficiency of residential greenness is a new risk factor, in addition to well-identified, modifiable risk factors for CKD, such as obesity, alcohol, and smoking.

We found that greenness affects not only mortality but also progression of CKD to ESRD. Although statistically significant associations were not shown in the main analysis, greenness reduced ESRD occurrence significantly in patients living in nonmetropolitan areas, indicating that a lack of green space contributes to progression of kidney disease, as well as to mortality in those with kidney disease. Moreover, our findings are consistent with previous studies showing that representative risk factors for CKD, such as DM, hypertension, obesity, and dyslipidemia, could be modified positively by greenness.

Our study has several strengths. First, no previous study has reported a direct examination of the effect of greenness on mortality in CKD patients. Second, we adjusted for the comorbidities of DM and hypertension, biochemical indices that are important for predicting mortality risk, and socioeconomic factors, such as population density, financial independence rate, and the number of hospital beds. In addition, we estimated the greenness effect by modifying models by the time-varied measurement of particulate matter concentrations. Third, no previous study has reported the effects of greenness on progression of CKD to ESRD.

This study had the following limitations. First, we obtained data based on residence at entry into the cohort, and changes in residence were not updated. NDVI values reflecting address relocation could provide more accurate exposure measures. Second, although the cohort data contained information on education and socioeconomic status in terms of three levels (low, middle, and high), this could not be used as a modifier in the main or stratified analyses because the response rate for these variables were 35.5% and 9.0%, respectively. In addition, relevant individual risk factors, such as physical activity and social engagement, which are considered as modulators [31], were not available for this cohort. A study conducted in China found that adults 65 years or older, living in the highest quartile of residential greenness, scored 28% more on activities of daily living than those living in the lowest quartile [32]. Thus, our study did not explore how much participants utilized the green space for exercise and relaxation. Third, unmeasured confounding factors of area-level socioeconomic status could have affected the association between greenness and clinical outcomes in CKD patients.

In conclusion, we report that greenness surrounding patients’ residences reduced the risk of mortality in CKD and ESRD patients and the progression from CKD to ESRD. This study suggests that a deficiency in residential greenness is a new modifiable risk factor for CKD.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

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**Authors’ contributions**

Conceptualization: HK, JPL
Data curation: YCK
Formal analysis: JJ
Funding acquisition: HK, JPL, JJ
Investigation, Visualization: JYP, JJ
Methodology: HL, EK
Project administration: YSK, HK, JPL
Writing–original draft: JYP, JJ
Writing–review & editing: All authors
All authors read and approved the final manuscript.

References


Causal effect of alcohol use on the risk of end-stage kidney disease and related comorbidities: a Mendelian randomization study

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**Background:** An inverse observational association between alcohol use and the risk of chronic kidney disease (CKD) or end-stage kidney disease (ESKD) has been reported. The causal effect of alcohol use on the risk of ESKD warrants additional investigation.

**Methods:** The study was an observational cohort study investigating the UK Biobank and performed Mendelian randomization (MR) analysis. Amounts of alcohol use were collected using a touchscreen questionnaire. In the observational analysis, 212,133 participants without prevalent ESKD were studied, and the association between alcohol use and the risk of prevalent CKD or incident ESKD was investigated. The genetic analysis included 337,138 participants of white British ancestry. For one-sample MR, an analysis based on a polygenic risk score (PRS) was conducted with genetically predicted alcohol intake. The MR analysis investigated ESKD outcome and related comorbidities.

**Results:** Lower alcohol use was observationally associated with a higher risk of prevalent CKD or incident ESKD. However, the genetic risk of CKD was significantly associated with lower alcohol use, suggesting reverse causation. A higher PRS for alcohol use was significantly associated with a higher risk of ESKD (per units of one phenotypical alcohol drink; adjusted odds ratio of 1.16 \([95\% \text{ confidence interval}, 1.02–1.31]\)) and related comorbidities, including hypertension, diabetes mellitus, obesity, and central obesity.

**Conclusion:** The inverse observational association between alcohol use and the risk of CKD or ESKD may have been affected by reverse causation. Our study supports a causal effect of alcohol use on a higher risk of ESKD and related predisposing comorbidities.

**Keywords:** Alcohol, Chronic kidney disease, End-stage kidney disease, Life style, Mendelian randomization
Introduction

End-stage kidney disease (ESKD), the terminal state of chronic kidney disease (CKD), is a major and global health problem [1,2]. As the prevalence of ESKD is increasing rapidly as populations’ age, the socioeconomic burden of ESKD is growing, with patients suffering from a heightened risk of death and impaired quality of life.

Alcohol use is a leading modifiable lifestyle factor associated with higher risks of mortality and various disabilities. Alcohol use is also prevalent in individuals with CKD, and heavy alcohol consumption has been identified in a non-negligible portion of individuals with impaired kidney function [3]. However, debate persists regarding the possible protective associations of moderate alcohol consumption with respect to some clinical conditions [4]. Similarly, moderate alcohol use was associated with a lower risk of CKD or ESKD in several previous reports [5–8] and in a meta-analysis of prospective cohorts [9]. In addition, light-to-moderate alcohol use is not discouraged by current guidelines for CKD patients [10]. However, as this inverse association between alcohol use and CKD or ESKD may mislead the public into assuming that alcohol use is relatively safe for the kidney, additional explanation of the clinical association is necessary. Such observational findings can be affected by reverse causation, particularly when studying a lifestyle factor that is recognized to be harmful to health. Thus, additional investigations of the causal effects of alcohol use on the risk of ESKD or related comorbidities are warranted.

Mendelian randomization (MR) is a useful tool to assess the causal effects of a modifiable environmental factor on complex diseases in observational cohorts [1]. Because the genetic instrument implemented in MR is determined in-born, the genetic exposure is minimally biased by reverse causation or confounding. The method has been used to suggest a causal linkage between alcohol use and the risk of cardiovascular disease as part of efforts to correct misunderstanding of the purported benefits of light-to-moderate alcohol use on cardiovascular health [11].

In this study, we assessed the causal effects of alcohol use on the risk of ESKD or related comorbidities in a UK Biobank prospective cohort. An inverse observational association between alcohol use and prevalent CKD was present in the studied cohort, but an MR analysis revealed that this may be the result of reverse causation. We also used MR to demonstrate that higher alcohol use may increase the risk of ESKD or related comorbidities, including hypertension, diabetes mellitus, or obesity.

Methods

Ethical considerations

The study was performed in accordance with the Declaration of Helsinki and approved by the Institutional Review Boards of Seoul National University Hospital (No. E-2005-182-1126) and the UK Biobank Consortium (application No. 53799). The requirement for informed consent was waived because we used data from an anonymized public database.

Study setting

The UK Biobank is a population-scale prospective cohort that recruited >500,000 participants aged 40 to 69 years from multiple centers in the UK from 2006 to 2010. The project assessed various socioeconomic and lifestyle factors and laboratory and anthropometric measurements. Details of the UK Biobank database have been previously described [12].

Study population

For the observational analysis, we screened 502,505 UK Biobank participants at the time of study approval (Fig. 1). We excluded those for whom information on phenotypic alcohol amounts was missing (n = 162,223), those without information on the studied covariates (n = 127,959), and those with a previous ESKD history, based on baseline estimated glomerular filtration rate (eGFR) of <15 mL/min/1.73 m² (n = 190), as we were examining incident ESKD cases as an outcome. After exclusion, 212,133 participants with complete information for the studied variables remained.

For the genetic analysis, we applied sample filters using information provided by the UK Biobank and included individuals of European ancestry who were not outliers in terms of heterozygosity or missing data and who had no sex chromosome aneuploidy or excess kinship. After exclusion, 337,138 participants remained for the genetic analysis.
Alcohol use

Self-reported amounts of alcohol use were collected using a standardized touchscreen questionnaire. The participants were asked to report their average weekly consumption of specific types of alcoholic drinks. The touchscreen questionnaire was accompanied by pictures of an example of a single unit of each drink type. We summed the self-reported amounts of each drink type to derive the average total alcohol use in units per week. Those with missing data for any of the alcohol types were excluded.

Clinical outcomes

In the clinical analysis, we first assessed the association between alcohol use exposure and the prevalence of CKD stage ≥ 3, determined by baseline eGFR, calculated by the Chronic Kidney Disease Epidemiology Collaboration equation, <60 mL/min/1.73 m² [10,13]. Next, we studied the incident ESKD outcomes during follow-ups. Incident ESKD information was algorithmically defined by the UK Biobank, including hospital electronic health records and death registries. The follow-up was censored on February 29, 2016, as complete hospital inpatient data were available only until that date in all three regions (England, Scotland, and Wales).

In the genetic analysis, we studied the total ESKD outcome, both prevalent and incident, of the genotyped participants as a binary variable, as there was no concern that disease occurrence would influence the genetic exposure, in contrast to conventional observational studies [14]. In MR, a genetic instrument can be used regardless of the timing of the outcome and baseline assessment, as the genotype is determined inborn. Details of the collection of the other covariates are described in the Supplementary Methods (available online).

Statistical methods for observational analyses

Observational associations between the ordinal alcohol use category and prevalent CKD were investigated by logistic regression analysis. The risk of incident ESKD was analyzed by Cox regression analysis. Details of the statistical methods for observational analyses are presented in the Supplementary Methods.

Genetic analysis

We performed an allele-score MR as a one-sample MR analysis [15]. Two-sample MR was not implemented because external genome-wide association study (GWAS) summary statistics for ESKD covering sufficient single nucleotide polymorphisms (SNPs) for the approach were absent. Details of the imputation process and genotyping have been described previously [16]. For a GWAS to determine the genetic instrument, we filtered out variants with allele frequency of <0.1%, and a p-value of <5 × 10⁻⁸ was applied to identify significant SNPs associated with the phenotype and the continuous variable for amounts of total alcohol intake, adjusting for age, sex, and the first 20 principal components by linear regression. After removing SNPs in linkage disequilibrium (R² < 0.1), the genetic instrument for alcohol amounts was determined. Genes containing or related to SNPs were searched using the Ensembl Variant Effect Predictor [17]. We tested whether the SNPs included in the genetic instrument were directly associated with the CKD or ESKD phenotype by GWAS to determine whether a disproportionate effect of an individual SNP would affect the overall results (a single-SNP analysis). If a single SNP reached a Bonferroni-corrected significance level associated with the outcome phenotypes, a reason for the result (e.g.,...
presence of a pleiotropic pathway) would need to be identified. We used the genetic instrument to calculate a polygenic risk score (PRS) with the regression effect sizes, betas of the GWAS results, and the gene dosage matrix, and the PRS was regressed to the phenotypes of interest. All of the above processes were performed in PLINK2.0 (version alpha 2.3) [18] and R (version 3.6.2; R Foundation for Statistical Computing, Vienna, Austria) [18]. The instrumental power was estimated by calculating F statistics in the regression model for a PRS of alcohol-intake amount and phenotypical self-reported alcohol intake.

First, we investigated whether there was a reverse causation between CKD and alcohol use—whether individuals with a genetic risk of CKD drink less alcohol. We constructed a genetic instrument for CKD stage ≥ 3, as determined by an eGFR of <60 mL/min/1.73 m² or a prevalent history of ESKD. The PRS for CKD was linearly regressed to phenotypic amounts of alcohol use, and a multivariable model adjusted for age, sex, hypertension, and diabetes mellitus, which are major factors related to kidney function, was also constructed. As large-scale GWAS results for ESKD were absent due to the low prevalence of the outcome, an analysis including PRS for ESKD was not performed.

Second, we investigated whether genetically predicted amounts of alcohol use were associated with a risk of ESKD or related comorbidities, lifestyles, and socioeconomic status, including CKD stage ≥ 3, hypertension, diabetes mellitus, obesity, central obesity, current smoking, average days of moderate physical activity, income grade, or the number of household members. The PRS, scaled to reflect one phenotypical unit of average alcohol consumption, was regressed by logistic or linear regression to the phenotypes, and age- and sex-adjusted effect sizes were plotted.

Third, we tested whether alcohol-use phenotype, numerical value, or stratification by the recommendation (≤2 drinks for males, ≤1 drink for females per day) [19] interacted with the causal effects of the alcohol-use PRS and risk of ESKD with interaction-term analysis.

Fourth, to minimize the effect of horizontal pleiotropy and test whether alcohol use had a direct causal effect on the risk of ESKD, we constructed a multivariable model adjusted for age, sex, and phenotypes significantly associated with the PRS for amounts of alcohol use.

Fifth, to further assess the independence and exclusion-restriction assumptions, a “negative-control approach” was utilized [1]. If the genetic effect from the PRS for alcohol use was through the phenotypical alcohol-intake amount, the association between a genetic predisposition for exposure and outcomes would be attenuated in those with low amounts of phenotypical alcohol intake. This is because an effect from a higher alcohol-intake trait would be absent in negative controls, and if the significance remained, this means that another pleiotropic pathway rather than phenotypical alcohol intake, mediated the genetic effects. This approach has been suggested previously to test important MR assumptions when demonstrating causal effects. In this analysis, the main PRS analysis was limited to those in the genetic analysis dataset with an alcohol intake of ≤7 drinks per week.

Finally, as we chose the one-sample MR method, the results may have been biased toward observational findings [20]. Although the direction of clinically observed associations was inverse, we minimized such bias by recalculating the regressed betas for the alcohol-amount phenotype and excluding CKD stage ≥ 3 cases from the genetic dataset. This approach, which calculates the association between the genetic instrument and the exposure of interest only in the controls, reportedly yields valid estimates of exposure, even in a one-sample MR [20]. We recalculated the PRS, which was again regressed to the ESKD or CKD outcome with multiple adjustments for phenotypes associated with the PRS for amounts of alcohol use.

Results

Clinical characteristics

Clinical characteristics according to ordinal categories of amounts of alcohol use are presented in Table 1. There were 3,694, 83,392, 67,522, and 57,525 participants who reported consuming an average of 0 or 1 drink, >1 and ≤7 drinks, >7 and ≤14 drinks, and >14 drinks per week, respectively. Those who reported higher amounts of alcohol use were more likely to be male, obese, or centrally obese. Baseline dyslipidemia, hypertension, or current smoking was particularly common in those reporting consuming >14 drinks per week. Those with an annual income of £18,000 were more common among those who reported 0 or 1 drink per week, but those who reported earning £52,000 to £100,000 or >£100,000 per year were more common among those who consumed large...
### Table 1. Baseline characteristics according to self-reported amounts of alcohol intake per week

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Alcohol intake (drink/wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 or 1</td>
</tr>
<tr>
<td>No. of participants</td>
<td>3,694</td>
</tr>
<tr>
<td>Alcohol use (time/wk)</td>
<td>1 (1–1)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>59 (51–64)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2,406 (65.1)</td>
</tr>
<tr>
<td>Male</td>
<td>1,288 (34.9)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td></td>
</tr>
<tr>
<td>Body mass index ≥ 30 kg/m²</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>760 (20.6)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td></td>
</tr>
<tr>
<td>Waist circumference ≥ 102 cm</td>
<td></td>
</tr>
<tr>
<td>Central obesity</td>
<td>1,119 (30.3)</td>
</tr>
<tr>
<td>Previous history of stroke, angina, or heart attack</td>
<td>178 (4.8)</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>134.0 (122.5–147.5)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>80.5 (73.5–87.0)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>154 (4.2)</td>
</tr>
<tr>
<td>Hemoglobin A1c (mmol/mol)</td>
<td>35.3 (32.9–37.9)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>566 (15.3)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.6 (4.9–6.4)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.5 (3.0–4.1)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.4 (1.2–1.7)</td>
</tr>
<tr>
<td>History of smoking</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>2,506 (67.8)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>990 (26.8)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>198 (5.4)</td>
</tr>
<tr>
<td>Moderate physical activity (day/wk)</td>
<td>3 (2–5)</td>
</tr>
<tr>
<td>No. of illnesses</td>
<td>1 (0–3)</td>
</tr>
<tr>
<td>No. of treatments received</td>
<td>2 (0–3)</td>
</tr>
<tr>
<td>Income grade (GBP)</td>
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</tr>
<tr>
<td>&lt;18,000</td>
<td>941 (25.5)</td>
</tr>
<tr>
<td>18,000–30,999</td>
<td>1,010 (27.3)</td>
</tr>
<tr>
<td>31,000–51,999</td>
<td>962 (26.0)</td>
</tr>
<tr>
<td>52,000–100,000</td>
<td>659 (17.8)</td>
</tr>
<tr>
<td>&gt;100,000</td>
<td>122 (3.3)</td>
</tr>
<tr>
<td>No. of household member</td>
<td>2 (2–3)</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>91.9 (81.8–99.1)</td>
</tr>
<tr>
<td>&lt;60 ml/min/1.73 m²</td>
<td>103 (2.8)</td>
</tr>
</tbody>
</table>

Data are expressed as number only, median (interquartile range), or number (%).

BP, blood pressure; eGFR, estimated glomerular filtration rate; GBP, Great Britain pound; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Obervational association between alcohol use and CKD or ESKD

Those who reporting consuming 0 or 1 alcoholic drink per week had more than 30% greater odds of being diagnosed...
with prevalent CKD than those who reported consuming >1 and ≤7 drinks per week (Table 2). Even among those who reported higher amounts of alcohol use, the odds for prevalent CKD were significantly lower, and this remained similar in multivariable models adjusted for various clinical, lifestyle, and socioeconomic factors. During a median of 7.0 years (interquartile range, 6.3–7.6 years) of follow-up, 135 participants progressed to ESKD in the clinical analysis dataset. Individuals consuming 0 or 1 drink per week had an approximately 3-fold risk of ESKD compared with those who reported >1 and ≤7 drinks per week of alcohol use, even after adjustment for multiple variables.

Reverse causation of genetically predicted CKD on amounts of alcohol use

The analysis revealed 849 SNPs with GWAS-significant p-values for the CKD stage ≥ 3 phenotype. After removing those with an R² value of <0.1, a total of 17 SNPs were identified as the genetic instrument for CKD (Supplementary Table 1, available online). When we regressed the PRS for CKD to the amounts of alcohol use, a higher PRS was significantly associated with lower phenotypical amounts of alcohol use, implying that the presence of CKD is a causative factor for low phenotypical alcohol use amount (Table 3).

Genetically predicted amounts of alcohol use and risk of ESKD and related factors

A total of 4,596 SNPs with GWAS-significant p-values were observed for the numerical amounts of alcohol-use phenotype. The 35 SNPs not in a linkage disequilibrium state (R² < 0.1) were identified as the genetic instrument for genetically predicted amounts of alcohol use (Table 4). The genetic in-

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### Table 2. Risk of prevalent CKD or incident ESKD according to amounts of alcohol use

<table>
<thead>
<tr>
<th>Alcohol use (drink/wk)</th>
<th>Univariable model</th>
<th>Multivariable model 1</th>
<th>Multivariable model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR or HR (95% CI)</td>
<td>p-value</td>
<td>Adjusted OR or HR (95% CI)</td>
</tr>
<tr>
<td>Prevalent CKD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 or 1</td>
<td>1.48 (1.21–1.81)</td>
<td>&lt;0.001</td>
<td>1.38 (1.13–1.70)</td>
</tr>
<tr>
<td>&gt;1 and ≤7</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>&gt;7 and ≤14</td>
<td>0.87 (0.80–0.93)</td>
<td>&lt;0.001</td>
<td>0.86 (0.79–0.93)</td>
</tr>
<tr>
<td>&gt;14</td>
<td>0.80 (0.73–0.87)</td>
<td>&lt;0.001</td>
<td>0.73 (0.67–0.79)</td>
</tr>
<tr>
<td>Incident ESKD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 or 1</td>
<td>2.95 (1.26–6.89)</td>
<td>0.010</td>
<td>2.62 (1.12–6.14)</td>
</tr>
<tr>
<td>&gt;1 and ≤7</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>&gt;7 and ≤14</td>
<td>1.15 (0.76–1.73)</td>
<td>0.52</td>
<td>1.38 (0.91–2.09)</td>
</tr>
<tr>
<td>&gt;14</td>
<td>1.17 (0.76–1.79)</td>
<td>0.48</td>
<td>1.29 (0.83–1.99)</td>
</tr>
</tbody>
</table>

For the prevalent CKD outcome, logistic regression analysis was performed (OR), and for the incident ESKD outcome, Cox regression analysis was performed (HR). Multivariable model 1 was adjusted for age, sex, history of diabetes mellitus, and hypertension. When analyzing the incident ESKD outcome, the baseline eGFR was additionally adjusted. Multivariable model 2 was adjusted for age, sex, body mass index, waist circumference, history of angina/heart attack/stroke, diabetes mellitus, hemoglobin A1c level, hypertension, systolic blood pressure (BP), diastolic BP, dyslipidemia, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, smoking (nonsmoker, ex-smoker, current smoker), average days of moderate physical activity per week, number of illnesses, number of treatments received, income grade (<£18,000, £18,000–£30,999, £31,000–£51,999, £52,000–£100,000, and >£100,000), and number of household members. CI, confidence interval; CKD, chronic kidney disease; ESKD, end-stage kidney disease; HR, hazard ratio; OR, odds ratio.

### Table 3. Genetic predisposition to chronic kidney disease and its association with alcohol-intake phenotype

<table>
<thead>
<tr>
<th>Alcohol use (drink/wk)</th>
<th>Univariable model</th>
<th>Multivariable model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp(β) (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>For numerical amounts of alcohol intake</td>
<td>0.96 (0.92–0.99)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Reported exp(β) and confidence interval values were from a linear regression model with amounts of alcohol use as the outcome variable and polygenic risk score (PRS) for chronic kidney disease stage ≥ 3 as the exposure variable. The effect sizes of one standard deviation increment of the PRS are reported. The multivariable model was adjusted for age, sex, diabetes mellitus, and hypertension. CI, confidence interval.
Table 4. Genetic instrument for the calculation of polygenic risk score of amounts of alcohol intake

<table>
<thead>
<tr>
<th>Chr</th>
<th>Position</th>
<th>SNP</th>
<th>Gene</th>
<th>Minor allele</th>
<th>Other allele</th>
<th>Minor allele frequency</th>
<th>Beta</th>
<th>Standard error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8078085</td>
<td>rs571188732</td>
<td>ERRF1</td>
<td>T</td>
<td>C</td>
<td>0.002</td>
<td>2.539</td>
<td>0.436</td>
<td>5.87 × 10⁻⁹</td>
</tr>
<tr>
<td>2</td>
<td>27739880</td>
<td>2:27739880_CT_C</td>
<td>-</td>
<td>CT</td>
<td>C</td>
<td>0.497</td>
<td>-0.224</td>
<td>0.029</td>
<td>1.74 × 10⁻¹⁴</td>
</tr>
<tr>
<td>2</td>
<td>45138325</td>
<td>rs539447</td>
<td>-</td>
<td>A</td>
<td>G</td>
<td>0.494</td>
<td>-0.180</td>
<td>0.028</td>
<td>2.49 × 10⁻¹⁰</td>
</tr>
<tr>
<td>2</td>
<td>45207824</td>
<td>rs503435</td>
<td>-</td>
<td>A</td>
<td>G</td>
<td>0.491</td>
<td>0.155</td>
<td>0.028</td>
<td>4.94 × 10⁻⁸</td>
</tr>
<tr>
<td>4</td>
<td>39368083</td>
<td>rs3736168</td>
<td>RFC1</td>
<td>C</td>
<td>T</td>
<td>0.489</td>
<td>-0.179</td>
<td>0.029</td>
<td>3.50 × 10⁻¹⁰</td>
</tr>
<tr>
<td>4</td>
<td>39426395</td>
<td>rs151010045</td>
<td>KLB</td>
<td>C</td>
<td>T</td>
<td>0.033</td>
<td>0.477</td>
<td>0.081</td>
<td>3.16 × 10⁻⁹</td>
</tr>
<tr>
<td>4</td>
<td>99691047</td>
<td>rs71612659</td>
<td>-</td>
<td>A</td>
<td>G</td>
<td>0.058</td>
<td>-0.426</td>
<td>0.064</td>
<td>3.03 × 10⁻¹¹</td>
</tr>
<tr>
<td>4</td>
<td>99973122</td>
<td>4:99973122_AATG_A</td>
<td>HZAZ1-AC</td>
<td>A</td>
<td>AATG</td>
<td>0.003</td>
<td>-2.935</td>
<td>0.427</td>
<td>6.33 × 10⁻¹²</td>
</tr>
<tr>
<td>4</td>
<td>100239319</td>
<td>rs1229984</td>
<td>ADH1B</td>
<td>T</td>
<td>C</td>
<td>0.022</td>
<td>-1.949</td>
<td>0.101</td>
<td>3.15 × 10⁻⁸</td>
</tr>
<tr>
<td>4</td>
<td>100270452</td>
<td>rs13125415</td>
<td>ADH1C</td>
<td>G</td>
<td>A</td>
<td>0.423</td>
<td>0.157</td>
<td>0.029</td>
<td>4.89 × 10⁻⁸</td>
</tr>
<tr>
<td>4</td>
<td>100284882</td>
<td>4:100284882_AT_A</td>
<td>-</td>
<td>A</td>
<td>AT</td>
<td>0.062</td>
<td>-0.349</td>
<td>0.060</td>
<td>4.37 × 10⁻⁹</td>
</tr>
<tr>
<td>4</td>
<td>100313619</td>
<td>rs574536742</td>
<td>-</td>
<td>A</td>
<td>C</td>
<td>0.001</td>
<td>-5.110</td>
<td>0.765</td>
<td>2.35 × 10⁻¹¹</td>
</tr>
<tr>
<td>4</td>
<td>100401757</td>
<td>rs148500703</td>
<td>AP001960.1</td>
<td>TAATT</td>
<td>TTATTT</td>
<td>0.019</td>
<td>-0.605</td>
<td>0.107</td>
<td>1.31 × 10⁻⁸</td>
</tr>
<tr>
<td>4</td>
<td>103198082</td>
<td>rs13135092</td>
<td>SLCO9A1</td>
<td>T</td>
<td>C</td>
<td>0.083</td>
<td>-0.336</td>
<td>0.052</td>
<td>2.33 × 10⁻⁸</td>
</tr>
<tr>
<td>7</td>
<td>73042085</td>
<td>rs62466318</td>
<td>MXI1</td>
<td>T</td>
<td>C</td>
<td>0.205</td>
<td>0.197</td>
<td>0.035</td>
<td>7.82 × 10⁻⁹</td>
</tr>
<tr>
<td>7</td>
<td>103906175</td>
<td>rs185752293</td>
<td>DCTN6</td>
<td>C</td>
<td>A</td>
<td>0.001</td>
<td>2.600</td>
<td>0.475</td>
<td>1.89 × 10⁻⁸</td>
</tr>
<tr>
<td>9</td>
<td>12412669</td>
<td>rs796759482</td>
<td>-</td>
<td>G</td>
<td>T</td>
<td>0.001</td>
<td>-5.110</td>
<td>0.765</td>
<td>2.35 × 10⁻¹¹</td>
</tr>
<tr>
<td>9</td>
<td>12412669</td>
<td>rs796759482</td>
<td>-</td>
<td>G</td>
<td>T</td>
<td>0.001</td>
<td>-5.110</td>
<td>0.765</td>
<td>2.35 × 10⁻¹¹</td>
</tr>
<tr>
<td>9</td>
<td>80061902</td>
<td>rs537067378</td>
<td>GNA14</td>
<td>T</td>
<td>C</td>
<td>0.001</td>
<td>2.406</td>
<td>0.417</td>
<td>5.59 × 10⁻⁹</td>
</tr>
<tr>
<td>10</td>
<td>57856312</td>
<td>rs187661602</td>
<td>-</td>
<td>G</td>
<td>T</td>
<td>0.001</td>
<td>4.113</td>
<td>0.752</td>
<td>2.38 × 10⁻⁸</td>
</tr>
<tr>
<td>10</td>
<td>47676170</td>
<td>rs7107356</td>
<td>AGBL2</td>
<td>A</td>
<td>G</td>
<td>0.494</td>
<td>-0.165</td>
<td>0.028</td>
<td>3.69 × 10⁻⁸</td>
</tr>
<tr>
<td>11</td>
<td>113413565</td>
<td>rs10891570</td>
<td>-</td>
<td>A</td>
<td>G</td>
<td>0.398</td>
<td>-0.163</td>
<td>0.029</td>
<td>2.69 × 10⁻⁸</td>
</tr>
<tr>
<td>11</td>
<td>116013220</td>
<td>rs561117150</td>
<td>-</td>
<td>A</td>
<td>G</td>
<td>0.002</td>
<td>2.035</td>
<td>0.370</td>
<td>3.51 × 10⁻⁸</td>
</tr>
<tr>
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<td>112522709</td>
<td>rs612409</td>
<td>-</td>
<td>G</td>
<td>A</td>
<td>0.499</td>
<td>0.159</td>
<td>0.029</td>
<td>2.19 × 10⁻⁸</td>
</tr>
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<td>74667953</td>
<td>rs35807116</td>
<td>AC090826.2</td>
<td>T</td>
<td>C</td>
<td>0.394</td>
<td>-0.160</td>
<td>0.029</td>
<td>2.86 × 10⁻⁸</td>
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<td>69720964</td>
<td>rs3169315</td>
<td>NFAT5</td>
<td>A</td>
<td>G</td>
<td>0.187</td>
<td>0.204</td>
<td>0.036</td>
<td>3.03 × 10⁻⁸</td>
</tr>
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<td>43498316</td>
<td>rs539386657</td>
<td>ARHGAP27</td>
<td>C</td>
<td>CA</td>
<td>0.429</td>
<td>0.168</td>
<td>0.030</td>
<td>2.63 × 10⁻¹²</td>
</tr>
<tr>
<td>17</td>
<td>43660196</td>
<td>rs2668683</td>
<td>DNA1P1</td>
<td>G</td>
<td>A</td>
<td>0.499</td>
<td>-0.182</td>
<td>0.033</td>
<td>5.90 × 10⁻⁹</td>
</tr>
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<td>rs35111772</td>
<td>CRHR1</td>
<td>CTTTTTTT</td>
<td>C</td>
<td>0.468</td>
<td>0.206</td>
<td>0.030</td>
<td>1.79 × 10⁻⁹</td>
</tr>
<tr>
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<td>43934256</td>
<td>rs2316770</td>
<td>MAPT-AS1</td>
<td>A</td>
<td>G</td>
<td>0.442</td>
<td>0.169</td>
<td>0.029</td>
<td>3.21 × 10⁻¹⁰</td>
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<td>rs55885927</td>
<td>ARL17B</td>
<td>C</td>
<td>T</td>
<td>0.033</td>
<td>-0.669</td>
<td>0.111</td>
<td>2.50 × 10⁻⁸</td>
</tr>
<tr>
<td>17</td>
<td>44573874</td>
<td>rs75104997</td>
<td>-</td>
<td>G</td>
<td>C</td>
<td>0.496</td>
<td>0.203</td>
<td>0.032</td>
<td>2.81 × 10⁻⁸</td>
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<td>44874453</td>
<td>rs1563304</td>
<td>WNT3</td>
<td>T</td>
<td>C</td>
<td>0.181</td>
<td>-0.206</td>
<td>0.037</td>
<td>1.29 × 10⁻⁸</td>
</tr>
<tr>
<td>18</td>
<td>32091959</td>
<td>rs571604652</td>
<td>DTNA</td>
<td>G</td>
<td>C</td>
<td>0.005</td>
<td>1.219</td>
<td>0.220</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>41237960</td>
<td>rs185843056</td>
<td>-</td>
<td>A</td>
<td>G</td>
<td>0.001</td>
<td>3.749</td>
<td>0.659</td>
<td></td>
</tr>
</tbody>
</table>
kidney function traits.

Higher genetically predicted amounts of alcohol use were significantly associated with a higher risk of ESKD, hypertension, diabetes mellitus, obesity, central obesity, current smoking, and a lower number of household members (Fig. 2). The association between the PRS and CKD stage ≥ 3 phenotype did not reach statistical significance, although the odds ratio [OR] was above 1. The average number of days per week of moderate physical and income grades were not significantly associated with the PRS for amounts of alcohol use.

In addition, there was no significant interaction between phenotypical amounts of alcohol use and the association with genetically predicted amounts of alcohol use and the risk of CKD (interaction p = 0.20) or ESKD (interaction p = 0.81). When alcohol use within the recommended level (an average of ≤2 drinks daily for males or ≤1 drink daily for females) was tested, the variable did not show a significant interaction with the association with CKD (interaction p = 0.996) or ESKD (interaction p = 0.27).

In the multivariable model adjusted for baseline age, sex, hypertension, diabetes mellitus, obesity, central obesity, current smoking, and lower number of household members, the genetically predicted amounts of alcohol use were still significantly associated with a higher risk of ESKD (per unit reflecting one phenotypical alcohol-amount use: adjusted OR, 1.16; 95% confidence interval [CI], 1.02–1.31; p = 0.02), implying a direct effect of alcohol use on the ESKD risk.

In the negative-control approach, the genetic effect from the alcohol intake PRS to ESKD was attenuated in those with low amounts of phenotypical alcohol intake, implying that alcohol-intake exposure mediated the genetic effect to ESKD.

### Polygenic risk score for amounts of alcohol use

<table>
<thead>
<tr>
<th>ESKD (adjusted)</th>
<th>ESKD</th>
<th>CKD stage 3 or higher</th>
<th>Hypertension</th>
<th>Diabetes mellitus</th>
<th>Obesity by BMI</th>
<th>Central obesity</th>
<th>Current smoking</th>
<th>Frequency of moderate PA</th>
<th>Income grades</th>
<th>No. of household members</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted OR or exp(β) (95% CI)</td>
<td>1.00</td>
<td>1.05</td>
<td>1.10</td>
<td>1.15</td>
<td>1.20</td>
<td>1.25</td>
<td>1.30</td>
<td>1.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2.** A higher polygenic risk score for amounts of alcohol use was associated with a higher risk of ESKD and related comorbidities.

Multivariable logistic or linear regression analysis was performed with the calculated polygenic risk scores based on 35 single nucleotide polymorphisms, and the age- and sex-adjusted effect sizes per unit of polygenic risk score scaled to reflect one phenotypical unit of alcohol consumption (continuous) increase are plotted. For the ESKD (adjusted) outcome, all identified phenotypes that were significantly associated with the polygenic risk score for alcohol amounts, including age, sex, hypertension, diabetes mellitus, obesity, central obesity, current smoking, and lower number of household members, were adjusted for the multivariable model, implying a direct effect from genetical predisposition for alcohol use amount to risk of ESKD. The dots indicate the odds ratio (OR) or exp(β), and the horizontal lines indicate the 95% confidence interval (CI).

BMI, body mass index; CKD, chronic kidney disease; ESKD, end-stage kidney disease; PA, physical activity.
Finally, when we obtained regressed betas for amounts of alcohol use of the genetic instrument only in those without CKD stage ≥ 3, the recalculated PRS was still significantly associated with a higher risk of ESKD (adjusted OR, 1.16; 95% CI, 1.02–1.31; p = 0.03), but still not with a risk of CKD (adjusted OR, 1.00; 95% CI, 0.97–1.04; p = 0.99).

**Discussion**

The inverse association revealed in this study between alcohol use and CKD could have been affected by reverse causation. Furthermore, genetically predicted higher amounts of alcohol use were significantly associated with the risk of ESKD and related factors, including hypertension, diabetes mellitus, obesity, central obesity, current smoking, and a low number of household members. The MR results support recommendations that alcohol be avoided, as it causally elevates the risk of ESKD and related comorbidities.

Our study showed that higher amounts of alcohol use can cause important predisposing comorbidities for CKD, including obesity, hypertension, or diabetes mellitus. The risk of ESKD was significantly higher according to higher genetically predicted amounts of alcohol use, and this association was not affected by whether alcohol use was within the recommended level or the amounts of alcohol-use phenotype. The MR results suggest a causal link between higher amounts of alcohol use and an increased risk of ESKD and that the use of alcohol may generally increase the risk of ESKD [1]. As recent evidence has shown that there is no “safe” level of alcohol use [2], this may be a reasonable recommendation for people overall or individuals with CKD, not only regarding the risk of ESKD but also considering the risk of general mortality.

Our analysis including the PRS is a one-sample MR method, and MR requires three assumptions to be met to suggest causal effects [21]. First, the genetic instrument should be strongly associated with the studied outcome. As the instrument was obtained from a GWAS, the assumption was met. Second, the genetic instrument should affect the outcome through the exposure of interest. Third, the association should not be the result of horizontal pleiotropy, implying the presence of confounders. We found that important comorbidity and lifestyle factors related to the risk of ESKD were significantly associated with the PRS for alcohol use. As the association between the PRS and risk of ESKD remained significant even after adjusting for comorbidities, the finding may not be the result of horizontal pleiotropy but possibly of vertical pleiotropy, which does not violate the MR assumption. Alcohol use may cause diabetes mellitus, hypertension, obesity or central obesity, or current smoking behavior, and, as one of the downstream endpoints from the comorbidities, ESKD. The effect was significant even after adjusting for the comorbidities, implying a direct causal effect from alcohol use on ESKD. In addition, the negative-control approach supported the absence of a horizontal pleiotropic pathway, suggesting the attainment of the important MR assumptions.

Previous studies have reported that alcohol can damage kidneys through increased oxidative stress injury associated with ethanol exposure [22–24]. In addition, heavy ethanol consumption reportedly leads to pathologic glomerular changes through activation of the renin-angiotensin-aldosterone pathway or blood pressure increments in vivo [25,26]. As the biological benefit of alcohol use is unlikely to present, the inverse observational association between alcohol use and the risk of CKD, which cannot be simply accepted to reflect causal effects, required further examination. Although few studies suggest that alcohol use over the recommended level elevates the risk of CKD or ESKD [27], not only moderate but also high amounts of alcohol use have been associated with a lower risk of incident CKD or ESKD in several reports [5–7,28] involving multiple ethnic backgrounds [5,6,28], including healthy individuals [7] and those with a certain proportion of metabolic risk factors [5]. However, even a single drink of alcohol is not safe with respect to the risk of mortality [2], an explanation for the inverse association is necessary to prevent misunderstandings of this observational association. For cardiovascular diseases, adverse causal effects of alcohol use on cardiovascular health have been demonstrated [11]. Our study indicates that CKD may reduce alcohol use; that is, people with a risk of CKD use lower amounts of alcohol. This suggests that the observational inverse association between alcohol use and the risk of CKD should be interpreted simply as those at risk for CKD tend to use less alcohol. Also, the previous observational findings were likely affected by various unmeasured clinical confounding effects (e.g., smoking, drug use, diet, medical...
compliance, or healthy behaviors) [3,28,29], and not discouraging alcohol intake because the risk of kidney function based on such observational results would be misleading.

Our study has several limitations and raises some questions. First, a CKD stage ≥ 3 outcome was not significantly associated with the PRS for alcohol use. This may be related to the fact that genetic instruments can explain only some of the impacts of the studied factor or that the effect size was too small to be detected in our study, which included a general population with a low prevalence of CKD. Furthermore, the assumption that alcohol use is associated with kidney hyperfiltration may have caused the null result [30]. As diabetes mellitus, hypertension, and obesity are important predisposing factors for CKD, and alcohol use is causally associated with these phenotypes, the result may not be misinterpreted. Second, the result has not been replicated by a two-sample MR. However, as no external large-scale GWAS for ESKD is available, and the issue of reverse causation or hyperfiltration remains for CKD outcomes, the current one-sample MR should be considered an appropriate method for this study. Third, as the information on alcohol use was self-reported, measurement bias may be present. However, the direction of the bias would not affect the positive finding that alcohol use affects the risk of ESKD, as diseased people would likely report lower amounts of alcohol use. Fourth, the utility of PRS risk estimation is currently limited by its simplicity. Further methodologic advances may be necessary to improve the comprehensiveness and to reduce the uncertainty involved in utilizing the PRS [31]. In addition, validation of the suggested PRS for alcohol-intake amount would be necessary to confirm the generalizability of the instrumented information. Last, the genetic analysis included only those of white British ancestry, undermining the generalizability of the results to other ethnic populations.

In conclusion, alcohol use causally increases the risk of ESKD and related comorbidities. As the issue of reverse causation is present in the form of an inverse association between alcohol use and the risk of CKD, the potential beneficial effects of alcohol use on this complex disease should not be erroneously assumed simply based on observed clinical associations. Healthcare providers may recommend avoiding alcohol use to reduce the risk of ESKD and predisposing comorbidities.

Conflicts of interest
All authors have no conflicts of interest to declare.

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1. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian


Age, diabetes mellitus, and dialysis modality are associated with risk of poor muscle strength and physical function in hemodialysis and peritoneal dialysis patients

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Background: Due to the poor outcomes associated with the impairment of physical function and muscle strength in patients on maintenance dialysis, it is important to understand the factors that may influence physical function and muscle strength. The aim of this study was to explore the factors associated with physical function in hemodialysis and peritoneal dialysis patients.

Methods: Patients with chronic kidney disease on dialysis for at least 3 months, aged 18 years old or above, were enrolled. Physical function was assessed by handgrip strength, gait and sit-to-stand tests, and the Short Physical Performance Battery (SPPB). Clinical and laboratory data were collected to verify the association with physical function parameters through binary logistic regression.

Results: One-hundred ninety patients on maintenance dialysis were included; 140 patients (73.7%) on hemodialysis and 50 (26.3%) on peritoneal dialysis. The mean age was 57.3 ± 14.9 years, 109 (57.4%) were male, and 87 (45.8%) were older than 60 years. The median SPPB was 8.0 points (6.0–10.0 points) and the mean ± standard deviation of handgrip strength was 24.7 ± 12.2 kg. Binary logistic regression showed that age, type of renal replacement therapy, diabetes mellitus, and serum creatinine were significantly associated with both higher 4-meter gait test times and lower SPPB scores. Only age and diabetes mellitus were associated with higher sit-to-stand test times, while age and ferritin were associated with lower handgrip strength.

Conclusion: Age, diabetes mellitus, serum creatinine, and hemodialysis modality are factors related to physical function in dialysis patients.

Keywords: Chronic renal insufficiency, Dialysis, Hand strength, Physical functional performance, Physical performance
Introduction

The prevalence of sarcopenia in chronic kidney disease (CKD) patients varies widely, ranging from 4.0% to 13.7% depending on the CKD stage, kidney replacement therapy (KRT), and the consensus version [1–3]. This number is even higher in CKD patients aged 60 years and above [3].

In this context, the assessment of physical function of CKD patients undergoing conservative treatment or who are on dialysis is extremely important. Deterioration of physical function is accelerated in patients with CKD, increasing the risk of worse outcomes, such as loss of independence, risk of morbidity, reduced quality of life, and reduced survival [4,5]. Recently, the association of poor physical function with mortality was verified in meta-analyses including nondialysis [5] and dialysis CKD patients, with an inverse association found between handgrip strength (HGS) and all-cause mortality [6].

Due to the importance of physical function and muscle strength for patients on maintenance dialysis, it is important to understand the factors that may influence these traits. As a consequence, patients at risk of diminished physical function and muscle strength may receive more attention from their health care team in order to improve or maintain such attributes. Therefore, our objective was to explore the factors associated with muscle strength and physical function in maintenance hemodialysis (HD) and peritoneal dialysis (PD) patients.

Methods

This was a cross-sectional analysis, which included participants with CKD on HD or PD from three protocols. These patients were either on (1) maintenance HD or PD in the Dialysis Unit from the Clinics Hospital of Botucatu Medical School (Botucatu, Brazil) or (2) maintenance HD in the Dialysis Service from the Presidente Prudente Regional Hospital (Presidente Prudente, Brazil). The three research protocols were approved by the respective Institution Research Ethics Committees (CAAE 71393717.7.0000.5411, 61634816.4.0000.5411, and 73640317.9.0000.5515) and the enrolled patients provided written informed consent.

The enrolled patients were on dialysis for at least 3 months and aged 18 years old or above. Patients who were bedridden, those with upper and lower limb sequelae, or amputees were excluded because they cannot perform the necessary physical performance assessments. Patients with other catabolic conditions were also excluded, such as neoplasia, final stage liver disease, severe heart diseases, or chronic obstructive pulmonary disease.

Age, sex, dialysis vintage, presence of diabetes mellitus (DM), dialysis adequacy (Kt/V), and routine laboratory tests (serum levels of urea, creatinine, albumin, cholesterol, triglycerides, hemoglobin, calcium, phosphorus, potassium, parathyroid hormone, iron, and ferritin) were collected from medical records.

Anthropometric evaluation consisted of actual body weight and height measurements for body mass index calculations. For this assessment, patients on PD had an empty peritoneal cavity and HD patients were evaluated after their dialysis session.

Muscle strength and physical function were evaluated in this study using a 4-meter gait test, sit-to-stand test, Short Physical Performance Battery (SPPB), and HGS test. All assessments were performed 30 minutes after the end of the HD session. Patients on PD were evaluated during routine care visits.

To assess the 4-meter gait test, patients had two attempts to walk a 4-meter course at their usual speed with static start. Each attempt was timed and the faster value was considered for the analyses [7]. The sit-to-stand test measures the amount of time the patient takes to rise five times from a straight-backed chair. Patients were instructed to stand up from the initial sitting position and sit down five times as quickly as possible, without using his or her arms [7]. Sit-to-stand tests require both muscle strength and endurance [8].

The SPPB consists of three different tests of lower-extremity function; balance test (ability to maintain for 10 seconds the following stand positions: feet together side-by-side, semi-tandem, and tandem), 4-meter gait test, and sit-to-stand chair test. A summary score ranging from 0 (worst performance) to 12 (best performance) was calculated [7].

HGS measurement was performed using a Jamar (Sammons Preston Rolyan, Bolingbrook, IL, USA) or Saehan hydraulic dynamometer (Saehan Corp., Changwon, Korea). These dynamometers are considered equivalents, with an intraclass correlation coefficient of >0.97 [9]. Patients were positioned with the dynamometer facing away from the body and the other arm extended to the side of the body. In the sequence, patients were instructed to hold the grip
for around 3 seconds with maximum force in response to a voice command. Three measurements were performed, with intervals of around 30 seconds between each; the highest value was considered for analysis. The evaluation of patients on HD was performed on the side opposite from their arteriovenous fistula or central access. Patients on PD were evaluated through the dominant limb [10].

**Statistical analysis**

Data are expressed as mean ± standard deviation or median (interquartile range), according to the variables’ distribution. Frequencies are expressed as percentages. In order to compare patients according to age, presence of DM, and method of dialysis, Student t test, Mann-Whitney test, or chi-square test were used.

The correlations between clinical and laboratory variables with muscle strength and physical function were assessed by Spearman or Pearson correlation coefficients. To address associated factors, four models of binary logistic regression were built, each one with a muscle strength or physical function parameter as a dependent variable (4-meter gait time, sit-to-stand test time, SPPB total score, and HGS) categorized by the respective median. HGS was categorized according to the median by each sex. Parameters correlated with the dependent variables (p < 0.2) in univariate analysis were selected to be included in the logistic regression models.

Statistical significance was considered when p < 0.05. Analyses were performed using IBM SPSS version 22.0 (IBM Corp., Armonk, NY, USA).

**Results**

One-hundred ninety patients on maintenance dialysis were enrolled, with 140 patients (73.7%) on HD and 50 (26.3%) on PD. The PD modality used by most patients was continuous cycling PD (CCPD) (60.0%, n = 30), followed by Nocturnal Intermittent PD modality (36.0%, n = 18), and continuous ambulatory PD modality (4.0%, n = 2). The majority of the patients (57.4%) were males, and 87 individuals (45.8%) were older than 60 years. Demographic, clinical, and laboratory characteristics, as well as physical function scores of the patients, are presented in **Table 1**.

Patients were compared according to dialysis method. Dialysis vintage, serum urea, creatinine, albumin, and potassi-
um were significantly higher in HD patients. Serum calcium, iron, ferritin, and high-density lipoprotein (HDL)-cholesterol were significantly lower in HD patients.

Comparison of physical function variables between patients according to age (younger or older than 60 years), presence of DM, and dialysis method (PD and HD) are presented in Table 2.

There was a significant correlation among all physical function parameters; the time of the 4-meter gait test was positively correlated with the sit-to-stand test (r = 0.17, p = 0.02) and negatively correlated both with the SPPB total score (r = -0.72, p < 0.001) and HGS (r = -0.41, p < 0.001). The sit-to-stand test was negatively correlated with SPPB total score (r = -0.15, p = 0.04). The SPPB total score was positively correlated with HGS (r = 0.47, p < 0.001). Correlations between physical function parameters and other variables are presented in Table 3.

For the binary logistic regression, times for the 4-meter gait test were categorized by the median, 5.22 seconds. Sex, age, type of KRT, dialysis vintage, DM, serum creatinine, urea, phosphate, and ferritin were included in the model. Sit-to-stand test times were categorized by the median of 17.56 seconds and SPPB was categorized by its median of 8 points. The same variables were included in each model; sex, age, type of KRT, DM, serum creatinine, urea, ferritin, albumin, and HDL-cholesterol.

HGS was categorized according to the median for each sex; 30 kg for male and 15 kg for female. The variables included in the model were sex, age, dialysis vintage, DM, Kt/V, serum creatinine, urea, calcium, potassium, hemoglobin, ferritin, albumin, and HDL-cholesterol.

The variables included in the final model were those that improved the model predictive capacity. In the final models, age, type of KRT (HD), DM, and serum creatinine were significantly associated with both a higher 4-meter gait test time and lower SPPB (models 1 and 3, respectively) (Table 4). Only age and DM were associated with a higher sit-to-stand test time (model 2), while age and ferritin were associated with a lower HGS (model 4) (Table 4).

**DISCUSSION**

The objective of this study was to identify the factors that are associated with muscle strength and physical function, assessed by four parameters in patients receiving dialysis treatment for at least 3 months. The results indicate that aging, DM, lower serum creatinine, and HD modality are factors related to poor muscle strength and physical function. Age was associated with all muscle strength and physical function tests performed in the study. DM, HD modality, and serum creatinine were associated with three of the four tests.

Aging is a well-known feature associated with worsening muscle strength and physical function in the general population [11]. Moreover, a natural decrease in muscle mass, strength, and performance occurs with aging; however, multiple other conditions contribute to such decrease [12]. Aging is considered a condition that leads to a proinflammatory state. The increased cytokine levels affect muscle protein synthesis, leading to muscle mass loss [13]. Lower muscle strength has been associated with increased interleukin-6 and C-reactive protein levels in older adults [14]. CKD uremia and dialysis are also known to promote inflammation, which may act synergistically with the effects of ‘inflammaging’ and lead to poor physical function.

In fact, among dialysis patients, older patients present worse physical function compared to younger patients [15,16]. Our results have shown age as an independent predictor of all parameters used to assess muscle strength and physical function in this study. Moreover, the decline in physical function related to aging often leads to loss of independence and ability to perform activities of daily life, as well as poorer quality of life [17]. Poor physical function is associated with increased risk of outcomes such as cognitive impairment, institutionalization, falls [18], disability [7], cardiovascular events [19], and mortality [7,20]. Therefore, elderly people on KRT should receive greater attention from their health care team and interventions should be proposed.

One of the comorbidities affecting physical function is DM. In our results, DM was associated with SPPB and the sit-to-stand and gait tests, but not with HGS. DM may affect muscle function through several mechanisms. Peripheral insulin resistance decreases the glucose uptake to muscle and reduces muscle tissue anabolic rates [13]. Also, changes in microvascularization decrease blood flow to the muscles. Moreover, other prevalent comorbidities (such as decreased vision, heart failure, neuropathy, and peripheral vascular disease) result in decreased physical activity and may also explain decreased physical function among the dialysis population [21,22]. However, the prevalence of these elements was not assessed in the current study.
Table 2. Comparison of clinical and physical function parameters between the groups according to age, diabetes mellitus, and dialysis method

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age (yr)</th>
<th>Diabetes mellitus</th>
<th>Dialysis method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;60 (n = 103)</td>
<td>≥60 (n = 87)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>46.7 ± 10.8</td>
<td>69.9 ± 7.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>24 (23.3)</td>
<td>36 (41.4)</td>
<td>0.008</td>
</tr>
<tr>
<td>Dialysis vintage (mo)</td>
<td>16.0 (7.2–42.9)</td>
<td>34.3 (11.0–65.6)</td>
<td>0.02</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.0 ± 4.9</td>
<td>26.9 ± 5.0</td>
<td>0.20</td>
</tr>
<tr>
<td>Balance test (score)</td>
<td>4.0 (4.0–4.0)</td>
<td>4.0 (1.0–4.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4-Meter gait test⁴</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (sec)</td>
<td>4.5 (3.9–5.7)</td>
<td>6.2 (4.7–7.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Score</td>
<td>4.0 (3.0–4.0)</td>
<td>3.0 (2.0–4.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;5.22 sec</td>
<td>36 (36.0)</td>
<td>57 (66.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sit-to-stand test²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (sec)</td>
<td>16.3 (13.0–19.6)</td>
<td>19.9 (16.6–23.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Score</td>
<td>1.0 (1.0–3.0)</td>
<td>1.0 (1.0–1.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;17.56 sec</td>
<td>34 (37.0)</td>
<td>45 (68.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SPPB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total score</td>
<td>9.0 (7.0–11.0)</td>
<td>7.0 (4.0–8.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;8 Points</td>
<td>27 (26.2)</td>
<td>53 (60.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low or weak handgrip strength (kg)</td>
<td>28.3 ± 12.8</td>
<td>20.4 ± 10.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low or weak handgrip strength ³</td>
<td>30 (29.1)</td>
<td>66 (75.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation, number (%), or median (interquartile range).

SPPB, Short Physical Performance Battery.

⁴Four patients were not able to perform the 4-meter gait test. ²Thirty-two patients were not able to perform the sit-to-stand test. ³Male, <30 kg; female, <15 kg.
Serum creatinine was a significant predictor of physical function in the models with the 4-meter gait test and SPPB as dependent variables. Serum creatinine may reflect muscle mass of patients on dialysis [23]. Muscle function declines at a rate different from that of muscle size [24], and the worse physical function of patients on HD compared to non-CKD elderly was not explained by muscle size [22]. Nonetheless, a recent systematic review discusses how the association between physical function and muscle size in CKD patients is still controversial [25]. The current study found that HD patients have a higher risk of reduced muscle strength and poor physical function

### Table 4. Binary logistic regressions with physical function parameters as dependent variables

<table>
<thead>
<tr>
<th>Model</th>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4-Meter gait test</td>
<td>Age</td>
<td>1.07 (1.04–1.11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>&gt;5.22 sec (n = 186)</td>
<td>Dialysis modality (HD)</td>
<td>5.13 (2.09–12.62)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetes mellitus</td>
<td>2.63 (1.19–5.79)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum creatinine</td>
<td>0.85 (0.74–0.97)</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>Sit-to-stand test</td>
<td>Age</td>
<td>1.07 (1.03–1.10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>&gt;17.56 sec (n = 157)</td>
<td>Diabetes mellitus</td>
<td>2.31 (1.04–5.11)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>1.77 (0.85–3.69)</td>
<td>0.13</td>
</tr>
<tr>
<td>3</td>
<td>SPPB</td>
<td>Age</td>
<td>1.12 (1.07–1.17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>&lt;8 points (n = 186)</td>
<td>Dialysis modality (HD)</td>
<td>20.13 (5.69–71.18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetes mellitus</td>
<td>4.73 (1.90–11.78)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum creatinine</td>
<td>0.74 (0.61–0.88)</td>
<td>0.001</td>
</tr>
<tr>
<td>4</td>
<td>Handgrip strength</td>
<td>Age</td>
<td>1.14 (1.10–1.19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>&lt;30 kg in male or &lt;15 kg in female</td>
<td>Ferritin</td>
<td>1.001 (1.00–1.003)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Cl, confidence interval; HD, hemodialysis; SPPB, Short Physical Performance Battery; OR, odds ratio.
compared to PD patients. In a systematic review, Purnell et al. [26] showed no significant differences in physical function in 76% of comparisons between these two KRT modalities. However, the majority of the included studies used 36-Item Short Form Survey (SF-36) domains to assess physical function; such methodology is considered a subjective assessment. A comparison of quality of life between HD and PD patients using SF-36 has not found differences between the KRT modalities [27]. Moreover, none of the studies included in the systematic review [26] used the same objective assessments used in the current study (gait test, sit-to-stand test, SPPB, or HGS).

The characteristics of each KRT modality may influence physical function through the daily routine imposed by the treatment. Most PD patients have some free time during the day, with one or two dialysate changes per day on CCPD modality. On the other hand, HD patients spend many hours, 3 days a week, with no activities during transit to the HD center and during the HD session. Moreover, frequent symptoms after HD sessions, such as bleeding, hypotension, dizziness, fatigue, etc., increase the need for rest. Thus, these factors related to HD therapy may favor a more sedentary lifestyle, and intradialytic interventions aimed at physical function improvement could be useful.

Another issue related to KRT modality is the assessment timing. In our study, HD patients were assessed after the HD session. At this time, hypotension, dizziness, and fatigue may affect the results of the physical function tests. However, before the dialysis session, the patients are overhydrated, which may also influence the results of the performance tests [28]. Pinto et al. [29] compared HGS before and after HD sessions and concluded that the HD procedure negatively affects HGS. Moreover, HGS variation was correlated with blood pressure variations. On the other hand, Leal et al. [30] found no difference between HGS values before and after HD sessions. Dialysis variables, such as ultrafiltration, inter-dialytic body weight gain, Kt/V, urea, and blood pressure were not correlated with HGS values [30]. Some studies assessed physical function before [31,32] or after [10,33] dialysis session, and others on nondialysis days [22]. Therefore, there is no standard for physical function assessment timing.

Assessments of muscle strength and physical function can be done by different methods in CKD. Sit-to-stand tests, 4-meter gait speed tests, SPPB, and HGS were chosen for this study based on the ease and practicality of performing these tests, even in dialysis unit facilities. These tests reflect common daily living activities, i.e., getting up from a chair or walking small distances. SPPB and HGS assessments were previously shown to be reliable in HD patients [31,32] and both have been recommended by the European Working Group on Sarcopenia in Older People 2 (EWGSOP2) for sarcopenia diagnosis [8]. However, there are no specific cutoffs for physical function assessments among the CKD population.

In a comparison between patients with low and appropriate muscle strength, Isoyama et al. [34] observed that those with low strength were older and had more comorbidities, such as cardiovascular disease and DM, lower serum creatinine, and higher inflammatory markers levels. Furthermore, HGS has been considered an independent predictor of all-cause mortality in HD and PD patients [10,34], which was recently confirmed by Hwang et al. [6] in a meta-analysis. They found that patients with low HGS had a 1.88-fold higher risk of death than those with higher HGS. In addition, a 1-kg unit increase in HGS was associated with a 5% reduction in the risk of mortality [6].

As poor physical function is associated with poorer outcomes in the dialysis population, interventions that improve physical function may decrease the risk of poor outcomes. Exercise training is the most investigated intervention, and several modalities have been tested.

A recent meta-analysis [35] reported that either aerobic or resistance exercise modalities could improve objective measures of physical function in patients undergoing dialysis. Additionally, intradialytic exercise improved physical function more efficiently than interdialytic exercise, supporting the hypothesis of intradialytic exercise interventions for HD patients [35]. Intradialytic resistance band exercise training and neuromuscular electrical stimulation were also effective interventions to enhance physical function in patients on HD [35–37]. It is important to highlight that previous studies have shown exercise interventions are safe and well-tolerated [36], with no significant changes in hemodynamic parameters [38] and no adverse events during the training session [39].

There are fewer studies with exercise interventions in PD. Although the physical function of these patients is better than in HD patients, exercise could bring other benefits to this population as well as to HD patients. In a randomized controlled trial in PD patients, a 12-week home-based Exercise training...
program that included aerobic and resistance exercise was effective in improving aerobic capacity, some domains of quality of life, serum albumin, and insulin resistance [40]. Combined interventions, such as nutritional supplementation and exercise, offer strategies to improve muscle strength and physical function. Martin-Alemány et al. [41] showed that the combination of oral nutritional supplementation with aerobic or resistance intradialytic exercise had better effects on physical function than supplementation alone in young HD patients. Due to the association of vitamin D status with muscle health and the high prevalence of vitamin D deficiency in CKD, Olvera-Soto et al. [42] added cholecalciferol supplements to a resistance exercise program intervention in stage 4 CKD patients. A significant increase in HGS was observed. Those strategies are still incipient in CKD research. Therefore, more trials are necessary to assess such effects.

A limitation of this study is the merging of three different data sets of HD and PD patients from two centers. Nonetheless, in all of these data sets, the assessments were obtained using the same protocol. The cross-sectional design may also be a limitation since it is not possible to demonstrate the relationship between cause and effect.

In conclusion, age, DM, and dialysis modality are factors related to muscle strength and physical function in dialysis patients. Thus, special attention should be given to patients with these characteristics, and specific interventions should be tested with the objective to improve muscle strength and physical function in both HD and PD patients.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Authors’ contributions

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32. Ortega-Pérez de Villar L, Martínez-Olmos FJ, Junqué-Jiménez A, et al. Test-retest reliability and minimal detectable change scores for the short physical performance battery, one-legged


This study aimed to investigate whether high body mass index (BMI) and presensitization to human leukocyte antigen (HLA) in kidney transplant recipients (KTRs) affected allograft outcomes.

Methods: From January 2010 to December 2018, 1,290 kidney transplantations (KTs) were performed at the Seoul St Mary’s Hospital. Of these, 682 cases of ABO-compatible living donor KT patients were enrolled. They were divided into four groups (low BMI-non-sensitized, high BMI-non-sensitized, low BMI-sensitized, and high BMI-sensitized) according to the median BMI value (22.7 kg/m\(^2\)) and HLA presensitization status (anti-HLA antibody mean fluorescence intensity > 3,000). Short-term and long-term allograft outcomes were compared between groups.

Results: In the high BMI-sensitized group, the decline in allograft function was higher than that in the other three groups. Death-censored graft loss (DCGL) rates were highest in the high BMI-sensitized group (4 of 21 [19.0%], p = 0.04). In the multivariable Cox regression hazard regression model analysis, the hazard ratio (HR) for DCGL was intensified when high BMI and presensitization statuses were combined (HR, 3.75; p = 0.03); these statuses significantly interacted with each other (p-value for interaction = 0.008).

Impact of high body mass index on allograft outcomes in kidney transplant recipients with presensitization to human leukocyte antigen

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Introduction

Obesity is a major public health problem that is increasing worldwide [1], and its prevalence in Korea increased from 29.2% in 2001 to 34.6% in 2018 [2]. Obesity and metabolic syndromes are recognized as risk factors for the development and progression of chronic kidney disease (CKD) by various mechanisms [3,4]. In a previous nationwide cohort study in Korea, obesity (body mass index [BMI] ≥ 25.0 kg/m²) was identified as an independent risk factor for CKD progression [5]. Moreover, obesity also adversely affects allograft function in kidney transplant (KT) recipients (KTRs). A study reported that recipient obesity was an independent risk factor for death-censored graft loss (DCGL) and biopsy-proven acute rejection (BPAR) [6]. Another study also reported that recipient obesity was an independent risk factor for overall graft loss [7]. Furthermore, a meta-analysis reported that recipient obesity had a marginally greater risk for DCGL [8].

Pretransplant sensitization to human leukocyte antigen (HLA) is a well-known risk factor associated with adverse allograft outcomes [9]. Presensitization to HLA is not only associated with a high rate of acute antibody-mediated rejection (ABMR) but also with the gradual development of chronic allograft tissue injury caused by humoral immune system activation. Indeed, the development of chronic ABMR was significantly higher in patients with presensitization to HLA compared to patients with low immunologic risk [10–12]. Moreover, both preformed persistent donor-specific antibody (DSA) and preformed cleared DSA showed an increased risk of graft loss [9]. Another study reported that preformed donor-specific anti-HLA antibodies (HLA-DSAs) with mean fluorescence intensity (MFI) > 3,000 had an increased risk of graft loss [13].

Therefore, both obesity and pretransplant sensitization to HLA in KTRs might contribute to the progression of chronic allograft tissue injury and adverse allograft outcomes, although the mechanisms are different. However, it has not been investigated whether both factors have an interactive effect on allograft outcomes. Hence, in this study, we analyzed the short- and long-term graft outcomes in KTRs with high BMI and presensitization to HLA and investigated the interaction between high BMI and HLA presensitization status.

Methods

Study design

This was a retrospective observational single-center study. Between January 2010 and December 2018, 1,290 KTs were performed at the Seoul St. Mary’s Hospital in Seoul, South Korea. Of these, 412 patients received a kidney from a deceased donor, 195 cases were ABO-incompatible KTs, and 1 KTR had both legs amputated; these cases were excluded from the study. Finally, 682 KTRs were included in this analysis. The distribution of BMI in the KTRs is presented in Supplementary Fig. 1 (available online), and the median BMI value was 22.7 kg/m². Patients with BMI of ≥ 22.7 kg/m² were categorized in the high BMI group, while others were classified in the low BMI group. The cases were defined as presensitized to HLA when the MFI value of HLA-DSA at baseline was higher than 3,000 [13] and as non-sensitized when the value was below 3,000. Based on the above classifications, KTRs were divided into four groups: low BMI-non-sensitized, high BMI-non-sensitized, low BMI-sensitized, and high BMI-sensitized as presented in Fig. 1. This study followed the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of Seoul St. Mary’s Hospital (No. XC15RIM0072K). As it was a retrospective study using data obtained from medical records, informed consent was waived by the IRB.

Human leukocyte antigen typing, human leukocyte antigen antibodies, and donor-specific antibody

HLA typing and HLA antibodies were measured as described previously [14,15]. Briefly, HLA-A, HLA-B, HLA-DR, and HLA-DQB1 typing was performed using deoxyribonucleic acid molecular typing with sequence-specific oligonucle-
otide probes with Lifecodes HLA SSO typing kits (Immucor, Stamford, CT, USA). Lifecodes LSA Class I and Class II kits (Gen-Probe Transplant Diagnostic Inc., Stamford, CT, USA) or LABScreen Single Antigen (One Lambda Inc., Thermo Fisher Scientific, Canoga Park, CA, USA) were used to detect HLA antibodies in the recipient sera. The manufacturer’s instructions were followed and 10 μL of each serum sample was used. The fluorescence intensities of the samples were measured using a Luminex 200 system (Luminex Corp., Austin, TX, USA).

Desensitization protocols for presensitized patients

The desensitization protocol in our center has been described previously [16–18]. Briefly, the desensitization protocol for HLA presensitized patients consisted of rituximab, total plasma exchange (TPE), and intravenous immunoglobulin (IVIG). Rituximab was administered 2 weeks to 1 month before the transplantation and TPE was performed seven times using 5% albumin and fresh frozen plasma. The control of TPE frequency was based on the MFI titer of HLA-DSAs. IVIG was administered at a dose of 100 mg/kg for 1 hour after every TPE. In patients with an HLA-DSA MFI titer of 1,000 to 3,000 or panel reactive antibody (PRA, Class I or Class II) of > 50% with no HLA-DSAs, only rituximab was administered before transplantation. In all patients who underwent desensitization, prophylactic agents were used to prevent Pneumocystis jirovecii pneumonia (PJP) and cytomegalovirus (CMV) infection. If the crossmatch (XM) test of T-cell complement-dependent cytotoxicity (CDC) was positive or HLA-DSAs were present, and the MFI of HLA-DSAs did not decrease adequately after three cycles of TPE, a bortezomib-based protocol was used, in which bortezomib was administered four times in addition to the desensitization protocol.

Clinical parameters and outcomes

The age, sex, height, and weight of the donor and estimated glomerular filtration rate (eGFR) based on the Chronic
Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation were collected as baseline characteristics. The age, height, and weight of the KTR and the Mosteller body surface area (BSA) ratio of donor to recipient, history of diabetes mellitus (DM) and hypertension (HTN), cause of end-stage renal disease (ESRD), previous dialysis modality, previous dialysis period, and previous KT history were collected as baseline demographic characteristics. Total cholesterol, triglyceride, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, hemoglobin A1c levels, and hepatitis C virus (HCV) seropositivity rates were obtained from pretransplant investigations. The results of the XM test using CDC and flow cytometry crossmatch (FCXM), HLA-DSA and MFI results by Luminex single antigen assay, and PRA titers were obtained as a pretransplant immunoassay. Transplantation information included mismatch number, type of induction therapy, the main immunosuppressant used, and drugs used for desensitization.

We analyzed the incidence of BPAR within 1-year of transplantation (early acute rejection), CMV infection, BK viremia, and PJP rates as short-term clinical outcomes in the four groups. The variables used for analyzing long-term clinical outcomes included BPAR incidence after 1-year of transplantation (late acute rejection), chronic active ABMR, and biopsy-proven calcineurin inhibitor (CNI) toxicity rates. DCGL and patient death rates were also analyzed.

CMV infection and BK viremia were screened with CMV real-time quantitative (RQ) polymerase chain reaction (PCR) and BK virus real-time (RT) PCR through blood tests at 1- to 2-month intervals until 1 year after transplantation. From 1 year after transplantation, screening was performed with CMV RQ-PCR and BKV RT-PCR every 6 months to 1 year. Moreover, CMV RQ-PCR and BKV RT-PCR tests were performed when renal function deterioration occurred or when the clinician determined that the tests were necessary.

Allograft kidney biopsy was performed in cases of unexpected renal allograft dysfunction (serum creatinine of 25% above the baseline), unexpected development of proteinuria, and development of de novo HLA-DSA. Allograft kidney biopsy findings were interpreted according to the Banff classification in 2009. BPAR was diagnosed with allograft biopsy as suitable for acute T-cell mediated rejection (TCMR) and acute ABMR criteria according to the Banff classification. Similarly, chronic active ABMR and biopsy-proven CNI toxicity were diagnosed with allograft biopsies according to the Banff classification [19]. Death-censored allograft survival duration was defined as the period from KT to dialysis or preemptive KT, except for patient death in a functioning allograft. Patient survival duration was defined as the period from KT to death due to any cause. The data of changes in allograft function based on serum creatinine levels were collected until 4 years after KT.

The primary outcome of this study was to compare the impact of BMI on DCGL in non-sensitized and sensitized patients. Secondary outcomes of this study were early acute rejection, CMV infection, BK viremia, PJP, late acute rejection, chronic active ABMR, biopsy-proven CNI toxicity, patient death rates, and eGFR based on the CKD-EPI equation [20].

Statistical analysis

All continuous variables were expressed as mean ± standard deviation. If the variables followed a normal distribution, an analysis of variance was performed. If the variables showed a non-normal distribution, a Kruskal-Wallis test was performed. Tukey or Dunnett T3 method was performed for post hoc analysis. All categorical variables were compared using the chi-square test or Fisher exact test and expressed as proportions. A multivariable Cox hazard regression model analysis was performed to determine the risk factors affecting DCGL and to investigate the interaction between high BMI and HLA presensitization. Cumulative survival rates were analyzed during the follow-up period in the four groups by Kaplan-Meier survival analysis. Causes of DCGL and patient death were compared using the chi-square test or Fisher exact test. The mean eGFR (CKD-EPI) and standard deviation in the four groups were evaluated. All statistical analyses were performed using IBM SPSS version 24 (IBM Corp., Armonk, NY, USA) and Microsoft Excel 2016 (Microsoft, Redmond, WA, USA).

Results

Comparison of baseline clinical and laboratory parameters according to body mass index and human leukocyte antigen presensitization status

The mean duration of follow-up of the patients included in this study was 61.8 months. The baseline characteristics of the four groups are shown in Table 1, 2. Among donor fac-
tors, there were no statistical differences among the groups in terms of old age, sex, and eGFR; however, the normal weight of the donors (BMI, 18.5–23.0 kg/m$^2$) was relatively higher in the low BMI-sensitized group (21 of 40, 52.5%; $p = 0.04$) than those in the other groups. Regarding the clinical demographics of recipients, the proportion of elderly patients was not significantly different among the four groups, but the proportion of male patients was higher in the non-sensitized groups, especially in the high BMI-non-sensitized group (235 of 319, 73.7%; $p < 0.001$). According to BMI categories, grossly obese patients were observed only in the high BMI-non-sensitized group (25 of 319, 7.8%; $p < 0.001$). The donor/recipient (D/R) BSA ratio was significantly lower in the high BMI-non-sensitized group than in other groups ($0.94 ± 0.13$, $p < 0.001$). The proportions of recipients with DM and HTN were higher in the high BMI groups ($p < 0.001$, for each). The mean triglyceride and hemoglobin A1c levels were significantly higher, and HDL-cholesterol levels were lower in both high BMI groups ($p < 0.001$, for each). Regarding factors that cause ESRD, previous renal diseases had high proportions of DM in the high BMI-sensitized group, and other causes showed various distributions between groups. Among the types of dialysis modality, the proportion of peritoneal dialysis was the highest in the high BMI-non-sensitized group (47 of 319, 14.7%; $p = 0.02$).

In the baseline immunologic test, the proportions with a positive XM test through both CDC and FCXM were significantly higher in the sensitized groups ($p < 0.001$ for each) than in the non-sensitized groups; however, no difference in the rate of positivity was seen within the sensitized groups according to BMI status. The MFI values of HLA-DSAs were significantly higher in the sensitized groups than in the non-sensitized groups. The proportion of previous KT history was the highest in the low BMI-sensitized group (13 of 40, 32.5%; $p < 0.001$). Antithymocyte globulin was used as induction therapy, and the proportion of desensitization therapy was significantly higher in the sensitized groups ($p < 0.001$ for each).

### Table 1. Comparison of baseline characteristics of donor factors among the four groups according to BMI and presensitization status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Low BMI-non-sensitized</th>
<th>High BMI-non-sensitized</th>
<th>Low BMI-sensitized</th>
<th>High BMI-sensitized</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>302</td>
<td>319</td>
<td>40</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Age, ≥ 65 yr</td>
<td>2 (0.7)</td>
<td>7 (2.2)</td>
<td>0 (0)</td>
<td>1 (4.8)</td>
<td>0.20</td>
</tr>
<tr>
<td>Male sex</td>
<td>146 (48.3)</td>
<td>129 (40.4)</td>
<td>20 (50.0)</td>
<td>13 (61.9)</td>
<td>0.08</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 18.5 (underweight)</td>
<td>12 (4.0)</td>
<td>8 (2.5)</td>
<td>0 (0)</td>
<td>1 (4.8)</td>
<td>0.45</td>
</tr>
<tr>
<td>18.5–23.0 (normal)</td>
<td>121 (40.1)</td>
<td>113 (35.4)*</td>
<td>21 (52.5)*</td>
<td>4 (19.0)*</td>
<td>0.04</td>
</tr>
<tr>
<td>23.0–25.0 (overweight)</td>
<td>75 (24.8)</td>
<td>88 (27.6)</td>
<td>6 (15.0)</td>
<td>8 (38.1)</td>
<td>0.19</td>
</tr>
<tr>
<td>25.0–30.0 (mildly obese)</td>
<td>78 (25.8)</td>
<td>95 (29.8)</td>
<td>12 (30.0)</td>
<td>5 (23.8)</td>
<td>0.69</td>
</tr>
<tr>
<td>≥ 30.0 (grossly obese)</td>
<td>16 (5.3)</td>
<td>15 (4.7)</td>
<td>1 (2.5)</td>
<td>3 (14.3)</td>
<td>0.23</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m$^2$)</td>
<td>114.3 ± 12.6</td>
<td>113.5 ± 13.1</td>
<td>117.3 ± 11.7</td>
<td>113.1 ± 10.5</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Data are expressed as number only, number (%), or mean ± standard deviation. BMI, body mass index; eGFR, estimated glomerular filtration rate.

* $p < 0.05$ vs. low BMI-sensitized group, † $p < 0.05$ vs. high BMI-non-sensitized group, and ‡ $p < 0.05$ vs. high BMI-sensitized group.

Comparison of short- and long-term clinical outcomes and allograft functions according to body mass index and presensitization status

Table 3 shows the short- and long-term clinical outcomes of the compared groups. In the short-term clinical outcomes, the rates of early ABMR were significantly higher in the sensitized groups ($p < 0.001$), but there was no significant difference within the sensitized groups according to BMI status. CMV infection and BK viremia rates tended to be higher in the sensitized groups but without statistical significance ($p = 0.08$ and $p = 0.18$, respectively). The median duration until CMV infection after transplantation was 33 days, and the median duration until BK viremia was 99 days. Early TCMR and PJP rates did not show significant differences between the four groups. In long-term clinical outcomes, the rate of late ABMR tended to be the highest in the high BMI-sensitized group, although the difference was not significant (2 of 21, 9.5%; $p = 0.15$). There were no significant differences between groups.
Table 2. Comparison of baseline characteristics of recipient factors among the four groups according to BMI and presensitization status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Low BMI-non-sensitized</th>
<th>High BMI-non-sensitized</th>
<th>Low BMI-sensitized</th>
<th>High BMI-sensitized</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>302</td>
<td>239</td>
<td>40</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Age, ≥65 yr</td>
<td>13 (4.3)</td>
<td>22 (6.9)</td>
<td>0 (0)</td>
<td>2 (9.5)</td>
<td>0.17</td>
</tr>
<tr>
<td>Male sex</td>
<td>138 (45.7)</td>
<td>235 (73.7)</td>
<td>10 (25.0)</td>
<td>5 (23.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 18.5 (underweight)</td>
<td>57 (18.9)</td>
<td>0 (0)</td>
<td>5 (12.5)</td>
<td>0 (0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>18.5–22.7 (normal)</td>
<td>245 (81.1)</td>
<td>0 (0)</td>
<td>35 (87.5)</td>
<td>0 (0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>22.7–25.0 (overweight)</td>
<td>0 (0)</td>
<td>145 (45.5)</td>
<td>0 (0)</td>
<td>12 (57.1)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>25.0–30.0 (mildly obese)</td>
<td>0 (0)</td>
<td>149 (46.7)</td>
<td>0 (0)</td>
<td>9 (42.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>≥30.0 (grossly obese)</td>
<td>0 (0)</td>
<td>25 (7.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>D/R BSA ratio</td>
<td>1.11 ± 0.16</td>
<td>0.94 ± 0.13</td>
<td>1.13 ± 0.16</td>
<td>1.06 ± 0.15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Laboratory findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>163.2 ± 52.8</td>
<td>156.0 ± 43.1</td>
<td>161.9 ± 40.0</td>
<td>154.0 ± 42.9</td>
<td>0.27</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>120.5 ± 67.7</td>
<td>148.8 ± 91.3</td>
<td>119.3 ± 65.1</td>
<td>147.6 ± 84.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>47.0 ± 16.4</td>
<td>37.4 ± 12.1</td>
<td>44.2 ± 11.3</td>
<td>38.2 ± 10.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>85.7 ± 35.2</td>
<td>85.1 ± 32.6</td>
<td>84.4 ± 30.0</td>
<td>79.9 ± 29.5</td>
<td>0.89</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
<td>5.4 ± 0.9</td>
<td>5.8 ± 1.1</td>
<td>5.4 ± 1.2</td>
<td>6.1 ± 1.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HCV seropositivity</td>
<td>8 (2.6)</td>
<td>6 (1.9)</td>
<td>0 (0)</td>
<td>1 (4.8)</td>
<td>0.58</td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>48 (15.9)</td>
<td>115 (36.1)</td>
<td>7 (17.5)</td>
<td>9 (42.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>177 (58.6)</td>
<td>238 (74.6)</td>
<td>18 (45.0)</td>
<td>14 (66.7)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Previous renal disease (cause of ESRD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>36 (11.9)</td>
<td>100 (31.3)</td>
<td>4 (10.0)</td>
<td>8 (38.1)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>21 (7.0)</td>
<td>38 (11.9)</td>
<td>2 (5.0)</td>
<td>2 (9.5)</td>
<td>0.14</td>
</tr>
<tr>
<td>Clinical glomerulonephritis</td>
<td>124 (41.1)</td>
<td>91 (28.5)</td>
<td>14 (35.0)</td>
<td>7 (33.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>4 (1.3)</td>
<td>18 (5.6)</td>
<td>4 (10.0)</td>
<td>1 (4.8)</td>
<td>0.008</td>
</tr>
<tr>
<td>Others</td>
<td>27 (8.9)</td>
<td>10 (3.1)</td>
<td>1 (2.5)</td>
<td>0 (0)</td>
<td>0.007</td>
</tr>
<tr>
<td>Unknown</td>
<td>90 (29.8)</td>
<td>62 (19.4)</td>
<td>15 (37.5)</td>
<td>3 (14.3)</td>
<td>0.003</td>
</tr>
<tr>
<td>Dialysis modality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>185 (61.3)</td>
<td>160 (50.2)</td>
<td>24 (60.0)</td>
<td>13 (61.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>Peritoneal dialysis</td>
<td>22 (7.3)</td>
<td>47 (14.7)</td>
<td>3 (7.5)</td>
<td>1 (4.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>Preemptive KT</td>
<td>95 (31.5)</td>
<td>112 (35.1)</td>
<td>13 (32.5)</td>
<td>7 (33.3)</td>
<td>0.82</td>
</tr>
<tr>
<td>Dialysis vintage (mo)</td>
<td>18.2 ± 33.7</td>
<td>16.6 ± 25.7</td>
<td>28.2 ± 46.9</td>
<td>12.3 ± 17.1</td>
<td>0.28</td>
</tr>
<tr>
<td>Presensitization status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crossmatch, CDC</td>
<td>4 (1.3)</td>
<td>1 (0.3)</td>
<td>11 (27.5)</td>
<td>6 (28.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Crossmatch, FC</td>
<td>12 (4.0)</td>
<td>5 (1.6)</td>
<td>20 (50.0)</td>
<td>10 (47.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PRA, &gt; 50%</td>
<td>65 (21.5)</td>
<td>57 (17.9)</td>
<td>36 (90.0)</td>
<td>17 (81.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DSA, any titer</td>
<td>20 (6.6)</td>
<td>18 (5.6)</td>
<td>40 (100.0)</td>
<td>21 (100.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MFI</td>
<td>1,646 ± 640</td>
<td>1,724 ± 574</td>
<td>7,730 ± 4,048</td>
<td>8,738 ± 4,061</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Previous KT history</td>
<td>36 (11.9)</td>
<td>21 (6.6)</td>
<td>13 (32.5)</td>
<td>3 (14.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mismatch number</td>
<td>2.9 ± 1.7</td>
<td>3.2 ± 1.7</td>
<td>3.4 ± 1.4</td>
<td>3.4 ± 1.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Induction therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basilixim</td>
<td>279 (92.4)</td>
<td>291 (91.2)</td>
<td>19 (47.5)</td>
<td>8 (38.1)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Anti-thymocyte globulin</td>
<td>23 (7.6)</td>
<td>28 (8.8)</td>
<td>21 (52.5)</td>
<td>13 (61.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Maintenance immunosuppressant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus/cyclosporine</td>
<td>287/15</td>
<td>304/15</td>
<td>39/1</td>
<td>21/0</td>
<td>0.68</td>
</tr>
<tr>
<td>Desensitization therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rituximab</td>
<td>64 (21.2)</td>
<td>50 (15.7)</td>
<td>37 (92.5)</td>
<td>20 (95.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Bortezomib</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (7.5)</td>
<td>1 (4.8)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data are expressed as number only, number (%), or mean ± standard deviation. BMI, body mass index; BSA, body surface area; CDC, complement-dependent cytotoxicity; D/R, donor/recipient; DSA, donor-specific antibody; ESRD, end-stage renal disease; FC, flow cytometry; HCV, hepatitis C virus; HDL, high-density lipoprotein; KT, kidney transplantation; LDL, low-density lipoprotein; MFI, mean fluorescence intensity; PRA, panel reactive antibody.

*p < 0.05 vs. high BMI-non-sensitized group, **p < 0.05 vs. low BMI-sensitized group, ***p < 0.05 vs. low BMI-non-sensitized group, ****p < 0.05 vs. high BMI-sensitized group. aBSA was calculated using the Mosteller body surface area equation.
Comparison of death-censored graft loss rate and its causes according to body mass index and presensitization status

In all, 44 DCGL events occurred; of these, 14 were in the low BMI-non-sensitized group (14 of 302, 4.6%), 22 in the high BMI-non-sensitized group (22/319, 6.9%), and four in the low BMI-sensitized group (4/40, 10.0%) and high BMI-sensitized groups (4/21, 19.0%), respectively (Table 4). There were no statistically significant differences in the causes of DCGL between the four groups. In the Kaplan-Meier survival analysis, the graft survival rate decreased significantly in the sensitized groups, and it was the poorest in the high BMI-sensitized group (Fig. 3, log-rank p = 0.007).

Table 5 shows the results of the multivariable Cox hazard regression model analysis. Model 1 adjusted baseline characteristics showed significant differences among the four groups, except for dialysis modality and vintage, and the variables were as follows: donor factors (age ≥ 65 years, sex, BMI categories, and eGFR), recipient factors (age ≥ 65 years, sex, D/R BSA ratio, triglyceride, HDL-cholesterol, hemoglobin A1c, HCV seropositivity, comorbidities, previous renal disease, previous KT history, PRA > 50%, and mismatch number). Model 2 included dialysis modality and vintage as adjusted variables. High BMI alone did not show a significant hazard ratio (HR) in any of the DCGL models. Presensitization status was observed as an independent risk factor for DCGL (univariable HR = 2.73, p = 0.01, adjusted HR = 2.90, p = 0.007 in model 1; and adjusted HR = 3.63, p = 0.004 in model 2). Interestingly, when high BMI and presensitization status were combined, HR increased further and was identified as a significant independent risk factor (univariable HR = 4.06, p = 0.008, adjusted HR = 3.82, p = 0.01 in model 1; and adjusted HR = 3.75, p = 0.03 in model 2). Moreover, high BMI and presensitization status showed a statistically significant interaction for DCGL (p-value for interaction = 0.008).

Comparison of patient death rate and its causes according to body mass index and presensitization status

Overall, 21 patients died; however, there was no significant difference in the rate of death between the four groups; 8
Figure 2. The eGFR during the follow-up period in the four groups according to BMI and HLA presensitization status. Note that the decrease in allograft function tended to be higher in the high BMI-sensitized group.

BMI, body mass index; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen.

Table 4. Comparison of death-censored graft loss, overall graft loss, and patient death rates among the four groups according to BMI and presensitization status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low BMI-non-sensitized (n = 302)</td>
<td></td>
</tr>
<tr>
<td>Death-censored graft loss</td>
<td>14 (4.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High BMI-non-sensitized (n = 319)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22 (6.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low BMI-sensitized (n = 40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 (10.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High BMI-sensitized (n = 21)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 (19.0)</td>
<td></td>
</tr>
<tr>
<td>Overall graft loss</td>
<td>20 (6.6)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>30 (9.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 (12.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 (19.0)</td>
<td></td>
</tr>
<tr>
<td>Patient death</td>
<td>8 (2.6)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>12 (3.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (2.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as number (%).

BMI, body mass index.

"p < 0.05 vs. high BMI-sensitized group, "p < 0.05 vs. low BMI-non-sensitized group.

Deaths occurred in the low BMI-non-sensitized group (8 of 302, 2.6%), 12 in the high BMI-non-sensitized group (12 of 319, 3.8%), 1 in the low BMI-sensitized group (1 of 40, 2.5%), and none in the high BMI-sensitized group (Table 4). When comparing the causes of death between the groups, there were no significant differences (Table 6).

Discussion

In this study, we analyzed the short- and long-term allograft outcomes according to the high BMI and HLA presensitization status of the recipients. We found that the high BMI-sensitized group had the most significant decline in allograft function among the four groups and the highest DCGL rates. Moreover,
Figure 3. Kaplan-Meier survival analysis among the four groups according to BMI and human leukocyte antigen presensitization status for death-censored graft loss. Cumulative graft survival rates were significantly lower in the high BMI-sensitized group than in the low BMI-non-sensitized group (log-rank p = 0.001) and high BMI-non-sensitized group (log-rank p = 0.02) in post hoc analyses.

Table 5. Cox proportional hazard ratio (HR) model analysis for death-censored graft loss

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariable HR (95% CI)</th>
<th>p-value for interaction</th>
<th>Model 1*; multivariable HR (95% CI)</th>
<th>p-value for interaction</th>
<th>Model 2*; multivariable HR (95% CI)</th>
<th>p-value for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low BMI</td>
<td>Reference</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>High BMI</td>
<td>1.60 (0.88–2.93)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Non-presensitization</td>
<td>Reference</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Presensitization</td>
<td>2.73 (1.27–5.88)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>High BMI &amp; presensitization</td>
<td>4.06 (1.45–11.36)</td>
<td>0.008</td>
<td>3.82 (1.36–10.73)</td>
<td>0.01</td>
<td>3.75 (1.12–12.62)</td>
<td>0.03</td>
</tr>
<tr>
<td>Mismatch number</td>
<td>1.28 (1.07–1.53)</td>
<td>-</td>
<td>1.28 (1.06–1.53)</td>
<td>-</td>
<td>1.34 (1.08–1.65)</td>
<td>-</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>0.90 (0.50–1.64)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.39 (0.19–0.81)</td>
<td>-</td>
</tr>
</tbody>
</table>

BMI, body mass index; CI, confidence interval.

*a Model 1 was adjusted with parameters showing significant differences among the four groups according to BMI and presensitization status. Excluding recipients with missing values, 673 (98.7%) were included in Model 1. The following parameters were used: donor factors (age ≥ 65 years, sex, BMI categories, estimated glomerular filtration rate), recipient factors (age ≥ 65 years, sex, donor/recipient body surface area ratio, triglyceride, high-density lipoprotein-cholesterol, hemoglobin A1c, hepatitis C virus seropositivity, comorbidities, previous renal disease, previous kidney transplantation history, panel reactive antibody > 50%, and mismatch number). *b Model 2 was adjusted with dialysis modality and dialysis vintage in addition to the variables included in Model 1. Excluding recipients with missing values, 448 (65.7%) were included in Model 2.

A high BMI with presensitization status was found to be an independent risk factor for DCGL. There was also a significant interaction between high BMI and pretransplant HLA sensitivity leading to adverse allograft outcomes.

In a previous study that analyzed BMI and allograft outcomes for 51,927 recipients from 1988 to 1997 in the United States Renal Database, the relative risk of graft loss (1.07, \( p = 0.047 \)) increased significantly when BMI ≥ 28.0 kg/m². The
Table 6. Comparison of the causes of graft loss and patient death among the four groups according to BMI and presensitization status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low BMI-non-sensitized</th>
<th>High BMI-non-sensitized</th>
<th>Low BMI-sensitized</th>
<th>High BMI-sensitized</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cause of graft loss</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute rejection</td>
<td>2 (14.3)</td>
<td>7 (31.8)</td>
<td>2 (50.0)</td>
<td>2 (50.0)</td>
<td>0.33</td>
</tr>
<tr>
<td>Chronic active TCMR/ABMR</td>
<td>2 (14.3)</td>
<td>4 (18.2)</td>
<td>0 (0)</td>
<td>1 (25.0)</td>
<td>0.77</td>
</tr>
<tr>
<td>BKVAN</td>
<td>1 (7.1)</td>
<td>1 (4.5)</td>
<td>0 (0)</td>
<td>1 (25.0)</td>
<td>0.47</td>
</tr>
<tr>
<td>Recurrent glomerulonephritis</td>
<td>3 (21.4)</td>
<td>1 (4.5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.27</td>
</tr>
<tr>
<td>Others</td>
<td>2 (14.3)</td>
<td>4 (18.2)</td>
<td>2 (50.0)</td>
<td>0 (0)</td>
<td>0.29</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (28.6)</td>
<td>5 (22.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.44</td>
</tr>
<tr>
<td>Cause of patient death</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac</td>
<td>1 (12.5)</td>
<td>2 (16.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.89</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>0 (0)</td>
<td>1 (8.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.68</td>
</tr>
<tr>
<td>Vascular</td>
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<td>2 (16.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.44</td>
</tr>
<tr>
<td>Infection</td>
<td>4 (50.0)</td>
<td>3 (25.0)</td>
<td>1 (100)</td>
<td>0 (0)</td>
<td>0.23</td>
</tr>
<tr>
<td>Malignancy</td>
<td>3 (37.5)</td>
<td>3 (25.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.68</td>
</tr>
<tr>
<td>Unknown</td>
<td>0 (0)</td>
<td>1 (8.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Data are expressed as number only or number (%).

ABMR, antibody-mediated rejection; BKVAN, BK virus-associated nephropathy; BMI, body mass index; TCMR, T-cell mediated rejection.

*Graft loss causes of others were postoperative bleeding and amyloidosis. *Graft loss causes of others were drug-induced nephropathy, sepsis, oxalate nephropathy, and thrombotic microangiopathy. *Graft loss causes of others were two cases of postoperative bleeding.

authors suggested the effects of various comorbidities and proinflammatory cytokines expressed in obesity were a reason for the poor allograft outcomes in obesity [21]. Additionally, a recent study reported that the risk of acute rejection may increase due to the relative underdosing of immunosuppressants in obese recipients [22]. However, in studies after 2000, obesity was not identified as a significant risk factor for graft loss (risk ratio, 0.99; 95% confidence interval, 0.83–1.19) [23]. Moreover, in a study that directly compared recipients from 1987 to 1999 and recipients from 2000 to 2016 in the Organ Procurement and Transplantation Network/United Network for Organ Sharing database, high BMI (≥ 30.0 kg/m²) was still an independent risk factor for graft loss, but the relative risk significantly decreased after 2000 [24]. The authors suggested that the recent development of an immunosuppressive regimen and general medical practice for comorbidities in transplant recipients may be the reason for this phenomenon [23,24].

The International Obesity Task Force of the World Health Organization (WHO) recommends that the Asian population sets the BMI value of obesity to 25.0 kg/m² and the BMI value of overweight to 23.0 kg/m² when analyzing comorbidity risk [25]. Previous studies that reported a worse allograft outcome in obesity (BMI ≥ 30.0 kg/m²) were not studies involving Asian populations [7,21,23,24]. We considered that setting the obesity criterion to BMI of 25.0 kg/m² according to the WHO recommendation would be appropriate for the Asian population in this study. Similar results were obtained when patients in this study were classified by using obesity (BMI ≥ 25.0 kg/m²), and the DCGL rate in the obesity-sensitized group was higher than that in the high BMI-sensitized group classified by BMI of ≥ 22.7 kg/m² (Supplementary Table 1, available online). Furthermore, the median BMI value of 22.7 kg/m² in this study, was close to the Asian population overweight cutoff value, and most patients in the high BMI group met the Asian overweight criteria.

In a recent study of 296,807 adult recipients from 2000 to 2019 in the Scientific Registry of Transplant Recipients database, the overall BMI reported a “J-shaped” risk profile for graft loss. However, in terms of graft loss and mortality, BMI has been reported to interact with various factors (recipient age, race/ethnicity, sex, and primary renal disease) of recipients. The authors emphasized the importance of personalized risk stratification rather than predicting recipient risk based on absolute BMI alone [26]. Accordingly, our study focused on the presensitization status among recipient factors, and the results showed a significant interaction between recipient BMI and presensitization status, which was an important finding.

In the comparison of baseline characteristics, the D/R BSA ratio was significantly lower in the high BMI-non-sensitized group in this study. Previously, a study had reported that
if the D/R BSA ratio is less than 0.8, the allograft outcome is adversely affected [27]. This donor and recipient BSA mismatch may have influenced the allograft outcome in this study, but a high BMI with presensitization status was found to be an independent risk factor when this variable was adjusted in multivariable regression analysis. The rates of DM and HTN as comorbidities were higher in the high BMI groups, and laboratory parameters, such as triglyceride, HDL-cholesterol, and hemoglobin A1c, also showed suitable differences in metabolic syndromes as expected [28]. As reported previously, the rate of previous KT history was higher in the sensitized groups [29]. Lastly, the rate of peritoneal dialysis before transplantation was significantly higher in the high BMI group than in the low BMI group. Weight gain is thought to occur as a consequence of glucose absorption from the peritoneal dialysate in patients undergoing peritoneal dialysis [30].

In the comparison of short-term outcomes, the rates of CMV infection and BK viremia tended to be higher in the HLA presensitized groups. This could be due to immunosuppression caused by desensitization therapy despite adequate prophylaxis in the sensitized groups. In a nationwide cohort study in Korea, desensitization therapy has also been found to be a significant risk factor for infection-related mortality [31]. Early ABMR rates were higher in the sensitized groups, and the rates of late ABMR also tended to be higher in the sensitized groups. These results were consistent with those of previous studies, which showed that the acute rejection rate is higher despite appropriate desensitization therapy in patients with HLA presensitization [17,31]. In the comparison of long-term outcomes, a significant decline in allograft function was seen in the high BMI-sensitized group. Additionally, the high BMI-sensitized group had the poorest outcome in adjusted allograft DCGL, and high BMI and presensitization showed significant interaction.

This could be due to several reasons. First, the nephron mass of the donated kidney might be relatively inadequate for recipients with a high BMI. Simply put, a physiologic mismatch between the metabolic demand of the recipient and the nephron mass of the donated kidney may have an adverse allograft outcome [32]. Brenner et al. [33] have previously proposed a nephron underdosing theory for chronic allograft failure. They suggested that the transplantation of kidneys with a relatively large nephron mass compared to the recipients’ metabolic demand might lead to tolerance for future immunologic challenges, ischemic events, and CNI toxicities. Hence, in high BMI recipients with high metabolic demand, kidneys with relatively small nephron mass might be more susceptible to immunologic damage when accompanied by presensitization, resulting in poor allograft outcomes. This is reinforced by the fact that the donated kidney weight/recipient body weight ratio in the high BMI group was significantly low in this study (2.6 ± 0.7 vs. 3.4 ± 0.8, p < 0.001; Supplementary Table 2, available online).

Second, obesity is known to induce alloimmune dysregulation by decreasing adiponectin levels and increasing leptin levels. Adiponectin is known to inhibit B-cell lymphopoiesis, macrophage activation, T-cell proliferative response, and responses of helper T-cell (Th)-1 and Th-2 [34], and serum adiponectin levels are reported to decrease with visceral obesity [35]. In contrast, leptin is known to increase T-cell response, proinflammatory cytokines, recruitment of inflammatory cells, and activities of neutrophils, macrophages, and natural killer cells [34], and is reported to be positively correlated with adipose tissue mass [36]. Previously, a cross-sectional analysis of obese patients and control groups in humans showed a good correlation between leptin level and leukocyte count [37]. Moreover, it has recently been reported that B cells also play an important role in obesity through adipose tissue infiltration and activation [38]. In this study, the relatively rapid decline of the allograft function in the high BMI groups, especially in the high BMI-sensitized group, is probably due to the systemic chronic inflammation in the high BMI group. The presensitization status may interact with chronic inflammation status caused by high BMI, resulting in a worse allograft outcome.

This study has some limitations. First, this was a single-center, retrospective study with a relatively small sample size of only 682 cases. The number of patients in the high BMI-sensitized group was only 21 and only four patients developed graft loss events. However, repeat analysis based on BMI of ≥ 25.0 kg/m² showed results that are consistent with previous studies (odds ratio for overall graft loss 1.85, p = 0.03; Supplementary Table 3, available online). Additionally, this study is important because it is the first to analyze the relationship between recipient BMI and presensitization status. The second limitation of the study was that it focused on the condition of the recipients before KT and did not analyze whether the patients developed obesity after KT. After KT, prednisolone and other immunosuppressive drugs are administered, which can lead to weight gain. It is not possible to exclude allograft outcomes that are affected by obesity.
developed after KT. In conclusion, high BMI and HLA sensitization before KT significantly affect long-term allograft outcomes in terms of the decline in allograft function and survival in KT recipients. Our results suggest that active reduction and careful monitoring of BMI might be necessary, especially in patients with high immunologic risk. In the future, studies with larger sample sizes are needed to further clarify the relationship between high BMI and presensitization status.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Authors’ contributions

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Formal analysis: YP, BHC
Funding acquisition: BHC
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References


Background: Recurrent glomerulonephritis (GN) is a common cause of allograft loss in kidney transplantation (KT), the most frequent of which is immunoglobulin A (IgA) nephropathy (IgAN). Galactose-deficient IgA1 (Gd-IgA1) plays a major role in the pathophysiology of IgAN, but the association between Gd-IgA1 and recurrent IgAN in kidney transplant recipients (KTRs) is uncertain. We aimed to evaluate the efficacy of Gd-IgA1 for prediction of recurrent IgAN and graft and patient survival according to Gd-IgA1 level.

Methods: We enrolled 27 KTRs who underwent allograft biopsy between 2009 and 2016 and measured the serum Gd-IgA1 level of each KTR. We divided the patients into two groups: nonrecurrent IgAN (patients with IgAN prior to KT who were not diagnosed with recurrent IgAN) and recurrent IgAN (patients with IgAN prior to KT who were diagnosed with recurrent IgAN).

Results: The mean serum Gd-IgA1 level was significantly higher in the recurrent IgAN group than in the nonrecurrent IgAN group (6,419 ± 3,675 ng/mL vs. 3,381 ± 2,844 ng/mL, p = 0.02). The cutoff value of serum Gd-IgA1 in receiver operating characteristic curve analysis was 4,338 ng/mL (area under the curve, 0.76; 95% confidence interval [CI], 0.57–0.95, p = 0.02). Serum Gd-IgA1 level was an independent factor for recurrent IgAN (odds ratio, 17.60; 95% CI, 1.33–233.03, p = 0.03). There was no significant difference in graft or patient survival between the two groups.

Conclusion: Serum Gd-IgA1 can be used as a diagnostic biomarker for recurrent IgAN in KT.

Keywords: Glomerulonephritis, Graft survival, Immunoglobulin A, Kidney transplantation, Survival
Introduction

One of the most common causes of allograft kidney loss is recurrent glomerulonephritis (GN), and immunoglobulin A (IgA) nephropathy (IgAN) is the most common cause of recurrent GN after kidney transplantation (KT) [1,2]. The pathophysiology of IgAN is the most potent of the “four hits” hypotheses [3], and galactose-deficient IgA1 (Gd-IgA1) plays a major role in the pathophysiology of IgAN [4]. The main mechanism of action of Gd-IgA1 is deposition with an autoimmune IgG complex in the mesangium and endothelium, subsequently damaging those cells [4]. The same mechanism can be assumed for recurrent IgAN after KT, but recurrent GN because of immunosuppressive drug administration does not have the same mechanism of action as IgAN of the general population. Considering that Gd-IgA1 can be measured in the laboratory, it can be used as a diagnostic and prognostic biomarker for IgAN, but it is unclear whether recurrent IgAN after KT is similarly observed based on this clinical pattern and whether de novo IgAN is observed. However, the association between Gd-IgA1 and occurrence of recurrent IgAN in kidney transplant recipients (KTRs) is uncertain. Therefore, in this study, we aimed to investigate whether Gd-IgA1 is effective as a diagnostic and prognostic marker for recurrent IgAN.

Methods

Study design

We enrolled 27 KTRs with stored samples in the Biobank of Keimyung University Dongsan Hospital and who underwent allograft biopsy between 2009 and 2016. There were 17 living donor KTRs (10 living-related donor KTRs and seven living-unrelated donor KTRs) and 10 deceased donor KTRs. The living-related donor KTRs comprised four parent-to-child and six sibling-to-sibling KTRs; two wife-to-husband KTRs were included in the living-unrelated donor KTRs. The patients were divided into the nonrecurrent IgAN group (patients with IgAN prior to KT who were not diagnosed with recurrent IgAN after KT [n = 14]) and the recurrent IgAN group (patients with IgAN prior to KT who were diagnosed with recurrent IgAN [n = 13]). The nonrecurrent IgAN group included KTRs with other recurrent GN issues, acute and chronic rejection, and calcineurin inhibitor (CNI) toxicity. We evaluated the clinical characteristics of the study population, status of immunosuppression at diagnosis, change in allograft function, severity of proteinuria at diagnosis, and graft and patient survival.

Demographic and clinical data

We investigated the age of donors and recipients at diagnosis of recurrent IgAN, sex of donors and recipients, KT type, frequency of KT, dialysis type prior to KT, dialysis vintage, causes of end-stage renal disease (ESRD), number of human leukocyte antigen (HLA) mismatches, immunosuppressant for induction and maintenance treatment, previous biopsy-proven acute rejection (BPAR), severity of proteinuria at diagnosis of recurrent IgAN, panel reactive antibody (PRA) > 50%, and donor-specific antibody positivity. Allograft biopsy was performed when allograft dysfunction (decreased estimated glomerular filtration rate [eGFR]) or persistent microscopic hematuria and proteinuria were observed. Allograft biopsy was analyzed using the Banff 2013 classification [5]. Allograft function was measured based on eGFR according to the guidelines of the Chronic Kidney Disease Epidemiology Collaboration within 1 month before diagnosis and at 12 months, 3 years, and 5 years after diagnosis [6]. Proteinuria was measured using the spot urine protein-to-creatinine ratio (g/g).

Serum Gd-IgA1 level was measured using the Gd-IgA1 assay kit (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) through the enzyme-linked immunosorbent assay [7]. The level of each serum Gd-IgA1 was obtained based on a standard curve with an appropriate regression curve on each plot (four-parameter logistics; optical density, 450 nm). The assay for Gd-IgA1 was performed in triplicate.

The Institutional Review Board (IRB) of Keimyung University Dongsan Hospital approved this study (No. 2017-02-030). The requirement for informed consent was waived by the IRB because use of patient data for research, except identifying personal information, was explained to all donor families and all recipients before KT. Therefore, as a retrospective medical record study, this study did not contain any distinguishable personal information, except clinical process and outcome.

Immunosuppression protocols

We administered basiliximab (Simulect, 20 mg on days 0 and
4; Novartis, Basel, Switzerland) for KTRs with low immunologic risk and antithymocyte globulin (Thymoglobulin, 1.5 mg/kg on day 0 and 1.0 mg/kg from day 1 to day 3; Genzyme, Cambridge, MA, USA) for KTRs with high immunologic risk as induction immunosuppressants. We administered cyclosporine (Sandimmune, 3 mg/kg, twice a day; Novartis AG, Basel, Switzerland) or tacrolimus (Prograf, 0.05 mg/kg, twice a day; Astellas Pharma Inc., Toyama, Japan) as a CNI, prednisolone (30 – 20 – 10 – 5 – 0 mg, once a day, on a stepdown regimen), and mycophenolate mofetil (CellCept, 750 or 1,000 mg, twice a day for 1 month after KT and subsequent 500 mg, twice a day; Hoffmann-La Roche Inc., Nutley, NJ, USA) as maintenance immunosuppressants. The treatment protocol for recurrent IgAN was use of an angiotensin receptor blocker at diagnosis; if it elicited no response, then 0.25 mg/kg per day of oral steroid was used.

Statistical analyses

Continuous variables were analyzed using the Mann-Whitney U test, and categorical variables were analyzed using the chi-square or Fisher exact test. Graft and patient survival rates were evaluated using the Kaplan-Meier analysis with log-rank test. Univariate and multivariate analyses with logistic regression analysis were performed to investigate the risk factors for development of recurrent IgAN. The p-values less than 0.05 were considered statistically significant. Statistical analysis was performed using the PASW Statistics (version 18.0; IBM Corp., Armonk, NY, USA).

Results

Baseline characteristics of the study population

The mean follow-up period for all patients was 147.5 ± 83.6 months.Recipient age at KT was significantly lower in the recurrent IgAN group than in the nonrecurrent IgAN group (32.8 ± 11.5 years vs. 41.9 ± 9.9 years, p = 0.04), but there was no significant difference in recipient age at diagnosis. There was also no significant difference in donor age at KT between the two groups. There were no significant differences in rates of recipient and donor sex, PRA > 50%, use of induction and maintenance immunosuppressants, or previous BPAR between the two groups. The proportion of living donor KT was higher in the recurrent IgAN group than in the nonrecurrent IgAN group. Moreover, the proportion of deceased donor KT was higher in the nonrecurrent IgAN group than in the recurrent IgAN group. The mean number of HLA mismatches was significantly lower in the recurrent IgAN group than in the nonrecurrent IgAN group (2.6 ± 1.9 vs. 3.9 ± 1.1, p = 0.05). There were no significant differences between the recurrent IgAN and nonrecurrent IgAN groups in the other clinical parameters (Table 1).

Comparison of clinical and laboratory parameters according to recurrent IgA nephropathy

The mean serum Gd-IgA1 level was significantly higher in the recurrent IgAN group than in the nonrecurrent IgAN group (6,419 ± 3,675 ng/mL vs. 3,381 ± 2,844 ng/mL, p = 0.02). The mean time between KT and allograft biopsy was longer in the recurrent IgAN group than in the nonrecurrent IgAN group (108.9 ± 83.3 months vs. 61.7 ± 69.2 months). The proportion of steroid use was significantly lower in the recurrent IgAN group than in the nonrecurrent IgAN group. Allograft function between diagnosis and 5 years after diagnosis and proteinuria at diagnosis did not differ between the two groups (Table 2).

Receiver operating characteristic curve analysis

The cutoff value of serum Gd-IgA1 in receiver operating characteristic curve analysis was 4,338 ng/mL (area under the curve, 0.76; 95% confidence interval [CI], 0.57–0.95; p = 0.02). The sensitivity and specificity were 76.9% and 78.6%, respectively. The positive predictive value and negative predictive value were 76.1% and 78.6%, respectively (Fig. 1A). There was no significant difference in death-censored graft survival rate after diagnosis of recurrent IgAN or other diseases according to Gd-IgA1 level (Fig. 1B).

Comparison of death-censored allograft survival and patient survival according to development of recurrent IgA nephropathy and factors related to development of recurrent IgA nephropathy

A total of 14 patients (51.9%; six patients [22.2%] in the recurrent IgAN group and eight patients [29.6%] in the nonrecurrent IgAN group) experienced graft failure. The causes of graft failure were chronic rejection (three [50.0%] and
Table 1. Comparison of clinical and laboratory parameters according to recurrent IgAN

<table>
<thead>
<tr>
<th>Variable</th>
<th>Recurrent IgAN</th>
<th>Nonrecurrent IgAN</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>13</td>
<td>14</td>
<td>0.04</td>
</tr>
<tr>
<td>Recipient age at KT (yr)</td>
<td>32.8 ± 11.5</td>
<td>41.9 ± 9.9</td>
<td>0.15</td>
</tr>
<tr>
<td>Recipient age at diagnosis (yr)</td>
<td>41.7 ± 10.0</td>
<td>47.1 ± 8.6</td>
<td>0.12</td>
</tr>
<tr>
<td>Recipient sex, male:female</td>
<td>6 (46.2):7 (53.8)</td>
<td>11 (78.6):3 (21.4)</td>
<td>0.12</td>
</tr>
<tr>
<td>Donor age at KT (yr)</td>
<td>34.3 ± 10.7</td>
<td>38.9 ± 11.6</td>
<td>0.31</td>
</tr>
<tr>
<td>Donor sex, male:female</td>
<td>7 (53.8):6 (46.2)</td>
<td>5 (35.7):9 (64.3)</td>
<td>0.45</td>
</tr>
<tr>
<td>Dialysis duration (mo)</td>
<td>24.6 ± 24.6</td>
<td>52.7 ± 63.0</td>
<td>0.16</td>
</tr>
<tr>
<td>Donor type, living:deceased</td>
<td>11 (84.6):2 (15.4)</td>
<td>6 (42.9):8 (57.1)</td>
<td>0.05</td>
</tr>
<tr>
<td>Living-related:living-unrelated</td>
<td>8:3</td>
<td>4:2</td>
<td></td>
</tr>
<tr>
<td>HLA mismatch (n)</td>
<td>2.6 ± 1.9</td>
<td>3.9 ± 1.1</td>
<td>0.05</td>
</tr>
<tr>
<td>PRA &gt; 50%</td>
<td>2 (15.4)</td>
<td>5 (35.7)</td>
<td>0.65</td>
</tr>
<tr>
<td>DSA</td>
<td>2 (15.4)</td>
<td>6 (42.9)</td>
<td>0.37</td>
</tr>
<tr>
<td>Induction immunosuppressant</td>
<td></td>
<td></td>
<td>0.52</td>
</tr>
<tr>
<td>Basiliximab</td>
<td>7 (53.8)</td>
<td>8 (57.1)</td>
<td></td>
</tr>
<tr>
<td>Antithymocyte globulin</td>
<td>0 (0)</td>
<td>2 (14.3)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>6 (46.2)</td>
<td>4 (28.6)</td>
<td></td>
</tr>
<tr>
<td>Maintenance immunosuppressant</td>
<td></td>
<td></td>
<td>0.39</td>
</tr>
<tr>
<td>Cyclosporine: tacrolimus</td>
<td>4 (30.8):9 (69.2)</td>
<td>2 (14.3):12 (85.7)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as number only, mean ± standard deviation, or number (%).

BPAR, biopsy-proven acute rejection; DSA, donor-specific antibody; HLA, human leukocyte antigen; IgAN, immunoglobulin A nephropathy; KT, kidney transplantation; PRA, panel reactive antibody.

Table 2. Comparison of clinical outcomes according to recurrent IgAN

<table>
<thead>
<tr>
<th>Variable</th>
<th>Recurrent IgAN (n = 13)</th>
<th>Nonrecurrent IgAN (n = 14)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd-IgA1 (ng/mL)</td>
<td>6,418 ± 3,675</td>
<td>3,381 ± 2,844</td>
<td>0.02</td>
</tr>
<tr>
<td>Time from KT to biopsy (mo)</td>
<td>108.9 ± 83.3</td>
<td>61.7 ± 69.2</td>
<td>0.12</td>
</tr>
<tr>
<td>Immunosuppressant at diagnosis</td>
<td></td>
<td></td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Cyclosporine: tacrolimus</td>
<td>2 (15.4):11 (84.6)</td>
<td>2 (14.3):12 (85.7)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>7 (53.8)</td>
<td>11 (78.6)</td>
<td>0.24</td>
</tr>
<tr>
<td>Steroid</td>
<td></td>
<td></td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Before diagnosis</td>
<td>2 (15.4)</td>
<td>9 (64.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>After diagnosis</td>
<td>8 (61.5)</td>
<td>8 (57.1)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>ARB</td>
<td></td>
<td></td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Before diagnosis</td>
<td>3 (23.1)</td>
<td>3 (21.4)</td>
<td></td>
</tr>
<tr>
<td>After diagnosis</td>
<td>8 (61.5)</td>
<td>5 (35.7)</td>
<td></td>
</tr>
<tr>
<td>Allograft function (CKD-EPI) (mL/min/1.73 m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within 1 mo before diagnosis</td>
<td>50.9 ± 25.2</td>
<td>45.9 ± 24.1</td>
<td>0.61</td>
</tr>
<tr>
<td>After diagnosis (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>53.3 ± 28.9</td>
<td>48.9 ± 25.1</td>
<td>0.27</td>
</tr>
<tr>
<td>2</td>
<td>48.9 ± 25.1</td>
<td>43.3 ± 22.8</td>
<td>0.58</td>
</tr>
<tr>
<td>3</td>
<td>51.1 ± 16.8</td>
<td>35.8 ± 22.8</td>
<td>0.09</td>
</tr>
<tr>
<td>4</td>
<td>51.4 ± 23.6</td>
<td>43.9 ± 29.6</td>
<td>0.57</td>
</tr>
<tr>
<td>5</td>
<td>44.4 ± 23.5</td>
<td>32.4 ± 23.2</td>
<td>0.35</td>
</tr>
<tr>
<td>Proteinuria at diagnosis (g/g)</td>
<td>1.7 ± 1.9</td>
<td>1.0 ± 0.8</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or number (%).

ARB, angiotensin receptor blocker; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; Gd-IgA1, galactose-deficient immunoglobulin A1; IgAN, immunoglobulin A nephropathy; KT, kidney transplantation.
six [75.0%] in the recurrent IgAN and nonrecurrent IgAN groups, respectively), recurrent IgAN (three [50.0%] and none in the recurrent IgAN and nonrecurrent IgAN groups, respectively), and patient death with graft function (none and one [12.5%] in the recurrent IgAN and nonrecurrent IgAN groups, respectively) (Table 3). The 10-year graft survival rate was 88.9% in the recurrent IgAN group and 69.6% in the nonrecurrent IgAN group, but there was no significant difference in overall death-censored graft survival between the two groups (Fig. 2A). There was no significant difference in death-censored graft survival rate after diagnosis of recurrent IgAN or other diseases between the two groups (Fig. 2B). One patient (3.7%) in the nonrecurrent IgAN group died due to cytomegalovirus pneumonia. The 10-year patient survival rate was 100% in the recurrent IgA group and 92.9% in the nonrecurrent IgAN group, but there was no significant difference in death-censored graft survival between the two groups.

Based on logistic regression analysis, serum Gd-IgA1 level was an independent factor for diagnosis of recurrent IgAN (odds ratio, 17.06; 95% CI, 1.33–233.03; p = 0.03) adjusting for recipient age, sex, living donor KT, dialysis vintage, mainte-

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**Figure 1.** The cutoff of Gd-IgA1 level and allograft outcome according to the Gd-IgA1 level. (A) ROC curves for serum Gd-IgA1 level. ROC AUC for recurrence of IgAN was 0.76 (0.57–0.95) for serum Gd-IgA1 (p = 0.023). (B) Comparison of death-censored graft survival rate after diagnosis of recurrent IgAN according to Gd-IgA1 level. AUC, area under the curve; Gd-IgA1, galactose-deficient-immunoglobulin A1; IgAN, immunoglobulin A nephropathy; ROC, receiver operating characteristic.

**Table 3.** Comparison of cause of allograft failure and patient death according to recurrent IgAN

<table>
<thead>
<tr>
<th>Variable</th>
<th>Recurrent IgAN (n = 13)</th>
<th>Nonrecurrent IgAN (n = 14)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Causes of graft failure</td>
<td></td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>Chronic rejection</td>
<td>3 (23.1)</td>
<td>6 (42.9)</td>
<td></td>
</tr>
<tr>
<td>Recurrent IgAN</td>
<td>3 (23.1)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>0 (0)</td>
<td>1 (7.1)</td>
<td></td>
</tr>
<tr>
<td>Patient death with a functioning graft</td>
<td>0 (0)</td>
<td>1 (7.1)</td>
<td></td>
</tr>
<tr>
<td>Causes of death</td>
<td></td>
<td></td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Cytomegalovirus pneumonia</td>
<td>0 (0)</td>
<td>1 (7.1)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as number (%). IgAN, immunoglobulin A nephropathy.
**Figure 2.** Allograft outcome of kidney transplant recipients according to the development of recurrent IgAN. Comparison of (A) death-censored overall graft survival rate and (B) death-censored graft survival rate after diagnosis of recurrent IgAN. IgAN, immunoglobulin A nephropathy; KT, kidney transplantation.

**Table 4.** Risk factors associated with recurrent IgA nephropathy in KT recipients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th></th>
<th></th>
<th>Multivariate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
<td>p-value</td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Recipient age at KT</td>
<td>0.92</td>
<td>0.85–1.00</td>
<td>0.05</td>
<td>1.06</td>
<td>0.92–1.24</td>
</tr>
<tr>
<td>Recipient male sex</td>
<td>0.23</td>
<td>0.04–1.25</td>
<td>0.09</td>
<td>0.06</td>
<td>0.01–1.20</td>
</tr>
<tr>
<td>Living donor KT</td>
<td>0.12</td>
<td>0.02–0.86</td>
<td>0.03</td>
<td>13.08</td>
<td>0.94–181.49</td>
</tr>
<tr>
<td>Dialysis vintage</td>
<td>0.99</td>
<td>0.96–1.01</td>
<td>0.20</td>
<td>1.01</td>
<td>0.98–1.04</td>
</tr>
<tr>
<td>Tacrolimus vs. cyclosporine</td>
<td>0.38</td>
<td>0.06–2.52</td>
<td>0.31</td>
<td>8.09</td>
<td>0.09–772.74</td>
</tr>
<tr>
<td>HLA mismatches</td>
<td>0.56</td>
<td>0.30–1.04</td>
<td>0.07</td>
<td>0.69</td>
<td>0.27–1.75</td>
</tr>
<tr>
<td>Time from KT to diagnosis</td>
<td>1.01</td>
<td>1.00–1.02</td>
<td>0.13</td>
<td>1.01</td>
<td>0.98–1.04</td>
</tr>
<tr>
<td>Gd-IgA1 &gt; 4,338 ng/mL</td>
<td>12.22</td>
<td>1.99–75.06</td>
<td>0.007</td>
<td>17.06</td>
<td>1.33–233.03</td>
</tr>
<tr>
<td>Steroid use</td>
<td>0.10</td>
<td>0.02–0.65</td>
<td>0.02</td>
<td>2.09</td>
<td>0.01–556.51</td>
</tr>
</tbody>
</table>

CI, confidence interval; Gd-IgA1, galactose-deficient-immunoglobulin A1; HLA, human leukocyte antigen; HR, hazard ratio; IgA, immunoglobulin A; KT, kidney transplantation.

Discussion

IgAN has been implicated in the most frequent, primary form of GN and is the most common cause of recurrent GN in KTRs. However, the clinical manifestations of recurrent GN include proteinuria and allograft dysfunction such as acute rejection, recurrent GN, or CNI toxicity. To identify the causes of these symptoms, allograft biopsy is currently the best diagnostic method [8]. However, allograft biopsy is invasive, requires hospitalization, and can lead to false diagnosis due to insufficient specimens. Therefore, several studies are being conducted to overcome these shortcomings; in the case of recurrent IgAN, the research has been conducted to identify the mechanism of IgAN in native kidney diseases [9]. In particular, Gd-IgA1, involved in the mechanism of IgAN, has been reported as a diagnostic biomarker [10]. We evaluated the usefulness of Gd-IgA1 as a diagnostic biomarker for recurrent IgAN in KTRs. Temurhan et al. [11] assessed the Gd-IgA1 level of recu-
rent IgAN in KT, nonrecurrent IgAN in KT, non-transplant IgAN patients, and healthy relatives. They found that, regardless of transplantation, Gd-IgA1 level was significantly higher in patients with recurrent IgAN and non-transplant IgAN than in those with nonrecurrent IgAN and healthy relatives (recurrent IgAN, 8,735 ± 10,854 ng/mL; non-transplant IgAN, 8,791 ± 8,700 ng/mL; nonrecurrent IgAN, 4,790 ± 6,089 ng/mL; healthy relatives, 2,615 ± 1,611 ng/mL). The results of our study are consistent with those of the above-mentioned study. Patients with recurrent IgAN after KT showed significantly higher Gd-IgA1 level than patients with nonrecurrent IgAN after KT.

Because Gd-IgA1 level is specifically elevated in IgAN patients according to pathogenesis of IgAN, IgAN can be predicted by Gd-IgA1 level, although the cause of ESRD is not confirmed through kidney biopsy. In addition, in KTRs, it is possible to indirectly evaluate the status of the allograft kidney for presence of acute or chronic rejection or CNI toxicity, although the Gd-IgA1 level is not elevated and the cause of ESRD is not IgAN, which can overcome the disadvantages of allograft biopsy.

In other words, when KTRs with IgAN as a cause of ESRD have allograft dysfunction or new-onset proteinuria or hematuria, we recommend measuring the Gd-IgA1 level with blood samples. If the Gd-IgA1 level is high, we can consider recurrent IgAN, which will lead us to use an angiotensin receptor blocker or steroid without allograft biopsy.

Interestingly, the proportion of living donor KT was higher in the recurrent IgAN group than in the nonrecurrent IgAN group. Some studies have reported that KT from a living-related donor is the most well-known risk factor for recurrent IgAN, and the incidence of IgAN in renal allografts is higher in living-related donor KT than in deceased donor KT. The results of our study are consistent with those of the above-mentioned studies [12,13]. In our study, multivariate logistic regression analysis showed an odds ratio of living donor KT for occurrence of recurrent IgAN of 13.08 (95% CI, 0.94–181.49, p = 0.055); therefore, the occurrence of recurrent IgAN is associated with living donor KT. However, there was no significant difference in allograft survival of patients with recurrent IgAN between living donor KT and deceased donor KT. These observations were not confirmed in other studies [14,15], and there was no speculated reason for the higher recurrence rate of IgAN in living donor KT than in deceased donor KT. Therefore, further large-scale studies are needed.

There is a lack of consensus regarding the role of steroids against occurrence of recurrent IgAN. Allen et al. [16] reported that corticosteroids reduced the occurrence of recurrent IgAN in KTR. In our study, the proportion of steroid use at diagnosis was significantly lower in the recurrent IgAN group compared with the nonrecurrent IgAN group, but steroid use was not a factor related to development of recurrent IgAN in multivariate analysis. Furthermore, steroid was used when there was no response to angiotensin receptor blocker, and steroid was not effective in most patients with recurrent IgAN.

Our study has some limitations. First, this was a retrospective study. Therefore, since a selection bias can develop, further large-scale prospective studies are needed. Second, we did not assess Gd-IgA1 level regularly after KT because examination for Gd-IgA1 was not commercialized for research purposes. In other words, we only measured Gd-IgA1 level at the time of allograft biopsy in blood samples stored in the Biobank for evaluation of allograft dysfunction. Third, because the sample size was small, the results of this study might not be representative. In particular, we cannot compare the difference in Gd-IgA1 level according to the effect of induction immunosuppressant because of small sample sizes. However, we did observe significant differences in serum Gd-IgA1 level that might indicate recurrent IgAN in KTRs with allograft dysfunction.

In conclusion, serum Gd-IgA1 can be effective for early detection of recurrent IgAN in KTRs. Therefore, serum Gd-IgA1 can be used as a diagnostic biomarker for recurrent IgAN in KT.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

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Authors’ contributions

Conceptualization, Project administration: SH
Data curation, Funding acquisition: WYP
Investigation: YK, JHP, KJ
Writing–original draft: WYP
Writing–review & editing: All authors
All authors read and approved the final manuscript.

Acknowledgments

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References

Hypersensitivity reactions to synthetic membranes continue to be observed [1] and typically disappear following replacement of the dialysis membrane with a different type, usually cellulose triacetate. Previous studies have described the low purification ability of β2-microglobulin and its limited use in online hemodiafiltration (OL-HDF) with symmetric cellulose dialyzers [2]. The high ultrafiltration coefficient (K_{UF}) and configuration of asymmetric cellulose triacetate (ACT) allow its use in OL-HDF. However, there is lack of evidence of the performance of ACT dialyzers.

The aim of our study was to evaluate small- and mid-sized molecule clearance and albumin leakage in postdilution OL-HDF using the ACT dialyzer. Nineteen adult patients with stage-5D kidney disease were enrolled in this study. The inclusion criteria were as follows: older than 18 years, completion of three 4-hour sessions weekly for at least 6 months, blood flow > 400 mL/min in the regular sessions, absence of hospital admission in the 4 weeks prior to the study and signed informed consent. Patients who failed to meet the inclusion criteria were excluded. Details of the dialyzer (Solacea dialyzer®, Nipro Medical Corp., Doral, FL, USA) are as follows: membrane surface area, 2.1 m²; K_{UF}, 76 mL/hr/mmHg; inner diameter of hollow fiber, 200 μm; and membrane thickness, 25 μm. Ultrafiltration was prescribed according to the patient’s needs, and 24 L of substitution volume was programmed. The prescribed dialysis features included a 4-hour duration, blood flow of 400 mL/min, and dialysate flow of 700 mL/min.

Pre- and postdialysis blood samples were collected at the mid-week dialysis session. Removal of urea (60 Da), creatinine (113 Da), β2-microglobulin (11.8 kDa), cystatin C (13 kDa), myoglobin (17.2 kDa), and prolactin (23 kDa) was estimated using the reduction ratio (RR) as follows.

\[ RR = \frac{(C_{pre} - C_{post})}{C_{pre}}, \]

where \( C_{pre} \) and \( C_{post} \) are the pre- and posttreatment concentrations, respectively. Posttreatment concentrations of mid-size molecules were corrected for the haemoconcentration using the Bergström and Wehle formula [3].

\[ \beta_2 \text{ microglobulin was measured using a nephelometric immunoassay, and myoglobin and prolactin were assessed via electrochemiluminescence. Albumin was measured using an autoanalyzer. Estimated albumin leakage (EAL) was estimated according to the following formula.} \]

\[ EAL = \left[ 15 \times \left( C_0 + C_{15} \right) / 2 + 15 \times \left( C_{15} + C_{30} \right) / 2 + 30 \times \left( C_{30} + C_{60} \right) / 2 + C_{60} \right] \times \left( C_{60} + C_{120} \right) / 240 \times UF + Sust + \left( Q_d \times 240 / 1000 \right) \]

where \( C \) is the albumin concentration in dialysate at the beginning \( (C_0) \) and also at 15 minutes \( (C_{15}) \), 30 minutes \( (C_{30}) \), 60 minutes \( (C_{60}) \), and 120 minutes \( (C_{120}) \) (mg/
L; UF, ultrafiltration (L); Sust, substitution volume (L); and Qd, dialysate flow (mL/min).

Written informed consent was obtained from all patients. All procedures were in accordance with the Declaration of Helsinki and its revisions. Descriptive results are expressed as the mean ± standard deviation for normally distributed continuous variables and the median and interquartile ranges for non-normally distributed continuous variables. Categorical variables are reported as percentages. All analyses were performed using IBM SPSS for Mac version 20.0 (IBM Corp., Armonk, NY, USA).

The baseline characteristics of the 19 patients included are shown in Table 1. All patients had a permanent native or prosthetic arteriovenous fistula. All patients completed the experimental sessions with no technical or clinical problems, no occurrences of blood circuit clotting, and no hypersensitivity reactions. The results are represented in Table 2.

Previous *in vitro* and *in vivo* studies have reported that an ACT membrane provides high diffusive transport rates with high solute clearance and good biocompatibility, but albumin leakage has not been addressed in these studies [4–6]. Although the acceptable upper limit of dialysis-related albumin loss remains unknown, it should be minimized because it may contribute to hypoalbuminemia and adversely affect the patient’s prognosis. In this study, we demonstrated that OL-HDF with ACT membrane albumin loss is in the lower range compared to synthetic dialyzers and the removal of small and medium-molecules is similar to the results with synthetic membranes under similar conditions [7]. Although our findings demonstrated less albumin leakage than other authors [8], this difference was probably related to the higher convective volume achieved in other studies.

Our study had several limitations. First, this study was developed as an acute study that focused on the results of one dialysis session. Therefore, prospective studies are needed to assess the clinical impact of the use of ACT membranes. Despite the small sample size and absence of a control group, our results are in line with other authors’ [4,5,8] and demonstrated efficacy in solute clearance and also achieved the recommended convective volume while minimizing albumin leakage.

In conclusion, ACT shows excellent behavior in OL-HDF. Although more prospective studies are needed, according to its clearance results and amount of albumin leakage, this dialyzer is not only an alternative for patients with an allergy to synthetic membranes but is also a good option for nonallergic hemodialysis patients.

**Conflict of interest**

All authors have no conflicts of interest to declare.

**Authors’ contributions**

Conceptualization: NM, AV
Investigation: LC, AV
Methodology: AS
Writing—original draft: AS
Writing—review & editing: LC
All authors read and approved the final manuscript.

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### Table 1. Baseline characteristics of the included patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>55.0 ± 17.3</td>
</tr>
<tr>
<td>Male sex</td>
<td>13 (68.4)</td>
</tr>
<tr>
<td>Dry weight (kg)</td>
<td>65.4 ± 14.2</td>
</tr>
<tr>
<td>Serum albumin (g/dL)</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.2 ± 1.2</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or number (%).

---

### Table 2. The performance of asymmetric cellulose triacetate dialyzer

<table>
<thead>
<tr>
<th>Performance</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total convective volume* (L)</td>
<td>27.4 ± 3.4</td>
</tr>
<tr>
<td>Dialysance (Kt/V)</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>Reduction ratio (%)</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>83.7 ± 5.2</td>
</tr>
<tr>
<td>Creatinine</td>
<td>76.4 ± 5.3</td>
</tr>
<tr>
<td>β2 microglobulin</td>
<td>79.3 ± 4.7</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>77.3 ± 4.7</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>76.6 ± 5.4</td>
</tr>
<tr>
<td>Prolactin</td>
<td>73.7 (67.3–77.5)</td>
</tr>
<tr>
<td>Estimated albumin loss (mg/session)</td>
<td>481.2 (384.8–596.7)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or median (interquartile range).

*Substitution volume + ultrafiltration.
References

1. Manuscript Submission

Manuscripts for *Kidney Research and Clinical Practice* (KRCP) should be submitted online at https://www.editorialmanager.com/krcp. All submissions to KRCP must conform to the International Committee of Medical Journal Editors (ICMJE) uniform requirements for manuscripts submitted to biomedical journals. Our requirements reflect those of the ICMJE, although we also have specific requirements for different types of article. For editorial questions, please contact us via e-mail (registry@ksn.or.kr), telephone (+82-2-3486-8736), or fax (+82-2-3486-8737).

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Please ensure that the following submission documents are also included, where applicable:

1. A cover letter. It must include your name, address, telephone and fax numbers, e-mail address, and state that all authors have contributed to the paper and have never submitted the manuscript, in whole or in part, to other journals.
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5. Articles where human subjects can be identified in descriptions, photographs or pedigrees must be accompanied by a signed statement of informed consent to publish (in print and online) the descriptions, photographs and pedigrees from each subject who can be identified.
6. The terms sex (when reporting biological factors) and gender (identity, psychosocial or cultural factors) should be correctly used. The sex and/or gender of study participants, the sex of animals or cells should be reported, and the methods used to determine sex and gender should be described. If the study was done involving an exclusive population, for example in only one sex, authors should justify why, except in obvious cases (e.g., ovarian cancer).
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These are expected to present major advances and important
new research results. Section headings should include Abstract, Introduction, Methods, Results, Discussion, Conflicts of interest, Acknowledgments (if applicable), and References. The text should be limited to 4,000 words (excluding tables, figures and references) and 40 references.

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These describe new developments of significance in the field of nephrology and highlight unresolved questions and future directions. Most reviews are solicited by the editors, but unsolicited submissions may also be considered for publication. Review articles should include Abstract, Introduction, brief main headings, and References. The text should be limited to 5,000 words (excluding tables, figures and references) and 100 references.

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These are manuscripts that are related to materials within the current issue; they raise challenging questions or explore controversies. The editor solicits such opinion pieces. The order of the submitted manuscript includes title page, integrated discussion, conflict of interest, acknowledgments (if applicable) and references. The text should be limited to 1,500 words and 10 references. A maximum of 2 figures or tables may be included.

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These present classic or unique images of common medical conditions in clinical nephrology. Images are an important part of much of what we do and learn in clinical practice. The text should be limited to 400 words. There should be no more than two figures. No tables or references are included.

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The title page should include article title, each author’s first and last names, positions (associate professor, fellow, student, etc.), and ORCID identifiers, and the institutions with which they are affiliated, short running title not exceeding 50 characters, separate word count for abstract and text, and details of the corresponding author (name, address, phone, and e-mail information). Funding sources should be included, and the individual contribution of each co-author must also be detailed (see relevant section 4.3 below).

3.2. Abstract and Keywords
Abstract should not exceed 250 words in original, review or special articles. It must be written for easy reading with no abbreviations. The abstract of the original article should be divided into four subsections: Background, Methods, Results, and Conclusion. Four to six keywords should be listed alphabetically below the abstract. For selecting keywords, refer to the Index Medicus Medical Subject Headings (available from: http://www.ncbi.nlm.nih.gov/mesh).

3.3. Main Text
The text for original articles, for example, should include the following sections: Introduction, Methods, Results, and Discussion. The Introduction should be as concise as possible, without subheadings. The Methods section should be sufficiently detailed. Subheadings may be used to organize the Results and Discussion. Each section should begin on a new page.

3.4. Acknowledgments
General acknowledgments for consultations, statistical analysis and so on should be listed after main body of text, before the References section, including the names of the individuals involved. All financial and material support for the research
3.5. References

References should be cited with Arabic numerals in square brackets. References are numbered consecutively in order of appearance in text. References are limited to those cited in text and listed in numerical order. List all authors if there are less than or equal to six authors. List the first three authors followed by “et al” if there are more than six authors. If an article has been published online but has not yet been given an issue or pages, the digital object identifier (DOI) should be supplied. Journal titles should be abbreviated in the style used in Index Medicus. Other types of references not described below should follow The NLM Style Guide for Authors, Editors, and Publishers (https://locatorplus.gov/cgi-bin/Pwebrecon.cgi?DB=local&v1=1&ti=1,1&Search_Arg=101318441&Search_Code=0359&CNT=1&SID=1). The authors may format the citations and references using the KRCP EndNote style file, but we generally recommend the authors to type the citation numbers and references manually.

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**Online publication but not yet in print:**


**Entire Book:**


**Book chapter:**


**Website:**


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Tables are numbered consecutively using Arabic numerals in the order of their citation in text. Table titles should be short and descriptive (e.g. Table 1. Demographic characteristics of patients). If numerical measurements are given, the unit of measurement should be included in the column heading. The statistical significance of observed differences in the data should be indicated by the appropriate statistical analysis. All nonstandard abbreviations should be defined in footnotes. Lower case letters in superscripts (\(^a, b, c\)…) should be used for special remarks.

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4. Ethical Considerations

4.1. Ethical Approval of Studies
For human or animal experimental investigations, appropriate institutional review board or ethics committee approval is required. Such approval and the approval number should be stated in the Methods section of the manuscript. For those investigators who do not have formal ethics review committees, the principles outlined in the Declaration of Helsinki as revised in 2013 should be followed (World Medical Association. Declaration of Helsinki: Ethical principles for medical research involving human subjects. Available at: https://www.wma.net/policiespost/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). For all relevant clinical transplant articles, KRCP requires authors state in the Methods section their adherence to the Declaration of Istanbul (Available at: http://www.declarationofistanbul.org/). Copies of written informed consent and Institutional Review Board (IRB) approval for clinical research should be kept. If necessary, the editor or reviewers may request copies of these documents to resolve questions about IRB approval and study conduct.

4.2. Conflicts of Interest
The corresponding author must inform the editor of any potential conflicts of interest that could influence the authors’ interpretation of the data. Examples of potential conflicts of interest include financial support from or connections to pharmaceutical companies, political pressure from interest groups, and academically related issues. Conflict of interest statements will be published at the end of the text of the article, before the References section. Please consult the Committee on Publishing Ethics guidelines (http://www.publicationethics.org/) on conflict of interest. All sources of financial support for the study should be stated in Acknowledgments (see relevant section 3.4 above).

4.3. Authorship
Authorship credit should be based on 1) conception or design, or analysis and interpretation of data; 2) drafting the article or revising it; 3) providing intellectual content of critical importance to the work described; and 4) final approval of the version to be published. Authors should meet above four conditions. The title page should include a list of each author’s role for the submitted paper.

4.4. Redundant Publication or Duplicate Submission
Submitted manuscripts are considered with the understanding that they have not been published previously in print or electronic format (except in abstract or poster form) and are not under consideration in totality or in part by another publication or electronic medium. Authors must state that neither the manuscript nor any significant part of it is under consideration for publication elsewhere or has appeared elsewhere in a manner that could be construed as a prior or duplicate publication of the same, or very similar, work.
When malpractices are found in an article submitted to KRCP, we will follow the flowchart by the Committee on Publication Ethics (COPE, https://publicationethics.org/resources/flowcharts) for settlement of any misconduct. Although the editors and referees make every effort to ensure the validity of published manuscripts, the final responsibility rests with the authors, not with KRCP, its editors, or the Korean Society of Nephrology.

5. Review Process
All submissions are sent to peer reviewers. Authors will usually be notified within 4 weeks by e-mail of whether the submitted article is accepted for publication, rejected, or subject to revision before publication. Revised manuscripts must be submitted online by the corresponding author. Failure to resubmit the revised manuscript within 3 months of the editorial decision is regarded as a withdrawal.

6. Visual Abstract Guidelines
Visual Abstracts are brief graphical summaries of Original Articles published online. They serve to summarize the work for readers and may be used in social media postings. Authors do not need to include a Visual Abstract with their initial submission but will be required to submit one at the revision stage for all original research articles. The submitted visual abstract will be reviewed along with the revised manuscript.
If the submission of visual abstract is delayed, there is inevitable delay in publication. Please submit it within the specified time.

6.1. Creating Your Visual Abstract
Select one of the visual abstract templates provided (https://www.krcp-ksn.org/file/KRCP_Visual_Abtracts_v1.0.pptx). There are multiple layouts to accommodate author preferences as well as graphical constraints. The visual abstract should
include a title, methods, outcome and a concluding sentence. Please fill in the template as it’s laid out and do not alter the basic components of the template.

Keep in mind the following:
• Avoid excessive detail and clutter and keep text to a minimum.
• Any descriptive text should be at least 12 pt font size.
• The visual abstract should be saved as an editable PowerPoint file as staff will add the article DOI and may edit the text for clarity.

6.2. Adding Visual Details
It is critical that you only use images for which you have permissions or rights. To avoid any potential problems, either use the copyright filter during an image search online or subscribe to an icon image bank. There are many image banks on the internet, which are free to use. The images used for visual abstract is recommended only open source, and the author is responsible for copyright issues of visual abstract. Researchers who frequently prepare visual abstracts may benefit from purchasing a subscription to access higher quality icons (e.g. Shutterstock, Getty Images, iStock, etc.).

Guiding principles:
• Select bold, solid color icons
• Avoid highly detailed icons as the intricacy may be lost in the small format
• Exclude trade names, logos, or images of trademarked items.
• Graphics should be 440 pixels wide by 350-365 pixels tall.

7. Peer Review
This journal operates blind review processes. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor’s decision is final. For more information, please refer to Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (Available at: http://www.icmje.org/icmje-recommendations.pdf).

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11. Data Sharing Policy

For clarification on data accuracy and reproducibility of the results, raw data or analysis data will be deposited to a public repository, for example, Harvard Dataverse (https://dataverse.harvard.edu/) after acceptance of the manuscript. Therefore, submission of the raw data or analysis data is mandatory when requested by reviewers. If the data is already a public one, its URL site or sources should be disclosed. If data cannot be publicized, it can be negotiated with the editor. If there are any inquiries on depositing data, authors should contact the editorial office.

12. After acceptance

12.1. Article-in-press publication

After the manuscript is finally accepted, it will be published online in PDF format through the English editing, author proofing and final editorial correction process. The corresponding author should promptly and appropriately respond to this editing process. Online publication will take place within several weeks depending on the proof process. A Digital Object Identifier (DOI) is allocated, making it fully citable and searchable by title, author name(s), and the full text. Since our journal is officially published every 3 months interval, the volume, issue, and page will be finally allocated sequentially according to the order of accepted articles.

12.2. Publication charges

In order to cover the costs of reviewing, copy editing, layout, and online hosting and archiving, KRCP charges an article processing fee upon acceptance of submitted original, review or special articles as follows:

- KRW 500,000 (Korea)
- USD 500 (rest of world)

There are no additional charges based on color, length, figures or other elements. No fee applies to correspondences and images in practice. The publication costs for invited papers are covered by the Korean Society of Nephrology. Payments are processed by a department unconnected to KRCP’s editorial board.

• Publication charge waiver policy

Our mission is to share the achievements in the nephrology field with researchers worldwide including the scientists in the low-income countries. We continue to apply the publication charge waiver policy to encourage the academic activity and support the limited funding for their research. To request a publication charge waiver, please send an application to registry@ksn.or.kr. Corresponding author from low-income countries could be waived. Waiver application must contain the manuscript number and country of corresponding author.
NESP®
Darbepoetin alfa

INDICATIONS
1. Renal anemia
2. Chemotherapy-induced anemia in solid cancer patients

DOSEAGE AND ADMINISTRATION
<Imediated/patients>
Initial dose
The usual dose of NESP in adult patients is 20 μg, to be administered as a single intravenous injection once weekly.

Initial dose at the switching from epoetin-a preparations: See Precautions related to Dosage and Administration

Maintenance dose
When correction of anemia is achieved, the usual dose of NESP in adult patients is 30-50 μg as darbepoetin alfa (parenteral recombinant), to be administered as a single injection once every two weeks subcutaneously or intravenously. If deviation of anemia is maintained by once every two weeks injection, the frequency of administration can be changed to once every four weeks with a initial dose set to twice-fold of the dose in the once every two weeks injection. In this case, the usual dose in adult patients is 60-100 μg administered as a single injection once every four weeks subcutaneously or intravenously. In all cases, the dose should be adjusted in view of the degree of anemia symptoms and the patients age, and should not exceed 100 μg as a single injection. The target of anemia correction is around 11 g/l of hemoglobin level.

<Precautions related to Dosage and Administration>
1. Initial dose at the switching from an epoetin-a preparation.
When NESP is started in substitution for an epoetin-a preparation, the dose and frequency of administration should be determined on the basis of the dose of the epoetin-a preparation that has been used. See the tables (package insert).
2. Patients who have been treated with an epoetin-a preparation before weekly or three times weekly. Calculations the total dose of the epoetin-a preparation administered during the week before the switching, and then determine the initial dose of NESP according to the table below. The treatment should be started on once weekly basis
3. Patients who have been treated with an epoetin-a preparation once weekly or once every two weeks. Calculate the total dose of the epoetin-a preparation administered during the two weeks before the switching, and then determine the initial dose of NESP according to the table below. The treatment should be started on once every two weeks basis. See the insert paper.
4. Dose adjustment
If dosing adjustment is required for example, when the increase in the hemoglobin concentration of the hematocrit levels can not be achieved in correction phases, or when the hemoglobin concentration is lower than the target range for successive maintenance phases, the dose should be increased or decreased according to the table below. Any dose increase should be performed stage by stage in principle.

PRECAUTIONS
See the package insert.

STORAGE
Store in a tight, light-proof container at 2-8°C and avoid freezing

PACKAGING
1 syringe, 10 syringes for NESP 20μg, 30μg, 50μg, 60μg, respectively

MANUFACTURED BY:
Takyo Pharmaceutical Co., Ltd.
1040-22 Makino Tokiyama-cho Gifu, Japan
Kyowa Hakko Kirin Co., Ltd.
100-1 Nakanoshima-cho, Tatsukokai, Gunma, Japan

IMPORTED BY:
KYOWA KIRIN

111, Aca Tower, 480, Noryangjin, Gangnam-gu, Seoul, 06229, Rep. of Korea
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MIRCERA exists because life is long.

It exists because CKD in a long life requires a long treatment.

It exists because we want to provide a prolonged stability of Hemoglobin levels along the long treatment.

It exists because we believe that a prolonged stability will overcome the long treatment and give longer hope to your longer life.

MIRCERA exists because we believe in the power of longer stability.

A long-lasting change caused by long-acting effects including Non-dialysis CKD, PD, and HD.

Purple Effect MIRCERA

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MIRCERA
methoxy polyethylene glycol–epoetin beta

Preclinical and clinical development of a novel erythropoietin stimulant, MIRCERA. What's the future for patients with ESRD? The future is now. MIRCERA (methoxy polyethylene glycol–epoetin beta) is a novel erythropoietin stimulating agent that targets the hematopoietic cells and increases the production of red blood cells, leading to an increase in hemoglobin levels.

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It exists because CKD in a long life requires a long treatment.

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Purple Effect MIRCERA

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MIRCERA pre-filled syringe
Presentation medicine, identification number 249

MIRCERA
methoxy polyethylene glycol–epoetin beta

Preclinical and clinical development of a novel erythropoietin stimulant, MIRCERA. What's the future for patients with ESRD? The future is now. MIRCERA (methoxy polyethylene glycol–epoetin beta) is a novel erythropoietin stimulating agent that targets the hematopoietic cells and increases the production of red blood cells, leading to an increase in hemoglobin levels.
4. Undesirable Effects

- Acute renal failure
- Dual blockade of RAAS is therefore not recommended. If dual blockade therapy is considered, it should be carefully monitored, and appropriate adjustments should be made to manage blood pressure. 5) Patients with ischemic or cerebrovascular disease. 6) Hepatic failure. 7) Hypertropic cardiomyopathy

5. Contraindications

- Hypersensitivity to the active substance,
- Renal artery stenosis or stenosis of the artery to a solitary kidney, who have been treated with ACE inhibitors, may have an increased risk of severe hypotension and renal insufficiency. In these patients, treatment should be started with extreme caution and with frequent monitoring of serum potassium. (13) Diabetic patients:
- Diabetic patients: ACEI and ARB should not be used concomitantly in patients with diabetic nephropathy. 17) Patients who administer Neprilysin(NEP) inhibitor or within 36 hours after discontinuation.

6. Interactions

- Combination with other antihypertensive medications should be avoided.
- ACE inhibitors should be stopped immediately, and, if appropriate, alternative therapy should be started. (2) Breastfeeding: Alternative treatments with better established safety profiles during breast-feeding are preferable.

7. Pregnancy and Breastfeeding

- Pregnancy or potential pregnancy, lactation. 7) The drug is usually not administered in case of combinations with diuretics.

8. Drive and use machine:

- CAD
- Hypertension

9. Mise and storage

- Store at 15-30°C, protected from light. Do not freeze.
- Unused portion should be discarded, and the remaining portion should be used within 2 years of manufacture.

10. Special care:

- Antidiabetic agents (insulins, oral hypoglycaemic agents), Baclofen, Non-potassium sparing diuretics, potassium-sparing diuretics, ACE inhibitors, ARBs, NSAIDs, heparins, immunosuppressant agents such as cyclosporin, and other drugs that may interact with potassium metabolism should be used with caution and with frequent monitoring of serum potassium. (13) Diabetic patients:
- Diabetic patients: ACEI and ARB should not be used concomitantly in patients with diabetic nephropathy. 17) Patients who administer Neprilysin(NEP) inhibitor or within 36 hours after discontinuation.

11. Administration and dosage

- Starting dose of 2.5mg once daily, depending on renal function and whether the 5 mg dose is well tolerated. - Elderly: 2.5mg once daily
- Renal impairment: Clcr ≥ 60 ml/min: 5 mg/day; 30 < Clcr < 60 ml/min: 2.5 mg/day; 15 < Clcr < 30 ml/min: 1.25 mg/day
- Perindopril should be used with extreme caution in patients with collagen vascular disease, immunosuppressant therapy, treatment with gold, and other immunosuppressant agents.

12. Pharmacological properties

- Antihypertensive agents, Vasodilators, Gliptines (linagliptine, saxagliptine, sitagliptine, vildagliptine), Tricyclic antidepressants, Antipsychotics, Anesthetics, Sympathomimetics, Gold.

13. Clinical studies

**ACERTIL ARGININE TAB. 5mg:** Light-green, rod-shaped film-coated tablet engraved with the company logo on one face. **ACERTIL ARGININE TAB. 10mg:** Green, round, biconvex, film-coated tablet engraved with 10 on one face and the company logo on the other face.

**DESCRIPTION**

- **Active Substance:** Argenine 5 mg / 10 mg.
- **Pharmaceutical Form:** Film-coated tablet.
- **Uses:** Indicated for the treatment of essential hypertension in adults.

**PHARMACOLOGICAL PROPERTIES**

- **Pharmacological Class:** Angiotensin-converting enzyme (ACE) inhibitor.
- **Mechanism of Action:** Converts angiotensin I into angiotensin II, a potent vasoconstrictor.
- **Clinical Effects:** Reduces blood pressure by decreasing renin release and decreasing circulating angiotensin II levels.

**INDICATIONS**

- **Hypertension:** Used for the treatment of essential hypertension in adults.

**CONTRAINDICATIONS**

- Hypersensitivity to the active substance or any component of the product.
- Moderate to severe renal impairment (creatinine clearance < 30 ml/min).
- Hypersensitivity to ACE inhibitors.
- Thrombocytopenia, aplastic anemia, or severe neutropenia.
- Primary aldosteronism.
- Angioedema, especially in patients with a history of angioedema.

**WARNINGS**

- **Hypotension:** May cause symptomatic hypotension, especially in patients with poor renal function or those taking other hypotensive agents.
- **Surgery or Anaesthesia:** Discontinue at least one day prior to surgery.
- **Volume Expansion:** May cause volume expansion and hypertension.

**ADVERSE REACTIONS**

- **Common (≥1/100, <1/10):** Headache, dizziness, vertigo, paraesthesia, visual disturbances, tinnitus, hypotension and effects on sense of taste.
- **Uncommon (≥1/1,000, <1/100):** Eosinophilia, hypoglycaemia, confusion, angina pectoris, arrhythmia, myocardial infarction, pulmonary oedema, phlebitis, hyperkalaemia, hypernatremia, acute renal failure, haemolytic anaemia in patients with a congenital deficiency of G-6PDH, confusion, angina pectoris, arrhythmia, myocardial infarction, pulmonary oedema, phlebitis, hyperkalaemia, hypernatremia, acute renal failure.

**PHARMACODYNAMICS AND PHARMACOKINETICS**

- **Onset of Action:** Within 1-2 weeks.
- **Duration of Action:** Unknown.
- **Metabolism:** Hepatic.
- **Excretion:** Renal.

**INTERACTIONS**

- **ACE Inhibitors & ARBs:** Increased risk of hypotension, hyperkalaemia and decreased renal function.
- **Diuretics:** May decrease the antihypertensive effect of ACE inhibitors.
- **Potassium-Sparing Diuretics:** May increase serum potassium levels.
- **Symptomatic Hypotension:** Initiation of therapy and dose adjustment should be performed under close medical supervision.

**DOSAGE AND ADMINISTRATION**

- **Hypertension:** Initially 2.5 mg once daily.
- **Congestive Heart Failure:** Initial dose: 2.5 mg once daily.
- **Acute Coronary Syndrome:** 5 mg once daily.
- **Adjuvant Treatment with Non-Potassium-Sparing Diuretics and Digitalis Treatment:** 2.5 mg once daily.

**PACKAGING**

- **Stability:** Store at 2-8°C.

**MEDICAL INFORMATION INQUIRY**

- **For your patients of Hypertension**
- **For your patients of CAD**
- **For your patients of Heart Failure**

**INITIATE ACERTIL® EARLY!**

**ACERTIL® 4mg, 8mg**

**ACERTIL® 5mg, 10mg**

**ARGinine**
Futhan is an anticoagulant during extracorporeal blood circulation in patients with bleeding complications or bleeding tendency.¹

- Due to its short half life (5~8 min), its anticoagulant activity is almost limited to extracorporeal circuit.²,³,⁴
- Increase of bleeding risk was not noted in HD patients with bleeding risk.⁵,⁶,⁷
- The filter-life is significantly prolonged during CRRT.⁸,⁹,¹⁰

Summary of Prescribing Information¹

PRODUCT NAME IN KOREA: Futhan for Inj. (nafamostat mesilate) • Futhan50 for Inj. (nafamostat mesilate) INGREDIENT: Futhan for Inj.: 1 vial contains 10mg of nafamostat mesilate • Futhan50 for Inj.: 1 vial contains 50mg of nafamostat mesilate INDICATION AND USAGE: 1. For improvement of acute symptoms of pancreatitis (acute pancreatitis, acute exacerbation of chronic pancreatitis, acute postoperative pancreatitis, ERCP–induced acute pancreatitis, traumatic pancreatitis) • Futhan for Inj., only 2. Disseminated intravascular coagulation (DIC) 3. To prevent coagulation of blood during extracorporeal blood circulation (ex. hemodialysis, plasmapheresis) in patients with bleeding complications or bleeding tendency. DOSAGE AND ADMINISTRATION: 3. To prevent coagulation of blood during extracorporeal blood circulation (ex. hemodialysis, plasmapheresis) in patients with bleeding complications or bleeding tendency. For priming, wash and fill the blood route with 20mg of nafamostat mesilate dissolved in 500mL of saline after dissolving in the small amount of 5% glucose solution or water for injection. After beginning of extracorporeal circulation, inject continuously at a rate of 20–50mg/hr as nafamostat mesilate dissolved in 5% glucose solution into anticoagulant injection line. The dosage should be appropriately adjusted according to the patient’s symptoms. The average dosage from clinical study is 35mg/hr as nafamostat mesilate. MANUFACTURED by Yuhan corporation. DISTRIBUTED by SK chemicals.

We provide one-stop service by building an integrated pipeline.

We always put the patient's health first and care for the whole life.

We devote for continuous product development and service improvement.

We work with therapists to find the optimal solution.
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조성
1바이알(30mL)중 에 kull주맙 300mg
효능·효과
1) 발작성 야간 혈색소뇨증(PNH : Paroxysmal Nocturnal Hemoglobinuria) 용혈을 감소시키기 위한 발작성 야간 혈색소뇨증(PNH : Paroxysmal Nocturnal Hemoglobinuria) 환자의 치료, 수혈이외의 치료가 없음을 의미하는 임상 증상이 있는 환자의 용혈에 임상적 이익이 확립되었다.
2) 비정형 용혈성 요독 증후군(aHUS : atypical Hemolytic Uremic Syndrome) 보체 매개성 혈전성 미세혈관병증을 억제하기 위한 비정형 용혈성 요독 증후군(aHUS : atypical Hemolytic Uremic Syndrome) 환자의 치료
사용제한 : 시가(Shiga) 톡신 생성 대장균에 의한 용혈성 요독 증후군(STEC-HUS) 환자 대상의 적용을 권장하지 않는다.
3) 전신 중증 근무력증(Generalized Myasthenia Gravis) 항아세틸콜린 수용체 항체 양성인 환자의 불응성 전신 중증 근무력증(Refractory gMG: Refractory Generalized Myasthenia Gravis)의 치료
4) 시신경 척수염 범주 질환(Neurologic Myelitis optica spectrum disorder) 항아쿠아포린-4(AQP-4) 항체 양성인 환자의 시신경 척수염 범주질환(NMOSD: Neuromyelitis optica spectrum disorder)의 치료
용법·용량
심각한 감염에 대한 위험을 줄이기 위해서 환자들은 최신의 백신 접종 지침(Advisory Committee on Immunization Practices(ACIP) recommendations)에 따라 백신 접종을 해야 한다.(사용상의 주의사항 1. 경고 항 참고) 이 약은 정맥투여되어야 하며 급속정맥투여(IV push) 또는 일시정맥투여(IV bolus)로 투여해서는 안된다. [성인] 1) 발작성 야간 혈색소뇨증(PNH) : 첫 4주간은 매 7일마다 600 mg, 네 번째 용량 투여 7일 후에 다섯 번째 용량으로 900 mg을 투여하고, 그 후부터는 매 14일마다 900 mg을 투여한다. 2) 비정형 용혈성 요독 증후군(aHUS) 및 불응성 전신 중증 근무력증(Refractory gMG) 및 시신경 척수염 범주질환(NMOSD) 환자 : 첫 4주간은 매 7일마다 900 mg, 네 번째 용량 투여 7일 후에 다섯 번째 용량으로 1200 mg을 투여하고, 그 후부터는 매 14일마다 1200 mg을 투여한다. <소아> 1) 비정형 용혈성 요독증후군(aHUS) 만 18세 미만의 aHUS 환자일 경우, 체중에 따라 권장 일정으로 투여한다. (제품정보 원문 용법·용량 [표 1] 만 18세 미만 환자에서의 권장투여법 참고) 이 약은 권장 투여량과 일정에 맞게 투여, 혹은 예정된 일정의 2일 전/후로 투여되어야 한다. <혈장교환요법 및 신선 동결혈장투여시> 성인 및 소아 비정형 요독증후군, 성인 불응성 전신 중증 근무력증 및 시신경 척수염 범주질환 환자에 대해 PE/PI(혈장 교환 요법(plasma exchange 또는 plasmapheresis), 또는 신선 동결 혈장 투여(fresh frozen plasma infusion))와 같은 부수적 시술을 받는 경우 추가 용량 투여가 필요하다. (제품정보 원문 용법·용량 [표 2] PE/PI 이후 이 약의 추가적 투여법 참고)
사용상의 주의사항
1. 경고
중대한 수막구균 감염 작용기전으로 인하여 이 약의 사용은 중대한 수막구균 감염(패혈증 그리고/또는 뇌수막염)에 대한 환자의 감수성을 증가시킨다. 이 약의 투여 환자에게서 치명적이고 생명을 위협하는 수막구균 감염이 발생하였다. 수막구균 감염은 어느 혈청군에 의해서도 발생할 수 있지만, 이 약의 투여 환자들은 흔하지 않은 혈청군(X 등)에 의한 질환이 발생할 수 있다. 감염의 위험성을 낮추기 위하여, 이 약의 치료가 지연됨으로 인한 위험성이 수막구균 감염 발생의 위험성보다 큰 경우를 제외하고는 모든 환자들은 반드시 이 약의 투여 시작 최소한 2주 전에 수막구균 백신을 투여 받아야 한다. 만약 접종 받지 않은 환자가 긴급히 이 약의 치료를 받아야 하면, 최대한 빨리 수막구균 백신을 투여 받도록 한다. 수막구균 백신 접종 후 2주 이내 이 약을 투여할 경우, 4가 수막구균 백신 접종 후 2주 동안 적절한 예방적 항생요법으로 치료 받아야 한다. 흔한 병원성 수막구균 혈청군을 예방하기 위하여 가능하다면 혈청군 A, C, Y, W135, B에 대한 백신이 권장된다. 환자들은 백신 사용을 위한 최신의 백신 접종 지침(Advisory Committee on Immunization Practices(ACIP) recommendations)에 따라 백신을 접종/재접종 받아야 한다. 백신 접종은 보체를 더욱 활성화시킬 수 있다. 결과적으로, PNH, aHUS, 불응성 gMG 및 NMOSD를 포함한 보체 매개 질환을 가진 환자들은 용혈(PNH의 경우)이나 혈전성 미세혈관병증(TMA; aHUS의 경우) 또는 중증 근무력증의 악화(불응성 gMG의 경우) 또는 재발(NMOSD의 경우)과 같은 그들의 기저 질환의 징후 및 증상이 증가하는 경험이 할 수 있다. 따라서, 지침에 따른 백신 접종 이후 질환의 증상에 대해 면밀히 관찰되어야 한다. 백신 접종은 수막구균 감염 위험을 줄일 수 있지만, 완전히 없애지는 않는다. 적절한 항생제 사용에 대한 공식 지침(예: 국내 성인 세균성 수막염의 임상진료지침 권고안 등)을 고려하여야 한다. 수막구균 감염은 초기에 발견하고 치료하지 않으면 급격히 치명적이고 생명을 위협하게 될 수 있다. 중대한 수막구균 감염을 치료받는 환자는 이 약의 투여를 중지하도록 한다.
2. 다음 환자에는 투여하지 말 것
1) 이 약의 주성분, 뮤린 단백질 또는 기타 구성성분에 과민반응이 있는 환자 2) 치료되지 않은 중대한 수막구균(Neisseria meningitidis) 감염 환자 3) 수막구균(Neisseria meningitidis) 백신을 현재 접종하지 않은 환자 또는 백신 접종 이후 2주 동안 적절한 예방적 항생요법으로 치료를 받지 않은 환자(이 약의 치료를 늦추는 것이 수막구균 감염을 일으키는 것보다 중대하지 않은 경우)
3. 다음 환자에는 신중히 투여할 것
1) 기타 전신 감염: 작용기전으로 인하여 이 약의 치료는 활성 전신 감염이 있는 환자들에게 주의하여 투여하여야 한다. 이 약은 말단 보체 활성을 차단하므로 환자들은 감염, 특히 Neisseria균 및 피낭성 세균(encapsulated bacteria) 감염에 대한 감수성이 증가할 수 있다. 파종성 임균 감염을 포함하는 N. meningitidis 외의 Neisseria 종에 의한 중대한 감염이 보고되었다. 잠재적인 중대한 감염과 그 증상 및 징후에 대한 인식을 높이기 위하여 환자용 정보 안내서의 정보를 환자에게 제공해야 한다. 임질 예방에 관해 환자에게 조언해야 하고 위험성이 있는 환자는 정기적인 검사를 권고한다. 더욱이, 면역력이 약화된 환자와 호중구 감소 환자에서 아스페르길루스 감염이 발생하였다. 이 약을 투여 받는 소아는 폐렴연쇄상구균(Streptococcus pneumoniae)과 인플루엔자 간균 B형(Haemophilus influenza type b(Hib))에 의한 중대한 감염을 일으킬 위험이 증가할 수 있다. 폐렴연쇄상구균(Streptococcus pneumoniae)과 인플루엔자 간균 B형(Haemophilus influenza type b(Hib))에 의한 감염을 예방하기 위해 최신의 백신 접종 지침에 따라 백신 접종을 받도록 한다. 전신 감염이 있는 환자에게 이 약을 투여할 때는 주의하도록 한다. 에 kull주맙에 안정되고 유지 요법을 받는 환자에게 추가적인 백신 접종이 필요한 경우, 이 약 투여에 따른 백신 접종 시기를 신중히 고려해야 한다.
4. 약물이상반응
시판 후 보고 및 완료된 임상시험에서 보고된 약물이상반응(발생률 1% 이상 발췌): 매우 흔하게(≥1/10) – 두통, 흔하게(≥1/100 ~ <1/10) - 폐렴, 상기도감염, 비인두염, 기관지염, 요로 감염, 구강 헤르페스, 백혈구감소증, 빈혈, 불면, 현기증, 미각이상, 고혈압, 기침, 입인두통, 설사, 구토, 구역, 복부통증, 발진, 탈모, 소양증, 관절통, 근육통, 열, 피로감, 인플루엔자 유사질환모든 임상시험에서, 가장 중대한 이상반응은 수막구균 패혈증이었고, 이는 이 약으로 치료받은 환자에서 수막구균 감염증의 흔한 증상이었다. 수막구균 패혈증의 징후와 증상에 대해 환자에게 알리고 즉시 의료 조치 받을 것을 환자에게 권고해야 한다. Neisseria gonorrhoeae, Neisseria sicca / subflava, Neisseria spp unspecified로 인한 패혈증을 포함하여 Neisseria 종의 다른 사례들이 보고되었다.
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\textsuperscript{1} Prograf®: Product Information (last updated 2020.05.14).

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