Kidney Health Plan 2033 in Korea: bridging the gap between the present and the future

Status and trends in epidemiologic characteristics of diabetic end-stage renal disease: an analysis of the 2021 Korean Renal Data System

Greenness and kidney? A review of epidemiological studies on the association between green space and kidney disease

Frequency of Fabry disease in chronic kidney disease patients including patients on renal replacement therapy in Korea

Significance of C4d expression in peritubular capillaries concurrent with microvascular inflammation in for-cause biopsies of ABO-incompatible renal allografts
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.

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The image on the front cover: Kim et al provided the kidney health plan 2033 in Korea. Schematic illustration for the three goals and four action plans to enhance kidney health in Korea. Please see the text for more details (pp. 8-19).
Cho et al. [1] conducted a study in Korea that is considered the first attempt to utilize plasma globotriaosylsphingosine (lyso-Gb3) concentration as a screening tool for Fabry disease (FD) in chronic kidney disease (CKD) patients, including those undergoing dialysis. The authors found a prevalence rate of FD in these patients to be 0.11%, consistent with rates reported in other studies. Previous research has indicated prevalence rates of approximately 0.2% to 0.95% for CKD patients and 0% to 1.69% for male hemodialysis patients [2, 3]. Utilizing lyso-Gb3 for screening purposes proves to be a valuable method for identifying FD cases within the CKD population with unknown etiology.

FD is a rare X-linked lysosomal disorder caused by a genetic mutation in the GLA gene. This gene codes for the enzyme α-galactosidase A (α-Gal A), and its deficiency leads to the accumulation of complex glycosphingolipids, especially globotriaosylceramide (Gb3) and its metabolites such as lyso-Gb3, in various tissues and organs. Fig. 1A shows the synthesis from ceramide to Gb3, which is useful in cellular membranes. The pathogenesis of Gb3 and lyso-Gb3 in FD patients depicted in Fig. 1B, Gb3 and lyso-Gb3 accumulation in various tissues leads to impairment of autophagy, which, in turn, induces mitochondrial dysfunction, increased apoptosis, and inflammatory immune responses [4]. Studies have demonstrated that Gb3 accumulation in various tissues leads to a dose-dependent increase in reactive oxygen species production in cultured vascular endothelial cells from individuals with FD [5]. Additionally, an in vitro study has shown the production of transforming growth factor beta 1 when normal podocytes are exposed to lyso-Gb3 [6] and plasma lyso-Gb3-induced proliferation of vascular smooth muscle cells, which leads to inflammation and fibrosis [7]. Furthermore, exposure to lyso-Gb3 has been shown to cause podocyte injury, glomerular fibrosis, and inflammation [6].

The clinical features of FD are diverse and can vary in severity depending on the age of onset and the degree of enzyme deficiency. Typical clinical features of this disorder include angiookeratoma, corneal verticillata, hypohidrosis, peripheral neuropathy, recurrent diarrhea, abdominal pain, ventricular hypertrophy, progressive kidney dysfunction, and cryptogenic stroke. FD phenotypes can be classified as classic, characterized by typical multiorgan involvement, and later-onset (or variant), predominantly affecting specific organs. Classic phenotype males showed that α-Gal A activity may be very low (or absent), develop symp-
Figure 1. Schematic representation elucidating the pathogenesis of Fabry disease. (A) Schematic representation of the synthesis from ceramide to globotriaosylceramide (Gb3). (B) Gb3 and its deacylated form, globotriaosylsphingosine (lyso-Gb3), which leads to increased apoptosis, oxidative stress, and inflammation. Lyso-Gb3 in the plasma activates transforming growth factor beta (TGF-β) and promotes the proliferation of vascular smooth muscle cells, thereby inducing inflammation and fibrosis. α-Gal A, α-galactosidase A.
toms earlier, and show a more severe presentation. But the pattern of organ-specific X-chromosome inactivation in heterozygous females showed that α-Gal A activity may be low-normal or variably deficient with variable symptoms [8]. Late-onset FD patients may present with nonspecific symptoms and signs.

Since FD can potentially be a cause of CKD with an unknown origin, it is recommended to test any patient suspected of having FD (Fig. 2). However, there may be differences in how this condition presents itself in males

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**Figure 2. Screening of Fabry disease in chronic kidney disease (CKD) with unknown origin.** For males suspected of Fabry disease, it is crucial to measure α-galactosidase A (α-Gal A) activity as a diagnostic indicator. Genetic testing serves as both a confirmatory and primary screening method for females. Additionally, evaluating plasma globotriaosylsphingosine (lyso-Gb3) levels can provide valuable insights into the assessment of females.
and females. In males suspected of having FD, it is important to measure α-Gal A activity. An α-Gal A activity level of less than 1% highly suggests classic FD. The diagnosis of FD involves measuring α-Gal A activity in plasma, dried blood spots (DBS), and leukocytes. While plasma is commonly used, some cases may yield inconclusive results, highlighting the need for accurate sample processing. DBS offers similar performance with easier collection and a lower cost, although it may lack accuracy in certain cases. Leukocytes provide a definitive diagnosis but require complex processing and are more expensive. Therefore, careful sample processing and selection are crucial, considering the advantages and disadvantages of plasma, DBS, and leukocytes.

On the other hand, in females suspected to have FD, the α-Gal A activity can vary and may fall within the normal range. Genetic testing is a confirmatory and primary screening method for females; however, it can be costly. Therefore, there is a need for biomarkers that can be used for FD screening and diagnosis in individuals with normal or borderline α-Gal A activity. Lyso-Gb3, a hydrophilic deacylated form of Gb3, has emerged as a promising biomarker. It is detected at high levels in the plasma of FD patients and shows superiority over Gb3 as a diagnostic and prognostic biomarker [9].

In disease progression, respond more rapidly than Gb3, and have been linked to clinical events and disease burden [7]. Because it is a smaller molecule, lyso-Gb3 may freely reach cells, which enables it to be detected more readily [9]. Lyso-Gb3 levels vary among individuals with different disease severity and genotypes, indicating their potential as an indicator of disease severity and different phenotypic presentations [9]. Even when typical symptoms of FD are present, diagnosing the condition can be challenging if the underlying genetic abnormality is unknown. Understanding the characteristics of lyso-Gb3, as mentioned earlier, would be beneficial for diagnosing such patients as well. Assessing plasma lyso-Gb3 levels can help evaluate disease severity and aid in the diagnosis of patients with genetic variants of unknown significance [10]. Hence, if there is a family history of FD in females, genetic testing should be performed next. However, in the absence of family history, it may be considered to perform genetic testing if there is an increase in lyso-Gb3 levels.

To summarize, early detection of FD is essential for improving patient outcomes and safeguarding the health of their family members. Therefore, it is crucial for physicians to have an effective screening tool. The lyso-Gb3 test, conducted by the researchers, is a laboratory test currently available for experimental purposes in domestic settings. The study’s findings underscore the significant role of lyso-Gb3 in FD screening and the establishment of screening criteria within the country, especially for females and late-onset FD. It is hoped that this research will pave the way for broader insurance coverage of lyso-Gb3 as a diagnostic technique for FD screening in the future.

Conflicts of interest

All authors have no conflicts of interest to declare.

Data sharing statement

The data presented in this study are available on request from the corresponding author.

Authors’ contributions

Conceptualization: SHK
Writing–original draft, Writing–review & editing: All authors
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The pathological diagnosis of antibody-mediated rejection (ABMR) in allograft kidneys has posed a significant challenge for renal pathologists. According to the latest version of the Banff classification, the diagnosis of ABMR (active and chronic active) requires three categories of evidence: histologic evidence of acute tissue injury, evidence of antibody interaction with endothelial cells, and serologic evidence (presence of circulating donor-specific antibodies [DSA]) [1]. Initially, evidence of antibody interaction referred to linear C4d staining in peritubular capillaries or medullary vasa recta. Based on subsequent studies, it has been revealed that microvascular inflammation (MVI), specifically glomerulitis and peritubular capillaritis, can substitute for C4d [2,3]. This discovery has led to the emergence of C4d-negative ABMR [4].

Although ABO-incompatible (ABOi) kidney transplantation is a significant advancement against the shortage of donor kidneys, from the perspective of diagnosing ABMR, ABOi kidney transplantation presents unique challenges. In a previous study, C4d positivity in peritubular capillaries was observed in 94% (diffuse in 66%) of the protocol biopsies without connection with ABMR [5]. Therefore, C4d status cannot be used as reliable evidence of antibody-mediated tissue injury. Moreover, the current Banff classification does not specifically address ABOi transplantation, and the molecular diagnostics suggested by the Banff meeting [6] are not yet widely available in most transplantation centers.

In this issue of Kidney Research and Clinical Practice, Cho et al. [7] retrospectively analyzed for-cause allograft renal biopsies. The researchers found that C4d positivity was associated with more pronounced glomerulitis, peritubular capillaritis, and MVI. Among cases with a moderate or higher degree of MVI, the group with positive DSA, and the group with negative DSA but positive C4d showed similar estimated glomerular filtration rates and graft survival. Based on these results, it can be inferred that if there is a moderate or higher degree of MVI and C4d is positive, even if DSA is negative, it is reasonable to consider it as ABMR and initiate appropriate treatment. This is the key message of this article.

Indeed, the limitations of this study are evident. Firstly, since it was based on a single center, the sample size included in the study was limited. As a result, it was not possible to draw conclusions regarding the group with only MVI in the absence of both DSA and C4d, which is a clinically significant question. It is also important to consider that the study focused solely on for-cause biopsies as the study population. This aspect should be taken into account when interpreting the results. Despite these limitations, this study has diligently performed its role in fitting a small
puzzle piece necessary to solve the challenging question of diagnosing ABMR in ABOi kidney transplantation. There are still numerous puzzle pieces that need to be filled. For instance, how should we approach ABOi transplant patients who exhibit acute tubular injury and C4d positivity but lack MVI [8]? We still need a clear conclusion on this matter. I hope for a future where research similar to this paper is conducted more actively, providing clear evidence for diagnosing ABMR in ABOi kidney transplantation patients and enabling appropriate treatment.

**Conflicts of interest**

The author is an Editorial Board member of *Kidney Research and Clinical Practice*, the official journal of the Korean Society of Nephrology, serving as an Associate Editor. There are no other conflicts of interest to be declared.

**Data sharing statement**

The data presented in this study are available on request from the corresponding author.

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Kidney Health Plan 2033 in Korea: bridging the gap between the present and the future

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In response to the increase in the prevalence of chronic kidney disease (CKD) in Korea, the growth of patients requiring renal replacement therapy and the subsequent increase in medical costs, the rapid expansion of patients with end-stage kidney disease (ESKD), and the decrease in patients receiving home therapy, including peritoneal dialysis, the Korean Society of Nephrology has proclaimed the new policy, Kidney Health Plan 2033 (KHP 2033). KHP 2033 would serve as a milestone to bridge the current issues to a future solution by directing the prevention and progression of CKD and ESKD, particularly diabetic kidney disease, and increasing the proportion of home therapy, thereby reducing the socioeconomic burden of kidney disease and improving the quality of life. Here, we provide the background for the necessity of KHP 2033, as well as the contents of KHP 2033, and enlighten the Korean Society of Nephrology’s future goals. Together with patients, healthcare providers, academic societies, and national policymakers, we need to move forward with goal-oriented drive and leadership to achieve these goals.

Keywords: Chronic kidney failure, Diabetic nephropathies, Kidney Health Plan 2033, Korean Society of Nephrology

Introduction

Chronic kidney disease (CKD) is a major public health issue that causes early death and poor quality of life. The global prevalence of CKD is approximately 10%; however, it varies by region, country, and study design, reaching up to 13.4% in systematic reviews and meta-analyses [1–3]. Furthermore, CKD has emerged as a leading cause of mortality, accounting for 1.2 million deaths globally. As a cause of death, it has ranked from being the 19th leading cause in 2013 to the 12th in 2017 and is expected to rank 5th in 2040 [3]. Aside from mortality, disease burden is an essential value in determining health policy. The burden of CKD is increasing in all aspects of disease burden, including...
incidence, prevalence, mortality, and disability-adjusted life years, particularly serious in low- and lower-middle-income countries [4–6].

As diabetes mellitus (DM) and hypertension are the leading causes of CKD in most areas of the world, CKD prevalence is expected to increase with the growing incidence of DM and increase in the elderly population. The current state of CKD in Korea is comparable with the global status of CKD. The Korean National Health and Nutrition Examination Survey (KNHANES) data shows that the prevalence of CKD (estimated glomerular filtration rate [eGFR] of <60 mL/min/1.73 m² and/or albuminuria) among adults aged >18 years in 2012 was 8.0%, which has increased to 8.4% by 2021 [7]. Moreover, based on the data from the United States Renal Data System (USRDS), the incidence of treated end-stage kidney disease (ESKD) in Korea (355 per million population [PMP] in 2020) was the fourth highest globally, and Korea (9.7 PMP) had the largest average yearly increases in the incidence of treated ESKD attributed to DM between 2010 and 2020 worldwide [8].

In response to the high ESKD incidence, particularly diabetic kidney disease (DKD), the disproportional expansion of hemodialysis (HD) patients, and the resulting increase in medical costs in Korea to date, the Korean Society of Nephrology (KSN) recently suggested the “Kidney Health Plan 2033 (KHP 2033)” for national kidney health. KHP 2033 established the objective of “leading a healthy society through improvement of public kidney health” and provided a vision of “active prevention, treatment, and management of CKD,” “reduction of socioeconomic burden,” and “increased quality of patient-centered treatment.”

In this special article, we first discuss the current state of CKD and ESKD in Korea, focusing on DKD prevalence and medical costs. Moreover, we provide current statistics on home therapy in Korea, including peritoneal dialysis (PD) and kidney transplants. Lastly, we suggest missions and action plans for KHP 2033 as a milestone to bridge the gap between the present and the future.

The current challenges in kidney health in Korea

Increasing chronic kidney disease and end-stage kidney disease prevalence

As stated in the KSN fact sheet, it is estimated that one in every nine adults in Korea has CKD, with a total patient population of around 4.6 million [9]. CKD has an extremely high severity that patients with CKD have a 7.2-fold higher risk of death than normal individuals. According to the data of the National Health Insurance Service and the Health Insurance Review and Assessment Service (HIRA), CKD was the disease with the highest medical cost per patient. In 2021, 8,369,000 Korean won (KRW; 7,692 US dollars) was spent for the treatment of one patient, which was more than twice the amount of 3,719,000 KRW (3,418 US dollars) for malignant disease, which ranks second in terms of medical cost per patient. As CKD progressed, the cost of treatment per person increased. Compared with a patient with hypertension, the cost was 3-, 5-, and 21-fold higher in patients with stages 3, 4, and 5 predialysis CKD, respectively. In 2021, the total cost of reimbursement on CKD was over 2.3 trillion KRW (2.08 billion US dollars) in Korea.

As reported by the 2022 Korean Renal Data System (KORDS) of the KSN, the number of patients with ESKD receiving renal replacement therapy (RRT) in Korea reached 127,068 (Fig. 1) [10]. Among them, 78% were receiving HD, and 3.0 trillion KRW (2.71 billion US dollars) was spent on dialysis treatment annually [11].

Compared with other countries, the increasing trend of CKD in Korea is serious. According to the 2022 Annual Report of the USRDS, comparing 2010 and 2020, the average annual increase in ESKD incidence was 18.8 PMP, the second highest in the world following Thailand (Fig. 2A). Particularly, the average annual increase in the number of patients with ESKD due to DM was 9.7 PMP, the highest among all countries surveyed. Among countries or regions with the highest percentage increase in 2019–2020 vs. that in 2010–2011, the prevalence of ESKD treated in Korea more than doubled over this period (2010 vs. 2020) (Fig. 2B) [8].

Diabetic kidney diseases: a lethal and progressive burden to the Korean society

In Korea, as DKD was the main cause of ESKD, we focused on the current status of DKD and ESKD epidemics. Furthermore, we analyzed the medical cost of HD patients depending on diabetic status based on HIRA data.
Diabetic kidney disease epidemiology in Korea: from big data

A study reported the DKD prevalence in Korea by analyzing the KNHANES V conducted in 2011 [12]. In this study, albuminuria was defined as >30 mg/g and CKD was defined as an eGFR of <60 mL/min/1.73 m². Among participants with DM, 26.7% and 8.6% had albuminuria and CKD, respectively. DM increased the risk of albuminuria and CKD by 2.5 and 1.8 times, respectively [12]. The age-standardized rate of DKD prevalence in 2015 was 12,400 per 100,000 individuals in patients with DM, 12,900 per 100,000 in males and 11,800 per 100,000 in females. In patients with DM aged ≥ 30 years, DKD prevalence increased from 8.4% to 12.4% between 2006 and 2015 [13]. Additionally, the incidence of ESKD among the diabetic population was 13.8-fold higher than that among the nondiabetic population [13]. In this study, as of 2015, the standardized incidence rates (per 100,000) of ESKD in diabetic and nondiabetic individuals were 109.0 and 7.9, and the standardized prevalence rates (per 100,000) were 860.8 and 39.3, respectively. Furthermore, DM is associated with a poor prognosis in patients with CKD. In a study with the KNOW-CKD cohort, patients with DM had higher risks of renal function deterioration, ESKD, cardiovascular complications, and death than those without DM [14].

The comparison of USRDS international data revealed that the incidence of ESKD due to DM was 177 PMP in 2020, the fourth highest among 45 countries and the third highest among 12 Asian countries. Between 2010 and 2020, the average annual increase in the incidence of diabetic ESKD was 9.7 PMP, which was the highest among 37 countries. Compared with 2010, the overall ESKD incidence percentage increased by 96.2% in 2020, showing a high correlation with the 84.6% increase in ESKD incidence due to DM. This suggests that DM has recently led to an increase in patients with ESKD in Korea [8].

Status of diabetic end-stage kidney disease: data from the Korean Renal Data System

As in other countries, DM is the most significant cause of ESKD in Korea. According to KORDS data, it has maintained the highest ranking since 1994 and accounted for...
Figure 2. Prevalence of treated ESKD in countries or regions with the largest percentage increase in prevalence, 2010 vs. 2020 (data from 2022 USRDS annual data report). (A) Average yearly change in the incidence of treated ESKD by country or region, 2010 vs. 2020. (B) Prevalence of treated ESKD in countries or regions with the largest percentage increase in prevalence, 2010 vs. 2020. ESKD, end-stage kidney disease.

Data sources: https://usrds-adr.niddk.nih.gov/2022/end-stage-renal-disease/11-international-comparisons
47.8% of the causes of ESKD in 2022 [10]. DM was followed by hypertension and glomerulonephritis, with percentages of 21.5% and 9.4%, respectively.

The trends observed in the epidemiologic characteristics of diabetic ESKD in Korea after analyzing the 2021 KORDS data (unpublished data) revealed that the proportion of diabetic and nondiabetic ESKD was 50.3% and 49.7%, respectively, in HD patients, whereas they were 45.5% and 54.5%, respectively, in PD patients. The trend for all-cause mortality showed a gradual decrease since 2001. The decreasing trend of mortality is similar in both HD and PD patients. In the analysis of the cause of death, cardiac origin was 38.4% in patients with diabetic ESKD, which was higher than 30.3% in patients with nondiabetic ESKD. Patients with diabetic ESKD had more comorbidities, including cardiac disease, vascular disease, and pneumonia, than those with nondiabetic ESKD (unpublished data).

Burden to the Korean society: an analysis from Health Insurance Review and Assessment Service data

1) Data source
To gather demographic and clinical data of individual HD patients, we used HD quality assessment data and HIRA claims data collected from October to December 2015. This analysis was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (IRB) of Hallym University Kangnam Sacred Heart Hospital (No. HKS 2021-11-043). The IRB waived the need for written informed consent from the patients because the identity of study participants was unknown in this study. Adult HD patients aged ≥18 years who received HD treatment ≥2 times weekly as outpatients were included in this assessment. Patients who were admitted during assessment or lost to follow up were excluded from the analysis [15]. Additionally, the HD quality assessment collected data from each HD facility through a web-based data-collection system [16,17].

The total costs of medical treatments were the sum of the reimbursed expenses, the data for which was extracted from the HIRA claims data between January 2016 and November 2021. Claims data generated at the hospital included the costs of the medical examination, medication, counseling, laboratory tests, procedures, and surgeries that may have been prescribed by the doctor. The total costs of medical treatments included the claim data for total visits to outpatient clinics and hospitalization [18]. We calculated the amounts paid by the provider and the individual patient per year to determine the total medical cost per person per year. In addition, the annual provider’s payment and patient’s payment were calculated, respectively, and the annual outpatient cost and cost of hospitalization were calculated. The exchange rate to US dollar presented in this study was the rate in 2019.

2) Characteristics of patients with diabetic end-stage kidney disease in Korea
Among the patients, the mean age of the diabetic ESKD group was 60.0 ± 12.8 years, and male participants comprised 58.4%, which was smaller than that of the nondiabetic ESKD group. Dialysis vintage was 4.2 ± 3.4 years in patients with diabetic ESKD, which was shorter than 6.6 ± 5.8 years in patients with nondiabetic ESKD. In the nondiabetic ESKD group, the causes of ESKD were hypertension (45.7%), unknown etiology (20.0%), and glomerulonephritis (19.5%). Patients with diabetic ESKD had lower serum levels of hemoglobin, albumin, calcium, phosphorus, and spKt/V than those with nondiabetic ESKD. Patients with diabetic ESKD had higher systolic blood pressure and pulse pressure than those with nondiabetic ESKD (Table 1).

3) Trend of using medical facilities in diabetic and nondiabetic end-stage kidney disease
In terms of medical insurance, Medical Aid was used for 20.9% of patients with diabetic ESKD and 24.3% of patients with nondiabetic ESKD. Medical Aid is a type of social security system in which the government provides medical care for low-income citizens who cannot maintain their livelihood or have difficulty making a living [19]. The number of patients with diabetic ESKD receiving dialysis treatment at general or tertiary hospitals was higher than that of patients with nondiabetic ESKD.

In all patients, the annual number of outpatient clinic visits was 119.7 ± 60.8 visits per year, and the annual number of emergency department visits was 0.8 ± 1.3 visits per year. The annual number of hospitalizations was 2.5 ± 3.4 times, and the mean duration of hospitalization was 28.0 ± 55.1 days. Diabetic ESKD had more annual outpatient clinic visits (124.5 ± 59.7 visits/yr vs. 116.3 ± 61.4 visits/yr, p < 0.001) and annual emergency department visits (1.0 ± 1.5 visits/yr vs. 0.6 ± 1.1 visits/yr, p < 0.001) than nondiabetic
ESKD. The annual number of hospitalizations was 3.2 ± 3.9 visits per year, which was higher than that of patients with nondiabetic ESKD (p < 0.001); the mean duration of hospitalization was longer (37.1 ± 63.1 days vs. 21.5 ± 47.7 days, p < 0.001) than that of patients with nondiabetic ESKD.

4) Comparison of medical costs in diabetic and nondiabetic end-stage kidney disease

Patients with diabetic ESKD had higher annual total medical costs than those with nondiabetic ESKD (p < 0.001). Furthermore, the annual cost of patient’s and provider’s payments was higher in patients with diabetic ESKD than in those with nondiabetic ESKD (p < 0.001). Additionally, medical costs due to hospitalizations and visits to outpatient clinics were higher in patients with diabetic ESKD (p < 0.001) (Fig. 3). However, no difference in medication cost was observed between the two groups (p = 0.67).

Home therapy: challenges and future directions in Korea

When CKD progresses and patients exhibit uremic symptoms, they are recommended to undergo RRT such as kidney transplantation, HD, and PD. Kidney transplantation is the most ideal type of RRT with the highest survival rate and the lowest cost in the long term. However, most patients undergo dialysis due to the lack of donors. Unlike HD patients, stable kidney transplant patients do not need to visit the hospital frequently, so kidney transplantation is a type of home therapy.

PD, another type of home RRT, requires fewer hospital visits and is more flexible than HD. Therefore, PD facilitates social activities, including working and studying, and is ad-

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**Table 1. Baseline characteristics of diabetic and nondiabetic ESKD**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Diabetic ESKD</th>
<th>Nondiabetic ESKD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>35,099</td>
<td>14,508</td>
<td>20,591</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>60.0 ± 12.8</td>
<td>62.2 ± 11.2</td>
<td>58.4 ± 13.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>20,543 (58.8)</td>
<td>17,482 (58.4)</td>
<td>3,061 (60.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dialysis vintage (yr)</td>
<td>5.6 ± 5.1</td>
<td>4.2 ± 3.4</td>
<td>6.6 ± 5.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cause of ESKD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>14,508 (41.3)</td>
<td>14,508 (100)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>9,420 (26.8)</td>
<td>0 (0)</td>
<td>9,420 (45.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>4,021 (11.5)</td>
<td>0 (0)</td>
<td>4,021 (19.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Others</td>
<td>3,040 (8.7)</td>
<td>0 (0)</td>
<td>3,040 (14.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Unknown</td>
<td>4,110 (11.7)</td>
<td>0 (0)</td>
<td>4,110 (20.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>29,808 (84.9)</td>
<td>12,953 (89.3)</td>
<td>16,855 (81.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>21,526 (61.3)</td>
<td>14,178 (97.7)</td>
<td>7,348 (35.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>12,083 (34.4)</td>
<td>5,846 (40.3)</td>
<td>6,237 (30.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>3,101 (8.8)</td>
<td>1,709 (11.8)</td>
<td>1,392 (6.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>5,142 (14.7)</td>
<td>2,453 (16.9)</td>
<td>2,689 (13.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>1,857 (5.3)</td>
<td>695 (4.8)</td>
<td>1,162 (5.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.72 ± 0.85</td>
<td>10.70 ± 0.78</td>
<td>10.74 ± 0.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.09 ± 0.34</td>
<td>3.97 ± 0.35</td>
<td>4.01 ± 0.34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.0 ± 0.8</td>
<td>8.9 ± 0.8</td>
<td>9.1 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>5.0 ± 1.3</td>
<td>4.8 ± 1.2</td>
<td>5.1 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Single-pool Kt/V</td>
<td>1.55 ± 0.28</td>
<td>1.52 ± 0.26</td>
<td>1.58 ± 0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.4 ± 3.4</td>
<td>23.0 ± 3.4</td>
<td>21.9 ± 3.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>141.2 ± 15.5</td>
<td>144.7 ± 15.1</td>
<td>138.7 ± 15.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>77.6 ± 9.6</td>
<td>76.6 ± 9.6</td>
<td>78.3 ± 9.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>63.6 ± 14.1</td>
<td>68.1 ± 14.6</td>
<td>60.4 ± 12.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as number only, mean ± standard deviation, or number (%).
ESKD, end-stage kidney disease; BP, blood pressure.
vantageous for the maintenance of patients’ independence and economic activities [20]. Furthermore, as the risk of hypotension or arrhythmia is low, and residual renal function is maintained longer than HD, PD is possible in small children or patients with poor vascular access [21]. These characteristics of PD might help improve patients’ quality of life [22].

Recently, in several domestic and international studies, the mortality rate of dialysis patients has been improved, particularly in PD; accordingly, the mortality rate of PD is lower than that of HD. In Korea, the mortality rate of PD has been lower than that of HD since 2019; this pattern has also been observed in countries, such as the United States and Australia [8,10,23,24]. Moreover, it is suggested as a pretransplant modality because patients with PD had better post-transplant outcomes than those with HD before transplantation [25,26].

Several studies have reported the effect of PD on the reduction of socioeconomic burden [20,27]. In one study, the authors analyzed the annual medical costs of dialysis patients in Korea in 2017. In this study, HD costs 22 million KRW/person (20,236 US dollars/person), which is 1.36-fold higher than that of PD, which costs approximately 16 million KRW/person (14,717 US dollars/person) [28]. The mortality rate of PD patients decreased; therefore, the socioeconomic burden from high mortality is also reduced. Furthermore, nonmedical costs, including transportation, dialysis machines, water purification, electricity, and patient time lost, are also reduced [29,30]. As patients can maintain their jobs during treatment, economic losses are further reduced [31]. In addition, PD patients had lesser psychological distress than HD patients during the COVID-19 pandemic when social distancing was required [32].

However, despite these advantages of PD, the number of patients with PD is decreasing compared to HD or kidney transplants. The PD rate among patients with ESKD is continuously decreasing from 28% in 2006 to 4.4% in 2021 [10]. There is a small number of patients who cannot receive PD, and a study has shown that approximately 78% of patients with ESKD can be considered for PD [33,34]. However, for various reasons, 83.6% of patients who started RRT in 2021 selected HD.
In Korea, several factors contribute to the reduced PD uptake in patients with ESKD, including healthcare policy or lack of incentives for PD, growing numbers of for-profit HD units, lack of manpower (particularly nurses) dedicated to PD, and lack of appropriate process for dialysis modality selection (shared decision-making). To expand home therapy in Korea, several aspects, including national government support, the development of technology, and educational programs for individual patients, should be considered.

The mission for the future: the Kidney Health Plan 2033

Recognizing the situation in Korea, the KSN proposed the KHP 2033 and has set three action goals for reducing the socioeconomic burden of dialysis and improving the quality of patient-centered treatment by preventing ESKD occurrence and increasing the home therapy rate. The three action goals to be achieved by 2033 are as follows: 1) a 10% reduction in the number of patients with CKD, 2) a 10% decrease in the proportion of patients with DKD in ESKD, and 3) an increase up to 33% of home therapy (PD + transplant) proportion in patients with ESKD. Each goal needs to be determined and then evaluated using the method described above. CKD prevalence reflects the proportion of patients with eGFR of <60 mL/min/1.73 m² or albuminuria among all patients included in the KNHANES. The second and third goals can be compared based on KORDS data.

What are the action plans of Kidney Health Plan 2033?

KSN set up four major areas, including patients, practice, partner, and policy (4Ps), for the effective achievement of the three action goals, and subsequently established action plans for each area. The overview of KHP 2033, including each action plan, is summarized in Fig. 4. For the development and implementation of detailed action plans, KSN cooperated with its committees, including the KORDS.

Figure 4. Kidney Health Plan 2033 overview. The Korean Society of Nephrology set three goals and four action plans to achieve those goals.

CKD, chronic kidney disease; DKD, diabetic kidney disease; ESKD, end-stage kidney disease; ESRD, end-stage renal disease.
registry, the Clinical Practice Guidelines, the Collaborative Studies, and the Public Relation.

KHP 2033 attempts to accurately grasp the current situation in Korea through different data sources. We identified and summarized domestic and foreign references related to CKD, ESKD, and DKD prevalence or incidence in Korea. We analyzed KORDS data to identify the status of ESKD and home therapy more specifically as well as examined costs related to dialysis in connection with the National Health Insurance Service (NHIS). Moreover, KSN conducted research on DKD using big data from national health insurance with the Korean Diabetic Kidney Disease Working Group, an affiliated organization, and supported the establishment of a DKD cohort. Accumulated data from KHP 2033 will be valuable basic information for patient education and policy proposals.

A significant achievement regarding clinical practice is the development of Korean DKD treatment guidelines [35]. This guideline reflects Korea’s special circumstances, including DKD epidemiology and reimbursement regulation by the NHIS. Particularly, this guideline recommends consultation with a nephrologist from the early stage of DKD (moderately increased albuminuria or eGFR below 60 mL/min/1.73 m²), emphasizing active and early intervention for DKD prevention.

KSN participates in and supports the “Pilot Project for Home Management of PD Patients” by the Ministry of Health and Welfare. Patients can receive professional educational counseling for dialysis management and receive close monitoring by medical staff through the pilot project. In the analysis of registered patients from 11 institutions, peritonitis and dialysis-related complications decreased following the pilot project implementation. Additionally, monitored patients had lower hospitalizations, emergency department visits, mortality, and medical costs than non-registered patients in the pilot project (unpublished results).

To improve public awareness of kidney disease, KSN continues to promote KHP 2033 through YouTube, newsletters, symposiums, and press conferences. Moreover, KHP 2033 plans to continue policy proposal activities, including policy forums and expert roundtables, to generate health policies that are impactful to the public.

The action plans and the progress of KHP 2033 toward each action goal are summarized in Table 2.

In summary, we focus on the following three significant aspects of kidney health problems in Korea: 1) the rapid increase in CKD and ESKD populations; 2) the constantly increasing diabetic ESKD incidence and increase in med-

### Table 2. Specific action plans for each action goal

<table>
<thead>
<tr>
<th>Action goal</th>
<th>Patients</th>
<th>Practice</th>
<th>Partner</th>
<th>Policy</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% reduction in the number of patients with CKD</td>
<td>Education program in KSN YouTube channel (I) Distribution of education material (I)</td>
<td>CKD guideline update (S)</td>
<td>Promotion for early nephrology referral (I) Cooperation in clinical trials with pharmaceutical companies (I)</td>
<td>KORDS data analysis (D) OKD fact sheet (D) Policy forum for the legislation of the OKD Management Act (D)</td>
</tr>
<tr>
<td>10% decrease in the proportion of DKD in ESKD</td>
<td>DKD self-management education (S)</td>
<td>Cooperative study on DKD (I) Development and distribution of DKD guideline (D)</td>
<td>Survey on DKD awareness and practice pattern (D) Multidisciplinary joint symposium (I)</td>
<td>Big data analysis for DKD epidemiology in Korea (I)</td>
</tr>
<tr>
<td>33% of home therapy (PD + transplant) in ESKD</td>
<td>SDM aid for dialysis modality selection (D) Education for home therapy (S)</td>
<td>Cooperative study on SDM for dialysis modality selection (I) Medical staff training for PD (I)</td>
<td>ISPD–KSN MOU (S) Cooperation with PD providers for remote patient management program (I)</td>
<td>Cooperation with the government for pilot project for home management of PD patients (I) Rationalization of PD reimbursement fee (S)</td>
</tr>
</tbody>
</table>

Status: D, done; I, in progress; S, suggested; SDM, shared decision-making. CKD, chronic kidney disease; DKD, diabetic kidney disease; ESKD, end-stage kidney disease; ISPD, International Society for Peritoneal Dialysis; KSN, Korean Society of Nephrology; KORDS, Korean Renal Data System; MOU, memorandum of understanding; PD, peritoneal dialysis.
ical costs associated with the aforementioned two factors; and 3) the decrease in the number of patients treated with home therapy, particularly PD. To address the current situation, we established the following three main goals: 1) a 10% decrease in the number of patients with CKD; 2) a 10% decrease in the proportion of patients with DKD in new patients receiving RRT; and 3) an increase of up to 33% in the proportion of patients with ESKD treated with home therapy, including PD and transplantation.

We presented KHP 2033 as a future vision for kidney health, with the goal of promoting kidney health for all. Our goals are to prevent the development of ESKD, particularly DKD, and increase the proportion of patients with ESKD receiving home therapy, thereby reducing the socioeconomic burden of kidney disease and improving the quality of patient-centered care.

The missions of KHP 2033 will be realized not only through the efforts of the nephrology society or patients but also through feasible and sustained efforts on the part of healthcare professionals and policymakers.

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Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

The data presented in this study are available upon reasonable request from the corresponding author.

Authors’ contributions

Conceptualization: CSL, SHP
Data curation, Formal analysis: DHK, YYH
Investigation, Project administration, Supervision: JJC, SL, HKL, JWC, SHK, SYH, CWP, EYL, DRC, SGK, CSL, SHP
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References


Status and trends in epidemiologic characteristics of diabetic end-stage renal disease: an analysis of the 2021 Korean Renal Data System

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Korean Renal Data System (KORDS) is a nationwide end-stage renal disease (ESRD) registry database operated by the Korean Society of Nephrology (KSN). Diabetes mellitus is currently the leading cause of ESRD in Korea; this article provides an update on the trends and characteristics of diabetic ESRD patients. The KORDS Committee of KSN collects data on dialysis centers and patients through an online registry program. Here, we analyzed the status and trends in characteristics of diabetic chronic kidney disease stage 5D (CKD 5D) patients using data from 2001 to 2021. In 2021, the dialysis adequacy of hemodialysis (HD) was lower in diabetic CKD 5D patients than in nondiabetic CKD 5D patients, while that of peritoneal dialysis (PD) was similar. Diabetic CKD 5D patients had a higher proportion of cardiac and vascular diseases and were more frequently admitted to hospitals than nondiabetic CKD 5D patients, and the leading cause of death was cardiac disease. From 2001 to 2020, diabetic CKD 5D patients had a higher mortality rate than nondiabetic CKD 5D patients, but in 2021 this trend was reversed. Diabetic PD patients had the highest mortality rate over 20 years. The mortality rate of diabetic HD patients was higher than that of nondiabetic HD patients until 2019 but became lower starting in 2020. There was a decreasing trend in mortality rate in diabetic CKD 5D patients, but cardiac and vascular diseases were still prevalent in diabetic CKD 5D patients with frequent admissions to hospitals. More specialized care is needed to improve the clinical outcomes of diabetic CKD 5D patients.

Keywords: Chronic renal insufficiency, Diabetes mellitus, Dialysis, Chronic kidney failure, Mortality

Introduction

Diabetic end-stage renal disease (ESRD) is a serious complication of diabetes mellitus (DM) that affects the quality of life and survival of patients. Diabetic nephropathy is the leading cause of ESRD, accounting for about 50% of all cases in developed countries [1]. The prevalence of DM in South Korea's overall population continues to rise, with the prevalence rate among adults aged 19 years and older estimated to be 13.9% in 2020 [2]. The Korean Renal Data System (KORDS) is a comprehensive, nationwide registry established by the Korean Society of Nephrology (KSN)
in 1985 that systematically collects and maintains data on ESRD patients receiving renal replacement therapy (RRT), chronic kidney disease stage 5D (CKD 5D), in South Korea. KORDS provides valuable information on the epidemiology, management, and outcomes of these patients. KORDS is a valuable resource that provides crucial epidemiological data, insights into treatment patterns and outcomes, informs healthcare policy and planning, promotes quality improvement in renal care, and fosters research collaborations both nationally and internationally. In this study, the Registration Committee of the KSN conducted a comparative analysis of the characteristics of diabetic CKD 5D patients and nondiabetic CKD 5D patients using data from the KORDS registry. The objective of this study was to gain a comprehensive understanding of the current status of patients with diabetic CKD 5D in South Korea to provide insights for frontline healthcare providers and inform healthcare policy-making related to DM and ESRD.

**Methods**

This study utilized KORDS, a nationwide registry updated on an annual basis [3,4]. To examine trends over time, we conducted a retrospective analysis of 20 years of data (2001 to 2021) for CKD 5D patients enrolled in KORDS. In addition, we utilized the most recent data available from 2021 to provide a current snapshot of the status of CKD 5D patients in Korea. More comprehensive data on KORDS can be found on the KSN website (http://www.ksn.or.kr). The study population comprised CKD 5D patients 19 years of age or older. The following exclusion criteria were applied: 1) Missing data or errors, including cases without dialysis start date or death date or those with death dates preceding the dialysis start date for deceased patients, or cases with enrollment dates preceding the dialysis start date for survivors; 2) Patients with a dialysis start date in 2001 or earlier; and 3) Individuals who had undergone kidney transplantation. The analysis encompassed changes in annual proportions and mortality across all patient groups. Meanwhile, a specific examination of the current status was specifically conducted for the subset of patients who initiated dialysis in the year 2021. Continuous variables are expressed as mean ± standard deviation or median (interquartile range, IQR), while categorical variables are presented as absolute values and percentages. Categorical variables were compared between groups using the chi-square test. Differences between groups for continuous variables were assessed using either a two-tailed Student t test or the Mann-Whitney U test as appropriate. Trends in mortality rates are presented for patients treated each year according to the number of patient-years at risk. Absolute mortality rates were presented per 1,000 person-years of follow-up and adjusted for age and sex. All statistical analyses of survival data were analyzed using R version 4.2.1 (R Foundation for Statistical Computing).

**Results**

**Trends in the prevalence and mortality rates of diabetic CKD 5D patients**

**Trends in the prevalence of diabetic CKD 5D patients**

Over the past two decades, DM has remained the leading cause of ESRD in South Korea. The proportion of diabetic CKD 5D has remained between 46.4% and 51.0% of all CKD 5D patients in the KORDS registry (Fig. 1A). The proportion of CKD 5D patients undergoing hemodialysis (CKD 5HD) has increased, while that of CKD 5D patients undergoing peritoneal dialysis (CKD 5PD) has decreased over 20 years (Fig. 1B). In 2001, over 40% of the total diabetic CKD 5D patient population were on PD. However, there has been a sustained decline in the proportion of patients undergoing PD, resulting in a decrease to less than 15% of diabetic CKD 5PD in 2021 (Fig. 1B). Diabetic CKD 5D patients accounted for about 50% of CKD 5HD patients over 20 years (Fig. 1C). The proportion of diabetic CKD 5D among CKD 5PD patients was over 50% in the mid-2000s, but has slowly declined to around 45% in 2021, whereas the proportion of diabetic CKD 5HD has remained relatively constant (Fig. 1D).

**Trends in mortality rates of diabetic CKD 5D patients**

Until the year 2020, patients with diabetic CKD 5D had a higher mortality rate than those with nondiabetic CKD 5D. However, in 2021, the mortality rate for nondiabetic CKD 5D patients increased slightly to 41.2 per 1,000 person-years, while the mortality rate for diabetic CKD 5D patients was 40.3 per 1,000 person-years, indicating a narrowing gap between the two groups (Fig. 2A).

Since 2001, diabetic CKD 5PD patients have consistently
**Figure 1.** Changes in the proportion of patients with diabetic CKD 5D. (A) Change in the ratio of patients with diabetic CKD 5D to those with nondiabetic CKD 5D. (B) Change in the proportion of patients with diabetic CKD 5HD vs. CKD 5PD. (C) Change in the ratio of patients with diabetic CKD 5HD to those with nondiabetic CKD 5HD. (D) Change in the ratio of patients with diabetic CKD 5PD to those with nondiabetic CKD 5PD. CKD 5D, chronic kidney disease stage 5D; CKD 5HD, CKD 5D patients undergoing hemodialysis; CKD 5PD, CKD 5D patients undergoing peritoneal dialysis; DM, diabetes mellitus.
had the highest mortality rate, and since 2013, nondiabetic CKD 5PD patients have had the lowest mortality rate. In the context of CKD 5HD, a consistent decline in the disparity of mortality rates was observed, with a significant turning point occurring in 2019 when the mortality rate for diabetic CKD 5HD patients (44.3/1,000 person-years) fell below that of their nondiabetic counterparts (44.7/1,000 person-years). This discrepancy continued to widen, and by 2021, the diabetic CKD 5HD patients exhibited a mortality rate of 40.3 per 1,000 person-years, while the nondiabetic CKD 5HD patients demonstrated a mortality rate of 45.4 per 1,000 person-years (Fig. 2B).

Age and sex distribution of diabetic CKD 5D patients

Among all CKD 5D patients in the KORDS registry in 2021, diabetic CKD 5D patients aged 60–69 years were the most prevalent (13.3%), followed by those aged 70–79 years (12.9%) and those aged 80 years or more (10.9%), as shown in Fig. 3A. The age distribution was also analyzed in diabetic CKD 5HD and diabetic CKD 5PD patients according to the RRT modality. The age distribution of both diabetic CKD 5HD patients and diabetic CKD 5PD patients showed a similar pattern; aged 60–69 years were most prevalent, followed by those aged 70–79 years and those aged 80 years or more (Supplementary Fig. 1, available online).

Upon examination of the age and sex distribution in diabetic CKD 5D patients, the sex distribution was significantly different in age subgroups (p < 0.001). There were more females than males under the age of 30 years with diabetic CKD 5D. However, starting from the 30s, the number of males surpasses that of females, and this difference became particularly significant in the 50s to 70s age group (Fig. 3B).

Status of diabetic CKD 5D patients starting renal replacement therapy in 2021

We analyzed the status of diabetic CKD 5D patients, focusing on incident dialysis patients in 2021.

Characteristics of incident diabetic CKD 5HD patients

Based on the data registered in the 2021 KORDS, the most common type of HD access for both diabetic and nondiabetic CKD 5HD patients was autologous arteriovenous fistula (AVF), followed by central venous catheterization (CVC) and arteriovenous graft (AVG). In nondiabetic CKD 5HD patients, the use of CVC was more prevalent, while in diabetic CKD 5HD patients, a higher proportion of patients utilized AVF and AVG (p < 0.001) (Fig. 4A).

AVFs were in the left forearm, left upper arm, right forearm, and right upper arm with decreasing frequency. The rate of AVF placement in the upper arm was significantly
higher in diabetic CKD 5HD patients than in nondiabetic CKD 5HD patients (p < 0.001) (Fig. 4B). The most common types of AVGs were, in order of prevalence, left loop, left straight, right loop, and right straight. The prevalence of types of AVG in diabetic and nondiabetic CKD 5HD patients was similar (p = 0.72), as depicted in Fig. 4C. The distribution of AVGs followed the order of left forearm, left upper arm, right forearm, and right upper arm, with similar distribution ratios observed for both diabetic and nondiabetic CKD 5HD patients (p = 0.58) (Fig. 4D).

Supplementary Fig. 2 (available online) provides additional analyses of HD access categorized by DM and sex. The type of HD access was different between diabetic and nondiabetic CKD 5HD male patients (p = 0.041) and female patients (p = 0.002) (Supplementary Fig. 2A, available online). The distribution of AVFs was different between di-
Figure 4. Characteristics of HD access of diabetic CKD 5HD patients. (A) Types of HD access for diabetic CKD 5HD patients in 2021. (B) Distributions of AVF among diabetic CKD 5HD patients in 2021. (C) Types of AVG among diabetic CKD 5HD patients in 2021. (D) Distributions of AVG among diabetic CKD 5HD patients in 2021. AVF, arteriovenous fistula; AVG, arteriovenous graft; CKD 5, chronic kidney disease stage 5; CKD 5HD, CKD 5D patients undergoing HD; CVC, central venous catheter; DM, diabetes mellitus; HD, hemodialysis. *p < 0.05.
abetic and nondiabetic CKD 5HD male patients (p = 0.001) and female patients (p = 0.003) (Supplementary Fig. 2B, available online). The type of AVFs showed similar ratios for diabetic and nondiabetic CKD 5HD male (p = 0.13) and female patients (p = 0.50) (Supplementary Fig. 2C, available online). The distribution of AVFs also showed similar ratios for diabetic and nondiabetic CKD 5HD male (p = 0.28) and female patients (p = 0.34) (Supplementary Fig. 2D, available online).

Supplementary Fig. 3 (available online) provides additional analyses of HD access categorized by DM and age subgroups. The type of HD access was statistically different between diabetic and nondiabetic HD patients aged 30–39 years (p = 0.02) and aged ≥80 years (p = 0.009) (Supplementary Fig. 3A, available online). The distribution of AVFs was statistically different between diabetic and nondiabetic HD patients aged 60–69 years (p < 0.001) (Supplementary Fig. 3B, available online). The type and distribution of AVFs showed similar ratios for diabetic and nondiabetic CKD 5HD patients in all age subgroups (Supplementary Fig. 3C, D; available online).

When comparing groups, the diabetic CKD 5HD group exhibited higher systolic blood pressure (median [IQR]: 150.0 [130.0–160.0] vs. 140.0 [130.0–150.0]) and lower diastolic blood pressure (80.0 [70.0–80.0] vs. 80.0 [70.0–83.5]) than the nondiabetic CKD 5HD group (both, p < 0.001). While there were no significant differences in hemoglobin (p = 0.10) or serum phosphorus (p = 0.39) levels between groups, the nondiabetic CKD 5HD group had higher levels of serum albumin (median [IQR]: 3.89 [3.50–4.10] vs. 3.80 [3.50–4.10]), calcium (8.60 [8.10–9.10] vs. 8.50 [8.00–8.90]), and intact parathyroid hormone (PTH) (164.0 [83.3–280.0] vs. 153.7 [82.0–249.9]) than the diabetic CKD 5HD group (all p < 0.001) (Fig. 5A).

Characteristics of incident diabetic CKD 5PD
Based on the data registered in the KORDS in 2021, continuous ambulatory peritoneal dialysis (CAPD) was more frequently used than automated peritoneal dialysis (APD) by both diabetic and nondiabetic CKD 5PD patients. Patients undergoing CAPD were more likely to be nondiabetic, whereas those undergoing APD were more likely to be diabetic (p = 0.03) (Fig. 6A).

Swan neck catheters and a swan neck with a coiled tip were the most common catheters used by diabetic and nondiabetic CKD 5PD patients. More nondiabetic CKD 5PD patients than diabetic CKD 5PD patients had a swan neck catheter (p = 0.03) (Fig. 6B).

In terms of PD catheterization technique, surgical catheterization was the most frequently utilized approach,
Figure 6. Characteristics of diabetic CKD 5PD patients. (A) PD modality types in diabetic CKD 5PD patients in 2021. (B) Types of PD catheters used in diabetic CKD 5PD patients in 2021. (C) Methods of PD catheter insertion in diabetic CKD 5PD patients in 2021. (D) The exit site infection rate and average incidence of peritonitis per patient and in diabetic CKD 5PD patients in 2021.

APD, automated peritoneal dialysis; CAPD, continuous ambulatory peritoneal dialysis; CKD 5PD, chronic kidney disease stage 5D patients undergoing peritoneal dialysis; DM, diabetes mellitus; PD, peritoneal dialysis.

*p < 0.05.
followed by trocar insertion. When comparing groups, the proportion of surgical insertions was higher in the diabetic CKD 5PD group, whereas the proportion of patients catheterized using a trocar was higher in the nondiabetic CKD 5PD group ($p < 0.001$) (Fig. 6C).

The rate of exit infections was slightly higher in nondiabetic CKD 5PD patients than diabetic CKD 5PD patients, with an overall rate of 1.6% in diabetic patients and 3.0% in nondiabetic patients ($p = 0.007$). However, no significant difference was observed in PD-related peritonitis between the two groups ($p = 0.142$) (Fig. 6D).

Median systolic and diastolic blood pressure, hemoglobin, calcium, phosphorus, and intact PTH levels in the blood did not differ significantly between diabetic and nondiabetic CKD 5PD patients, but serum albumin levels were slightly lower in diabetic CKD 5PD patients than nondiabetic CKD 5PD patients (median [IQR]: 3.50 [3.32–3.90] vs. 3.70 [3.40–4.00], $p = 0.049$) (Fig. 5B).

**Dialysis adequacy in diabetic CKD 5HD and CKD 5PD patients**

The distribution of single pooled Kt/V within each group revealed a median of 1.39 (IQR, 1.22–1.58) for diabetic CKD 5HD patients and a median of 1.45 (IQR, 1.25–1.66) for nondiabetic CKD 5HD patients, indicating a statistically significant difference ($p < 0.001$) (Fig. 7A). In CKD 5PD patients, the median weekly Kt/V, encompassing both urine and dialysate, was 2.15 (IQR, 1.70–2.72) for diabetic CKD 5PD patients and 2.16 (IQR, 1.79–2.69) for nondiabetic CKD 5PD patients, which showed no significant difference ($p = 0.86$) (Fig. 7B).

**Cause of deaths, comorbidities, and hospitalization of diabetic CKD 5D patients**

**Cause of death of diabetic CKD 5D patients**

In 2021, the leading cause of death among all diabetic CKD 5D patients registered in the KORDS was cardiac disease. However, cardiac disease and others that caused death had similar frequencies among nondiabetic CKD 5D patients. The proportion of deaths due to cardiac disease was not different between diabetic and nondiabetic CKD 5D patients ($p = 0.23$). However, the proportion of deaths due to vascular disease was higher in diabetic CKD 5D patients compared to nondiabetic CKD 5D patients ($p = 0.01$) (Fig. 8A).

The proportions of cause of deaths were also analyzed by RRT modality (Fig. 8A). Among CKD 5HD patients, the proportion of deaths attributed to cardiac disease was not statistically different between diabetic and nondiabetic patients ($p = 0.23$). However, diabetic CKD 5HD patients had a higher mortality rate due to vascular disease than nondiabetic CKD 5HD patients ($p = 0.006$). Among CKD 5PD patients, the proportion of deaths due to vascular disease was slightly higher in diabetic patients ($p = 0.04$).

CKD 5D, chronic kidney disease stage 5D; CKD 5HD, CKD 5D patients undergoing hemodialysis; CKD 5PD, CKD 5D patients undergoing peritoneal dialysis; CKD-MBD, chronic kidney disease-mineral bone disorder; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HD, hemodialysis; PD, peritoneal dialysis.

*p < 0.05.
5PD patients, the proportion of deaths attributed to cardiac disease, vascular disease, and infection was not statistically different between diabetic and nondiabetic patients (p = 0.92, p = 0.72, and p = 0.36, respectively). No significant differences were observed between diabetic and nondiabetic patients in CKD 5HD or CKD 5PD groups for other causes of deaths.

**Comorbidities and hospitalization in diabetic CKD 5D patients**
Cardiac disease was the most prevalent comorbidity in both diabetic and nondiabetic CKD 5D patients, followed by vascular disease. The prevalence of cardiac and vascular disease was higher in diabetic CKD 5D patients than nondiabetic CKD 5D patients (both p < 0.001) (Fig. 8B). The prevalence of pneumonia was higher (p = 0.02), while that of malignancy was lower (p < 0.001) in diabetic CKD 5D patients than nondiabetic CKD 5D patients.

Furthermore, the rate of hospitalization within the past year for diabetic CKD 5D patients was higher at 41.8% compared to 31.7% for nondiabetic CKD 5D patients (p < 0.001). The proportions of infection and cardiac disease as the cause of hospitalization were both higher for diabetic CKD 5D patients compared to nondiabetic CKD 5D patients (both p < 0.001) (Fig. 8C).

**Discussion**

Based on our analysis of the KORDS data from 2001 to 2021, we present several key findings regarding diabetic CKD 5D in Korea.

First, diabetic CKD 5D remains the most common cause of CKD 5D in Korea, accounting for approximately 50% of cases over the past 20 years. This finding is consistent with the global trend, where DM has been identified as the leading cause of ESRD.

Second, the proportion of diabetic CKD 5PD patients has decreased over the past 20 years from approximately 41.1% in 2001 to 14.6% in 2021. This trend is likely due to various factors including changes in clinical practice, provider or patient preferences, and accessibility to different dialysis modalities. Studies have discussed factors influencing the choice of PD in diabetic CKD 5D patients, such as potential impact on glycemic control, risk of peritonitis, and comorbidities that could complicate treatment [5–11].

Considering the high cardiovascular mortality and potential dialysis-induced cardiac injury associated with HD [12], PD remains a viable alternative. Our analysis indicates that nondiabetic CKD 5PD patients exhibited lower mortality rates than those who received HD, with the former subgroup displaying the lowest mortality rate among all analyzed groups. Consequently, optimal patient selection and effective management of PD may result in superior outcomes for diabetic CKD 5D patients relative to HD, emphasizing the importance of shared decision-making [13] between healthcare providers and patients when selecting the most appropriate RRT modality [14–17]. Nonetheless, further efforts are imperative to improve the survival outcomes of diabetic CKD 5PD, as this subgroup continues to exhibit the highest mortality rate among the four groups.

Third, diabetic CKD 5D patients were found to have a higher prevalence of comorbid cardiac and vascular diseases and a higher rate of hospitalization than nondiabetic ESRD patients. The greater prevalence of comorbidities and prior cardiovascular events in diabetic patients prior to initiating dialysis may account for this increased risk [18]. This underscores the importance of comprehensive management of diabetic CKD 5D patients, including control of cardiovascular risk factors and timely intervention for complications.

Fourth, dialysis adequacy differed between diabetic and nondiabetic CKD 5D patients. Specifically, diabetic CKD 5HD patients had lower dialysis adequacy than nondiabetic CKD 5HD patients, whereas there was no significant difference in dialysis adequacy between diabetic and nondiabetic CKD 5PD patients. Multiple factors may be involved in the difference, including comorbidities, access problems, or residual renal function, but the reason is unclear because of limited data. Clinical practice guidelines recommend maintaining a Kt/V level above 1.4 due to the association between lower values and increased morbidity in CKD 5HD patients [19,20]. In this study, the median Kt/V of diabetic CKD 5HD patients was 1.39. This finding suggests that a significant proportion of patients may have Kt/V levels below the recommended threshold, given that this value represents only the median value for the study population. Therefore, active management of these patients is necessary to improve survival rates and address gaps in outcomes.

Lastly, it is promising to observe that the mortality for
patients with diabetic CKD 5D, adjusted for age and sex, has decreased continuously over the last two decades, ultimately achieving parity in 2021 with nondiabetic CKD 5D patients. Nevertheless, when analyzed by RRT modality, it is evident that diabetic CKD 5PD patients still have the highest mortality rate, while nondiabetic CKD 5PD patients have the lowest mortality rate. Based on these observations, it appears that the primary underlying cause of death among patients with diabetic CKD 5D is DM itself rather than the PD modality itself. This suggests that there is a significant unmet need in addressing the impact of DM on outcomes in this patient population.

In conclusion, analysis of the KORDS registry data provided valuable insights into the epidemiologic characteristics of diabetic CKD 5D in Korea. There was a decreasing trend in mortality rate in diabetic CKD 5D patients, but cardiac and vascular diseases remained prevalent in diabetic CKD 5D patients in addition to frequent hospital admissions. More specialized care is needed to improve the clinical outcomes of diabetic CKD 5D patients. Further research to elucidate the factors contributing to the observed trends and to develop effective strategies for the prevention and management of diabetic CKD 5D is warranted.

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Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

The data presented in this study are available upon reasonable request from the corresponding author.

Authors’ contributions

Conceptualization: YKK, HEY
Data curation, Formal analysis: SAJ
Funding acquisition, Methodology: HEY
Investigation: KMK, THB, YAH, SDH, SRC, HL, JHK, SHK, THK, HSK, CYY, KK, SHA, HEY
Writing–original draft: KMK, HEY
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High prevalence of renal salt wasting induced by haptoglobin-related protein without signal peptide is linked to new syndrome of salt wasting in Alzheimer disease

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The subject of hyponatremia is undergoing significant changes after developing a more pathophysiologic approach that is superior to the ineffective volume approach and can more effectively identify the different causes of hyponatremia. This new approach identified cerebral salt wasting (CSW) in 24 (38%) of 62 hyponatremic patients from the medical wards of the hospital with 21 showing no evidence of cerebral disease to support our proposal to change CSW to renal salt wasting (RSW). RSW had to be differentiated from the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) because of diametrically opposite therapeutic goals of water-restricting water-logged patients with SIADH or administering salt water to volume-depleted patients with RSW. Both syndromes present with identical clinical parameters that require a difficult protocol to make such a differentiation possible. We describe rat clearance studies demonstrating natriuretic activity in the plasma of patients with neurosurgical and Alzheimer diseases (AD) and eventually identify the protein as haptoglobin-related protein without signal peptide, which can serve as a biomarker to simplify diagnosis of RSW and delivery of the proper management to improve clinical outcomes. We also discuss the introduction of a new syndrome of RSW in AD and its implications. The high prevalence of RSW and identification of the natriuretic factor have created debates over the existence of RSW with none questioning or addressing the pathophysiologic data that identified patients with RSW. We also discuss the potentially large group of patients with RSW who are normonatremic.

Keywords: Cerebral salt wasting, Haptoglobin-related protein without signal peptide in renal salt wasting, Hyponatremia, Renal salt wasting, Renal salt wasting in Alzheimer disease

Introduction

The reported high prevalence of renal salt wasting (RSW) without clinical evidence of cerebral disease has created an urgency to change the nomenclature of this syndrome from cerebral salt wasting (CSW) to RSW [1,2]. This change in nomenclature goes beyond its scientific accuracy because the absence of clinical evidence of cerebral disease would perpetuate the inappropriate water restriction of these volume-depleted patients with RSW, who were erroneously misdiagnosed as being water-logged by the syndrome of inappropriate secretion of antidiuretic hormone (SIADH).
Inappropriate water restriction of volume-depleted patients with RSW was common because CSW or more appropriately RSW, was considered to be nonexistent or very rare by internists despite its perception as being common among neurosurgeons, neurologists, and critical care physicians. The high prevalence of RSW without clinical evidence of cerebral disease supports our proposal to change CSW to RSW, which will be used throughout this manuscript [2]. However, differentiating SIADH from RSW has been elusive in part because of the perceived rarity of CSW and failure to address the identical clinical parameters that are shared by SIADH and RSW. Both syndromes present with hyponatremia; hypouricemia with high fractional excretion (FE) of uric acid; normal renal, adrenal, and thyroid function; concentrated urine where urine osmolality (Uosm) is higher than plasma osmolality; and urine sodium concentration (UNa) usually >30 mmol/L (Fig. 1).

Figure 1. Identical clinical characteristics of SIADH and RSW. The only difference is the volume status, being increased in SIADH and decreased in RSW.

RSW, renal salt wasting; SIADH, secretion of antidiuretic hormone.

Historical evolution of cerebral salt wasting from being nonexistent, rare to common: failure of volume approach

CSW had an inauspicious beginning as previously discussed because the three hyponatremic patients in the first report of salt wasting with cerebral diseases failed to prove salt wasting in 1950 [3,4]. When the seminal study on SIADH was first published in 1957, the ability to diagnose SIADH without the ability to determine plasma antidiuretic hormone levels by equating their findings to studies of SIADH induced in normal subjects by daily injections of arginine vasopressin rightfully captured the respect and admiration of the medical community [5,6]. They demonstrated that a hyponatremic patient with concentrated urine and high UNa was consistent with SIADH and not RSW by critically determining increased extracellular volume by the sulfate space [5]. SIADH has since been regarded as a major cause of hyponatremia in nonedematous hyponatremic patients. This dominance of SIADH persisted, in part, because the evaluation of hyponatremic patients utilized an approach that had little or no credibility by categorizing hyponatremic patients according to the state of their extracellular volume. This volume approach persisted despite our awareness that we cannot accurately determine the volume status of patients by usual clinical criteria [7,8]. Moreover, SIADH was erroneously designated as a form of euclidean hyponatremia, despite studies demonstrating them to be consistently hypervolemic [5,9–11].
**Pathophysiologic approach to evaluate hyponatremia and hyponatremia-related patients**

It is clear that the volume approach and prevailing controversies need to be reassessed by methods that will rapidly differentiate SIADH from RSW and vastly improve clinical outcomes. We developed and tested a new pathophysiologic approach over a 30-year period that has the potential to improve our understanding and treatment of hyponatremic and hyponatremia-related diseases, defined as normonatremic patients with RSW who will develop hyponatremia if they increased water intake.

**Fractional excretion rates of urate to identify causes of hyponatremia**

Urate is freely filtered at the glomerulus and is transported exclusively in the proximal tubule by reabsorbing and secretory transporters where there is a net reabsorption of 89% to 96% of the filtered load or 4% to 11% excretion of the filtered load that is referred to as FE of urate (FEurate) [1,12]. There is an expanding literature on the contribution of determining FEurate to identify the different causes of hyponatremia and hyponatremia-related diseases. FEurate has been especially useful in identifying patients with a reset osmostat (RO), Addison disease, SIADH, and RSW [13,14]. FEurate can also differentiate SIADH from RSW by its unique relationship to serum sodium. The increased FEurate that is >11% will decrease to a normal range of 4% to 11% in SIADH as compared to being persistently increased in RSW after correction of the hyponatremia (Fig. 2) [1,9,15–20]. Determinations of FEurate have effectively identified many causes of hyponatremia but need to be further investigated, especially psychotropic drugs that induce hyponatremia by an upregulation of the V2 receptor to ADH, which may thus induce an SIADH-like effect on FEurate as noted with hydrochlorothiazide [21,22].

The effect of saline on FEurate has become an issue in the recent debate over the existence of RSW, which contested our proposal that saline has a meager effect on FEurate [23]. They cite an increase in FEurate to 18.7% after receiving an extremely large amount of hypertonic saline that increased FENa sodium to an incredibly high 14.5% which may be almost impossible to attain in any clinical setting [24]. They failed to cite a more appropriate study where isotonic saline increased FENa sodium from 1.04% to 4.43% and FEurate from 7.98% to only 9.76% [25]. The highest FENa sodium we achieved while volume repleting a salt wasting patient with isotonic saline was 2.86% when FEurate was 49% [20]. Further insight into the effect of isotonic saline infusions on FEurate can be appreciated by a study in which we failed to correct the hyponatremia in a patient suspected of having RSW after infusing large volumes of isotonic saline with a baseline FEurate of 28%. We corrected the hyponatremia by infusing 1.5% hypertonic saline and demonstrated a progressive reduction in FEurate to 7% (Fig. 3) [13]. It, thus, appears that isotonic saline infusions only modestly increase FEurate to levels that do not come close to levels seen in RSW or SIADH. As will be discussed later, volume depletion can significantly reduce FEurate induced by extracellular volume depletion or a uricosuric agent.

**Effect of isotonic saline on urine osmolality and serum sodium in syndrome of inappropriate secretion of antidiuretic hormone and renal salt wasting**

The effect of isotonic saline infusions on Uosm and serum sodium concentration has well-established physiologic explanations that can differentiate SIADH from RSW. The response is based on the unresponsiveness or inappropriateness of ADH response to osmolar or volume stimuli in SIADH as compared to an appropriate response to both stimuli in RSW. The ADH response in RSW follows the principle that the volume stimulus for ADH secretion is more potent than the osmolar stimulus (Fig. 4) [26]. ADH levels will thus remain increased as long as the patient is volume-depleted, but when the volume stimulus is removed by infusing isotonic saline, the coexistent hypo-osmolality will inhibit ADH secretion, excrete dilute urines and correct the hyponatremia [1,9,20]. We decided to infuse isotonic saline to determine its effects on Uosm and serum sodium concentrations. Because of the difficulty in differentiating SIADH from RSW, we determined blood volume studies by radioisotope dilution methods using 51 chromium-labeled red blood cells and radiiodinated serum albumin and plasma renin and aldosterone to levels to ascertain that we were indeed studying cases of SIADH and RSW.
Two unequivocal cases of SIADH had increased blood volume and decreased plasma renin and aldosterone levels and one with RSW had decreased blood volume and increased plasma renin and aldosterone levels [19,20]. As noted in Fig. 5A, Isotonic saline failed to dilute the urine or correct the hyponatremia in the two patients with increased blood volume and reduced plasma renin and aldosterone levels as seen in SIADH [9]. In the patient with RSW, isotonic saline diluted the urine to 151 mOsm/kg 13 hours after initiation of isotonic saline infusions when plasma ADH was undetectable (Fig. 5B) [20]. We present this patient who provided valuable insights into RSW by virtue of the certainty with which the diagnosis of RSW was made and the credibility of the physiologic outcomes and unusual findings that were noted [20].

This is a 76-year-old female who was admitted with a hip...
fracture without clinical evidence of cerebral disease. A diagnosis of SIADH was made and she was water-restricted to 750 mL/day for 10 days without correction of her hyponatremia. She was referred to nephrology for further evaluation. At the time she was seen by nephrology, she had a serum sodium of 129 mmol/L, uric acid of 3.4 mg/dL, creatinine of 0.8 mg/dL, plasma renin of 8.63 ng/mL/hr, aldosterone of 16.5 ng/dL, atrial natriuretic peptide (ANP) of only 35 pg/dL, normal thyroid and adrenal function, Uosm of 321 mOsm/kg, UNa of only 6 mmol/L and FEurate of 29.6%. Her baseline blood volume as determined by 51 chromium-labeled red blood cells and radioiodinated serum album revealed a 7% reduction in blood volume. She was then infused with isotonic saline at a rate of 125 mL/hr and every urine was collected separately for the following 48 hours. As noted in Fig. 5B, Uosm increased from a baseline of 321 to 690 mOsm/kg and gradually decreased to a nadir of 151 mOsm/kg with a gradual increase in serum sodium to 138 mOsm/L 48 hours after initiation of isotonic saline infusion. Sixteen hours after initiating isotonic saline infusion, she awoke feeling much better and was very hungry. Because of the certainty of the diagnosis of RSW, there are many messages that characterize RSW and the physiology associated with the results obtained.

1. Isotonic saline removed the more potent volume stimulus to permit the hypo-osmolality to inhibit ADH secretion to a point where ADH was undetectable when the urine was diluted at 151 mOsm/kg. Because a dilute urine signified the removal of pure water from the body, serum sodium normalized to 138 mmol/L 48 hours after initiation of isotonic saline infusion. These data have been challenged in the recent debate on RSW claiming that the Uosm of 151 mOsm/kg did not attain a maximum dilution of 50 mOsm/kg, suggesting that ADH was still present in the plasma and not undetectable as reported [23]. This is correct in a normal subject, but this is precisely what you would expect in a patient with RSW. The Uosm of 151 mOsm/kg in the absence of ADH is consistent with the
Figure 5. Effects of isotonic infusions on Uosm and serum sodium concentrations. (A) In a patient with unequivocal syndrome of inappropriate secretion of antidiuretic hormone based on an increase in blood volume determined by radioiodinated serum albumin and 51 chromium-labeled red blood cells, decreased plasma renin and aldosterone levels. Note the failure of isotonic saline to dilute the urine or correct the hyponatremia. Graph taken from Maesaka et al. (Kidney Int 2009;76:934-938) according to the Creative Commons License. (B) In a patient with unequivocal renal salt wasting based on a decreased blood volume determined by radioiodinated serum albumin and 51 chromium-labeled red blood cells, increase in plasma renin, aldosterone, and antidiuretic hormone (ADH) levels at baseline. Note the progressive decrease in Uosm after initiation of isotonic saline and eventual normalization of serum sodium within 48 hours. Plasma ADH was undetectable when the Uosm was 152 mOsm/kg. Uosm, urine osmolality.
dictum that patients with RSW have a free water clearing defect. This is best explained by the infusion of hypotonic saline to normal subjects after they had attained the minimum Uosm of 50 mOsm/kg by ingesting water (Fig. 6). The hypo-osmolality of the hypotonic saline maintained ADH at a suppressed level throughout the study as the infusion of hypotonic saline increased extracellular volume and increased urinary sodium or solute excretion. As solute excretion increased with the continued infusion of hypotonic saline, Uosm progressively increased to levels approaching plasma osmolality in the absence of ADH (Fig. 6). Since the excretion of free water represents solute-free water, the progressive increase in solute excretion or osmolar clearance will progressively increase Uosm to approach plasma osmolality (Fig. 6) [27].

2. As noted in the two unequivocal cases of SIADH, isotonic saline infusion does not dilute urine or correct the hyponatremia in SIADH.

3. The UNa at baseline of only 6 mmol/L is considered to be inconsistent with RSW or SIADH but its presence in this case of RSW can be explained by the low salt intake by loss of appetite while being water restricted for an erroneous diagnosis of SIADH for 10 days. The reader is encouraged to read about the concept of escape in different clinical situations [28]. There are two phases in RSW. The first is the initiation phase where sodium excretion exceeds sodium intake to create a volume-depleted state. The patient then transitions to the equilibrated state where sodium input equals sodium output by undergoing hormonal, hemodynamic, and neural adjustments [28]. Resolution of the initiation phase must occur at some point because a daily output of sodium exceeding salt intake will otherwise lead to the total removal of all exchangeable sodium from the body. We have called this transition from the initiation to the equilibrated state as RSW escape because all exchangeable sodium would be eliminated from the body if there is no escape from the initiation phase. Mineralocorticoid escape has been a perfect counterpart of the escape phenomenon [29]. The patient must have been in the equilibrated stage when first seen by nephrology [28].

4. The low sodium intake must have reduced medullary sodium content to diminish the ability to concentrate the urine despite high levels of ADH with a baseline Uosm of 321 mOsm/kg. Infusion of isotonic saline rapidly increased sodium delivery to the distal tubules because of a major defect in proximal tubule sodium transport as will be discussed later. This rapid delivery of sodium to the distal tubule increased medullary sodium content or strengthened the medullary concentrating ability to increase Uosm to 690 mOsm/kg (Fig. 5B).

5. The baseline FEurate of 29.6% while in a volume-depleted state increased progressively to 64% after initiation of isotonic saline, suggesting that the volume depletion had decreased the effect of the uricosuric factor in plasma to reduce FEurate. Isotonic saline increased the effectiveness of the uricosuric factor to increase FEurate further in RSW as compared to a meager effect of infusing isotonic saline on FEurate in a normal euvoletic patient as noted earlier [25].

6. The ANP of a low normal 35 pg/mL is an unlikely cause of RSW as proposed in a number of publications. As will be discussed later, the physiology of the identified protein,
HPRWSP, has vastly different physiologic effects that essentially rule out ANP as a possible cause of RSW [1,30,31].

7. Potassium excretion has been proposed to increase significantly during the period of volume repletion by infusion of isotonic saline. This would be expected because potassium secretion in the distal nephron is influenced by the delivery of sodium, water, and anions. Contrary to this proposal, potassium excretion actually decreased from what was observed at baseline [20]. This difference in potassium excretion might be explained by the higher baseline renin and aldosterone levels that decreased while being volume-repleted, suggesting that aldosterone plays a major role in the handling of potassium by the kidneys.

8. The absence of cerebral disease in this patient is one of many RSW patients without clinical evidence of cerebral disease. Based on these and subsequent supporting data, we advocate changing cerebral to RSW, a very important change in nomenclature [2].

There are ample data to conclude that isotonic saline does not induce excretion of dilute urines or correct the hyponatremia in SIADH. In contrast, isotonic saline infusion in RSW eliminated the more potent volume stimulus and permitted the coexisting hypo-osmolality to inhibit ADH secretion, induce the excretion of dilute urines and correct the hyponatremia in RSW [26]. These data in SIADH are consistent with comments made by Bartter and Schwartz [32], “a striking and consistent finding in patients with SIADH is the persistence of hyponatremia even when large quantities of sodium are administered” or by Janicic and Verbalis [33] who state that “volume expansion does not correct the hyponatremia” in SIADH. Verbalis [34] also presented a case of SIADH who was misdiagnosed as having CSW when the hyponatremia did not respond to isotonic saline but corrected the hyponatremia by water restriction. In addressing the controversy over the existence and prevalence of RSW, there are two editorials that comment on the rarity of RSW by citing studies of questionable credibility on the hyponatremia associated with subarachnoid hemorrhage (SAH) [34,35]. Both editorials cite retrospective and prospective studies by a single group. The credibility of the retrospective study was severely damaged by proposing that 4.8% had a combination of SIADH and RSW in the same patient without defining how they arrived at such an unlikely diagnosis [36]. In the prospective study of 49 hyponatremic patients with SAH, the diagnosis of SIADH met the criteria included in Fig. 1 without attempting to differentiate it from RSW. All 49 patients corrected their hyponatremia in a median of 3 days while on isotonic saline infusions without ever being water restricted, receiving hypertonic saline or the ADH V2 receptor inhibitor. These 49 patients had RSW and not SIADH as suggested by these authors and both editorials [34,35,37]. As a final comment, none of the criticisms about the existence and prevalence of RSW questioned the validity of our pathophysiologic approach or provided data to prove them to be wrong [23,34,35,38].

Because patients with SAH were often misdiagnosed as having SIADH, there was an increase in ischemia and brain infarction with increased morbidity and mortality when water was restricted [39]. The infusion of isotonic saline has, thus, become the standard of care for patients with SAH. As a result of this paradigm shift, we can expect to see less hyponatremia in these patients because patients must have sufficient water intake to induce hyponatremia unless they are undergoing desalination where UNa far exceeds the intake sodium concentration [40]. Water intake must substantially exceed the insensible water losses of approximately 500 mL that occur daily. This was exemplified by the early studies on the effects of daily pitressin injections on normal humans and dogs where hyponatremia was only possible when they increased water intake [6,41].

**Pathophysiologic approach uncovers the high prevalence of renal salt wasting without cerebral disease**

Determinations of FEurate and response to isotonic saline infusions in 62 hyponatremic patients in the general medical wards of the hospital unexpectedly found RSW to be much more common than previously perceived [1].

1. Seventeen (27%) had SIADH. Isotonic saline infusion did not dilute or correct the hyponatremia in 11 patients. High baseline FEurate normalized to <11% in five patients after correction of their hyponatremia.

2. Nineteen (31%) had a RO. All 19 patients had a normal FEurate of 4% to 11% with eight excreting a spontaneously excreted dilute urine.

3. Twenty-four (38%) had RSW. Isotonic saline infusions induced the excretion of dilute urine in 19 patients with two having undetectable plasma ADH levels when the
urine was diluted. Ten patients who excreted dilute urines received 5% dextrose in water infusions to prevent serum sodium from increasing greater than 6 mmol/L in 24 hours to diminish the likelihood of inducing osmotic demyelination [42]. Eleven had a persistently increased FEurate after correction of their hyponatremia. Twenty-one of the 24 patients with RSW had no clinical evidence of cerebral disease to support our proposal to change cerebral to RSW [2]. It is our hope that this change in nomenclature will come to fruition in the future to improve clinical outcomes. The perceived rarity of RSW and absence of cerebral disease would have led to water-restricting these patients for an erroneous diagnosis of SIADH. The increase in morbidity and mortality associated with hyponatremia may thus be in part iatrogenic [43].

4. One due to Addison disease
5. One due to hydrochlorothiazide

Instructive case

A 71-year-old male with advanced large B-cell lymphoma presented with a 9.1 kg weight gain over a 6-week period, had bilateral leg edema and postural hypotension and reflex tachycardia; lying blood pressure and pulse were 95/65 mmHg and 109 beats/min that decreased to 76/56 mmHg and 138 beats/min, respectively, on standing [13]. His baseline serum sodium was 115 mmol/L; creatinine, 0.9 mg/dL; blood urea nitrogen, 22 mg/dL; uric acid, 6.8 mg/dL; FEurate, 22.7%, Uosm, 308 mOsm/kg; and UNa only 10 mmol/L. There were no clinical signs or symptoms of cerebral disease. Because of the high FEurate and postural hypotension and reflex tachycardia, he was considered to have RSW with complete obstruction of the inferior vena cava by the lymphoma, which was later confirmed by a computed tomography scan. He was thus started on infusions of isotonic saline, which induced excretion of dilute urine 14 hours after initiation of isotonic infusion with a Uosm of 140 mmol/L and undetectable plasma ADH. Because his serum sodium had increased by 5 mmol/L over 5 hours, he was started on 5% dextrose and water to limit the increase in serum sodium to less than 6 mmol/L/24 hr to reduce the possibility of inducing osmotic demyelination [42].

The presence of ascites, pleural effusion, decreased cardiac output, and UNa of only 10 mmol/L were construed to be consistent with prerenal azotemia due to heart failure. This prompted the decision by others to stop the isotonic saline infusion and start an intravenous infusion of furosemide, despite warnings that this would be harmful in a patient with RSW. What followed was an abrupt increase in urine output that led to severe hemodynamic instability that required infusion of large volumes of isotonic saline. The keys to the diagnosis of RSW were the postural hypotension with reflex tachycardia and the very important FEurate of 22.7% which dramatically contrasted it to heart failure where the FEurate would be expected to be <4% (Fig. 2). This case illustrates the value of utilizing the algorithm that was key to arriving at the accurate diagnosis and treatment of this very instructive case (Fig. 2).

The clinical absence of cerebral disease added another compelling case of RSW to support changing cerebral to RSW and the UNa of only 10 mmol/L can be seen in a patient with RSW as discussed above.

Rat clearance studies demonstrating natriuretic activity in plasma of patients with neurosurgical and Alzheimer diseases

As part of our quest to identify physiologic mechanisms that contribute to clinical diseases, we developed strategies to identify a natriuretic protein that might cause RSW. We decided that an animal model would be most informative because it allowed many competing variables to be expressed as compared to an in vitro model where a limited number of variables can be expressed and controlled. We performed rat clearance studies by infusing plasma from prospective patients with RSW. The most likely candidates were neurosurgical patients where blood volume studies demonstrated RSW to be very common and in AD where the reported hypouricemia might be consistent with a high FEurate and RSW [44–46]. Because uric acid is transported exclusively in the proximal tubule, a natriuretic factor must have a dominant effect on solute transport in the proximal tubule [12]. We elected to study lithium transport, which has been used as a marker of proximal tubular sodium transport by being transported on a one-to-one basis with sodium in the proximal tubule with little or no transport in the distal tubule [47]. On the other hand, sodium is vigorously transported in the distal tubule. In two separate rat clearance studies, we infused the plasma of 21
patients with various types of neurosurgical diseases and 18 patients with advanced AD, which we surmised might improve our chances of demonstrating the presence of a natriuretic factor [44,45]. The infusion of plasma in both studies revealed identical features. There were no changes in blood pressures or glomerular filtration rates and FENa sodium increased from the control of 0.3% and 0.33% to 0.59% and 0.63% in the neurosurgical and AD groups, respectively. FEliithium increased from the control of 22.3% and 27.2% to 36.6% and 41.7% in the neurosurgical and AD groups, respectively [44,45]. Interestingly FEliithium in the patients with fairly advanced AD progressively increased from an elevated level at a mini-mental status examination (MMSE) score of 12 to zero (Fig. 7). Attempts to identify the protein were abandoned because of limitations in protein analysis in 1993.

Identification of natriuretic protein in sera of patients with neurosurgical and Alzheimer diseases

Identification of the natriuretic factor(s) in the plasma of patients with neurosurgical and ADs became possible when the analysis of proteins had developed to a point where every protein could be identified with precision in small sample sizes. We modified the protocol of the previous rat renal clearance studies by injecting 0.5 mL of serum in 1.5 minutes instead of intraperitoneal injections of 0.5 mL serum followed 90 minutes later by a constant infusion of 2 mL administered over a 3-hour period [1,44,45]. We found natriuretic activity in the sera of normonatremic patient with evidence of RSW due to SAH and another with AD. We subjected the sera with natriuretic activity and control to mass spectrometry and SWATH (sequential window acquisition of all theoretical mass spectra) analysis and subjected the results to RCProtein (fold-change of protein) to estimate semiquantitatively the relative change of a specific protein in the active sera as compared to a control serum [1]. Seventeen proteins in the sera with natriuretic activity were increased at least two-fold over the control sample with the highest levels noted for haptoglobins and haptoglobin-related protein (HPR). Recombinant samples of HPR with signal peptide, haptoglobin Hp 1-1, Hp 2-2, kininogen, thrombospondin, PROZ, alpha 1 microglobulin/bikunin, and retinol-binding protein had no natriuretic activity. A review of our analytical data revealed that HPR found in the active sera did not possess the signal peptide. Infusion of HPRWSP resulted in a robust dose-response increase in FENa sodium and urine flow rate (Fig. 8) [1]. The protocol using only 0.5 mL of serum showed an increase

Figure 7. Graph demonstrating FEliithium excretion rates in rats infused with plasma from patients with AD and multi-infarct dementia at different MMSE scores.

AD, Alzheimer disease; FEliithium, fractional excretion of lithium; MMSE, mini-mental status examination; NS, not significant.

Figure 8. Effect of increasing dose of HPRWSP on FENa and UFR. A dose-response graph showing how increasing the dose of HPRWSP progressively increased FE of sodium and UFRs in rats. HPRWSP, haptoglobin-related protein without signal peptide; FENa, fractional excretion of sodium; UFR, urine flow rate.
in FENa sodium, FElithium, and urine flow rates with no effect on FEpGlucose, FEpPhosphate, or FEurate, but the data are based on a single pass of the active serum to the kidneys. The protein can increase some or all of these solutes if there is a constant infusion of HPRWSP.

**Clinical application of haptoglobin-related protein without signal peptide as biomarker and inhibitor to haptoglobin-related protein without signal peptide in hyponatremic and nonhyponatremic renal salt wasting patients**

The high prevalence of RSW in hyponatremic patients in the general wards of the hospital justifies future efforts to develop methods to rapidly identify patients with RSW to select the proper mode of therapy to improve clinical outcomes. This can be accomplished by developing HPRWSP as a reliable biomarker of RSW, which is presently underway. The major causes of hyponatremia are dominated by SIADH, RO, RSW, and possibly medications and congestive heart failure. It would be anticipated that HPRWSP will be increased only in the RSW patients. The problem is to set criteria for determining HPRWSP in an expanding list of nonhyponatremic patients with RSW. The combination of the increasing use of isotonic saline in neurosurgical units, especially SAH, the common occurrence of RSW by blood volume studies in these patients, and the demonstration of natriuretic activity in the plasma of patients with various neurosurgical diseases justify determining HPRWSP blood levels to identify RSW in every neurosurgical patient and possibly in critical care intensive care units. Because HPRWSP with signal peptide had no natriuretic activity, it will be important to develop methods to determine levels of HPRWSP as a biomarker of suspected patients with RSW.

**A new syndrome of renal salt wasting in Alzheimer disease**

As noted above, FENa and FElithium increased significantly when the plasma of AD patients was injected into rats [45]. The significant but modestly higher FEurate of 9.7% in the AD patients as compared to 6.6% in control patients was much lower than the 18.7% noted in the 24 hyponatremic RSW patients recruited from the general medical wards of the hospital [1,45]. The comparatively lower FEurate of 9.7% in the AD patients suggests that these AD patients were more volume-depleted than the RSW patients in the general wards of the hospital as noted above.

Because there was a dose-response of FENa/FElithium to increasing doses of plasma with natriuretic activity and HPRWSP, blood levels of HPRWSP must have been progressively increasing as MMSE scores decreased from 12 to 0 in AD patients [1,45]. It appears that most or all patients with AD become progressively volume-depleted as they become more demented. Future studies intend to correlate plasma levels of HPRWSP with the onset and magnitude of RSW at different stages of dementia.

**Up- and downregulation of haptoglobin-related protein without signal peptide and need to develop an inhibitor of haptoglobin-related protein without signal peptide**

It appears that HPRWSP is normally produced at low levels, which appears to be upregulated under certain comorbid conditions. The duration of upregulation appears to have been of short duration in the 24 RSW patients in the general medical wards of the hospital. Most of their RSW appeared to subside as their comorbid conditions were successfully treated [1]. Only one patient continued to have RSW for 2 months after being discharged from the hospital. Treatment of all of these patients was simple as they responded favorably to isotonic saline infusions. In those that had significant volume depletion over an extended period of time, isotonic saline infusions significantly reduced quality of life by inducing polyuria and nocturia every 2 hours. In AD, however, there appears to be a permanent HPRWSP upregulation that will expose all organs of the body, especially of brain, to this protein. There is thus a need to develop an inhibitor to HPRWSP to simplify the management of all RSW patients, especially in AD where all organs appear to be permanently exposed to the putative effects of a relatively unknown protein. This would warrant investigations on the biologic effects of HPRWSP, especially on the brain. It would also be interesting to inhibit HPRWSP in all RSW AD patients to not only eliminate the need for isotonic saline infusions but also to determine its effect on the brain and possibly other organs of the body.
Conclusions

We hope we have successfully reviewed the state of confusion that exists in the area of hyponatremia and hypo-natremia-related conditions. We focused on the evolution and substantive body of work on a new approach which demonstrated a high prevalence of RSW based on sound pathophysiologic principles, that have not been challenged.

We also hope we successfully justified changing cerebral to RSW and explaining the difficulty and therapeutic importance of differentiating SIADH from RSW. We encourage the reader to carefully review the valuable teaching points of the two cases presented and descriptions of a new syndrome of RSW in AD. We also describe the studies leading to identifying HPRWSP as the natriuretic peptide that causes RSW in hyponatremic and nonhyponatremic conditions and how it and its inhibitor can have optimistic diagnostic and therapeutic outcomes.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

This is a review manuscript that provides no data other than those found exclusively in the literature where all such concerns would be applicable.

Authors’ contributions

Conceptualization: JKM
Investigation, Visualization: LJI, CG, NM
Project administration: JKM
Writing–original draft: JKM
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Crosstalk mechanisms between glomerular endothelial cells and podocytes in renal diseases and kidney transplantation

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The glomerular filtration barrier (GFB), composed of endothelial cells, glomerular basement membrane, and podocytes, is a unique structure for filtering blood while detaining plasma proteins according to size and charge selectivity. Structurally, the fenestrated endothelial cells, which align the capillary loops, are in close proximity to mesangial cells. Podocytes are connected by specialized intercellular junctions known as slit diaphragms and are separated from the endothelial compartment by the glomerular basement membrane. Podocyte-endothelial cell communication or crosstalk is required for the development and maintenance of an efficient filtration process in physiological conditions. In pathological situations, communication also has an essential role in promoting or delaying disease progression. Podocytes and endothelial cells can secrete signaling molecules, which act as crosstalk effectors and, through binding to their target receptors, can trigger bidirectional paracrine or autocrine signal transduction. Moreover, the emerging evidence of extracellular vesicles derived from various cell types engaging in cell communication has also been reported. In this review, we summarize the principal pathways involved in the development and maintenance of the GFB and the progression of kidney disease, particularly in kidney transplantation.

Keywords: Extracellular vesicles, Glomerular filtration barrier, Kidney transplantation, MicroRNAs, Podocyte-endothelial cell crosstalk

Introduction

The glomerular capillary wall acts as a filtration barrier and exhibits selective permeability. The barrier comprises an inner layer of glomerular endothelial cells (GECs), a glomerular basement membrane (GBM), and an exterior layer of visceral epithelial cells named podocytes. GECs, facing the capillary lumen, are very flat cells with numerous fenestrations that facilitate and regulate the functions of filtration [1]. Podocytes, facing the urinary space, are terminally

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differentiated cells characterized by their large cell bodies, long major processes, and smaller foot processes. The foot processes derived from adjacent podocytes interdigitate with each other, forming a slit diaphragm to cover the capillaries. Between the GEC and podocyte layers, there is a condensed network of extracellular matrix (ECM) called GBM, which is composed preeminently of two heterotrimeric proteins, type IV collagen and laminin, as well as sulfated proteoglycans [2].

Since the high selectivity of the glomerular filter is achieved by the collaboration between each component of the glomerular filtration barrier (GBF), the podocytes and GECs crosstalk is essential not only for the development and maintenance of an efficient filtration process in physiological conditions but also has a fundamental role in promoting or delaying disease progression. In the glomerular microenvironment, abnormality or health of one cell type can influence the nearby cells by signaling molecules and extracellular vesicles (EVs). Secreted growth factors and signaling peptides, which may have an autocrine effect on the same cell type or a paracrine effect on nearby cells, serve as crosstalk effector molecules by binding to their specific receptors and activating signaling [3]. The ECM is crucial for depositing secreted ligands, the development of concentration gradient, and the presentation of ligands to cell surface receptors [4]. In addition to growth factors and signaling peptides, there is growing evidence implying an important role for EVs in cell communication [5]. This review will focus on recent data concerning the crosstalk between GECs and podocytes in physiological and pathological conditions. In particular, we will focus on the role of vascular endothelial growth factor A (VEGF-A), angiopoietins (ANGPTs), CXCL12/CXCR4/CXCR7, endothelin-1 (ET-1), interleukin-6 (IL-6), and EVs.

Vascular endothelial growth factor A signaling

Vascular endothelial growth factor A signaling in renal diseases

VEGF-A is a key factor for angiogenesis in multiple organ systems, including the formation and maintenance of the microvascular beds of the kidney. In Fig. 1, the role of VEGF on glomerular development is presented.

Podocytes begin to express all VEGF-A isoforms at the S-shaped stage of glomerular formation. During the capillary loop stage, premature podocytes express VEGF-A, encouraging the migration of VEGF receptor 2 (VEGFR-2) positive endothelial cell (EC) precursors in the renal mesenchyme. ECs move into the vascular cleft, proliferate, and differentiate in close proximity to the podocytes that produce VEGF-A [6]. Research in mice has demonstrated that decreased VEGF-A signaling from podocytes causes a loss of ECs’ migration and proliferation, which eliminates the GFBs’ functionality and reduces mice survival [7]. In the mature glomerulus, renal thrombotic microangiopathy (TMA) is caused by postnatal podocyte-specific VEGF-A deletion in mice and VEGF-A inhibition in humans, highlighting the significance of a sufficient level of VEGF-A within the mature kidney for maintaining the normal function of renal microvasculature [8].

Beyond the VEGF-A signaling to the glomerular endothelium, there is also an autocrine pathway involving the soluble form of VEGFR-1 (sFlt1) released by podocytes. Through binding to glycosphingolipids in lipid rafts, sFlt1 initiates an intracellular signaling cascade, facilitating actin reorganization and cell adhesion. Interestingly, severe proteinuria and renal failure are caused by the deletion of sFlt1 from podocytes [9].

Numerous kidney diseases involve VEGF signaling. According to recent animal research and clinical observations, endothelial dysfunction in preeclampsia may be brought on by the placenta’s excessive release of sFlt1 into the mother’s bloodstream. In this contest, VEGF-A might be trapped by sFlt1, leading to the reduction of free VEGF-A in circulation. Rats receiving an adenovirus expressing sFlt1 developed proteinuria, glomerular endotheliosis, and hypertension [10]. Podocyte-specific VEGF-A haploinsufficiency in mice causes proteinuria, endotheliosis, and, finally, the loss of ECs, similar to the characteristic renal lesions found in preeclampsia [11]. In humans, sFlt1 levels begin to rise at least 5 weeks before the onset of preeclampsia and remain elevated [12]. The finding that therapy with neutralizing VEGF-A antibodies can be associated with glomerular endothelial damage, endotheliosis, and proteinuria further supports this correlation [13].

The kidney and brain are especially affected by TMAs, a group of related illnesses in which the development of intracapillary and intra-arteriolar platelet thrombi results in end-organ ischemia and infarction. Hemolytic uremic
syndrome (HUS), a kind of TMAs, is characterized by the formation of fibrin-platelet thrombi and damage to the ECs, including ballooning, detachment, and endotheliosis. It is essential to highlight that individuals taking anti-VEGF drugs for cancer may experience kidney histology abnormalities that resemble TMAs [13].

Furthermore, it appears that VEGF has a role in developing diabetic nephropathy (DN). The glomerulus displays higher VEGF-A levels in the early angiogenic stage of DN. Experimental models of early diabetes have revealed glomerular overexpression of VEGF-A and its receptors [14], and markers of DN can be attenuated by blocking VEGF-A in rodents [15]. Moreover, transgenic overexpression of VEGF-A in podocytes causes the GBM to be thickened, proteinuria, and DN hallmarks [16]. As mentioned before, the sFlt1 acts as an antagonist of VEGF-A through sequestering circulating VEGF-A. In experimental diabetes, inducible overexpression of sFlt1 in podocytes of mice results in a reduction of albuminuria and amelioration of glomerular alterations [17]. In contrast to these findings, it has also been observed that the specific deletion of podocyte-VEGF-A accelerates renal damage in an experimental model of diabetes [18] and a decrease of VEGF-A expression in human diabetes [19]. These findings revealed that, depending on

**Figure 1. The role of vascular endothelial growth factor (VEGF) on glomerular development.** (A) At the S-shaped stage, podocyte progenitor cells start to produce VEGF-A, which draws the VEGF-A receptor (VEGF-Ar) expressing endothelial cells (ECs) to migrate. ECs, in turn, produce platelet-derived growth factor (PDGF)-B, which attracts mesangial cells (MCs), through binding with the PDGF-A receptor (PDGF-Ar). (B) The ECs start forming precursors of the capillary lumen; meanwhile, mesangial progenitor cells begin to envelop primordial ECs. The process of glomerular capillary lumen formation continues through EC apoptosis. (C) The MCs attach to the ECs and result in the formation of capillary loops. (D) Mature glomerulus.
the signal intensity, there might be a delicate balance between the protective and harmful effects of VEGF-A.

VEGF signaling is probably involved also in crescentic glomerulonephritis (CGN) and membranoproliferative glomerulonephritis (MPGN). High serum and urine levels of VEGF are reported in patients with CGN [20]. In human MPGN, VEGFR-1, VEGFR-2, and neuropilin-1 are expressed in mesangial cells (MCs), and VEGF-A can induce MC proliferation [21].

Vascular endothelial growth factor A signaling in kidney transplantation

Particularly interesting is the potential role of VEGF in kidney transplantation. Earlier investigations showed that human chronic allograft nephropathy (CAN) and experimental models both exhibit elevated expression of VEGF in the interstitial cell [22]. According to Malmström et al. [23], the chronic allograft damage index (CADI) score was correlated with the total intra graft as well as interstitial inflammatory cell expressions of VEGF and VEGFR-1. PTK787’s inhibition of the VEGF receptor significantly reduced both the CADI score and fibrosis. This finding suggested that elevated VEGF activity may facilitate alloimmune-induced inflammatory responses, ultimately resulting in fibrotic changes [23]. Moreover, VEGF may accelerate allograft vasculopathy by enhancing smooth muscle cell (SMC) migration either directly or by increasing the production of platelet-derived growth factor (PDGF) by surrounding cells [24]. In addition to its direct atherogenic effects, VEGF is crucial in controlling the mobilization, homing, and differentiation of vascular progenitor cells. Circulating VEGFR-2 positive progenitor cells transform into ECs or SMC when stimulated with VEGF or PDGF-BB, respectively, which could hasten the onset of allograft atherosclerosis [25]. It seems that the effects of VEGF on renal allografts are time-dependent. A study from Ozdemir et al. [26] demonstrated that in the short term after kidney transplantation, both tubular and interstitial VEGF expression acts as a protective signal on renal allografts. However, over the long term, interstitial fibrosis (IF) and, consequently, poor graft outcomes would be more likely in patients with marked tubular and interstitial VEGF expression [26].

Endothelin-1 signaling

Endothelin-1 signaling in renal diseases

The human kidney expresses all three ET family members, ET-1, ET-2, and ET-3, albeit ET-1 is the most common isoform [27]. ETA receptors (ETAR) and ETB receptors (ETBR) are two G-protein-coupled receptors that ET-1, ET-2, and ET-3 bind to, whereas ET-3 has a low affinity for the ETAR at physiological concentrations [28]. In the vasculature, ETAR predominantly mediates vasoconstriction and mitogenesis, whereas ETBR mainly mediates vasodilation and inhibition of growth and inflammation.

The human kidney expresses a high density of ETBR [29]. By engaging in ETBR, ET-1 releases vasodilators in an autocrine or paracrine manner. Moreover, the kidney, liver, and lung endothelial ETBR play a critical role in scavenging ET-1 from the plasma [30]. The activation of ETBR in medullary epithelial cells, which lowers salt and water reabsorption, is the third important role of ET-1 [31]. ETAR and ETBR are also present in human and rat podocytes and MCs [32]. ET signaling in podocytes is involved in different renal diseases, such as DN [33], proliferative lupus nephritis (LN) [34], and focal segmental glomerulosclerosis (FSGS) [35]. Lenoir et al. [33] showed that in mice with podocyte-specific double deletion of the alleles of ETAR and ETBR, diabetes-induced glomerulosclerosis and podocyte loss are avoided. Additionally, they discovered that ET-1 could directly activate the nuclear factor kappa B and β-catenin pathways in podocytes, which promotes the development of diabetic glomerulosclerosis and the loss of podocytes [33].

Results from the histological examination of LN samples pointed to a correlation between the width of the foot process and the pathological score of GEC damage. More ET-1 was secreted when GECs were exposed to a podocyte-conditioned medium stimulated with immunoglobulin G (IgG) from LN patients (PCM-LN). A redistribution of cytoskeleton F-actin and a marked decrease in nephrin was noted when podocytes were exposed to an endothelial-conditioned medium stimulated with PCM-LN (ECM-PCM-LN). It should be emphasized that the anti-ETAR antibody could block these effects, demonstrating that GECs and podocytes communicate among themselves through ET signaling [34].
Moreover, ET-1 plays a role in FSGS, frequently accompanied by proteinuria and a steady decline in glomerular function. Podocyte damage, podocyte depletion, and glomerular capillary segment collapse are symptoms of FSGS. Studies performed by Daehn et al. [35] and Ebeors et al. [36] demonstrated that in transgenic mice and BALB/c mice with adriamycin-induced glomerulosclerosis, podocyte-specific activation of transforming growth factor-beta (TGF-β) can cause ET-1 release by podocytes and enhance ETAR expression in nearby ECs. The paracrine ETAR activation by ET-1 led to degradation of the glomerular endothelial surface layer, mitochondrial oxidative stress, and dysfunction of GECs. ETAR antagonism prevented all of these consequences. Albuminuria, glomerulosclerosis, and podocyte apoptosis were, in turn, promoted by endothelial dysfunction [35,36].

**Endothelin-1 signaling in kidney transplantation**

Ischemia-reperfusion injury (IRI) and acute and chronic rejection after kidney transplantation can all trigger the innate and adaptive immune response. ET-1 synthesis in vitro is impacted by a variety of cytokines that are released by infiltrating activated mononuclear cells. Tumor necrosis factor-alpha (TNF-α) can increase the ET-1 messenger RNA (mRNA) and ET-1 protein release in rat MCs [37]. In bovine ECs, interferon-gamma cotreatment rat MCs [38]. TGF-β can stimulate ET-1 secretion in cultured MCs [39], endothelial [40], glomerular epithelial [41], as well as tubular epithelial cells (TECs) [42]. These results suggest that different cytokines and growth factors can regulate ET expression in the allograft during the immunological response to alloantigenic stimuli. Also, it was discovered that upregulation of ET-1 and its receptors in experimental and human kidney transplantation. Recent research has shown that chronic renal allograft rejection in rats results in a considerable overexpression of ET-1, ET-3, and their receptors. Moreover, in these renal allografts, inhibiting ETs reduced chronic rejection, suggesting a potential role for ETs in the pathogenesis of CAN [43]. CAN is associated with a higher level of ET-1 in human kidney transplantation [44]. Medial SMCs and SMCs within the neointima both displayed elevated expression of ETAR in intrarenal arteries with transplant renal arteriosclerosis. These results suggest that increased ETAR expression may enhance the local proliferative and vasoconstrictive effects of ET-1 in human kidney allografts [45].

**Paracrine signaling between vascular endothelial growth factor A, endothelial nitric oxide synthase/nitric oxide, and endothelin-1**

It has been shown that nitric oxide (NO) has antithrombogenic properties, inhibiting EC activation/injury brought on by cytokines and promoting vasodilation, all of which are protective for the vascular system. The main sites of endothelial NO synthase (eNOS) expression in rodents and humans include the medullary vasa recta, glomerular and peritubular capillaries, afferent/efferent arterioles, and the endothelium of intrarenal arteries [46,47]. Mice lacking eNOS displayed podocyte damage and aberrant mitochondria [48]. The protection of podocytes from TNF-α induced loss of synaptopodin by conditioned medium from eNOS-overexpressing microvascular ECs in vitro suggests that healthy GECs protect podocytes from inflammatory insults in a paracrine manner by secreting protective mediators [49]. Furthermore, the preservation of glomerular integrity may rely on the paracrine signaling between VEGF-A, eNOS/NO, and ET-1 in podocytes and GECs. Under physiological conditions, through binding to its receptors VEGFR-1 and VEGFR-2 expressed on GECs, VEGF-A synthesized by podocytes can induce eNOS activation in GECs and subsequently increase NO production [8]. The increase of NO may negatively regulate the amount of VEGF-A produced by podocytes [50]. The glomerular cells control the proper VEGF-A production through this crosstalk, preventing excessive vascular growth while maintaining VEGF-A availability.

In addition to NO, VEGF-A also regulates ET-1 production by GECs. A study by Collino et al. [51] revealed that podocyte VEGF-A blockade causes ET-1 release from GECs. High levels of ET-1 prevent the formation of NO, while low levels of ET-1 promote its production [52]. In addition to cytoskeleton rearrangement, ET-1 produced from GECs can also result in a decrease of nephrin in podocytes [34]. Conversely, NO has protective effects on podocytes and lowers the expression of ET-1 [49]. An illustration of crosstalk between GECs and podocytes in the VEGF-A-eNOS/NO-ET-1 axis is shown in Fig. 2.
Angiopoietin signaling

Angiopoietins in renal diseases

ANGPTs belong to the vascular growth factor family, including ANGPT1, ANGPT2, ANGPT3, and ANGPT4. ANGPT1 works as a Tie2 receptor agonist, supporting an anti-inflammatory, pro-survival, and anti-permeability phenotype of the vasculature. Contrarily, ANGPT2, secreted by ECs in response to proinflammatory stimuli, prevents Tie2 from being phosphorylated and thus breaks up the protective Tie2 signaling. Hence, it appears that the equilibrium between ANGPT1 and ANGPT2 is what controls signaling through Tie2.

Much like the VEGF-VEGFR paracrine pathway, ANGPT1 is expressed by podocytes and MCs, while its target Tie2 is expressed by GECs. ANGPT1 is essential for maintaining healthy glomeruli, and signaling of the ANGPT1/Tie2 pathway appears essential for maintaining the filtration barrier both during the normal development of the kidneys and during pathological circumstances.

Global deletion of ANGPT1 before the embryonic day (E) 12.5 causes vascular abnormalities that lead to early embryonic death. The glomerulus of mice with induced ANGPT1 deletion at E10.5 showed abnormalities, including dilated capillary loops, an unorganized GBM structure, and MC reductions, while podocytes appeared intact. The global ANGPT1 deletion induced later did not result in any obvious phenotype [53]. This indicates that ANGPT1/Tie2 signaling is required for the development of the vascula-
ture, including glomerular capillaries, but not necessary for quiescent vessels. In pathological conditions like diabetes, ANGPT1-deficient diabetic mice displayed higher proteinuria, mesangial matrix expansion, and glomerulosclerosis compared to diabetic controls. Albuminuria and GEC proliferation were delayed by the repletion of the glomerular ANGPT1 in diabetic mice with selective podocyte-specific overexpression of ANGPT1 [54].

Moreover, a decrease in the endothelial survival markers VEGF-A and ANGPT1 and an increase in ANGPT2 were temporally associated with the loss of glomerular capillaries in a mouse model anti-GBM glomerulonephritis [55]. These findings imply that ANGPT1/Tie2 signaling is not only crucial for maintaining GFB function but also has a remarkable capacity to regulate the glomerular capillary response to damage. Contrarily, the overexpression of ANGPT2 in podocytes increases GEC apoptosis and albuminuria, indicating that ANGPT2 may compete with ANGPT1 [56].

Angiopoietins in kidney transplantation

Increased ANGPT2 levels (the natural Tie2 antagonist) have been demonstrated to correlate with mortality in kidney transplant recipients, suggesting that an unbalanced ANGPT/Tie2 system may be detrimental to renal transplantation [57]. Ma et al. [58] showed that ANGPT1 was downregulated in a rat model of CAN, whereas ANGPT2 and Tie2 were increased. These changes have a strong correlation with the Banff score. Exogenous delivery of a PEGylated synthetic Tie2 agonistic peptide can enhance graft function in a mouse major histocompatibility complex-mismatched renal transplant model by controlling endothelial activation and the transmigration of deleterious inflammatory cells into the interstitium of the transplant [59].

**CXCL12/CXCR4/CXCR7 signaling**

**CXCL12/CXCR4/CXCR7 signaling in renal diseases**

Homeostatic chemokine CXC chemokine ligand 12 (CXCL12; stromal cell-derived factor 1) signals through its receptors CXCR4 and CXCR7 [60]. After being stimulated by their same ligand, CXCL12, both receptors can activate multiple cell signaling pathways and/or scavenge CXCL12 from the extracellular space. This promotes the development of organs and the preservation of homeostasis. In the kidney, podocytes can produce CXCL12, which acts on CXCR4 expressed by GECs and carries out an essential role in podocyte-EC cross-communication. Nephrogenesis, particularly in the formation of the renal vasculature, strongly benefits from CXCL12/CXCR4/CXCR7 signaling [61]. Similar renal phenotypes with abnormal blood vessel development, like the ballooning of glomerular capillaries and altered conformation of the renal vasculature, were seen in mice lacking either CXCL12 or CXCR4. Deletion of CXCR7 in mice, which also leads to a reduction of CXCR4 expression, replicated the phenotype of the CXCR4 deficient mice, suggesting that CXCR7 closely controls CXCL12/CXCR4 mediated signaling between podocytes and glomerular capillaries [62]. Moreover, CXCL12 has a role in several kidney diseases, including renal cell carcinoma, DN, LN, diarrhea-associated HUS, and acute kidney injury (AKI) [63].

**CXCL12/CXCR4/CXCR7 signaling in kidney transplantation**

According to Hoffmann et al. [64], compared to healthy transplant kidneys, the expression of CXCL12 was considerably higher in transplants with persistent fibrotic lesions. Indeed, CXCL12/CXCR4 induces renal TECs-mesenchymal transition (EMT) with the involvement of the Wnt pathway [65]. Renal allograft fibrosis can be successfully mitigated using a CXCR4 antagonist or a neutralizing antibody [66]. In IRI-induced renal transplantation damages, anti-CXCL12 antibodies could reduce IRI and chronic rejection [67]. On the other hand, studies also revealed that CXCL12 is essential for CXCR4-positive cells, such as hematopoietic stem cells and a fraction of mesenchymal stem cells (MSCs), to home and migrate to the kidney, representing a potential prevention for IRI-induced acute/chronic rejection and maintaining renal function [68].

**Interleukin-6 signaling pathway**

**Interleukin-6 signaling pathway in renal diseases**

IL-6 is a pleiotropic cytokine that, in addition to immunological and inflammatory responses, also controls hematopoiesis, metabolism, and organ development. Fig. 3
summarizes the two signaling pathways of IL-6: the classical and trans-signaling pathways. The classical pathway is activated by the binding of IL-6 with the membrane-bound IL-6R (mIL-6R, also named CD126 or gp80), while the trans-signaling is induced by the interaction of IL-6 with a soluble form of IL6R (sIL-6R). It’s important to note that there is a naturally produced isoform of Gp130 called soluble Gp130 (sGp130), which is identified in the bloodstream at relatively high concentrations (100–400 ng/mL in human plasma) [69]. sGp130 functions as a specific inhibitor of the IL-6 trans-signaling pathway because it can interact with the IL-6/sIL-6R [70]. It is generally accepted that IL-6 classical signaling is anti-inflammatory and trans-signaling is proinflammatory [71], although there is a debate [72].

Renal resident cells, such as podocytes, ECs, MCs, and TECs, can release IL-6 under certain conditions. Since only podocytes express the mIL-6R, which indicate that only podocytes can respond to IL-6 via both classical and

Figure 3. Interleukin-6 (IL-6) signaling pathway. (A) The classical pathway is activated by binding IL-6 with the membrane-bound IL-6 receptor (mIL-6R). This complex then establishes a connection with two Gp130 molecules and starts the signaling process. (B) The trans-signaling pathway is induced by soluble form of the IL-6 receptor (sIL-6R). sIL-6R binds to IL-6, which consequently activate Gp130. (C) Soluble form of Gp130 (sGp130), which is found naturally produced and is detected in the circulation, can interact with the IL-6/sIL-6R complex, acting as a specific inhibitor of the IL-6 trans-signaling pathway. Both classical and trans-signaling pathways activation leads to the downstream intracellular signal transduction.

AKT, a serine/threonine protein kinase; ERK, extracellular signal-regulated kinase; JAK, Janus kinase; MAPK, mitogen-activated protein kinase kinase; m-TOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa B; PI3K, phosphatidylinositol 3-kinase; RAS, rat sarcoma; RAF, rapidly accelerated fibrosarcoma; STAT, signal transducer and activator of transcription.
trans-signaling pathway, while in ECs, MCs, and TECs, the IL-6 trans-signaling pathway is predominant. IL-6 signaling is involved in many kidney diseases, such as IgA nephropathy, LN, DN, AKI, and chronic kidney disease [73].

Usually, different pathological stimuli can induce renal resident cells secreting IL-6, which in turn triggers the growth of MCs, the recruitment of inflammatory cells, and the overexpression of the angiotensin (Ang) II type 1 receptor in ECs with the consequence of Ang II-induced vasoconstriction, reactive oxygen species production, and endothelial dysfunction [74].

**Interleukin-6 signaling pathway in kidney transplantation**

It is widely known that IL-6 has a role in both acute and chronic kidney allograft rejection. Renal expression of IL-6 was elevated with decreased intragraft Foxp3+ Tregs after allograft rejection in a mouse kidney transplant model [75]. Furthermore, the absence of donor-produced IL-6 increased the survival of the renal allograft. It was correlated with higher levels of intragraft Tregs and lower levels of circulating anti-graft alloantibodies, indicating that selective inhibition of donor IL-6 signaling may prevent both humoral and cellular rejection [76]. In an experimental model of CAN, IF and tubular atrophy were demonstrated to be mediated by intragraft B-cell production of chemokines and cytokines, including IL-6. This finding indicates that IL-6 may play a potential role in causing CAN [77]. Renal allograft rejection in human kidney transplant patients is accompanied by increased IL-6 levels in the blood, urine, and biopsy tissue [78,79]. Rejection occurred in renal allograft recipients who developed high blood IL-6 and IL-17 levels during tolerance induction utilizing a mixed chimerism method, but recipients without high IL-6/IL-17 experienced long-term survival without rejection [80]. What’s more noteworthy is that donor genotypes for IL6 and IL6R, but not recipient genotypes, serve as an independent predictive biomarker for biopsy-confirmed renal allograft rejection [81].

It’s important to note that IL-6 signaling seems to have a protective role by boosting the repair process in some pathological circumstances, such as the ischemia-reperfusion–induced AKI model. By an underlying anti-oxidative stress mechanism, stimulation of IL-6 trans-signaling dramatically lowers kidney damage and protects renal function [82]. More interestingly, Kuravi et al. [83] proposed the existence of a crosstalk between podocytes and ECs via IL-6, which was demonstrated in a podocyte-EC coculture system. Particularly, TNF-α stimulated podocytes to release IL-6, which increased the expression of suppressor of cytokine signaling 3 in glomerular endothelium and induced IL-6’s immunosuppressive effect, hence limiting the migration of neutrophils to the endothelium [83].

**Extracellular vesicles**

**Extracellular vesicles: a novel frontier in renal diseases**

Recently, cell-cell communication mediated by EVs is an emerging biological concept. Almost all cells secrete EVs, which are divided into exosomes and microparticles based on their size. Contrary to signaling molecules secreted by the cells, which are well-defined proteins with specific roles, EVs contain a concentrated complex of molecules, including lipids, nucleic acids, proteins, glycans, and metabolites. These molecules can exert contemporarily synergistic or antagonistic functions. The EVs cargo is protected from enzymatic degradation in the extracellular environment and can be delivered to distant cells. Moreover, EVs-mRNA can be horizontally transferred to the target cells and translated into the corresponding protein [84]. Therefore, cell-to-cell communication, including podocytes and GECs bidirectional crosstalk through EVs, is an intriguing research topic. Wu et al. [85] found that high glucose (HG) causes GECs to undergo the endothelial-to-mesenchymal transition (EndMT), and HG-treated cells with the EndMT produce more exosomes than normal glucose-treated GECs. They demonstrated that exosomes originating from GECs undergoing EndMT might be taken up by podocytes and can cause the podocyte to undergo epithelial-to-mesenchymal transition (EMT) and barrier failure. Moreover, their study revealed that TGF-β1 mRNA is more abundant in exosomes from HG-treated GECs and likely causes EMT and malfunctioning of podocytes via canonical Wnt/β-catenin signaling. Their findings imply that renal fibrosis in DN is contributed by the paracrine communication between cells undergoing the EndMT and podocytes via exosomes. Therefore, protecting GECs from the EndMT and inhibiting TGF-β1-containing exosome release from GECs could be a new therapeutic strategy to prevent renal fibrosis.
fibrosis in DN [85]. According to recent research by Medica et al. [86], an L-selectin–based mechanism was primarily responsible for the internalization of EVs produced from endothelial progenitor cells (EPCs) in both GECs and podocytes. By modifying gene expression and triggering the release of growth factors like VEGF-A and hepatocyte growth factor, EVs improved the development of capillary-like structures and cell migration in GECs. EPC-derived EVs defended GECs against apoptosis in the presence of cytokines such as IL-6, TNF-α, and complement protein C5a by reducing oxidative stress and blocking leukocyte adherence by limiting the production of adhesion molecules (ICAM-1, VCAM-1, E-selectin). In podocytes, EVs reduced apoptosis and blocked the loss of nephrin brought on by cytokines and C5a. More intriguingly, EPC-derived EVs protected podocytes from apoptosis and a change in perm selectivity linked to inflammation-mediated damage in a coculture system of GECs/podocytes that simulated GFB. Moreover, pretreating EVs with RNase rendered their protective actions ineffective, indicating the critical role of RNA transfer from EVs to injured glomerular cells. Their findings suggested that the EPC-derived EVs protected GFB integrity against complement- and cytokine-induced damage, indicating a potential role as therapeutic agents for drug-resistant glomerulonephritis [86].

Extracellular vesicles in kidney transplantation

Recently, the relevance and role of EVs in renal transplantation have attracted increasing attention. Finding the diagnostic and prognostic biomarkers for evaluating donor kidney quality, graft function, and kidney allograft rejection were the main goals of the EV investigations. Turco et al. [87] demonstrated that specific populations of EVs derived from renal parenchymal cells identify kidney structural changes (nephron hypertrophy and nephrosclerosis) through profiling urinary EVs in 138 kidney donors at the time of live-donor transplantation, which may allow clinicians to assess donor kidney health and predict graft function. Using proteome and micro RNA (miRNA) analysis, Lozano-Ramos et al. [88] found that urinary EVs from live donors had higher concentrations of the miR-326 (which targets the B-cell lymphoma 2–related apoptotic pathway) than those from deceased donors. It’s interesting to note that EVs can also be found in donors’ preservation fluid both after circulato-

Glutamatergic signaling

Given the intricate structure of podocytes and the continual stimulation and stress brought on by blood pressure and contents, these cells probably need a precise and quick modality of communication among themselves and with the other glomerular cells as well. Studies from our group have demonstrated that podocytes possess glutamate-containing vesicular structures that undergo spontaneous and regulated exo-endocytosis [95]. Deletion of Rab3A, a small GTPase that controls glutamate exocytosis, or blocking the glutamate ionotropic N-methyl-D-aspartate receptor (NMDAR) with specific antagonists can alter glutamatergic signaling in podocytes, which can cause significant cytoskeletal reorganization, nephrin redistribution, and an elevated urinary albumin/creatinine ratio in mice [96]. These findings imply that the GFB’s integrity is sustained by glutamatergic signaling mediated by podocytes. Moreover, using a mouse podocyte-EC coculture system, which mimics the
Glomerular endothelial cells-podocytes crosstalk

In vitro, we demonstrated the existence of a crosstalk between those two cell types via glutamate signaling. We confirmed that the ionotropic glutamate receptor NMDAR and the metabotropic receptor Grm1 are present in mouse GECs in vivo as well as the ECs in culture. The addition of glutamate to the endothelial side of the coculture potently increased albumin permeability and the same effect was obtained by adding to the podocyte medium alpha-latrotoxin, a substance known to induce glutamate release from podocytes. In addition, both treatments caused increased endothelial p44/42 mitogen-activated protein kinase (MAPK). Preincubation of ECs with the NMDAR antagonist MK-801 was able to prevent albumin leakage from the GFB model and abolish 44/42-MAPK activation induced both by glutamate and by alpha-latrotoxin [97]. Those results suggested that excessive activation of EC glutamate signaling can result in the alteration of GFB permeability.

**Conclusion**

Much evidence suggests that the cell-cell communication between resident cells in the glomerulus through signaling molecules is involved in glomerular homeostasis, renal diseases, and kidney transplants (Fig. 4; Supplementary Table 1 and 2, available online). Our understanding of glomerular cross-communication has substantially expanded with the increased use of cell-specific transgenic models and new techniques, such as genomics, transcriptomics, proteomics, and metabolomics. However, much research must be conducted on discovering novel signaling cascades, particularly those released by EVs from various cell types. The identification of these mediators as well as a better understanding of already established crosstalk molecules, may lead to the recognition of new targets for managing kidney transplantation and the prevention and treatment of glomerular disorders.

**Figure 4.** The overall signaling pathways between podocytes and glomerular endothelial cells (GECs). Through binding to vascular endothelial growth factor (VEGF) receptor (VEGFR)-1/2 expressed on GECs, VEGF-A synthesized by podocytes can induce endothelial nitric oxide synthase (eNOS) activation in GECs and subsequently increase nitric oxide (NO) production. The increase of NO may negatively regulate the amount of VEGF-A produced by podocytes. VEGF-A can inhibit endothelin-1 (ET-1) production by GECs through the activation of eNOS. High levels of ET-1 inhibit NO production. ET-1 released from GECs can activate ET receptor (ETR) A and B on podocytes. Angiopoietin 1/2 (ANGPT1/2) produced by podocytes can bind with the Tie2 expressed on GECs. CXCL12 secreted by podocytes can activate CXCR4/7 on GECs. On podocytes, interkeukin-6 (IL-6) can bind with the membrane-bound IL-6 receptor (mIL-6R) and activate the classical signaling pathway. Moreover, IL-6 also can form a complex with soluble form of the IL-6 receptor (sIL-6R) and activate the trans-signaling pathway. On GECs, since mIL-6R is absent, only IL-6 trans-signaling can be activated. Extracellular vesicles (EVs) released by both podocytes and GECs act as bidirectional crosstalk mediators. GBM, glomerular basement membrane.
Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

The data presented in this study are available on request from the corresponding author.

Authors’ contributions

Conceptualization: DM
Funding acquisition: GC
Resources: ML, SA
Software: MI, PM
Supervision: CA, GC
Writing–original draft: ML, SA
Writing–review & editing: ML, SA, CC, CEC, SM
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Introduction

Kidney disease has been recognized as a crucial public health concern due to the increased risk of early death, poor quality of life, as well as the high cost of treatment [1–3]. The Global Burden of Disease 2019 study estimated that 1.43 million deaths and 41.5 million disability-adjusted life-years were attributable to chronic kidney disease (CKD; defined as an estimated glomerular filtration [eGFR] less than 60 mL/min per 1.73 m²), with increases of 28.8% and 20.1% since 2010, respectively [4]. In South Korea, approximately 11% of adults (around 4.6 million people) were estimated to have CKD in 2017, and the total medical care expenses for CKD were estimated at 1,707 billion Korean won (about $1.3 billion) [5]. In the United States, about 15% of adults were estimated to have CKD in 2021 [6], and the total Medicare fee-for-service spending for beneficiaries with CKD exceeded $100 billion in 2018 [7].

Recently, numerous studies have consistently reported the health benefits of green space, especially in relation to circulatory and endocrine diseases, which are major risk factors for kidney disease. Previous studies have reported that higher exposure to greenness may be related to a lower risk of hypertension, diabetes, and cardiovascular diseases, by increasing physical activities and social engagements [8–10] and reducing noise, extreme temperatures, and air pollution exposures [11,12]. In addition, greenness may alleviate air pollution concentration and extreme temperature events [13,14], and recent epidemiological studies identified that exposure to air pollution and extreme tem-

Keywords: Acute kidney injury, Chronic kidney disease, End-stage kidney disease, Green space
temperatures are associated with kidney diseases [3,15–18]; these results imply that reduced air pollution and extreme temperature events by green space can be directly or indirectly linked to the reduced risk of kidney diseases (Fig. 1). Despite these plausible mechanisms, however, studies on the relationship between greenness and kidney disease were limited [19].

In this review, we discuss the epidemiological evidence on the contribution of greenness exposures to kidney diseases. We also suggest important findings and limitations of previous studies and propose points that should be carefully addressed in future studies to reduce some of the knowledge gaps.

**Epidemiological studies on greenness and kidney diseases**

A literature search was performed using electronic databases PubMed, MEDLINE, Google Scholar, and Web of Science for published studies from 1990 to March 2023. We searched for relevant studies using the keywords ‘greenness and kidney disease, ‘ ‘green space and kidney disease, ‘ ‘open space and kidney disease, ‘ ‘green and kidney disease, ‘ ‘green and chronic kidney disease, ‘ limiting the search to research articles addressing human and published in English. Finally, we summarized a total of seven studies (Table 1).

**Greenness indices in epidemiological studies**

In general, previous epidemiological studies measured a level of residential greenness using two representative satellite-based vegetation indices: normalized difference vegetation index (NDVI) and enhanced vegetation index (EVI). The NDVI is calculated as the difference between near-infrared reflectance (NIR) and red reflectance (RED) divided by their sum: \( \text{NDVI} = \frac{\text{NIR} - \text{RED}}{\text{NIR} + \text{RED}} \) [20]. Thus, the calculated NDVI ranges from -1 (non-vegetated areas) to +1 (full-vegetated areas) with higher values indicating denser vegetation. The concept and calculation procedure of EVI is similar to NDVI; however, EVI corrects potential distortions in the reflectance due to the particles in the air, ground cover below the vegetation, and canopy background noise, and it is more sensitive in areas with dense vegetation [21]. The equation of EVI is as follows: \( \text{EVI} = G \times \left[ \frac{(\text{NIR} - \text{RED})}{(\text{NIR} + C_1 \times \text{RED} - C_2 \times \text{BLUE} + L)} \right] \).

![Figure 1. Directed acyclic graphs regarding the association between greenness and kidney disease.](image-url)
### Table 1. Summarized information on studies reviewed in this study

<table>
<thead>
<tr>
<th>Study region</th>
<th>Study year</th>
<th>Study design</th>
<th>No. of patients</th>
<th>Major greenness exposure</th>
<th>Major kidney outcome</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nationwide, United States</td>
<td>2000–2008</td>
<td>Prospective cohort</td>
<td>108,630</td>
<td>NDVI</td>
<td>Mortality related to kidney diseases</td>
<td>HR, 0.63 (95% CI, 0.38–1.04) for 0.1 increase in NDVI (250 m buffer)</td>
</tr>
<tr>
<td>Taipei, Taiwan</td>
<td>2009</td>
<td>Cross-sectional</td>
<td>21,656</td>
<td>Distance to open space</td>
<td>CKD (eGFR ≤60 mL/min/1.73 m²)</td>
<td>OR, 1.071 (95% CI, 1.007–1.138) for Distance to open space (100 m)</td>
</tr>
<tr>
<td>Metropolitan areas, Republic of Korea</td>
<td>2001–2016</td>
<td>Prospective cohort</td>
<td>64,565</td>
<td>NDVI (June to August)</td>
<td>Mortality in patients with CKD or ESRD</td>
<td>1) Mortality of CKD patients HR, 0.96 (95% CI, 0.93–1.00) for 0.1 increase in NDVI (1,250-m buffer)</td>
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<td>2) Mortality of ESRD patients HR, 0.91 (95% CI, 0.87–0.97) for 0.1 increase in NDVI (1,250-m buffer)</td>
</tr>
<tr>
<td>Nationwide, China</td>
<td>2007–2010</td>
<td>Cross-sectional</td>
<td>47,204</td>
<td>NDVI</td>
<td>CKD (eGFR ≤60 mL/min/1.73 m² or ACR &gt;30 mg/g)</td>
<td>OR, 0.79 (95% CI, 0.73–0.86) for IQR increase in NDVI (1,000 m)</td>
</tr>
<tr>
<td>New Taipei City, Taiwan</td>
<td>2007–2009</td>
<td>Cross-sectional</td>
<td>40,375</td>
<td>Distance to Small Public Urban Green Spaces (SPOUS) (February to April, August to October)</td>
<td>CKD (eGFR ≤60 mL/min/1.73 m²)</td>
<td>OR, 1.144 (95% CI, 1.059–1.237) for Distance to SPOUS &gt;200 m vs. ≤200 m</td>
</tr>
<tr>
<td>Bangkok, Thailand</td>
<td>2009–2019</td>
<td>Prospective cohort</td>
<td>2,022</td>
<td>NDVI</td>
<td>eGFR</td>
<td>(Linear) 1.03% (95% CI, 0.33–1.74) for IQR increase in NDVI</td>
</tr>
<tr>
<td>Massachusetts, United States</td>
<td>2000–2016</td>
<td>Retrospective cohort</td>
<td>1,462,949</td>
<td>EVI</td>
<td>First hospitalization with diagnostic codes for kidney diseases (total kidney disease, CKD, and AKI)</td>
<td>1) Total: HR, 0.95 (95% CI, 0.93–0.97) for 0.1 increase in an average (January, April, July, and October) NDVI in the 250-m areas around each participant’s address.</td>
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<td>2) CKD: HR, 0.98 (95% CI, 0.95–1.01)</td>
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<td>3) AKI: HR, 0.94 (95% CI, 0.92–0.97) for 0.1 increase in EVI (zip code)</td>
</tr>
</tbody>
</table>

ACR, urinary albumin to creatinine ratio; AKI, acute kidney injury; CI, confident interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; EVI, enhanced vegetation index; HR, hazard ratio; IQR, interquartile range; NDVI, normalized difference vegetation index; OR, odds ratio.

G is a gain factor, and BLUE is the reflectance in the blue band measured by a satellite. L is the canopy background adjustment, and C₁ and C₂ are the coefficients of the aerosol resistance terms using the blue and red bands. In other words, EVI indicates a ratio between RED and NIR values, while reducing the background and atmospheric noises, thus the valid range is −1 to +1, with higher values meaning higher vegetation [22].

**Literature review**

To our knowledge, a study by James et al. [23] in 2016 first suggested the potential association between greenness and kidney disease. From the Nurses’ Health Study prospective cohort in the United States with 108,630 female participants from 2000 to 2008 (627,008 person-years), the study found that women living in areas with higher greenness showed lower mortality risk, with a hazard ratio (HR) of 0.63 (95% confidence interval [CI], 0.38–1.04) for a 0.1 increase in an average (January, April, July, and October) NDVI in the 250-m areas around each participant’s address.

Since 2019, studies showing the linkage between exposure to green space and the prevalence/incidence of kidney disease have begun to emerge [24]. A cross-sectional study in New Taipei City including 21,656 participants reported that the association between proximity to open space (majorly consists of green space) and adult renal function, with an odds ratio (OR) of 1.071 (95% CI, 1.007–1.138) for 100-m increase in a distance to open space.

In 2021, a multihospital-based prospective cohort study
in South Korea examined the association between residential greenness and clinical outcomes of CKD patients [25]. The study constructed a large-scale cohort including 64,565 patients diagnosed with CKD from multiple metropolitan hospitals, and an average NDVI during the summer (June to August) corresponding to each participant’s residential address was used as an exposure to green space. For CKD patients, HRs were 0.96 (95% CI, 0.93–1.00; mortality) and 1.01 (95% CI, 0.98–1.04; progression to end-stage kidney disease [ESKD]) for 0.1 increase in NDVI (1,250-m buffer). Mortality of ESKD patients showed an evidently negative association with NDVI (1,250-m buffer) with an HR of 0.91 (95% CI, 0.87–0.97).

In 2022, two cross-sectional studies on CKD and green space were conducted in East Asia. Liang et al. investigated the relationship between residential NDVI and the presence of CKD based on the China National Survey of Chronic Kidney Disease with 44,876 individuals from 2007 to 2010 [26]. They found a negative association between all-season NDVI and CKD presence with an OR of 0.79 (95% CI, 0.73–0.86) for 0.26 (an interquartile range) increase in NDVI. Chien et al. [27] also performed an ecological study including 40,375 participants older than 30 years from the health screening program from 2007 to 2009 in the Metropolitan area in New Taipei City. They defined the urban open green space incorporating parks, green, plazas, public schools, and sports venues, as exposure to green space, and found the OR of CKD prevalence comparing the distance to small public urban open space over 200 m and 200 m or less was 1.14 (95% CI, 1.06–1.24).

In 2023, two cohort studies on greenness and kidney disease were published. A cohort study for electricity generating authority of Thailand employees with 2,022 participants aged 25 to 55 years in the Bangkok Metropolitan region showed that the interquartile increase in all-season NDVI was related to a higher eGFR, with 1.03% increase in eGFR (95% CI, 0.33–1.71) [28]. However, they did not find an association between eGFR and the EVI, which is an optimized satellite-driven vegetation index that corrects distortions in the reflectance [21].

Another cohort study was performed in Massachusetts, the United States. Lee et al. [19] constructed a longitudinal retrospective cohort including 1,462,949 Medicare beneficiaries living in Massachusetts from 2000 to 2016 (9.8 million person-years). This study used annual-average EVI for each beneficiary’s residential zip code as exposure and the first hospital admission for kidney diseases (total kidney disease, CKD, and acute kidney injury [AKI]) as outcome variables based on the International Classification of Diseases diagnostic codes. They found a negative association between greenness and the first hospitalization for the total kidney disease (HR, 0.95; 95% CI, 0.93–0.97), and the association was more pronounced in AKI (HR, 0.94; 95% CI, 0.92–0.97) compared to CKD (HR, 0.98; 95% CI, 0.95–1.01).

**Discussion**

This study reviewed a total of seven epidemiological studies on greenness and kidney disease. Although there were differences in statistical significance, all studies showed a beneficial association between higher exposure to green space and kidney disease. NDVI, a satellite-based vegetation index, was majorly used to measure exposure to greenness, and the prevalence or incidence of CKD was addressed as a kidney disease outcome.

As mentioned in the Introduction section, higher exposure to green space can alleviate the risk of kidney disease by increasing physical and social activities [8–10] and reducing environmental hazards such as air pollution and heat [11,12]. Previous studies identified that decreased physical activities and poor mental health may be associated with lower kidney function [29–31]. Furthermore, recent epidemiological studies consistently reported that exposure to extreme temperatures and air pollution was associated with a higher risk of kidney disease. Cohort studies in the United States reported that exposure to air pollution and heat increases the risks of incident CKD [17,18] or hospitalization for CKD [15] and AKI [19,32]. Korean studies based on the National Health Insurance System also showed that exposure to heat and air pollutants increases hospital admissions related to kidney disease [16,33]. Furthermore, previous studies suggested that the beneficial effects of green space on cardiovascular diseases [12,34], which are major causes of kidney diseases, should be majorly addressed in the beneficial roles of greenness on kidney health [19,25]. These results from multiple studies support biological mechanisms and epidemiological evidence of the beneficial effects of greenness on kidney health.

Nevertheless, several points should be discussed further. First, previous studies have respective limitations in
relation to their study design. Relevant studies in Taiwan and China [24,26,27] were cross-sectional studies, which are limited in assessing the association between exposure and incidence of health outcomes [35]. James et al. [23] designed the study to examine the association between green space and various causes of mortality, not only for kidney disease-related deaths. The cohort study of Park et al. [25] was based on a large-scale prospective cohort, but covered patients enrolled at metropolitan hospitals, thus there were limitations in addressing nonmetropolitan areas, particularly rural areas which are generally more vulnerable to medical services [36]. Paoin et al. [28] also conducted a prospective cohort study; however, their study included only employees in a certain company with limited age distribution (25 to 55 years), thus the generalizability of the study result may be less than in other population-based or multilocation cohort studies. The study by Lee et al. [19], which is the latest in this review, performed a population-based cohort with the largest sample size (1.4 million beneficiaries) to achieve more generalizable results on greenness and kidney disease. Nevertheless, because this study was based on a retrospective cohort in Massachusetts based on Medicare claim data, it was limited in reflecting other regions in the United States and addressing sufficient individual-level variables that might be crucial confounders [19].

Second, indices for greenness exposure that previous studies used were heterogeneous among studies, and evaluations for association with kidney disease were also sensitive to the greenness indices. Most of the studies in this review used NDVI [23,25–28], which is a standardized satellite-based index based on reflectance. However, measuring NDVI can be sensitive to distortions in the reflectance caused by particles in the air, ground coverage below the vegetation, and areas with a large amount of chlorophyll [21], thus it might be not optimized in certain circumstances. Paoin et al. [28] showed that the association with eGFR differed from the results of NDVI when EVI (that corrects the limitations of NDVI) was used as exposure. Furthermore, we should note that different buffer sizes to measure NDVI can increase the uncertainty of association assessment. Studies in this review used different buffer sizes: 250 m, 1,250 m [23,25], and 1,000 m [26]; and the evaluated association between greenspace and kidney outcomes was not robust to the buffer sizes [25]. Therefore, various sensitivity analyses and further studies to find the optimal greenness index and buffer sizes are required.

Third, most of the studies in this review addressed CKD or gradual changes in eGFR as kidney outcomes. This is a common characteristic in epidemiological studies regarding greenness as well as air pollution [3,19] because the effects of greenness are generally considered gradual effects [11]. However, interestingly, a previous study found that the association with greenness was more evident in AKI outcomes than in CKD outcomes and conjectured that reducing extreme temperature events by greenness can be related to AKI, which is an acute event [19]. This study suggested dehydration which is one of the major risk factors of AKI [33,37] and the well-known association between short-term exposures to extreme temperatures and hospitalization for heart failures, respiratory events, and urinary tract infections [38,39] might play an important role to explain the relationship between greenness and AKI. Therefore, future studies should address various kidney outcomes that are potentially associated with greenspace based on plausible mechanisms.

Fourth, potentially different exposure levels and health effects of green space depending on socioeconomic characteristics should be addressed importantly in future studies. Many studies in this review were performed in urban areas [24,25,27,28], thus there is a knowledge gap in explaining whether the beneficial effects of green space exist in rural areas. In addition, urbanicity is closely related to socioeconomic and environmental status as well as demographic compositions [40]. In general, it could be expected that the population living in urban areas showed higher average socioeconomic status (e.g., income) and levels of medical services, which may be beneficial for kidney health [19]. Previous studies reported that higher socioeconomic status populations are more likely to live in areas with higher levels of health-promoting greenspace (such as urban parks that can increase recreational and physical activities in neighborhoods) [41,42], and a previous study also showed that the beneficial effects of green space on kidney disease were more prominent in people with higher socioeconomic status than in people with lower socioeconomic status [19]. Concurrently, however, urban areas generally have higher air pollution levels and extreme heat events [43], and in some areas, urban areas also showed a larger population % below the poverty level and lower ac-
accessibility to healthcare services due to the large amounts of population [44]. A recent study in Massachusetts also showed that the beneficial impacts of green space on kidney disease were observed in both urban and rural areas (the differences were not statistically significant) and mentioned that the results should be interpreted carefully, in relation to the complexity of urbanicity [19]. Thus, the urban-rural disparities in relation to greenness and kidney disease should be examined in-depth in the future.

Fifth, most of the relevant studies were performed in Asian regions [24–28], thus more various study regions should be addressed to bring more generalizable effects of greenness. In particular, most studies reviewed in this article were conducted in countries or regions with temperate climates. Although we were not able to find a global or multicountry study that addressed the climate and kidney disease at a large scale, earlier studies have consistently reported kidney disease has seasonality [37] and climatic factors like extreme temperatures [33], and these findings strongly suggest the impacts of greenness on kidney disease can be highly associated with study regions which have different weather and environments. Therefore, more studies in various regions should be performed to address these knowledge gaps.

Lastly, most previous studies that investigated the association between greenness and kidney disease did not address advanced statistical models that can consider complicated spatiotemporal correlations among study subjects. Previous studies investigating kidney diseases in the entire United States showed that there were complex spatial patterns in the occurrence and prevalence of kidney diseases [3,15]. Further, in many countries, the prevalence of kidney disease has been increasing or showing evident temporal patterns [7,16]. However, we were unable to find previous studies on greenness and kidney disease performing spatiotemporal models (such as the Integrated Nested Laplace Approximation, called INLA). Thus, it is difficult to exclude the possibility of estimation bias due to the spatial and temporal autocorrelation in previous results measuring the association between green space and kidney disease, and the usage of advanced statistical models that can address this issue should be considered weightily in future studies.

**Conclusion**

Recently, the word “Green nephrology” has been raised very actively [45]. Of course, this concept mainly addresses more environmental conditions (energy consumption, water, high volumes of waste, etc.) for dialysis, but in a wide sense, the benefit of green space on kidney health can be closely related to the environmental sustainability of kidney care, which is a major aim of “Green nephrology”. Thus, in order to contribute to more sustainable strategies to address kidney disease, the ultimate goal of studies on greenness and kidney should be to identify the relationship between greenness and kidney disease at both experimental and epidemiological levels and provide scientific evidence for medical and public health implications to alleviate the medical and economic burden due to kidney diseases.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

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**Data sharing statement**

The data presented in this study are available on request from the corresponding author.

**Authors’ contributions**

Conceptualization, Funding acquisition: WL
Data curation: JP, HY
Writing – original draft: JP, WL
Writing – review & editing: HY, WL
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25. Park JY, Jung J, Kim YC, et al. Effects of residential greenness on clinical outcomes of patients with chronic kidney dis-


Frequency of Fabry disease in chronic kidney disease patients including patients on renal replacement therapy in Korea

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Background: Fabry disease (FD) is an X-linked lysosomal storage disorder caused by the deficient activity of α-galactosidase (α-Gal A), affecting multiple organs including kidney. In this study, we aimed to determine the prevalence of FD in patients with chronic kidney disease (CKD) including those on renal replacement therapy in Korea.

Methods: This is a national, multicenter, observational study performed between August 24, 2017 and February 28, 2020. Patients with the presence of proteinuria or treated on dialysis were screened by measuring the α-Gal A enzyme activity using either dried blood spot or whole blood, and plasma globotriaosylsphingosine (lyso-GL3) concentration. A GLA gene analysis was performed in patients with low α-Gal A enzyme activity or increased plasma lyso-GL3 concentration.

Results: Of 897 screened patients, 405 (45.2%) were male and 279 (31.1%) were on dialysis. The α-Gal A enzyme activity was measured in 891 patients (99.3%), and plasma lyso-GL3 concentration was measured in all patients. Ten patients were eligible for a GLA gene analysis: eight with low α-Gal A enzyme activity and two with increased plasma lyso-GL3 concentration. The GLA mutations were analyzed in nine patients and one patient was found with a pathogenic mutation. Therefore, one patient was identified with FD, giving a prevalence of 0.1% (1 of 897) in this CKD population.

Conclusion: Although the prevalence of FD in the CKD population was low (0.1%), screening tests are crucial to detect potential diseases in patients with relatives who can benefit from early treatment.

Keywords: Alpha-galactosidase, Chronic renal insufficiency, Fabry disease, Genetic testing, Globotriaosyl lysosphingolipid, Lyso-GL3

Introduction

Fabry disease (FD) is a recessive X-linked lysosomal storage disorder caused by the deficient activity of α-galactosidase A (α-Gal A) enzyme as a result of mutations in the GLA gene. The enzyme deficiency results in the progressive
accumulation of glycolipids, specifically globotriaosylceramide (GL-3) and its derivatives such as globotriaosylsphingosine (lyso-GL3), in the vascular endothelium and other tissues. This leads to damage in multiple organs, including kidneys, heart, and cerebrovascular system, and ultimately, premature death.

It is difficult to recognize patients with FD due to its rarity and nonspecific symptoms. Certain treatment strategies, such as enzyme replacement therapy (ERT), can attenuate the disease progression of FD. Hence, it is crucial to screen high-risk populations, such as patients with chronic kidney disease (CKD). Screening of families of patients with FD provides an opportunity for early diagnosis and treatment of FD. The European Renal Best Practice group has recommended FD screening for CKD patients with an unknown etiology [1]. Several screening studies have been performed outside of Korea, with the majority of patients being either male or on dialysis. Additionally, most of the studies have screened FD only via an α-Gal A enzyme test, even though plasma lyso-GL3 has been considered a more useful biomarker. There also have been a few screening studies in Korea, but those were performed in cardiology patients or used serum GL-3 in patients treated on dialysis [2–4]. Therefore, this is the first study in Korea that evaluated the prevalence of FD in adult patients with CKD including those on renal replacement therapy (RRT) by means of two screening tests, α-Gal A enzyme activity and level of lyso-GL3. In addition, the study included both male and female patients, as well as patients with CKD.

Methods

Study design

This was a national, multicenter, observational study conducted from August 24, 2017 to February 28, 2020 using a cross-sectional and retrospective study design. Six sites were selected considering the geography, urban/rural practice, and regional population size of South Korea. All centers were university hospitals; four located in Seoul, and one respectively in Busan and Gwangju. All investigators were nephrologists who were instructed to enroll consecutive patients in the study to prevent potential bias during selection of the patients.

Both outpatients and inpatients were recruited if they met the following inclusion criteria. Male patients aged between ≥19 and ≤60 years and female patients aged ≥19 years, who were on dialysis, or whose urine protein creatinine ratio (PCR) was ≥150 mg/g or urine albumin creatinine ratio (ACR) was ≥30 mg/g in two consecutive tests conducted during different visits, were included. With regard to CKD, only the presence of albuminuria or proteinuria was considered, taking into account that albuminuria/proteinuria is the early sign of Fabry nephropathy [5,6]. Kidney transplant patients were also included if their pre-transplant condition met the inclusion criteria. For patients retrospectively enrolled in this study, their diagnostic tests must have been performed after January 1, 2016. Patients with confirmed etiology on kidney biopsy or who were considered to have typical diabetic nephropathy, as determined by the responsible investigator, were excluded.

After informed consent was obtained, blood sample was collected and transported directly to Seoul Clinical Laboratories. Screening for FD was performed by measuring both α-Gal A enzyme activity and plasma lyso-GL3 concentration before conducting the GLA gene test (Fig. 1). Patients with α-Gal A enzyme activity of ≤35 nmol/hr/mg protein in whole blood or ≤2.35 µmol/hr/L on a dried blood spot (DBS) test, or with lyso-GL3 of >1.74 ng/mL in plasma, were eligible to undergo a GLA gene test to confirm the diagnosis of FD. Informed consent for gene test was obtained when a patient had abnormal results in screening tests. A single blood sample collection for screening was used for all required tests to minimize patient stress and the risk of missing data. All laboratory tests were performed at Seoul Clinical Laboratories.

Demographic data (age, sex, and ethnicity) and the type of RRT were recorded. Clinical and laboratory data, if available, were collected in the patient records. FD-related symptoms were also collected as follows: 1) cardiac dysfunction including angina, arrhythmia, congestive heart failure, left ventricular hypertrophy, and myocardial infarction; 2) neurologic symptoms including pain, depression, and anxiety; 3) ocular symptoms including corneal and lenticular opacities and vasculopathy; 4) pulmonary symptoms including airway obstruction; 5) gastrointestinal symptoms including dyspepsia; 6) ears, nose, and throat (ENT) symptoms including hearing loss; 7) dermatologic symptoms.

This trial was registered with the Korean Research-based
Measurement of α-galactosidase A activity

In female patients, α-Gal A enzyme activity was measured using whole blood. Leukocytes were isolated [7], and α-Gal A enzyme activity was measured with a fluorometer (1420 Multi-label Counter; PerkinElmer). The substrate and inhibitor used were 4-methylumbelliferyl-α-D-galactopyranoside (Sigma-Aldrich) and N-acetylgalactosamine (Sigma-Aldrich), respectively. Enzymatic activity was evaluated using the calibration curve of 4-methylumbelliferone (Sigma-Aldrich) and expressed as nmol/hr/mg protein. In male patients, α-Gal A enzyme activity was measured from dried venous blood spots on a Whatman 903 filter paper (GE Healthcare Life Sciences). The separation and detection of α-Gal A was performed using a high-performance liquid chromatography system (HPLC system; Agilent 1200 series, Agilent) and a flow injection analysis-tandem mass spectrometry system (FIA-MS/MS, API 4000, SCIEX) operated in a multiple reaction monitoring mode using NeoLS-DTM MSMS KIT (PerkinElmer). The enzyme activity was calculated and expressed as µmol/hr/L.

Measurement of plasma lyso-GL3

Plasma lyso-GL3 was measured by liquid chromatography-tandem mass spectrometry. HPLC was performed with Agilent 1200 and Unison UK-C18 column (2.0 × 50 mm, 3 μm; Imtakt), followed by an MS/MS analysis (API 4000).

Genetic analysis

Genetic analysis was performed with the Sanger sequencing method. Seven exons and flanking intron sequences of the GLA gene were amplified via polymerase chain reaction using ProFlex PCR System (Applied Biosystems) and sequenced using a 3500Dx Genetic Analyzer (Applied Biosystems) according to the manufacturer’s instruction. Analysis of the sequences was done with SeqScape v2.7 (Applied Biosystems). The references used for the identification of mutation and the determination of pathogenicity are as follows: the Human Genome Mutation Database (www.hgmd.org), the Fabry Database of Fabry disease mutations (http://fabry-database.org/), the American College of Medical Genetics standards and guidelines for the interpretation of sequence variants [8], and the International Fabry disease Genotype-Phenotype Database (dbFGP) (www.dbfgp.org).

Figure 1. Flow diagram and outcomes of Fabry disease (FD) screening in patients with chronic kidney disease (CKD) of unknown etiology.

α-Gal A, α-galactosidase A; DBS, dried blood spot; lyso-GL3, globotriaosylsphingosine.

One male patient with abnormal α-Gal A enzyme activity was lost to be followed up without informed consent for a GLA gene test.
Statistical analysis

All the variables were expressed using descriptive statistics with the aid of SAS version 9.4 (SAS Institute). Continuous variables were shown as mean ± standard deviation, and categorical variables were expressed as numbers and percentages.

Results

Baseline characteristics

A total of 902 patients were screened, of which five were excluded since they did not give consent to an amended informed consent form, thus 897 patients were eligible and included in this analysis. Of the total 897 patients, 405 (45.2%) were males and 492 (54.8%) were females and 279 of 897 patients (31.1%) were on dialysis; of the 279 patients who were on dialysis, 196 patients (70.3%) were on hemodialysis, and the remaining 83 (29.7%) were on peritoneal dialysis. The proportion of patients who were on dialysis in the male and female groups was 34.1% and 28.7%, respectively. Of the 42 patients with kidney transplants, 36 patients were on dialysis at the time of screening. And 50 of 897 patients (5.6%, 25 treated on dialysis) had kidney biopsy findings that were not informative to conclude a specific disease. The mean age of the male and female patients was 46.8 ± 9.5 years and 55.5 ± 13.5 years, respectively; almost all of the patients were Korean (888 of 897, 99.0%). The demographic and baseline characteristics are shown in Table 1. Values of estimated glomerular filtration rate (eGFR) were reported in 478 of 618 (77.3%) of nondialysis patients. Blood pressure and body mass index were reported in 856 of 897 (95.4%) and 849 of 897 (94.6%) of all patients, respectively.

FD-related symptoms were reported in patients at only two sites; therefore, these results needed to be interpreted with caution. The most frequently reported symptom was cardiac dysfunction (21 of 879, 2.3%), followed by gastrointestinal and ocular symptoms (12 of 897, 1.3%) each. Dermatologic, neurologic, and ENT symptoms, were respectively reported in seven patients (0.8%) (data not shown).

Table 1. Demographic characteristics and screening results of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>405</td>
<td>492</td>
<td>897</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>46.8 ± 9.5</td>
<td>55.5 ± 13.5</td>
<td>51.6 ± 12.6</td>
</tr>
<tr>
<td>Patients on dialysis</td>
<td>138 (34.1)</td>
<td>141 (28.7)</td>
<td>279 (31.1)</td>
</tr>
<tr>
<td>Patients not on dialysis</td>
<td>267 (65.9)</td>
<td>351 (71.3)</td>
<td>618 (68.9)</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>196 (56.6 ± 36.6)</td>
<td>282 (62.7 ± 37.3)</td>
<td>478 (60.2 ± 37.1)</td>
</tr>
<tr>
<td>Proteinuriaa (mg/gCr)</td>
<td>195 (1.207 ± 1.600)</td>
<td>280 (1.165 ± 2.158)</td>
<td>475 (1.182 ± 1.947)</td>
</tr>
<tr>
<td>Albuminuriaa (mg/gCr)</td>
<td>74 (925 ± 1.267)</td>
<td>77 (970 ± 1.184)</td>
<td>151 (948 ± 1.222)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>131.8 ± 19.0</td>
<td>127.7 ± 17.9</td>
<td>129.6 ± 18.5</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>82.2 ± 12.4</td>
<td>76.3 ± 13.0</td>
<td>79.0 ± 13.0</td>
</tr>
<tr>
<td>Ethnicity of patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Korean</td>
<td>402 (99.3)</td>
<td>486 (98.8)</td>
<td>888 (99.0)</td>
</tr>
<tr>
<td>Others</td>
<td>3 (0.7)</td>
<td>6 (1.2)</td>
<td>9 (1.0)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.4 ± 4.1</td>
<td>23.6 ± 4.1</td>
<td>24.4 ± 4.2</td>
</tr>
<tr>
<td>Patients with abnormal α-Gal A enzyme activityb</td>
<td>4 (1.0)</td>
<td>4 (0.8)</td>
<td>8 (0.9)</td>
</tr>
<tr>
<td>Patients with abnormal plasma lyso-GL3c</td>
<td>0 (0)</td>
<td>2 (0.4)</td>
<td>2 (0.2)</td>
</tr>
<tr>
<td>Patients included in gene analysis</td>
<td>3 (0.7)</td>
<td>6 (1.2)</td>
<td>9 (1.0)</td>
</tr>
</tbody>
</table>

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

α-Gal A, α-galactosidase A; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; lyso-GL3, globotriaosylsphingosine; SBP, systolic blood pressure.

aSome patients reported both values of proteinuria and albuminuria. bAbnormal α-Gal A enzyme activity was ≤35 nmol/hr/mg protein in whole blood (female patients) or ≤2.35 µmol/hr/L on a dried blood spot test (male patients). cAbnormal plasma lyso-GL3 was >1.74 ng/mL.
Screening tests: α-galactosidase A enzyme activity and plasma lyso-GL3 concentration

In total, α-Gal A enzyme activity was measured in 99.3% (891 of 897) of the patients using either DBS (for male patients) or whole blood (for female patients). All 405 male patients underwent a DBS test, with the results reported as normal or abnormal without enzyme activity measurement. Four male patients, all on dialysis, showed an abnormal DBS test (≤2.35 μmol/hr/L). Whole blood α-Gal A enzyme activity was measured in 98.7% of female patients (486 of 492). Four female patients, of whom three were on dialysis, showed a decreased enzyme activity, with a mean value of 32.3 ± 1.64 nmol/hr/mg protein. The mean value of normal results was 67.9 ± 19.15 nmol/hr/mg protein (range, 36.3–139.8 nmol/hr/mg protein).

The plasma lyso-GL3 concentration was measured in all 897 patients. Two patients, who were not on dialysis and with normal enzyme results, showed an increased plasma lyso-GL3 concentration of 3.57 and 5.66 ng/mL, compared to the mean value of normal concentration which was 1.001 ± 0.045 ng/mL. However, eight patients with decreased enzyme activity, four in DBS and four in whole blood, exhibited normal plasma lyso-GL3 concentrations, as shown in Table 2. Therefore, 10 patients were eligible for a GLA gene test to confirm FD. All these patients were of Korean ethnicity, and none of them were kidney transplant recipients. The clinical and laboratory data of patients with abnormal screening results are summarized in Table 2. The mean age of the patients eligible for a gene test was 44.0 ± 8.9 years, and seven patients were on dialysis. Only nine patients were subjected to a gene test, because one patient did not submit the informed consent for gene analysis. Among those nine patients, two female patients with an elevated lyso-GL3 concentration had a kidney biopsy done, which will be discussed later.

GLA gene analysis and a case presentation

Mutation analysis of the GLA gene was performed in 1% (9 of 897) of the total study population, and FD was diagnosed in one patient, resulting in a prevalence of 0.11% (1 of 897). Gene analysis detected a missense mutation in exon 5 of the GLA gene with the c.778G>C variant, resulting in an amino acid change of p.Gly260Arg, which was reported to be pathogenic and related to a classic phenotype at dbFGP (Fig. 2). This mutation was found in a 50-year-old female patient, not on dialysis, with an increased plasma lyso-GL3 concentration of 5.66 ng/mL and normal enzyme activity of 56.6 nmol/hr/mg protein. Her eGFR was 147.5 mL/min/1.73 m², and she had high PCR results of 980 and 1,200 mg/g. She did not have an underlying disease, such as hypertension or diabetes mellitus (DM), but suffered from unexplained acroparesthesia. Her electrocardiogram (ECG) showed T-wave inversion in anterolateral and inferior leads (Fig. 3) without abnormal findings on the echocar-

Table 2. Summary of 10 patients eligible for gene test

<table>
<thead>
<tr>
<th>Sex/age (yr)</th>
<th>Dialysis</th>
<th>α-Gal A enzyme activity</th>
<th>Lyso-GL3 (ng/mL)</th>
<th>Gene mutation (classification)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/32</td>
<td>Yes</td>
<td>Abnormal</td>
<td>-</td>
<td>1 Normal</td>
</tr>
<tr>
<td>Male/37</td>
<td>Yes</td>
<td>Abnormal</td>
<td>-</td>
<td>1 NA</td>
</tr>
<tr>
<td>Male/48</td>
<td>Yes</td>
<td>Abnormal</td>
<td>-</td>
<td>1 Normal</td>
</tr>
<tr>
<td>Female/31</td>
<td>Yes</td>
<td>Abnormal</td>
<td>31*</td>
<td>1 Normal</td>
</tr>
<tr>
<td>Female/44</td>
<td>Yes</td>
<td>Abnormal</td>
<td>31.6*</td>
<td>1 Normal</td>
</tr>
<tr>
<td>Female/50</td>
<td>No</td>
<td>Abnormal</td>
<td>51.6</td>
<td>5.66 c.778G&gt;C; p.Gly260Arg (pathogenic)</td>
</tr>
<tr>
<td>Female/43</td>
<td>No</td>
<td>Abnormal</td>
<td>44.9</td>
<td>3.57 Normal</td>
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<tr>
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<td>Abnormal</td>
<td>34.7*</td>
<td>1 c.-10C&gt;T (benign)</td>
</tr>
<tr>
<td>Male/55</td>
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<tr>
<td>Female/57</td>
<td>Yes</td>
<td>Abnormal</td>
<td>31.9*</td>
<td>1 c.-10C&gt;T (benign)</td>
</tr>
</tbody>
</table>

α-Gal A, α-galactosidase A; DBS, dried blood spot; lyso-GL3, globotriaosylsphingosine; NA, not analyzed. α-Gal A DBS activity ≤ 2.35 μmol/hr/L is reported as abnormal.

*α-Gal A enzyme activity ≤ 35 nmol/hr/mg protein in whole blood is abnormal.
diagram (left ventricular mass index of 82.9 g/m², relative wall thickness of 0.39, and septal wall thickness of 10 mm), and electron microscopy of her kidney biopsy demonstrated myeloid bodies in podocytes, glomerular and mesangial cells, and vessels. The patient underwent ERT.

Family screening

Family screening was performed, which revealed that her 26-year-old son had the same mutation (Fig. 4). He presented acroparesthesia and hypohidrosis. This c.778G>C (p.G260R) hemizygote patient showed abnormal α-Gal A enzyme activity on a DBS test (0.15 µmol/hr/L) as well as a remarkably increased plasma lyso-GL3 concentration of 116 ng/mL and an increased plasma GL-3 concentration of 13.6 µg/mL (normal, 3.9–9.9 µg/mL). He had albuminuria (ACR of 40.68 mg/g) at the time of screening, which elevated to 87.0 mg/g after 2 years. However, the patient rejected further evaluation regarding FD.

Discussion

During the study period, 897 patients were screened. One patient was identified with FD, exhibiting a 0.11% prevalence of FD in the CKD population in South Korea. To the best of our knowledge, three screening studies for the prevalence of FD have been conducted in Korea so far [2–4]. One of them screened 480 patients on dialysis using serum GL-3 but did not observe any patient with FD. The other two studies included patients with hypertrophic cardiomyopathy and male patients with left ventricular hypertrophy. Regarding Fabry nephropathy, most screening studies have been performed outside of Korea, and most of them included male patients or patients who were on dialysis. Doheny et al. [9] reanalyzed the prevalence results of previous screening studies conducted between 1995 and 2017 with regard to pathogenicity of GLA gene mutation. The results reported that the prevalence of FD was 0.21% and 0.15% for male and female patients, respectively, on hemodialysis, and 0.25% and 0% for male and female patients, respectively, who received renal transplants [9]. Also, a screening study that involved 1,453 CKD patients (not on dialysis) was performed in Turkey, which reported the prevalence of FD as 0.2% (0.4% in male, 0% in female) [10]. Although male and female patients were recruited to the present study in a nonselective way including both CKD patients with and without dialysis, the prevalence rate was similar to previous reports, albeit slightly low [9,10]. Differ-
Figure 3. Electrocardiogram of our Fabry disease patient shows T-wave inversion in anterolateral and inferior leads.

Figure 4. Pedigree of the index patient (arrow). Index patient had seven siblings and one son. Index patient and her son had the same GLA gene mutation. GLA gene test was recommended to her siblings.

ence in estimated prevalence rates could be attributed to the selection of study population, screening methods used, and the choice of cutoff values [11].

Renal involvement in FD often begins with hyperfiltration followed by proteinuria and a decline in the glomerular filtration rate, ultimately leading to end-stage renal disease and a decreased life expectancy [5,6]. The age at which ERT is initiated is an important factor related to progressive loss of kidney function; additionally, screening is essential for early diagnosis [12–14]. The European Renal
Best Practice Group recommends screening for FD in male patients below 50 years old and in female patients of any age with unexplained CKD [1]. However, after the introduction of dialysis and renal transplantation, the average age at death was reported to be 50 to 57 years in male patients and 64 to 72 years in female patients [15]. Therefore, the present study recruited male CKD patients aged ≤60 years to encompass possible undiagnosed FD patients. Renal manifestations are nonspecific, and the progression of CKD in Fabry nephropathy resembles that of diabetic nephropathy [11]. Hypertension and DM, the most common causes of CKD, are also frequent in patients with FD [16]. Multiple symptoms of DM may overlap with those of FD, thus making the diagnosis more difficult. Moreover, FD can be diagnosed in patients with diabetic and hypertensive nephropathy. Patients with dual pathology are observed, although it is rare [10,17]. The present study did not assess the prevalence of hypertension and DM, and patients with known diabetic nephropathy were excluded as decided by the responsible physician. This might have affected the prevalence rate of FD in the CKD population observed in this study.

This study identified only one female patient with FD. The low prevalence might be caused by the presence of 55% female patients in the study population. Because the values of α-Gal A enzyme activity in heterozygous female patients are divergent, and up to one-third showed an enzyme activity within the normal range due to a random X-chromosome inactivation [10,18]. Similarly, clinical symptoms in female patients are more diverse, from asymptomatic to severe, often at a later age in comparison to the symptoms observed in male patients, which are related to residual enzyme activity. Therefore, GLA gene mutation analysis is suggested as a primary screening tool in female patients, whereas enzyme activity measurement is recommended in male patients [1]. The latter method has been primarily used in the majority of screening studies partly due to its convenience and cost-effectiveness, in addition to avoiding unnecessary gene analysis [10]; nevertheless, gene mutation test is essential to diagnose FD, even though it is not always an ideal approach. Nearly 1,000 GLA gene mutations have been reported, and there are many gene variants of unknown significance (GVUS) [19]. For individuals with a GVUS of GLA gene, enzyme activity, plasma lyso-GL3, and/or evidence of GL-3 accumulation in an affected organ need to be studied, as they can help in the diagnosis of FD. Earlier studies have indicated the availability of reliable biomarkers for FD that can identify candidates for gene analysis more easily [20–25]. This study measured the α-Gal A enzyme activity using DBS in male patients, which is convenient and less expensive, and has a nearly 100% negative predictive value in male patients [1,26,27]. However, it used whole blood (leukocyte) in female patients, the gold standard for measuring the enzyme activity, because of a higher false-negative value with using DBS compared to using leukocytes in females [18]. The plasma lyso-GL3 was also measured in all patients, regardless of the results of enzyme activity.

Interestingly, no patient showed concurrent abnormal results in terms of both α-Gal A enzyme activity and plasma lyso-GL3. However, FD was diagnosed only in a patient with elevated plasma lyso-GL3. This result is in line with a previous study [20] where the investigators measured plasma lyso-GL3 concentration and plasma α-Gal A enzyme activity for screening of FD and identified 13 patients with FD (seven male and six female patients) out of 2,360 screened patients. Likewise, all patients with FD were detected in the group with elevated plasma lyso-GL3 [20]. Of note, a female patient with FD with elevated plasma lyso-GL3 displayed a normal α-Gal A enzyme activity. Nonpathogenic mutations or GVUS were found in patients with a low α-Gal A enzyme activity and normal plasma lyso-GL3. Although not all patients were genotyped and there were differences in the cutoff values and the method of α-Gal A enzyme measured [20], the results were similar to those obtained in the present study. In this study, a nucleotide substitution at c.-10C>T was also identified in two female patients with a low α-Gal A enzyme activity and normal plasma lyso-GL3, which is considered as a benign germline variant (https://www.ncbi.nlm.nih.gov/clinvar/RCV000335296/). This mutation was in exon 1 of the 5’ untranslated region upstream of the coding sequence and this region is often involved in the regulation of protein translation. This variant was classified as benign as per American College of Medical Genetics and Genomics guidelines. However, to determine its clinical significance, additional test like complex intronic haplotype (c.-10C>T, c.369+990C>A, c.370-81_370-77delCAGCC, c. 640-16A>G, c.1000-22C>T) needs to be done. Routine GLA gene analysis which examines exons and flanking introns cannot detect a deep intronic muta-
tion, although pseudoxon-activating mutations are often located deep in the introns [28,29].

In addition, the study by Maruyama et al. [20] evaluated patients screened positive for lyso-GL3 and having normal α-Gal A enzyme activity and a negative result in the gene test, and lamellar bodies were found in kidney or endomyocardial biopsies. Similarly, in our study, kidney biopsy from a female patient with elevated plasma lyso-GL3 (3.57 ng/mL) and negative gene test (Table 2) exhibited signs of FD with a large amount of laminated electron-dense bodies observed in the podocyte cytoplasm. She had eGFR of 81.8 mL/min/1.73 m² and proteinuria (PCR) of 990 and 830 mg/g. Her ECG showed a right bundle branch block and blood pressure was 109/75 mmHg at the time of inclusion. She did not present FD-related symptoms and there was no information about her medication history. However, it is suggested that this patient needs to be closely monitored for disease progression and the possible diagnosis of FD in due course of time.

Plasma lyso-GL3 analysis has been shown to be a more sensitive and specific test than α-Gal A enzyme activity assays for the diagnosis of FD, especially in heterozygous female patients [20–24]. Plasma lyso-GL3 is also suggested as a useful biomarker for therapeutic monitoring [30]. However, it has been proved that the values of lyso-GL3 increase with age in female patients, and the normal levels of lyso-GL3 cannot confirm the absence of FD. Recently, Baydakova et al. [25] suggested the α-Gal A/lyso-GL3 ratio as a novel biochemical criterion to increase sensitivity for diagnosis of FD in female patients. Although evaluating the role of plasma lyso-GL3 is out of the scope of this study, the results support the view that the measurement of both α-Gal A enzyme activity and lyso-GL3 helps to improve the screening ability for the diagnosis of FD.

There are a few limitations in the present study. First, the sample size is moderate, and the proportion of RRT in screened patients is not representative of CKD patients in South Korea. Second, not all participants have undergone gene analysis, which leaves the possibility of missing unrecognized FD patients. Third, α-Gal A enzyme activity or plasma lyso-GL3 can remain within normal range in female patients with FD. However, in this study, a dual-screening method was used involving both α-Gal A enzyme activity and lyso-GL3, which makes it less likely to overlook adult female patients with FD. Fourth, as only two institutions reported FD-related symptoms, there are limitations to interpreting the data. Fifth, since patients considered to have typical diabetic nephropathy were excluded on the discretion of the responsible physician, there is a possibility that patients who have both diabetes and FD might be excluded.

In the present study, the prevalence of FD in CKD patients via screening tests was low (0.11%); however, identification of one patient provides an opportunity for early treatment to patient’s relatives. We also determined that both α-Gal A enzyme activity and plasma lyso-GL3 concentration tests are needed to identify patients who are eligible for gene analysis. Further studies are required to examine the relationship between plasma lyso-GL3 concentration and the GLA gene mutation. In patients with CKD of unknown etiology, clinicians should consider FD as a differential diagnosis.

Conflicts of interest

Tae-Hyun Yoo is the Editor-in-Chief 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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Data sharing statement

Qualified researchers may request access to person-level data and related study documents including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan, and dataset specifications. Person-level data will be anonymized, and study documents will be redacted to protect the privacy of trial participants. Further details on Sanofi’s data sharing criteria, eligible studies, and process for requesting access can be found at https://www.clinicalstudydatarequest.com.
**Authors’ contributions**

Conceptualization, Methodology: EC, YJK  
Data curation: EC  
Funding acquisition: YJK  
Investigation: JTP, THY, SWK, CWP, SSH, YHK  
Writing—original draft: All authors  
Writing—review & editing: All authors  
All authors read and approved the final manuscript.

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**References**


Significance of C4d expression in peritubular capillaries concurrent with microvascular inflammation in for-cause biopsies of ABO-incompatible renal allografts

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Background: Pathologic diagnosis of antibody-mediated rejection (ABMR) in ABO-incompatible (ABOi) transplantation patients is often challenging because patients without ABMR are frequently immunopositive for C4d. The aim of this study was to determine whether C4d positivity with microvascular inflammation (MVI), in the absence of any detectable donor-specific antibodies (DSAs) in ABOi patients, could be considered as ABMR.

Methods: A retrospective study of 214 for-cause biopsies from 126 ABOi kidney transplantation patients was performed. Patients with MVI score of ≥2 and glomerulitis score of ≥1 (n = 62) were divided into three groups: the absolute ABMR group (DSA-positive, C4d-positive or C4d-negative; n = 36), the C4d-positive group (DSA-negative, C4d-positive; n = 22), and the C4d-negative group (DSA-negative, C4d-negative; n = 4). The Banff scores, estimated glomerular filtration rates (eGFRs), and graft failure rates were compared among groups.

Results: C4d-positive biopsies showed higher glomerulitis, peritubular capillaritis, and MVI scores compared with C4d-negative specimens. The C4d-positive group did not show significant differences in eGFRs and graft survival compared with the absolute ABMR group.

Conclusion: The results indicate that C4d positivity, MVI score of ≥2, and glomerulitis score of ≥1 in ABOi allograft biopsies may be categorized and treated as ABMR cases.

Keywords: ABO-incompatible, C4d, Kidney transplantation, Transplant rejection
tations [1–9]. With the increase in ABOi kidney transplants, the diagnosis of rejection in ABOi allograft biopsies has become crucial for ensuring better clinical outcomes, including graft survival. Pathologic evaluation of biopsied tissue is essential for diagnosing graft rejection along with the presence of donor-specific antibodies (DSAs) [10]. Linear C4d expression in peritubular capillaries is indicative of antibody-vascular endothelial cell interactions and a surrogate for DSAs in antibody-mediated rejection (ABMR) [10–13]. Therefore, C4d expression in peritubular capillaries is the currently adopted feature of ABMR according to the Banff classification system, the most widely used kidney allograft pathology scoring system [10,14]. However, the significance of C4d staining in ABOi renal allograft remains unclear because it can be observed in these allografts even without histologic evidence of ABMR [15–18], which hinders the diagnosis of ABMR in ABOi allografts. Although potentially due to accommodation, C4d expression in ABOi allografts is not necessarily indicative that complement activation is absent. The Banff Kidney Meeting Report recommends the use of molecular diagnostics when ABOi allografts show microvascular inflammation (MVI) scores of ≥2 without detectable DSAs; however, molecular diagnostics are not widely available in daily clinical practice. Furthermore, the usefulness of these tools in the diagnosis of ABMR in ABOi patients, especially with negative DSAs, has yet to be validated [19]

In the present study, the C4d staining pattern in for-cause biopsies was evaluated and whether C4d positivity with MVI and no detectable DSAs in ABOi patients should be considered ABMR determined.

**Methods**

**Patients included in the study and their clinical parameters**

From February 2009 to January 2016, a total of 501 patients underwent ABOi renal transplant at Asan Medical Center (Seoul, Republic of Korea). During follow-up, a total of 214 for-cause biopsies were performed on 126 patients that were included in this study. Exclusion criteria were the following: occurrence of polyomavirus nephropathy, recurrence of previous nephropathy, and only undergoing protocol biopsies and/or zero-hour biopsies.

Several parameters were collected and assessed from electronic medical records such as age at transplantation, sex, posttransplantation time (time elapsed since kidney transplantation until for-cause biopsy), the initial cause of renal failure, donor age, human leukocyte antigen (HLA) mismatch status between donor and recipient, baseline isoagglutinin titer, ABO group, body mass index (BMI), pathologic diagnosis, graft survival, and follow-up periods. The Institutional Review Board of Asan Medical Center approved this retrospective study (No. 2021-0702). The need for written consent was formally waived due to the retrospective and anonymous nature of the study.

**Histological evaluation of pathologic parameters and patient grouping**

Slides of biopsied material were stained with hematoxylin and eosin, periodic acid-Schiff, methenamine silver, Masson’s trichrome, and C4d and SV40 immunohistochemical (IHC) staining and independently evaluated and graded using the Banff 2017 criteria [10] by two nephropathologists. IHC staining was performed on 4-μm-thick sections from 10% formalin-fixed, paraffin-embedded blocks. For the IHC protocol, the rabbit polyclonal anti-C4d antibody (1:32 dilution; Cell Marque) and anti-SV40 antibody (1:32 dilution; Cell Marque) in the Ventana BenchMark XT autostainer (Ventana Medical Systems) were used following the manufacturer’s protocols. A representative image of the C4d staining is shown in Fig. 1.

For each biopsy, the MVI score was calculated as the sum of the glomerulitis (g) score and the peritubular capillaritis (ptc) score. C4d positivity was defined as a C4d score of >0. Patients with MVI score of ≥2 and g score of ≥1 were divided into three groups: absolute ABMR group (MVI score of ≥2, g score of ≥1, C4d-positive or C4d-negative, DSA-positive), C4d-positive group (MVI score of ≥2, g score of ≥1, C4d-positive, DSA-negative), and C4d-negative group (MVI score of ≥2, g score of ≥1, C4d-negative, DSA-negative). In patients with multiple biopsies, the highest MVI score was used for group distribution.

**Desensitization and immunosuppressive protocols**

The desensitization protocol for ABOi kidney transplantation consisted of rituximab administration combined with plasmapheresis [20]. A single dose of 200 mg of rituximab
was administered 7 days before the first plasmapheresis, which was performed 3 to 14 days prior to surgery, until the isoagglutinin titer decreased to $\leq 1:4$. Postoperative plasmapheresis was performed when the isoagglutinin titer was $\geq 1:16$. Tacrolimus, mycophenolate mofetil, and methylprednisolone were administered 7 to 10 days before surgery. As an induction therapy, basiliximab (anti-CD25 monoclonal antibody) was administered on the day of the surgery and 4 days after the procedure.

Treatment regimens for antibody-mediated rejection

To treat ABMR, intravenous methylprednisolone was administered for 3 days, 500 mg per day, followed by plasmapheresis daily or every other day for a maximum of nine sessions based on changes in the DSA titer. Intravenous immunoglobulin (IVIG) was administered at a dose of 100 to 300 mg/kg after each plasmapheresis session. Finally, a single dose of rituximab, 200 mg or $375 \text{ mg/m}^2$, was administered after plasmapheresis and IVIG. All patients in the three aforementioned groups were subjected to this treatment regimen.

Donor-specific antibodies

Blood samples were collected 1 week prior to the biopsy for DSA screening in 97 patients (158 biopsies). DSAs were screened using the Luminex single antigen bead assay, with LABScreen Single Antigen HLA Class I and Class II (One Lambda, Inc.). The cutoff for DSA presence was a mean fluorescence intensity of $>1,000$. In 10 biopsies from seven patients, the donor HLA class II was not available for analysis. DSAs were not tested in 57 biopsies from 49 patients.

Statistical analysis

All data analyses were conducted using the IBM SPSS version 24.0 (IBM Corp.). The chi-square test and Fisher exact test were used for comparison of categorical variables. The Mann-Whitney U test and Student t test were used for comparison of continuous variables. Graft survival was calculated using the Kaplan-Meier method and compared between groups using the log-rank test. For graft function, estimated glomerular filtration rate (eGFR) was measured and the mean sequential changes of eGFR were compared between groups and plotted.

Multivariable regression analysis was performed using the Cox proportional hazards model. Probability values of $<0.05$ were considered statistically significant.

Results

Clinical characteristics of the patients

The clinical characteristics of the 126 study patients who underwent for-cause biopsy are summarized in Table 1. The mean age at the time of transplant was $51.6 \pm 11.2$
years, with a mean BMI of 22.85 ± 3.05 kg/m². Among the patients, 87 (69.0%) were male. The most common cause of end-stage renal disease (ESRD) was diabetic nephropathy (24.5%) followed by immunoglobulin A nephropathy (13.5%). All donors were living donors (related or unrelated) with a mean age of 47.9 ± 8.6 years. The mean number of HLA mismatches was 3.84 ± 1.43. Thirty-four patients (27.0%) showed baseline isoagglutinin titers of ≥1:128. The mean number of biopsies was 1.7 ± 1.3 and the mean follow-up duration was 5.9 ± 2.8 years. The posttransplantation time until the first biopsy was 18.3 ± 23.0 months. Twenty-two patients (17.5%) experienced graft failure (e.g., restarting dialysis, retransplantation) and 11 patients (8.7%) died during the follow-up period.

**Association between C4d expression and Banff scores**

Each biopsy specimen was characterized either as C4d-positive or C4d-negative and compared based on the Banff scores, presence of DSAs, and posttransplantation time until biopsy (Table 2). Among the 214 biopsies, 162 (75.7%) showed C4d positivity. The g, ptc, and total MVI (g + ptc) scores were significantly higher in the C4d-positive biopsies than in C4d-negative biopsies. The proportion

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**Table 1. Clinical characteristics of ABOi recipients who underwent for-cause biopsy**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Absolute ABMR group</th>
<th>C4d-positive group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>126</td>
<td>36</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Age at transplantation (yr)</td>
<td>51.6 ± 11.2</td>
<td>50.2 ± 12.7</td>
<td>54.1 ± 7.8</td>
<td>0.16</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.85 ± 3.05</td>
<td>22.81 ± 3.25</td>
<td>22.92 ± 2.78</td>
<td>0.89</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>0.39</td>
</tr>
<tr>
<td>Male</td>
<td>87 (69.0)</td>
<td>24 (66.7)</td>
<td>17 (77.3)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>39 (31.0)</td>
<td>12 (33.3)</td>
<td>5 (22.7)</td>
<td></td>
</tr>
<tr>
<td>Cause of renal failure</td>
<td></td>
<td></td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>31 (24.5)</td>
<td>14 (38.9)</td>
<td>5 (22.7)</td>
<td></td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>17 (13.5)</td>
<td>6 (16.7)</td>
<td>2 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Hypertensive nephropathy</td>
<td>11 (8.7)</td>
<td>2 (5.6)</td>
<td>3 (13.6)</td>
<td></td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>6 (4.8)</td>
<td>0 (0)</td>
<td>3 (13.6)</td>
<td></td>
</tr>
<tr>
<td>FSGS</td>
<td>2 (1.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Unknown/other</td>
<td>59 (46.8)</td>
<td>11 (30.6)</td>
<td>5 (22.7)</td>
<td></td>
</tr>
<tr>
<td>Donor age (yr)</td>
<td>47.9 ± 8.6</td>
<td>48.0 ± 11.1</td>
<td>46.3 ± 11.1</td>
<td>0.57</td>
</tr>
<tr>
<td>HLA mismatch</td>
<td>3.84 ± 1.43</td>
<td>3.94 ± 1.41</td>
<td>3.86 ± 1.32</td>
<td>0.84</td>
</tr>
<tr>
<td>Baseline isoagglutinin titer, ≥1:128</td>
<td>34 (27.0)</td>
<td>7 (19.4)</td>
<td>7 (31.8)</td>
<td>0.29</td>
</tr>
<tr>
<td>ABO group</td>
<td></td>
<td></td>
<td></td>
<td>0.92</td>
</tr>
<tr>
<td>A to B</td>
<td>28 (22.2)</td>
<td>8 (22.2)</td>
<td>7 (31.8)</td>
<td></td>
</tr>
<tr>
<td>A to 0</td>
<td>23 (18.3)</td>
<td>8 (22.2)</td>
<td>5 (22.7)</td>
<td></td>
</tr>
<tr>
<td>B to A</td>
<td>24 (19.0)</td>
<td>10 (27.8)</td>
<td>4 (18.2)</td>
<td></td>
</tr>
<tr>
<td>B to 0</td>
<td>16 (12.7)</td>
<td>3 (8.3)</td>
<td>2 (9.1)</td>
<td></td>
</tr>
<tr>
<td>AB to A</td>
<td>16 (12.7)</td>
<td>4 (11.1)</td>
<td>3 (13.6)</td>
<td></td>
</tr>
<tr>
<td>AB to B</td>
<td>16 (12.7)</td>
<td>1 (2.8)</td>
<td>1 (4.5)</td>
<td></td>
</tr>
<tr>
<td>AB to O</td>
<td>2 (1.6)</td>
<td>2 (5.6)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>No. of biopsies</td>
<td>1.71 ± 1.30</td>
<td>2.17 ± 1.45</td>
<td>1.64 ± 0.90</td>
<td>0.01</td>
</tr>
<tr>
<td>Follow-up (yr)</td>
<td>5.9 ± 2.8</td>
<td>4.9 ± 2.4</td>
<td>5.4 ± 2.3</td>
<td>0.48</td>
</tr>
<tr>
<td>Posttransplantation time until first biopsy (mo)</td>
<td>18.3 ± 23.0</td>
<td>29.0 ± 27.8</td>
<td>24.7 ± 28.2</td>
<td>0.55</td>
</tr>
<tr>
<td>Graft failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dialysis restart/retransplantation</td>
<td>22 (17.5)</td>
<td>7 (19.4)</td>
<td>6 (27.3)</td>
<td>0.49</td>
</tr>
<tr>
<td>Death from any cause</td>
<td>11 (8.7)</td>
<td>4 (11.1)</td>
<td>4 (18.2)</td>
<td>0.45</td>
</tr>
</tbody>
</table>

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

ABMR, antibody-mediated rejection; ABOi, ABO-incompatible; FSGS, focal segmental glomerulosclerosis; HLA, human leukocyte antigen; IgA, immunoglobulin A.
of cases with MVI score of ≥2 and g score of ≥1 was also significantly higher in the C4d-positive biopsies (50.0% vs. 26.9%, p = 0.004). Other Banff scores, including t, i, v, ct, ci, and posttransplantation time were not significantly different between C4d-positive and C4d-negative biopsies.

C4d-positive biopsies were further divided in diffuse C4d-positive specimens (C4d score of 3, n = 44) and focal C4d-positive specimens (C4d score of 1 or 2, n = 118). Diffuse C4d positivity was accompanied with significantly higher MVI (2.8 vs. 2.2, p < 0.001), g (1.32 vs. 1.02, p < 0.001), and ptc (1.48 vs. 1.30, p < 0.001) scores and a higher proportion of cases with lower ci (1.17 vs. 1.04, p < 0.001) and ct (1.10 vs. 0.94, p < 0.001) scores compared with the C4d-negative specimens. The posttransplantation time until biopsy was significantly shorter in diffuse C4d-positive specimens than in the C4d-negative or focal C4d-positive specimens.

**Associations between donor-specific antibodies and Banff scores and clinical outcomes**

Each patient was defined as DSA-positive or DSA-negative and Banff scores and clinical outcomes were compared (Table 3). Among 126 patients, 49 (38.9%) were positive for DSAs and 77 (61.1%) were negative. DSA-positive patients had a significantly higher total MVI (3.56 vs. 1.36, p < 0.001), g (1.60 vs. 0.58, p < 0.001), ptc (1.96 vs. 0.78, p < 0.001), and i (1.68 vs. 1.20, p = 0.001) scores than DSA-negative patients. Furthermore, C4d scores and other Banff scores including t, v, ct, and ci were not significantly different between the two groups.

Among DSA-positive patients, 12 (24.5%) lost their graft function during the follow-up period and 6 (12.2%) died. Among DSA-negative patients, 10 (13.0%) lost their graft function and five (6.5%) died.

**Clinicopathological characteristics based on microvascular inflammation, C4d positivity, and donor-specific antibody status**

C4d positivity in the diagnosis of antibody-mediated rejection in ABO-incompatible patients

Among the 126 study patients, 62 (49.2%) had MVI score of ≥2 with at least mild g (≥1). Among the 62 patients, 36

| Table 2. Differences in DSA status and histological features according to C4d positivity |
|----------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Variable                               | C4d-positive     | C4d score, 1 or 2 | C4d score, 3     | C4d-negative     | p-value          |
|                                        | (n = 162)        | (n = 118)         | (n = 44)         | (n = 52)         |                  |
|                                        |                  |                  |                  |                  | C4d-positive vs. |
|                                        |                  |                  |                  |                  | C4d-negative vs. |
|                                        |                  |                  |                  |                  | C4d-positive vs. |
|                                        |                  |                  |                  |                  | C4d-negative vs. |
|                                        |                  |                  |                  |                  | C4d-negative     |
|                                        |                  |                  |                  |                  |                  |
| DSA                                    |                  |                  |                  |                  |                  |
| DSA-negative                           | 54 (33.3)        | 42 (35.6)        | 12 (27.3)        | 16 (30.8)        | 0.61             |
| DSA-positive                           | 66 (40.7)        | 51 (43.2)        | 15 (34.1)        | 16 (30.8)        | 0.098            |
| Class I                                | 26 (16.0)        | 14 (11.9)        | 12 (27.3)        | 5 (9.6)          |                  |
| Class II                               | 52 (32.1)        | 41 (34.7)        | 11 (25)          | 11 (21.2)        |                  |
| NA or ND                               | 42 (25.9)        | 25 (21.2)        | 17 (38.6)        | 20 (38.5)        |                  |
| MVI score                              | 2.44 ± 2.12      | 2.18 ± 2.00      | 2.80 ± 2.17      | 1.46 ± 2.02      | <0.001           |
| Glomerulitis                           | 1.10 ± 1.14      | 1.02 ± 1.14      | 1.32 ± 1.12      | 0.58 ± 1.02      | 0.004            |
| Peritubular capillaritis               | 1.35 ± 1.23      | 1.30 ± 1.21      | 1.48 ± 1.28      | 0.89 ± 1.18      | <0.001           |
| MVI ≥ 2 and g ≥ 1                     | 81 (50.0)        | 55 (46.6)        | 26 (59.1)        | 14 (26.9)        | 0.004            |
| Other Banff scores                     |                  |                  |                  |                  | 0.22             |
| t                                       | 1.53 ± 1.06      | 1.55 ± 1.03      | 1.45 ± 1.15      | 1.23 ± 1.04      | 0.08             |
| i                                       | 1.43 ± 1.01      | 1.47 ± 0.98      | 1.34 ± 1.10      | 1.23 ± 1.10      | 0.22             |
| v                                       | 0.19 ± 0.54      | 0.16 ± 0.47      | 0.25 ± 0.69      | 0.14 ± 0.53      | 0.09             |
| ci                                      | 1.17 ± 0.91      | 1.27 ± 0.92      | 0.89 ± 0.81      | 1.04 ± 0.77      | 0.36             |
| ct                                      | 1.10 ± 0.94      | 1.20 ± 0.96      | 0.80 ± 0.85      | 0.94 ± 0.83      | 0.31             |
| Posttransplantation time until biopsy (mo) | 25.40 ± 25.10    | 28.02 ± 24.71    | 18.43 ± 25.20    | 19.70 ± 22.3     | 0.15             |

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

DSA, donor-specific antibody; MVI, microvascular inflammation; NA, not available; ND, not done.
patients (58.1%) were DSA-positive and diagnosed with active ABMR regardless of the C4d positivity status. Among the 26 (41.9%) DSA-negative patients, 22 (84.6%) met the diagnostic criteria for active ABMR based on C4d positivity and were categorized into the C4d-positive MVI score of ≥2 group.

Clinicopathological characteristics
Baseline data of the absolute ABMR group and the C4d-positive MVI score of ≥2 group are summarized in Table 1. Significant differences were not observed in the clinical characteristics between the two groups including age, BMI, sex, cause of ESRD, donor age, number of HLA mismatches, proportion of baseline isoagglutinin titer of ≥1:128, ABO group of the donor and recipient, mean follow-up period, and posttransplantation time until first biopsy. In contrast, the number of for-cause biopsies was significantly higher in the absolute ABMR group than in the C4d-positive MVI score of ≥2 group (2.17 ± 1.45 vs. 1.64 ± 0.90, p = 0.01). In the absolute ABMR group, seven patients (19.4%) lost their graft function during the follow-up period and four (11.1%) died. In the C4d-positive MVI score of ≥2 group, six patients (27.3%) lost their graft function and four (18.2%) died.

Banff scores of the absolute ABMR and C4d-positive MVI score of ≥2 groups are summarized in Table 4; MVI (4.80 vs. 3.91, p = 0.003) and ptc (2.53 vs. 2.00, p = 0.008) scores were significantly higher in the absolute ABMR group than in the C4d-positive MVI score of ≥2 group. Other Banff scores including g, t, i, v, ct, and ci did not significantly differ between the two groups. Among the C4d-positive MVI score of ≥2 group, nine cases (40.9%) showed diffuse C4d positivity (C4d score, 3) and 13 (59.1%) showed focal C4d positivity (C4d score, 1 or 2).

In the absolute ABMR group, recurrence of ABMR was observed in 14 patients (38.9%), and in the C4d-positive group, was only observed in four patients (18.1%) but without statistical significance (p = 0.69).

Graft function and survival in the absolute antibody-mediated rejection and C4d-positive groups
Graft function was measured at 3 and 6 months and then yearly after transplant by analyzing eGFR according to

---

Table 3. Banff scores in DSA-positive and DSA-negative patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>DSA-positive (n = 49)</th>
<th>DSA-negative (n = 77)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVI score</td>
<td>3.56 ± 2.02</td>
<td>1.36 ± 1.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glomerulitis</td>
<td>1.60 ± 1.14</td>
<td>0.58 ± 0.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peritubular capillaritis</td>
<td>1.96 ± 1.16</td>
<td>0.78 ± 1.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C4d score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>12 (24.5)</td>
<td>21 (27.3)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12 (24.5)</td>
<td>21 (27.3)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>15 (30.6)</td>
<td>11 (14.3)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10 (20.4)</td>
<td>24 (31.2)</td>
<td></td>
</tr>
<tr>
<td>Other Banff scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>1.56 ± 1.02</td>
<td>1.39 ± 1.09</td>
<td>0.25</td>
</tr>
<tr>
<td>i</td>
<td>1.68 ± 0.95</td>
<td>1.20 ± 1.04</td>
<td>0.001</td>
</tr>
<tr>
<td>v</td>
<td>0.26 ± 0.70</td>
<td>0.17 ± 0.38</td>
<td>0.22</td>
</tr>
<tr>
<td>ci</td>
<td>1.16 ± 0.94</td>
<td>0.99 ± 0.90</td>
<td>0.34</td>
</tr>
<tr>
<td>ct</td>
<td>1.22 ± 0.87</td>
<td>1.08 ± 0.87</td>
<td>0.22</td>
</tr>
<tr>
<td>Restart dialysis/retransplantation</td>
<td>12 (24.5)</td>
<td>10 (13.0)</td>
<td>0.097</td>
</tr>
<tr>
<td>Death from any cause</td>
<td>6 (12.2)</td>
<td>5 (6.5)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or number (%).
DSA, donor-specific antibody; MVI, microvascular inflammation.
the Modification of Diet in Renal Disease equation. The eGFR sequential changes in the two groups are illustrated in Fig. 2. The mean eGFR of the absolute ABMR group at 3 months and 7 years after transplant was 60.55 ± 14.4 mL/min/1.73 m² and 39.80 ± 20.34 mL/min/1.73 m², respectively. In the C4d-positive group, the mean eGFR at 3 months and 7 years was 60.11 ± 17.3 mL/min/1.73 m² and 38.17 ± 15.92 mL/min/1.73 m², respectively. Throughout the follow-up period, eGFR was not significantly different between the two groups.

Graft survival in both groups is plotted as Kaplan-Meier curves in Fig. 3. The 5-year graft survival rate for the absolute ABMR group was 79.1% and for the C4d-positive group was 84.0%. The log-rank test indicated no significant difference in graft survival between the groups (p = 0.40).

Patient age (p = 0.03) and MVI score of ≥4 (p = 0.04) were associated with graft loss and patient death based on multivariable Cox proportional hazards regression analysis (Table 5).

**Table 4. Banff scores in the absolute ABMR and the C4d-positive MVI score of ≥2 groups**

<table>
<thead>
<tr>
<th>Score</th>
<th>Absolute ABMR (n = 36)</th>
<th>C4d-positive group (n = 22)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVI scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.80 ± 1.15</td>
<td>3.91 ± 0.97</td>
<td>0.003</td>
</tr>
<tr>
<td>Glomerulitis</td>
<td>2.24 ± 0.83</td>
<td>1.91 ± 0.75</td>
<td>0.13</td>
</tr>
<tr>
<td>Peritubular capillaritis</td>
<td>2.56 ± 0.73</td>
<td>2.00 ± 0.82</td>
<td>0.008</td>
</tr>
<tr>
<td>Other Banff scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>1.68 ± 0.94</td>
<td>1.73 ± 1.03</td>
<td>0.85</td>
</tr>
<tr>
<td>i</td>
<td>2.00 ± 0.91</td>
<td>1.73 ± 0.83</td>
<td>0.26</td>
</tr>
<tr>
<td>v</td>
<td>0.28 ± 0.66</td>
<td>0.14 ± 0.48</td>
<td>0.30</td>
</tr>
<tr>
<td>ci</td>
<td>1.11 ± 0.77</td>
<td>1.18 ± 0.85</td>
<td>0.74</td>
</tr>
<tr>
<td>ct</td>
<td>1.00 ± 0.85</td>
<td>1.14 ± 0.88</td>
<td>0.56</td>
</tr>
<tr>
<td>C4d score</td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>0</td>
<td>8 (22.2)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13 (36.1)</td>
<td>9 (40.9)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8 (22.2)</td>
<td>4 (18.2)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7 (19.4)</td>
<td>9 (40.9)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or number (%). ABMR, antibody-mediated rejection; MVI, microvascular inflammation.

Graft function and survival in the C4d-negative group (MVI score of ≥2, g score of ≥1, C4d-negative, DSA-negative)

The number of cases in the C4d-negative group was too small (n = 4) to statistically compare their prognostic differences with other groups. The mean eGFR of the four cases in the C4d-negative group was similar to the mean eGFR in the absolute ABMR group and the C4d-positive group (Supplementary Table 1, available online). None of the four patients restarted dialysis or underwent retransplantation.

**Mild g only with C4d positivity**

Among the 214 biopsies, 10 (4.7%) specimens from nine patients showed mild g (g = 1), no ptc, and C4d positivity. Among these nine patients, four underwent another biopsy due to poor graft function and three of the four patients had an MVI score of ≥2. Additional biopsy was not performed in the other five patients because signs of graft function deterioration were not observed.

**Discussion**

ABOi kidney transplantation is currently considered a viable option for ESRD patients and produces similar outcomes to ABO-compatible kidney transplantation [1–8]. ABMR is one of the main causes of graft loss in ABOi kidney transplantation [15,18,21,22], and as the number of ABOi kidney transplantations increases, accurate diagnosis of ABMR in ABOi allograft has become particularly important. In ABMR, DSAs interact with the donor endothelium activating the classical complement pathway, which leads to graft injury. C4d is a split product of the C4 component of the classical complement pathway and does not have a known biological function; however, C4d staining in peritubular capillaries was shown correlated with the pres-
Figure 2. Mean serum eGFR in the absolute ABMR group and the C4d-positive group.
ABMR, antibody-mediated rejection; eGFR, estimated glomerular filtration rate.

Figure 3. Graft survival in the absolute ABMR group and the C4d-positive group during the months after transplantation. Graft survival was calculated using the Kaplan-Meier method and compared with the long-rank test. ABMR, antibody-mediated rejection.
ence of DSAs and is considered evidence for antibody-tissue interactions [11,13,23]. Accordingly, C4d staining was incorporated in the 2003 Banff classification as an ABMR diagnostic marker and recognized as a DSA equivalent in the 2017 Banff classification [10,24].

However, in ABOi allografts, C4d positivity has been considered irrelevant to ABMR or MVI [11,15–18,25]. Haas et al. [17] considered C4d deposition without rejection a sign of accommodation in ABOi allografts [17,26]. The unclear significance of C4d staining in ABOi allografts results in diagnostic difficulties; in the present study, allograft biopsies of 62 patients with MVI score of ≥2 and g score of ≥1, 36 (58.1%) were DSA-positive and the remaining 26 (41.9%) were DSA-negative and required DSA-equivalent evidence to be diagnosed with ABMR. In cases of biopsies from ABOi allografts with a positive MVI score (g + ptc > 0), C4d positivity, and no DSAs, the 2017 Banff classification recommends molecular testing (i.e., the ABMR classifier) [10]. However, the cost and technical complexity of these tests render their application in daily clinical practice difficult. Furthermore, the validation of molecular testing in the diagnosis of ABMR in ABOi has not yet been clarified.

In the present study, 75.7% of the 214 biopsy specimens showed C4d positivity, a similar number to previous reports [15–18,25]. However, unlike previous studies, C4d positivity was associated with higher g and ptc scores and higher rate of MVI score of ≥2 + g score of ≥1, possibly because only for-cause biopsies were collected for this study, while in previous studies, both for-cause and protocol bi-

**Table 5. Multivariable Cox proportional hazard analysis for patient survival**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hazard ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.04 (1.01–1.08)</td>
<td>0.03</td>
</tr>
<tr>
<td>Donor age</td>
<td>1.02 (0.98–1.06)</td>
<td>0.64</td>
</tr>
<tr>
<td>Sex</td>
<td>1.56 (0.55–4.38)</td>
<td>0.56</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.99 (0.85–1.18)</td>
<td>0.51</td>
</tr>
<tr>
<td>HLA mismatch ≥ 3</td>
<td>0.963 (0.73–1.28)</td>
<td>0.20</td>
</tr>
<tr>
<td>Baseline isoagglutinin titer, ≥1:128</td>
<td>1.14 (0.45–2.88)</td>
<td>0.49</td>
</tr>
<tr>
<td>Presence of donor-specific antibody</td>
<td>1.54 (0.63–3.77)</td>
<td>0.83</td>
</tr>
<tr>
<td>MVI score ≥4</td>
<td>2.55 (1.05–6.17)</td>
<td>0.04</td>
</tr>
<tr>
<td>C4d-positivity</td>
<td>0.69 (0.24–1.96)</td>
<td>0.18</td>
</tr>
<tr>
<td>Acute T-cell–mediated rejection</td>
<td>2.12 (0.64–7.01)</td>
<td>0.39</td>
</tr>
</tbody>
</table>

CI, confidence interval; HLA, human leukocyte antigen; MVI, microvascular inflammation.

In the present study, C4d-positive cases were further divided into diffuse C4d-positive cases (C4d, 3) and focal C4d-positive cases (C4d, 1 or 2). Diffuse C4d-positive cases showed significantly higher MVI scores and significantly lower ct and ci scores. This may be because diffuse C4d-positive cases had a shorter posttransplantation duration (i.e., fewer days between transplantation and biopsy day).

Among the absolute ABMR and C4d-positive groups, multivariable Cox proportional hazards regression analysis was performed and patient age and MVI score of ≥4 were associated with poor outcome. This result is consistent with previous studies in which higher MVI score was associated with poor graft outcome [12,25,28].

In addition, the C4d-positive group did not show significant differences from the absolute ABMR group in terms of eGFR and graft survival. We suggest that ABOi allografts with MVI and C4d positivity without identifiable DSAs may be classified as ABMR and should be treated as such.

Several limitations should be mentioned. Only for-cause biopsies were included in the study and zero-hour biopsies and protocol biopsies were excluded; however, we believe this may be more appropriate for interpreting ABOi allograft biopsy in cases of graft deterioration. Furthermore, this study included biopsies and patients from a single center which limited the sample size. In addition, only four C4d-negative, MVI score of ≥2, g score of ≥1, C4d-negative, DSA-negative cases were included in this study, which did not allow statistical comparison with the other groups. Furthermore, molecular diagnostics in the C4d-positive MVI score of ≥2 cases was not performed. A multicenter study is required to further confirm the validity of our results.
In summary, the results indicate that cases of ABOi allograft biopsies that are C4d-positive, with MVI score of ≥2 and g score of ≥1 may be categorized and treated as ABMR cases. Larger studies and molecular research are required to determine the prognostic effect of C4d positivity/negativity in MVI score of ≥2 and g score of ≥1 cases in ABOi allograft.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Data sharing statement**

The data presented in this study are available on request from the corresponding author.

**Authors’ contributions**

Conceptualization: HC, SKP, HG
Data curation: HC, CHB, SKP, HG
Formal analysis: HC, HG
Methodology: CHB, SKP
Supervision: HK, HG
Validation: HC, HK
Writing–original draft: HC
Writing–review & editing: HK, HG
All authors read and approved the final manuscript.

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**References**


Association between volume status assessed by bioelectrical impedance analysis, lung ultrasound, or weight change and mortality in patients with sepsis-associated acute kidney injury receiving continuous kidney replacement therapy

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3The Graduate School, Yonsei University, Seoul, Republic of Korea

**Background:** Fluid overload is an independent risk factor of mortality in patients with acute kidney injury (AKI) receiving continuous kidney replacement therapy (CKRT). However, the association between fluid status, as assessed by bioelectrical impedance analysis (BIA) or lung ultrasound, and survival in patients with AKI requiring CKRT has not been established.

**Methods:** We analyzed 36 participants with sepsis-associated AKI who received CKRT at a tertiary hospital. The main exposures were volume surrogates: 1) overhydration normalized by extracellular water (OH/ECW, L/L) assessed by BIA, 2) the number of B-lines measured by lung ultrasound, and 3) weight change ([body weight at CKRT initiation – body weight at admission] × 100/body weight at admission). The primary outcome was the 28-day mortality.

**Results:** Seventeen participants (47.2%) died within 28 days. There were no significant correlations between OH/ECW and weight change ($R^2 = 0.040, p = 0.24$), number of B-lines and OH/ECW ($R^2 = 0.056, p = 0.16$), or weight change and number of B-lines ($R^2 = 0.014, p = 0.49$). Kaplan-Meier analyses revealed that patients in the highest tertile of OH/ECW showed a significantly lower cumulative 28-day survival probability than the others (the lowest + middle tertiles). The survival probability of participants in the highest tertile of the number of B-lines or weight change did not differ from that of their counterparts. In a multivariate Cox proportional hazard model, the hazard ratio for the highest tertile of OH/ECW was 3.83 (95% confidence interval, 1.04–14.03).

**Conclusion:** Volume overload assessed using BIA (OH/ECW) was associated with the 28-day survival rate in patients with sepsis-associated AKI who received CKRT.

**Keywords:** Acute kidney injury, Bioelectrical impedance analysis, Continuous kidney replacement therapy, Sepsis
**Introduction**

Acute kidney injury (AKI) is a frequent and serious complication among critically ill patients treated in intensive care units (ICUs) [1,2]. It has been reported that AKI increases the risk of death to approximately 60% to 80% [2-4]. Sepsis is the most common cause of AKI in patients admitted to ICUs [5,6]. Patients with sepsis-associated AKI often require kidney replacement therapy (KRT), and continuous KRT (CKRT) is the preferred modality for those patients [7,8].

Patients with sepsis-associated AKI who required CKRT had the highest risk of mortality [9,10]. Accordingly, several studies have been conducted to identify risk factors that may independently influence clinical outcomes in those patients. Recently, the fluid status of patients with sepsis-associated AKI receiving CKRT, as assessed by body weight change or cumulative fluid balance, has been suggested as an independent risk factor for survival [11-13].

Critically ill patients with AKI are often complicated by fluid overload, and accurate assessment of fluid status is essential for the appropriate management of those patients [11,14]. Bioelectrical impedance analysis (BIA) and lung ultrasound have recently been introduced to evaluate patients’ fluid status [15-18]. BIA estimates a patient’s body composition using electrical resistance, and the number of B-lines acquired via lung ultrasound confers information on the water content in the lungs [15,18]. However, the association between fluid status evaluated using BIA or lung ultrasound and survival in patients with sepsis-associated AKI receiving CKRT has rarely been investigated.

Therefore, we examined the association between fluid status evaluated using BIA or lung ultrasound and mortality in patients with sepsis-associated AKI receiving CKRT.

**Methods**

**Study design and participants**

This prospective, observational study was conducted at a tertiary care hospital (Severance Hospital, Seoul, Republic of Korea) between April 2014 and February 2015. This study was approved by the Institutional Review Board of Severance Hospital and was conducted in accordance with the provisions of the Declaration of Helsinki (No. 4-2014-0791). All participants and/or substitute decision makers were informed of the study and provided written informed consent.

Participants were eligible for enrollment if they were 20 years or older and satisfied following three criteria: 1) diagnosis of sepsis according to consensus conference criteria suggested by the Society of Critical Care Medicine/American College of Chest Physicians [19]; 2) AKI at a greater level than the ‘injury’ stage according to the RIFLE (Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease) criteria [20]; and 3) AKI not explained other than sepsis. Briefly, if a patient had a suspected infection and met the criteria for systemic inflammatory response syndrome (two or more of the following: body temperature of <36 °C or >38 °C, heart rate of >90 beats/min, respiratory rate of >20 breaths/min or PaCO$_2$ <32 mmHg, or white blood cell count of <4.0 × 10$^3$ μL or >12.0 × 10$^3$μL) in two consecutive measurements, the diagnosis of sepsis was established. Infection was diagnosed if the causative organisms were isolated by culture studies or was clinically suspected when patients satisfied one of the following criteria: 1) white blood cells in normally sterile fluid, 2) perforated viscus, or 3) infection focus detected on radiological examination. AKI at a greater level than the ‘injury’ stage was defined as a more than two-fold increase in serum creatinine level compared with baseline or urine output of <0.5 mg/kg/hr over 12 hours according to the RIFLE criteria. The exclusion criteria were as follows: 1) patients older than 80 years; 2) patients who had already been undergoing KRT due to kidney failure; 3) patients who had been diagnosed with malignancy and had a life expectancy of less than 3 months; 4) patients who had an intracardiac device, including a pacemaker, implantable cardioverter-defibrillator, or cardiac resynchronization therapy; 5) patients without volume overload according to BIA measurements; and 6) patients with generalized exfoliative skin disease. Finally, 36 participants were enrolled (Fig. 1).

**Data collection and measurements**

Demographic data, such as age, sex, body weight, and medical history, were collected from electronic medical records at the time of enrollment. Clinical and biochemical data were collected at CKRT initiation. The Acute Physiology and Chronic Health Evaluation (APACHE) II score was
determined upon ICU admission. Serum creatinine levels were measured using an isotope-dilution mass spectrometry-tractable method. Body weights were measured using a scale. As a surrogate for volume status, we assessed weight change (%), defined as the difference in body weight at CKRT initiation from the body weight at the time of admission, which was normalized by the body weight at the time of admission. Weight change (%) was calculated using the following formula:

\[
\text{Weight change (\%)} = \frac{\text{Body weight at CKRT initiation (kg)} - \text{Body weight at admission (kg)}}{\text{Body weight at admission (kg)}} \times 100
\]

**Bioelectrical impedance analysis measurement and lung ultrasound**

The amount of fluid overload was assessed by BIA using the Body Composition Monitor (Fresenius Medical Care) according to the manufacturer’s instructions within 6 hours of CKRT initiation. Briefly, the participants were removed from the metallic devices or accessories. A pair of electrodes was placed on the dorsum of the hand and the foot on the ipsilateral side. Overhydration (OH) and extracellular water (ECW) were measured by BIA. Simultaneously, lung ultrasound was performed using a portable ultrasound scanner (GE Logiq Book XP; General Electric Health Care) with a 2 to 3.6 MHz phased array cardiac assessment EM 3S-RS probe. The number of B-lines was measured at four points on the thorax: the intercostal spaces between the third and fourth ribs and the sixth and seventh ribs on each midclavicular line. The numbers were recorded at each point and summed to obtain the number of B-lines. OH normalized by ECW (OH/ECW, L/L) and the number of B-lines acquired using lung ultrasound were adopted as surrogates for patient volume status.

**Continuous kidney replacement therapy procedure**

CKRT was initiated at the discretion of a consulting nephrologist without considering the patient’s eligibility for this study. Generally, CKRT is prescribed in patients with AKI at a stage greater than the injury stage classified by the RIFLE criteria, with the presence of significant volume overload, intractable hyperkalemia (potassium of >6.5 mEq/L), or severe acidemia (pH <7.2). Vascular access for CKRT was made in the internal jugular or femoral veins using a 14-French double-lumen catheter. CKRT was performed using Prismaflex machines (Gambro) with ST100 (surface area, 1.0 m²) filter sets. The effluent volume was set to achieve a clearance of 30 to 40 mL/kg/hr, with a blood flow rate of 150 to 200 mL/min. Changes in the maintenance of CKRT, blood flow rate, replacement fluid flow, or ultrafiltration rate in each patient were determined by both the consulting nephrologist and the attending physician.

Patients remained on CKRT until kidney function recov-
ered, transferred to conventional hemodialysis, withdrew
CKRT as part of life support, or died. The decision to wean
patients from CKRT was first made by the nephrologist
when the patient recovered hemodynamic stability for
undergoing intermittent hemodialysis or had considerable
urine output (>1,000 mL/day).

Exposure and outcome

The exposure of interest was the volume status of the par-
ticipants. To measure the volume status, we used OH/ECW
(L/L), number of B-lines, and weight change (%). Partici-
pants were classified according to tertiles of OH/ECW (L/ L), number of B-lines, or weight change (%). The primary
outcome was death from any cause within 28 days of CKRT
initiation.

Statistical analysis

To investigate the association between surrogates for vol-
ume status and 28-day mortality in patients with sepsis-as-
associated AKI receiving CKRT, we first compared survival
among the groups (the lowest + middle tertiles vs. the
highest tertile) using Kaplan-Meier analyses with the log-
rank test. The Cox proportional hazards model was used to
examine the association between a surrogate that showed
a significant association with the primary outcome in the
Kaplan-Meier analysis. We made incremental adjustments
with the following variables: Model 1 is a crude model.
Model 2 was adjusted for age, sex, diabetes, and chronic
kidney disease. In Model 3, APACHE II score and total se-
rum CO\textsubscript{2} levels were added. The results of Cox propor-
tional hazards regression are presented as hazard ratios (HRs)
and 95% confidence intervals (CIs).

Data were analyzed using PASW Statistics version 18.0
(IBM Corp.). Statistical significance was set at p < 0.05.

Results

Baseline characteristics

Table 1 presents the baseline characteristics of the 36 par-
ticipants according to tertiles of volume overload (OH/ ECW).
The mean age was 64.6 years (standard deviation, 14.6 years) and 61.1% were male. Overall, participants in
the highest tertile of volume overload (OH/ECW) were
more likely to have a higher left atrial volume index and
APACHE II scores. There were no significant differences in
other demographic factors, medical histories, or laboratory
values between the tertiles. The baseline characteristics of

Table 1. Baseline characteristics of the participants according to volume status (OH/ECW)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Volume overload (OH/ECW, L/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low (≤0.18)</td>
</tr>
<tr>
<td>No. of patients</td>
<td>36</td>
<td>12</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>64.6 ± 14.6</td>
<td>62.4 ± 15.2</td>
</tr>
<tr>
<td>Male sex</td>
<td>22 (61.1)</td>
<td>8 (66.7)</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>23.0 ± 3.0</td>
<td>22.2 ± 3.4</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>14 (38.9)</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>5 (13.9)</td>
<td>3 (25.0)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>101.4 ± 23.9</td>
<td>100.0 ± 17.5</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>46.3 ± 7.3</td>
<td>48.0 ± 5.7</td>
</tr>
<tr>
<td>LAVI (mL/m\textsuperscript{2})</td>
<td>32.8 ± 17.3</td>
<td>27.6 ± 8.2</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>2.9 ± 1.7</td>
<td>3.6 ± 2.2</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.7 ± 0.9</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>Total CO\textsubscript{2} (mmol/L)</td>
<td>19.2 ± 5.9</td>
<td>22.1 ± 3.7</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>6.0 ± 6.4</td>
<td>3.4 ± 4.2</td>
</tr>
<tr>
<td>APACHE II</td>
<td>24.1 ± 6.5</td>
<td>21.6 ± 4.4</td>
</tr>
</tbody>
</table>

Data are expressed as number only, mean ± standard deviation, or number (%).
APACHE, Acute Physiology and Chronic Health Evaluation; BMI, body mass index; ECW, extracellular water; LAVI, left atrial volume index; LVEDD, left ventricu-
lar end-diastolic diameter; OH, overhydration.
the participants according to tertiles of volume overload estimated by the number of B-lines or weight changes are summarized in Supplementary Table 1, 2 (available online).

**Correlations between volume overload surrogates**

Supplementary Fig. 1 (available online) shows the correlations among the three markers of volume overload. OH/ECW, the number of B-lines, and weight change did not show significant correlations between OH/ECW and weight change ($R^2 = 0.040; p = 0.24$), the number of B-lines and OH/ECW ($R^2 = 0.056; p = 0.16$), or weight change and the number of B-lines ($R^2 = 0.014, p = 0.49$).

**Association between volume assessed by bioelectrical impedance analysis, lung ultrasound, or weight change and patient survival**

A total of 17 patients (47.2%) died within 28 days of CKRT initiation. Kaplan-Meier curves revealed that the cumulative 28-day survival probability was significantly lower for patients in the highest tertile of OH/ECW compared to other tertiles ($p = 0.02$). However, the survival rate at 28 days after CKRT initiation did not significantly differ between groups classified according to the number of B-lines ($p = 0.45$) or weight change ($p = 0.44$) (Fig. 2).

Next, the association between volume overload assessed using BIA (OH/ECW) and patient survival was evaluated using multivariate Cox proportional hazard models. In the unadjusted model, the HR for the risk of 28-day mortality was 2.93 (95% CI, 1.11–7.73) for the highest tertile compared with the lowest + middle tertiles (model 1 in Table 2). The association between OH/ECW and the primary outcome was maintained after adjusting for demographic factors and comorbidities (model 2 in Table 2). Further adjustment for the APACHE II score and serum total CO$_2$ level did not change the increased risk in the highest tertile of OH/ECW. The corresponding HR for the highest tertile was 3.83 (95% CI, 1.04–14.03) (model 3 in Table 2).

**Discussion**

In this prospective observational study, we showed that

![Figure 2. Kaplan-Meier curves showing patient 28-day survival.](image)

Cumulative survival probability within 28 days according to overhydration (OH)/extracellular water (ECW) categories (A), the number of B-lines categories (B), and weight change categories (C). Log-rank tests were used for comparison between groups.

| Table 2. Hazard ratios (HR) for 28-day mortality according to volume overload (OH/ECW) |
|----------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Volume overload, OH/ECW (L/L)   | Model 1                        | Model 2                        | Model 3                        |
|                                  | HR (95% CI)                    | p-value                        | HR (95% CI)                    | p-value                        | HR (95% CI) | p-value |
| Low + middle tertiles (%0.31)   | 1.00                          | -                              | 1.00                          | -                              | 1.00        | -       |
| High tertile (>0.31)            | 2.93 (1.11–7.73)              | 0.03                           | 4.92 (1.41–17.22)             | 0.01                           | 3.83 (1.04–14.03) | 0.04    |

Model 1: unadjusted; model 2: adjusted for age, sex, diabetes, and chronic kidney disease; model 3: model 2 + APACHE II score and serum total CO$_2$ level. APACHE, Acute Physiology and Chronic Health Evaluation; CI, confidence interval; ECW, extracellular water; OH, overhydration.
volume status evaluated using BIA was significantly associated with 28-day survival in patients with sepsis-associated AKI receiving CKRT. We did not find a significant association between the volume status assessed using lung ultrasound or weight change and 28-day mortality in these patients. Our findings suggest that fluid overload at the time of CKRT initiation is associated with worse 28-day survival in patients with sepsis-associated AKI receiving CKRT. In addition, volume assessment using BIA was found to have prognostic value in predicting adverse outcomes in these patients.

Recent studies have revealed that the mortality rate observed in critically ill patients with AKI is >50%; poor survival has been attributed to multiple complications associated with AKI, such as fluid overload, electrolyte imbalance, bleeding, and infection [21,22]. Fluid overload often precedes and follows the diagnosis of AKI [23,24]. The impact of fluid overload on survival in AKI patients has been evaluated in several studies. Bouchard et al. [11] found that fluid overload, defined as >10% increase in body weight relative to baseline, was associated with mortality among critically ill patients with AKI in a multicenter observational study involving more than 600 patients in North America. In a Finnish AKI study involving critically ill patients receiving KRT (FINNAKI study), cumulative fluid accumulation was revealed to be an independent risk factor for 90-day mortality [25]. Additionally, Woodward et al. [26] found that the fluid balance from admission to CKRT initiation was associated with in-hospital mortality in critically ill patients with AKI requiring CKRT.

Accurate volume status assessment is considered a key element in the management of patients receiving CKRT and risk stratification of those patients [14,27]. Conventionally, body weight measurement, review of daily fluid balance, physical examination, and chest radiograph have been used to estimate fluid overload in those patients. However, these approaches typically yield inconsistent results and are considered to be fairly inaccurate [28-30]. Recently, several measures have been introduced to more precisely evaluate the volume status of patients [29]. Among these, BIA and lung ultrasound are simple and relatively reliable methods for estimating body fluid status, particularly for detecting fluid overload [15-18]. Nonetheless, information regarding the prognostic implications of volume status assessed by BIA or lung ultrasound in patients with AKI requiring CKRT is relatively scarce.

In the present study, we performed analyses to investigate the relationships between three volume surrogates (OH/ECW, number of B-lines, and weight change) and the association between volume surrogates and 28-day survival in patients with sepsis-associated AKI receiving CKRT. Our findings showed that the volume status assessed using various measures did not show a significant correlation. Moreover, participants with higher OH/ECW had a higher risk of 28-day mortality, whereas the number of B-lines or weight change was not significantly associated with 28-day mortality. In line with our findings, an ambispective cohort study including 152 patients with AKI treated with either intermittent hemodialysis or CKRT showed that OH/ECW before the initiation of KRT was significantly associated with patient survival [31]. However, this study did not collect information regarding volume status estimated by lung ultrasound or weight change; thus, they could not examine the relationship between surrogates for volume overload and the association between volume status surrogates and patient outcomes in the same cohort. Therefore, our results may provide novel insights into the measurement of volume status and the prognostic value of volume surrogates in patients with AKI requiring CKRT. In other words, the severity of fluid overload may be estimated differently depending on the methods employed, and volume assessment using BIA can provide additional information on patient prognosis.

Our study has several limitations. First, due to the observational nature of this study, the possibility of residual confounding factors cannot be excluded. However, after adjusting for various factors that may contribute to patient outcomes, we consistently found the association between volume status assessed via BIA and 28-day survival. Second, our study was conducted at a single center. Third, our results may be underpowered because of the relatively small number of participants. Fourth, our cohort consisted of only Korean participants, which limits the generalizability of the study. Thus, our study results cannot be directly extrapolated to patients with ethnic backgrounds other than Korean.

In conclusion, our study revealed that the extent of volume can vary significantly depending on the measurement methods used. Among the fluid overload surrogates investigated in this study, only OH/ECW was associated with 28-
day survival in patients with sepsis-associated AKI receiving CKRT. Our findings require further validation in larger cohorts and well-designed clinical trials to determine the optimal ultrafiltration strategy to improve survival in patients with sepsis-associated AKI who require CKRT.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

This study was supported by a research fund by the Yonsei University College of Medicine. The sponsor had no role in the study design, data collection, or analysis.

**Data sharing statement**

The data presented in this study are available on request from the corresponding author.

**Authors’ contributions**

Conceptualization: CHP, SGH, SJK, SWK
Data curation: CHP, SGH, HWK
Formal analysis, Visualization: CHP, SGH, HWK, SWK
Funding acquisition: SGH
Investigation, Methodology: CHP, SGH, JTP, SHH, SJK, SWK
Project administration, Resources, Supervision, Validation: JTP, SHH, SJK, SWK
Writing-original draft: CHP, SGH, SJK, SWK
Writing-review & editing: All authors
All authors read and approved the final manuscript.

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**References**

The mediating role of the left ventricular mass index on the relationship between the fluid balance and left ventricular diastolic function in patients with chronic kidney disease

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Background: The pathophysiological mechanism of cardiovascular disease in patients with chronic kidney disease (CKD) is complicated. Mediation analysis is an important statistical tool for gaining insight into the complex mechanisms of exposure-outcome effects. We investigated the potential mediating role of the left ventricular mass index (LVMI) on the association between fluid balance (overhydration/extracellular water, OH/ECW) and left ventricular diastolic function (E/e′ ratio) in patients with CKD not yet on dialysis.

Methods: Bioimpedance spectroscopy, echocardiography, and laboratory evaluations were performed on 425 consecutive patients on the same day. The patients were classified into two groups according to the estimated glomerular filtration rate corresponding to CKD stages 3 and 5. Mediation analysis was performed using the PROCESS macro and bootstrapping methods.

Results: OH/ECW and LVMI were positively correlated with the E/e′ ratio in both the CKD stages 3 and five groups. In CKD stage 5, there was a statistically significant association between OH/ECW and LVMI, whereas no correlation was observed in CKD stage 3. In the mediation analysis, LVMI positively mediated the relationship between OH/ECW and E/e′ ratio when controlling for confounders in patients with CKD stage 5 (B = 2.602; Boot 95% confidence interval, 1.313–4.076).

Conclusion: In our analysis, the indirect effect of mediators was significant in patients with advanced CKD. Therefore, our study suggests that further research on several other risk factors may be needed to determine the underlying mechanisms of association between the associated factors in all CKD stages.

Keywords: Chronic kidney diseases, Diastole, Fluid balance, Impedance, Left ventricular hypertrophy

Introduction

Cardiovascular disease is the leading cause of death and hospitalization in patients with chronic kidney disease (CKD), regardless of the cause. Structural and functional myocardial abnormalities are common in patients with CKD because of high blood pressure, fluid overload, and nontraditional risk factors associated with CKD. The myocardial mass represented by left ventricular (LV) mass to body surface area (BSA) index (LV mass index, LVMI) is
one of the sensitive indicators of cardiac structural changes [1]. LV diastolic dysfunction (LVDD), represented by mitral peak Doppler E-wave to peak mitral annulus velocity ratio (E/e’ ratio), is a commonly observed functional impairment [2].

Although both changes are commonly associated with poor outcomes in patients with end-stage kidney disease (ESKD) [2–4], the clinical characteristics of LV hypertrophy (LVH) and LVDD in CKD differ depending on the CKD stage [5,6]. In patients with CKD, the mechanism of LVDD is complex and is mainly associated with LVH, which is a physiological response to pressure and volume overloads. In a longitudinal observational study, patients with more advanced CKD showed greater increases in LV mass and volume than those with early-stage CKD. Additionally, cardiac remodeling did not affect LV systolic function, whereas LVDD was aggravated in progressive CKD [7].

Euvolemia in patients with renal insufficiency from the early to late stages is important not only for short-term fluid management but also for the long-term prevention of cardiovascular disease; this is because fluid overload is a predictor of mortality and morbidity [8]. Although the relationship between fluid overload and cardiac impairment is complex, fluid overload is a critical step in the pathophysiological pathway of congestive heart failure in patients with ESKD. In general, LVH and LVDD are known to precipitate and/or cause heart failure [9]. Most previous studies on structural and functional abnormalities of the heart related to fluid overload have focused on patients on dialysis, and relatively few studies have focused on patients with CKD. We previously reported that biomarkers reflecting LVH and LVDD are associated with fluid overload in patients with CKD [10,11].

During the CKD progression course, fluid imbalance, which may be related to hemodynamics and structural and functional changes in the heart, can occur and evolve in complex patterns, either individually or simultaneously. Nevertheless, most of the studies, just like what we have analyzed before, have investigated a single relationship between fluid balance and cardiac structural and functional impairments, or have evaluated the degree of influence of single factors on the relationships. Analysis in this manner implies a limitation in that the role of multiple parameters cannot be adequately explained. The mediation effect represents the influence of the predictor on the dependent variable through the mediator [12]. The causal structure behind the relationship between the predictor and dependent variables can be understood using the statistics of the mediating effect analysis. With this theoretical background in mind, we investigated the potential mediating role of LV mass in the association between fluid balance and LV diastolic function in patients with CKD.

**Methods**

**Patients and data collection**

Since 2014, we registered consecutive patients with CKD in a bioimpedance cohort after receiving approval (No. CR319143 for CKD stage 3 and No. CR316024 for CKD stage 5) from the Institutional Review Board of Yonsei University Wonju Severance Christian Hospital. Using the estimated glomerular filtration rate (eGFR), patients with CKD stages 3 and 5 were analyzed. All patients underwent simultaneous bioimpedance spectroscopy (BIS), echocardiography, and laboratory evaluation at the time of enrolment. The CKD stage 5 group consisted of dialysis-naïve patients only. This means that all the aforementioned tests were performed prior to the initiation of renal replacement therapy in patients with CKD stage 5. Therefore, our study was a retrospective observational analysis of a prospective cohort database of patients with stages 3 and 5 CKD. All patients provided written informed consent before participating in the study. This study was conducted in accordance with the principles of the Declaration of Helsinki.

**Conventional echocardiographic study**

Echocardiography was performed in harmonic imaging mode using a 3-MHz transducer and a commercial ultrasound system (GE Vivid E9; GE Healthcare). LV mass was calculated following the American Society of Echocardiography recommendations [13] using the following equation:

$$LV\ mass = 0.8 \times [1.04 \times \{IVS + L\ VID + PWT\}^3 - (LVID)^3] + 0.6 \ g$$

where $IVS$ is the interventricular septum, $LVID$ is the LV internal diameter, and $PWT$ is inferolateral wall thickness. All measurements were performed at end-diastole. To correct for BSA, LVMI was calculated by dividing LV mass by BSA.
using the following formula: 

$$\text{BSA} = (0.007184 \times \text{weight}^{0.425} \times \text{height}^{0.725}) \text{m}^2.$$ 

Transmitral inflow velocities were measured using pulsed-wave Doppler in the apical four-chamber view, with the sample volume placed at the mitral valve leaflet tips. Transmitral early diastolic (E-wave) velocities were measured. Tissue Doppler imaging in the apical four-chamber view was used to measure LV myocardial velocities with the sample volume placed at the septal mitral annulus. We measured the peak early (e′) diastolic mitral annular velocity and calculated the E/e′ ratio [14]. The left atrial volume index (LAVI), LV end-diastolic volume (LVEDV), and LV ejection fraction were measured according to previously mentioned recommendations. Echocardiography was performed by trained cardiologists who were blinded to the patient’s information.

**Assessment of the volume status**

Whole-body BIS was performed using BCM (Body Composition Monitoring; Fresenius Medical Care AG & Co.). The BCM utilizes alternating electric currents across 50 discrete frequencies covering the frequency spectrum from 5 to 1,000 kHz and measures the impedance of each current. Disposable electrode patches placed on the wrist and ankle were used for all the measurements. The validity of BIS in the general and dialysis populations has been demonstrated in comparison to gold standard methods. Extracellular water (ECW), intracellular water, and total body water were automatically provided by the BCM using the equations of Moissl et al. [15] based on the Hanai mixture theory adjusted for body mass index (BMI). A three-compartment BIS model separates body weight into normally hydrated lean tissue mass, normally hydrated adipose tissue mass, and fluid overload, which is commonly described as the overhydration (OH) compartment [16]. Extracellular fluid overload, presented as positive or negative OH, can be calculated from the difference between the actual measured ECW and the expected ECW [17]. Because specific OH values vary in clinical relevance according to the patient body size, OH/ECW was primarily used to determine the volume status as relative OH in our study. In the same context, the patient’s BMI was recalculated using the following formula: 

$$\text{corrected BMI (cBMI, kg/m}^2\text{)} = (\text{body weight – OH})/\text{height}^2.$$ 

**Statistical analysis**

Categorical variables are expressed as frequencies and percentages, and continuous variables are reported as means with standard deviations. Based on the eGFR, all patients were divided into two groups: CKD stage 3 and CKD stage 5. Patient characteristics between groups were tested using a chi-square test and a two-sample t test, as appropriate. Pearson correlation analysis was used to examine correlations between the E/e′ ratio and other variables, such as laboratory findings, echocardiographic parameters, and markers of volume status in each group. Statistical analyses were performed using the IBM SPSS version 25.0 (IBM Corp.). Statistical significance was defined as $p < 0.05$.

Finally, mediation analysis was performed using the PROCESS macro and bootstrapping for SPSS (PROCESS version 4.1; Andrew F. Hayes) [18]. The bootstrap test complements the limited ability of the Sobel test, which relies on the assumption of normality [19,20]. The statistical significance of the mediating effect was determined using the confidence intervals (CI) of bootstrapping estimation techniques; when 0 was included in the bootstrap 95% CI, the indirect effect was considered nonsignificant. Recently, this method has been recommended more than Baron and Kenny’s method [12], which requires the Sobel test [19] to verify the indirect effect of the mediation analysis. Therefore, in this study, bootstrap analyses were used to verify the significance of the indirect effect of LVMI on the relationship between OH/ECW and E/e′ ratio in each group. A total of 5,000 bootstrap samples were repeatedly extracted to estimate the indirect effect, adjusted for age, sex, diabetes prevalence, cBMI, and eGFR. Graphs were generated using the Prism software (version 5.02; GraphPad Software).

**Results**

**Characteristics of the study patients**

The clinical characteristics of both groups according to the CKD stage are presented in Tables 1 and Fig. 1. The mean ages of male and female patients were 60.80 ± 12.92 years and 61.09 ± 13.60 years, respectively. Males accounted for 263 patients (61.9%). There was no statistical difference in the E/e′ ratio, LVMI, and OH/ECW between men and
women with CKD stage 3. In CKD stage 5, LVMI and OH/ECW were not significantly different between men and women, whereas the E/e’ ratio (14.55 ± 5.10 vs. 16.52 ± 6.24, p = 0.002) was significantly higher in women.

Although the OH/ECW (5.54% ± 6.45% vs. 0.00% ± 5.54%, p < 0.001) of the diabetic patients was higher than that of the nondiabetic patients with CKD stage 3, E/e’ ratio and LVMI were not different. In CKD stage 5, E/e’ ratio (16.28 ± 5.60 vs. 13.86 ± 5.49, p < 0.001) and OH/ECW (18.56% ± 14.81% vs. 9.96% ± 13.87%, p < 0.001) were significantly greater in patients with diabetes compared to those patients without, while LVMI was not different. In our study, fluid overload, defined as an OH/ECW greater than 15% [21], was present in six patients (6.0%) with CKD stage 3, whereas 152 patients (44.4%) presented with CKD stage 5. Meanwhile, LVDD with an E/e’ ratio >15 was present in

Data are expressed as number only, mean ± standard deviation, or number (%).
cBMI, corrected body mass index; CKD, chronic kidney disease; DBP, diastolic blood pressure; ECW, extracellular water; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LAVI, left atrial volume index; LDL-C, low-density lipoprotein cholesterol; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; OH, overhydration; SBP, systolic blood pressure; tCO2, total carbon dioxide.

women with CKD stage 3. In CKD stage 5, LVMI and OH/ECW were not significantly different between men and women, whereas the E/e’ ratio (14.55 ± 5.10 vs. 16.52 ± 6.24, p = 0.002) was significantly higher in women.

Although the OH/ECW (5.54% ± 6.45% vs. 0.00% ± 5.54%, p < 0.001) of the diabetic patients was higher than that of the nondiabetic patients with CKD stage 3, E/e’ ratio and LVMI were not different. In CKD stage 5, E/e’ ratio (16.28 ± 5.60 vs. 13.86 ± 5.49, p < 0.001) and OH/ECW (18.56% ± 14.81% vs. 9.96% ± 13.87%, p < 0.001) were significantly greater in patients with diabetes compared to those patients without, while LVMI was not different. In our study, fluid overload, defined as an OH/ECW greater than 15% [21], was present in six patients (6.0%) with CKD stage 3, whereas 152 patients (44.4%) presented with CKD stage 5. Meanwhile, LVDD with an E/e’ ratio >15 was present in

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>CKD stage 3</th>
<th>CKD stage 5</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>425</td>
<td>83</td>
<td>342</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>60.91 ± 13.17</td>
<td>61.86 ± 11.92</td>
<td>60.68 ± 13.47</td>
<td>0.468</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td>Male</td>
<td>263 (61.9)</td>
<td>62 (23.6)</td>
<td>201 (76.4)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>162 (38.1)</td>
<td>21 (13.0)</td>
<td>141 (87.0)</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Yes</td>
<td>255 (60.0)</td>
<td>43 (16.9)</td>
<td>212 (83.1)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>170 (40.0)</td>
<td>40 (23.5)</td>
<td>130 (76.5)</td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>140.56 ± 19.06</td>
<td>130.89 ± 15.39</td>
<td>142.91 ± 19.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79.20 ± 12.00</td>
<td>75.48 ± 12.55</td>
<td>80.10 ± 11.10</td>
<td>0.002</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>66.51 ± 12.63</td>
<td>71.61 ± 13.43</td>
<td>65.27 ± 12.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>cBMI (kg/m²)</td>
<td>24.27 ± 4.13</td>
<td>26.30 ± 3.93</td>
<td>23.78 ± 4.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OH/ECW (%)</td>
<td>12.87 ± 14.65</td>
<td>2.87 ± 6.60</td>
<td>15.29 ± 15.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LAVI (mL/m²)</td>
<td>39.22 ± 12.18</td>
<td>27.02 ± 7.77</td>
<td>39.70 ± 11.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEDV (mL)</td>
<td>142.62 ± 35.02</td>
<td>126.67 ± 30.68</td>
<td>146.49 ± 34.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E/e’ ratio</td>
<td>14.65 ± 5.56</td>
<td>11.72 ± 3.91</td>
<td>15.36 ± 5.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVMI (g/m²)</td>
<td>110.62 ± 29.92</td>
<td>89.80 ± 24.09</td>
<td>115.67 ± 29.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>61.27 ± 7.56</td>
<td>62.78 ± 4.56</td>
<td>60.90 ± 8.09</td>
<td>0.005</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>9.90 ± 2.20</td>
<td>13.38 ± 1.72</td>
<td>9.06 ± 1.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tCO2 (mmol/L)</td>
<td>19.34 ± 4.70</td>
<td>25.64 ± 3.24</td>
<td>17.81 ± 3.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>6.30 ± 0.85</td>
<td>7.20 ± 0.51</td>
<td>6.08 ± 0.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.65 ± 0.61</td>
<td>4.36 ± 0.36</td>
<td>3.48 ± 0.54</td>
<td>0.83</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>148.70 ± 44.07</td>
<td>162.01 ± 35.34</td>
<td>145.47 ± 45.40</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>138.91 ± 76.97</td>
<td>175.37 ± 87.97</td>
<td>130.06 ± 71.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>40.05 ± 13.30</td>
<td>46.52 ± 13.44</td>
<td>38.47 ± 12.80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>81.34 ± 36.99</td>
<td>79.84 ± 30.00</td>
<td>81.71 ± 38.53</td>
<td>0.68</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>81.33 ± 38.93</td>
<td>76.98 ± 32.09</td>
<td>82.40 ± 40.39</td>
<td>0.26</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.01 ± 1.17</td>
<td>9.39 ± 0.40</td>
<td>7.68 ± 1.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phosphate (mg/dL)</td>
<td>5.45 ± 1.83</td>
<td>3.27 ± 0.50</td>
<td>5.98 ± 1.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>7.59 ± 2.37</td>
<td>6.77 ± 1.83</td>
<td>7.79 ± 2.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>14.18 ± 15.38</td>
<td>44.34 ± 7.10</td>
<td>6.85 ± 2.60</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
nine patients (10.8%) with CKD stage 3. Among patients with CKD stage 5, 144 (42.1%) had LVDD.

**Correlation of volume status, echocardiographic findings, and serum chemistry with E/e’ ratio**

In all patients, LVMI and OH/ECW were positively correlated with E/e’ ratio (Table 2). LAVI and LVEDV were also positively associated with the E/e’ ratio. However, no consistent correlation was observed for the other measured variables compared to the other groups. To perform mediation analysis (Fig. 2), it is first necessary to show significant correlations between predictor and mediator, between predictor and dependent variable, and between mediator and dependent variable. The dependent variable was the E/e’ ratio. Predictors and mediators were OH/ECW and LVMI, respectively. In our analysis, the three criteria for significant correlations were not fulfilled in patients with CKD stage 3. OH/ECW was not significantly associated with LVMI (p = 0.06). However, OH/ECW was positively associated with LVMI (r = 0.229, p < 0.001) in patients with CKD stage 5.

**Mediating effect of left ventricular mass index**

The indirect effect for all patients with CKD was statistically significant because 0 was not included in the bootstrap 95% CI (B = 2.980; Boot 95% CI, 1.729–4.416). However, there were no consistent findings in the subgroup analysis. This was statistically significant only in CKD stage 5. LVMI

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**Table 2. Correlation of E/e’ ratio with variables in each group**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>CKD stage 3</th>
<th>CKD stage 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation coefficient</td>
<td>p-value</td>
<td>Correlation coefficient</td>
</tr>
<tr>
<td>Age</td>
<td>0.193</td>
<td>&lt;0.001</td>
<td>0.129</td>
</tr>
<tr>
<td>SBP</td>
<td>0.219</td>
<td>&lt;0.001</td>
<td>0.050</td>
</tr>
<tr>
<td>DBP</td>
<td>0.019</td>
<td>0.69</td>
<td>-0.035</td>
</tr>
<tr>
<td>Body weight</td>
<td>-0.076</td>
<td>0.12</td>
<td>0.040</td>
</tr>
<tr>
<td>cBMI</td>
<td>-0.019</td>
<td>0.70</td>
<td>0.160</td>
</tr>
<tr>
<td>OH/ECW</td>
<td>0.348</td>
<td>&lt;0.001</td>
<td>0.312</td>
</tr>
<tr>
<td>LAVI</td>
<td>0.498</td>
<td>&lt;0.001</td>
<td>0.275</td>
</tr>
<tr>
<td>LVEDV</td>
<td>0.272</td>
<td>&lt;0.001</td>
<td>0.262</td>
</tr>
<tr>
<td>LVMI</td>
<td>0.402</td>
<td>&lt;0.001</td>
<td>0.363</td>
</tr>
<tr>
<td>LVEF</td>
<td>-0.255</td>
<td>&lt;0.001</td>
<td>-0.090</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>-0.289</td>
<td>&lt;0.001</td>
<td>-0.204</td>
</tr>
<tr>
<td>tCO₂</td>
<td>-0.251</td>
<td>&lt;0.001</td>
<td>-0.036</td>
</tr>
<tr>
<td>Total protein</td>
<td>-0.236</td>
<td>&lt;0.001</td>
<td>-0.084</td>
</tr>
<tr>
<td>Albumin</td>
<td>-0.283</td>
<td>&lt;0.001</td>
<td>-0.163</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.027</td>
<td>0.58</td>
<td>-0.077</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>-0.109</td>
<td>0.02</td>
<td>-0.068</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-0.067</td>
<td>0.18</td>
<td>-0.049</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.042</td>
<td>0.39</td>
<td>-0.014</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>0.042</td>
<td>0.39</td>
<td>0.074</td>
</tr>
<tr>
<td>Calcium</td>
<td>-0.279</td>
<td>&lt;0.001</td>
<td>-0.140</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.252</td>
<td>&lt;0.001</td>
<td>0.193</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.141</td>
<td>0.004</td>
<td>0.045</td>
</tr>
<tr>
<td>eGFR</td>
<td>-0.268</td>
<td>&lt;0.001</td>
<td>0.027</td>
</tr>
</tbody>
</table>

cBMI, corrected body mass index; CKD, chronic kidney disease; DBP, diastolic blood pressure; ECW, extracellular water; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LAVI, left atrial volume index; LDL-C, low-density lipoprotein cholesterol; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; OH, overhydration; SBP, systolic blood pressure; tCO₂, total carbon dioxide.
mediated the relationship between OH/ECW and E/e’ ratio even after controlling for age, sex, diabetes prevalence, cBMI, and eGFR (B = 2.602; Boot 95% CI, 1.313–4.076). The indirect effect of LVMI on CKD stage 3 was not significant (B = 4.841; Boot 95% CI, -0.0002 to 11.435). Table 3 presents the results of the mediation analysis for each group.

**Discussion**

In patients with CKD, LVDD and LVH are common and are closely related to increased cardiovascular mortality. In the general population, LVH which is a physiological response to pressure overload has been reported as one of the pathogenetic mechanisms of LVDD. However, it is not clear whether cardiac structural changes occur prior to functional changes in CKD or vice versa. Previous studies have shown different results depending on the CKD stage at the time of evaluation and the evaluation tools used, such as echocardiography and cardiac magnetic resonance [22–24]. Certain pathophysiological factors may have different degrees of impact on the occurrence and exacerbation of cardiac structural and functional abnormalities at different stages of CKD, regardless of traditional or nontraditional CKD-related factors.

Fluid balance is particularly important because it can promote hemodynamic stability and minimize cardiovascular complications in patients with CKD. Fluid overload is associated with cardiovascular mortality and may be a relevant target for improving outcomes in patients undergoing hemodialysis [25]. Previous studies on the link between cardiac abnormalities and fluid imbalance have mostly focused on patients with ESKD undergoing dialysis [26]. As an extension of this point, fluid overload in patients with CKD is the same factor [8]. However, to prevent cardiovascular complications, it is essential to consider several factors rather than finding and controlling only one factor.

It is well known that the prevalence of LVH increases as kidney function decreases [27]. It is not yet clear which one plays a more important role in the development of LVH at each stage of CKD, making it difficult to generalize its evolution in CKD. Therefore, compared to the pathophysiology in the general population, the pathophysiology of LVH in patients with CKD is very complicated. In patients with advanced CKD, fluid overload has also been reported as a risk factor for LVH [11,28,29]. If the heart loses its elasticity as a result of thickened heart walls and is unable to pump as much force as necessary, fluid overload may fur-
ther accelerate heart failure at any stage of CKD. Although the pathogenesis of LVDD is complex, the clinical course and patterns of LVDD are also determined by the CKD stage. LVDD can be caused by various other factors, with or without LVH. Among them, fluid overload acts as an independent risk factor for LVDD and as a promoting factor for heart failure with preserved ejection fraction in patients with CKD 

We have previously reported an association between biomarkers reflecting LVH and LVDD and fluid overload in patients with CKD stage 5 [10,11]. Our previous studies did not examine each other’s relationship on one axis by integrating all three factors (fluid balance, LV mass, and LV diastolic function). These studies analyzed the meaning of a single factor using only dependent, independent, and control variables. From the perspective of uremic cardiomyopathy, knowing the exact time when cardiac structural or functional changes can be a very important point in the treatment and prevention of complications. However, more importantly, because complications are not determined only by a single factor, a truly preventive approach is possible only when numerous factors are understood and judged in a complex manner. Statistically, multiple regression is an objective method that can empirically verify the influential factors. However, there is a limit to confirming the relationship between the statistically significant factors. Since the introduction of the moderation-mediation analysis, the relationship between the associated factors has been analyzed. Recently, analysis using the PROCESS macro and bootstrapping technique [18] instead of Baron and Kenny’s method [12,30], which is often used in mediation analysis, has been accepted as a more advanced concept.

In a prospective longitudinal study over 1 year, advanced CKD stages 4 and 5 were more associated with larger cardiac changes, including increased LV mass and decreased diastolic function, compared with CKD stage 3 [7]. This study was performed to determine the importance of baseline CKD stages in predicting longitudinal changes in cardiac structure and function and not to evaluate which factors were involved. In our study, we first confirmed that CKD stage 5 had statistically significant differences in LVMI, OH/ECW, and E/e´ ratio compared with CKD stage 3 (Table 1). We confirmed that LVMI and OH/ECW had a statistically significant relationship with the E/e´ ratio (Table 2). The purpose of our study was to investigate the statistical significance of the latent mediating effect of the mediator on the association between the predictor and the dependent variable. Mediation analysis is possible only when the aforementioned three parameters have a significant correlation with each other. OH/ECW was positively correlated with LVMI (p < 0.001) in CKD stage 5, whereas there was no correlation with each other in CKD stage 3 (p = 0.06). This means that it was linked to the mediating analysis results that LVMI had no indirect effect on CKD stage 3. Considering the results reported by Cai et al. [7], it can be assumed that the pathophysiology of cardiac impairment becomes more complex as CKD progresses to an advanced stage. Our study demonstrated that mediation analysis could be a method for investigating complexity.

This study has some limitations. First, the number of

| Table 3. Bootstrapping results with confidence intervals for the lower and upper limits |
|----------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Group       | Effect          | Label | Estimate | SE  | 95% confidence interval | |
| CKD stage 3 and 5 | Indirect (Booted) | a × b | 2.980 | 0.680 | 1.729* | 4.416* |
|             | Direct          | c    | 10.268 | 1.893 | 6.547 | 13.988 |
|             | Total           | c + (a × b) | 13.248 | 1.963 | 9.461 | 17.035 |
| CKD stage 3 | Indirect (Booted) | a × b | 4.841 | 2.961 | -0.0002* | 11.435* |
|             | Direct          | c    | 18.469 | 6.874 | 4.776 | 32.161 |
|             | Total           | c + (a × b) | 23.310 | 7.064 | 9.241 | 37.374 |
| CKD stage 5 | Indirect (Booted) | a × b | 2.602 | 0.713 | 1.313* | 4.076* |
|             | Direct          | c    | 9.563 | 2.054 | 5.523 | 13.603 |
|             | Total           | c + (a × b) | 12.165 | 2.085 | 8.064 | 16.266 |

Covariates included age, sex, diabetes prevalence, corrected body mass index, and estimated glomerular filtration rate.

CKD, chronic kidney disease; LLCI, lower limit confidence interval; SE, standard error; ULCI, upper limit confidence interval.

*a Bootstrapping results with standard error and 95% confidence intervals for the lower and upper limits.

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patients with CKD stage 3 was relatively small compared to that of patients with CKD stage 5. It could not be determined whether this had an effect on the analysis results for CKD stage 3. Additionally, the proportion of men was relatively higher than that of women. There was also a difference in the sex ratio between the two groups. Therefore, this may have affected the LV mass. Although sex was used as a correction factor in the analysis, sufficient consideration was not given to sex-specific differences in LV mass. Second, we did not measure these parameters in patients with CKD stage 4. Therefore, the significance of the mediation analysis on whether there was an indirect effect could not be confirmed for CKD stage 4. Third, we know that the association between fluid overload and diastolic dysfunction cannot be determined by a single echocardiographic study. While a concrete consensus on the treatment of diastolic dysfunction has not yet been established, blood pressure control, heart rate control, improvement of myocardial ischemia, and blood volume control are being emphasized [31]. Therefore, our study provides an opportunity to examine the relationship between fluid balance and a well-known risk factor for LVDD. Fourth, other variables that may mediate fluid balance may also exist, since our patients were accompanied by complicated combinations of risk factors, such as increased uremic toxin, systemic inflammation, and long-term hemodynamic instability. Furthermore, there may have been unmeasured confounding factors. These factors were not sufficiently considered in this analysis. Fifth, since the serial assessment of the predictor, mediator, and dependent variables was not performed over time, the role of the three factors according to the amount of change could not be identified. Finally, we are well aware that mediation analysis methods are limited in their ability to account for all of the complexity. It is clear that the results of statistical analysis can only support a hypothesis and do not prove it. Despite these limitations, the strength of our study is that it provided meaningful information that the indirect effect of the mediator may be different at different CKD stages. To our knowledge, this is the first study to attempt such an analysis.

Considering our results, we suggest that the evaluation of structural and functional cardiac abnormalities and volume status should be performed regularly and simultaneously across all stages of CKD. Further analyses of several other risk factors could provide insight into the mechanisms underlying the associations between associated factors, which could lead to the tailored application of treatment strategies and, hence, improve cardiovascular outcomes during the progression of CKD.

Conflicts of interest

All authors have no conflicts of interest to declare.

Data sharing statement

The data presented in this study are available upon reasonable request from the corresponding author.

Authors’ contributions

Conceptualization: BGH
Data curation: BGH, JYL, JSK, JWY
Formal analysis: BGH, SWP
Project administration: BGH, JYL, JSK, JWY, SWP
Writing–original draft: BGH, SWP
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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Background: Although several therapeutic agents have been evaluated for the treatment of coronavirus disease 2019 (COVID-19), there are lack of effective and proven treatments for end-stage renal disease (ESRD). The present study aims to evaluate the effectiveness of regdanvimab on mortality in COVID-19–infected patients on hemodialysis (HD).

Methods: We conducted an observational retrospective study in 230 COVID-19–infected patients on HD, of whom 77 (33.5%) were administered regdanvimab alone or in combination with dexamethasone or remdesivir during hospitalization (regdanvimab group) and 153 patients (66.5%) were not (no regdanvimab group). The primary outcome was in-hospital mortality. We compared mortality rates according to the use of regdanvimab and investigated the factors associated with mortality.

Results: Fifty-nine deaths occurred during hospitalization, 49 in the no regdanvimab group (32.0%) and 10 in the regdanvimab group (13.0%), and the mortality rate was significantly higher in the no regdanvimab group than that in the regdanvimab group (p = 0.001). Multivariate Cox regression analysis showed that malignancy (p = 0.001), SPO\textsuperscript{2} of <95% at admission (p = 0.003), and administration of antibiotics and regdanvimab (p = 0.007 and p = 0.002, respectively) were significantly associated factors with mortality.

Conclusion: Regdanvimab administration is beneficial in improving prognosis in hospitalized COVID-19 patients on HD. Considering the vulnerability to infection and high mortality of ESRD patients, regdanvimab may be considered as a therapeutic option in COVID-19 patients on HD.

Keywords: COVID-19, Renal dialysis, Mortality, Regdanvimab
**Introduction**

Coronavirus disease 2019 (COVID-19), caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread globally since December 2019. The urgent need for effective treatments has led to unprecedented research efforts, and treatment guidelines for COVID-19 are being revised and updated based on results from clinical studies that evaluated therapeutic agents and approaches [1–3]. Among therapeutic agents, regdanvimab (Regkirona, Celltrion Healthcare) is a recombinant neutralizing monoclonal antibody that received final approval in September 2021 in South Korea for the treatment of COVID-19 [4]. Early treatment with regdanvimab has been shown to reduce the severity of disease and associated hospitalization or intensive care unit (ICU) admittance in COVID-19 patients with mild-to-moderate symptoms [5,6].

Patients with end-stage renal disease (ESRD) on hemodialysis (HD) are more vulnerable to this viral epidemic due to inevitable regular visits to dialysis units and contact with susceptible patients. Moreover, those on ESRD generally have many comorbidities and are in an immunocompromised state. Previous studies have shown that patients with ESRD have a higher mortality rate compared with those of the general population [7–9]. Regdanvimab is generally considered for use in adult patients with moderate symptoms or elderly patients aged >50 years with mild symptoms and at least one underlying medical condition, including obesity, cardiovascular disease, chronic lung disease, diabetes mellitus, and chronic liver disease; patients on immunosuppressive agents; and patients with chronic kidney disease [10]. Based on these indications, regdanvimab is considered for use in ESRD patients in the current clinical setting; however, data on the usage and effectiveness of regdanvimab in ESRD patients are still lacking.

To further clarify the clinical evidence of regdanvimab use in ESRD patients, the present study aims to evaluate the real-world effectiveness of regdanvimab on mortality in COVID-19–infected patients on HD.

**Methods**

This study was approved by the Institutional Review Board of Hallym University Kangnam Sacred Heart Hospital (No. HKS 2021-07-013). The need to obtain informed consent was waived due to the retrospective nature of the study.

**Study design and participants**

We retrospectively recruited all hospitalized, COVID-19–infected patients on HD who were admitted and treated at Good Samaritan Bagae Hospital (Pyeongtaek, Republic of Korea) from December 1, 2020 to November 30, 2021. Among the 338 patients, we finally analyzed the data of 230 patients who received dexamethasone, remdesivir, and regdanvimab and excluded 108 patients who received only conservative treatment without these drugs. Of them, 153 patients did not receive regdanvimab during hospital stay and 77 patients received regdanvimab alone or in combination with dexamethasone or remdesivir (Fig. 1). The diagnosis of COVID-19 was confirmed using real-time reverse transcription-polymerase chain reaction assays using samples from the upper or lower respiratory tract.

**Clinical management**

All hospitalized patients received symptomatic care, including oxygen, antipyretics, and antitussive agents. Therapeutic agents including dexamethasone, antibiotics, and antiviral agents were administered to the selected patients within 1 to 2 days after admission according to hospital protocols and clinician decision.

In our center, regdanvimab was considered for patients with at least one risk factor, with a one-week duration since disease onset, and with no need for supplemental oxygen. Risk factors included obesity, cardiovascular disease, chronic lung disease, type 1 or type 2 diabetes mellitus, and chronic liver disease. In some cases, regdanvimab was considered for patients requiring low-concentrated oxygen according to the clinician’s judgment. The physician explained the possible side effects of regdanvimab, and only patients who consented to its use were prescribed.

**Data collection and mortality**

The electronic medical records of all participating patients were reviewed. Baseline characteristics were investigated on the day when the patients were admitted to the institution after COVID-19 infection was confirmed. The clinical information of interest included age, sex, body mass index...
(BMI), underlying kidney disease, use of renin-angiotensin system blockers, type of dialysis unit before admission, underlying disease, initial symptoms, and saturation of partial pressure oxygen (SPO$_2$) at admission. Baseline laboratory data were also collected. Information on treatment included antiviral or antibiotic therapy, corticosteroid therapy, admission to ICU, use of mechanical ventilation (MV), use of continuous renal replacement therapy (CRRT), and oxygen supply. In-hospital mortality, defined as all-cause mortality during hospitalization for COVID-19 infection, was set as the primary endpoint, and information on the duration of hospitalization was also investigated.

**Statistical analysis**

Study participants were categorized into the regdanvimab group and no regdanvimab group. The t test was used for parametric estimation, and the Wilcoxon rank-sum test was for nonparametric estimation. Categorical variables were compared using the chi-square test. The Kaplan-Meier survival curve was used to determine the differences in mortality between the groups, and the statistical significance was assessed using the log-rank test. Univariate and multivariate Cox regression analyses were used to explore the factors associated with mortality. All statistical analyses were performed using IBM SPSS version 23.0 (IBM Corp.), and p-values less than 0.05 were considered statistically significant.

**Results**

**Baseline characteristics**

In total, 230 COVID-19–infected patients on HD were included in the study, of whom 77 (33.5%) were administered regdanvimab alone or in combination with dexamethasone or remdesivir during hospitalization and no regdanvimab group was not.
Table 1. Comparison of baseline characteristics between the groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Regdanvimab group</th>
<th>No regdanvimab group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>230</td>
<td>77</td>
<td>153</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>67.9 ± 12.1</td>
<td>68.2 ± 11.1</td>
<td>67.8 ± 12.6</td>
<td>0.79</td>
</tr>
<tr>
<td>Male sex</td>
<td>139 (60.4)</td>
<td>47 (61.0)</td>
<td>92 (60.1)</td>
<td>0.51</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>23.5 ± 4.3</td>
<td>23.5 ± 3.7</td>
<td>23.9 ± 4.9</td>
<td>0.61</td>
</tr>
<tr>
<td>Kidney disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>132 (57.9)</td>
<td>42 (54.5)</td>
<td>90 (58.8)</td>
<td>0.33</td>
</tr>
<tr>
<td>Hypertension</td>
<td>67 (29.1)</td>
<td>25 (32.5)</td>
<td>42 (27.5)</td>
<td>0.26</td>
</tr>
<tr>
<td>PKD</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>7 (3.0)</td>
<td>3 (3.9)</td>
<td>4 (2.6)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>22 (9.6)</td>
<td>6 (7.8)</td>
<td>16 (10.5)</td>
<td></td>
</tr>
<tr>
<td>Underlying disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>132 (57.4)</td>
<td>42 (54.5)</td>
<td>90 (58.8)</td>
<td>0.33</td>
</tr>
<tr>
<td>Hypertension</td>
<td>149 (64.8)</td>
<td>51 (66.2)</td>
<td>98 (64.1)</td>
<td>0.41</td>
</tr>
<tr>
<td>CAOD</td>
<td>41 (17.8)</td>
<td>16 (20.8)</td>
<td>25 (16.3)</td>
<td>0.26</td>
</tr>
<tr>
<td>CHF</td>
<td>9 (3.9)</td>
<td>4 (5.2)</td>
<td>5 (3.3)</td>
<td>0.35</td>
</tr>
<tr>
<td>CVA</td>
<td>29 (12.6)</td>
<td>10 (13.0)</td>
<td>19 (12.4)</td>
<td>0.53</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>13 (5.7)</td>
<td>3 (3.9)</td>
<td>10 (6.5)</td>
<td>0.31</td>
</tr>
<tr>
<td>Malignancy</td>
<td>16 (7.0)</td>
<td>4 (5.2)</td>
<td>12 (7.8)</td>
<td>0.33</td>
</tr>
<tr>
<td>Symptom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>94 (40.9)</td>
<td>25 (32.5)</td>
<td>69 (45.1)</td>
<td>0.009</td>
</tr>
<tr>
<td>Cough</td>
<td>51 (22.2)</td>
<td>14 (18.2)</td>
<td>37 (24.2)</td>
<td>0.20</td>
</tr>
<tr>
<td>Sputum</td>
<td>35 (15.8)</td>
<td>11 (14.3)</td>
<td>24 (15.7)</td>
<td>0.44</td>
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<tr>
<td>Sore throat</td>
<td>23 (10.0)</td>
<td>5 (6.5)</td>
<td>18 (11.8)</td>
<td>0.12</td>
</tr>
<tr>
<td>Rhinorrhea</td>
<td>12 (5.2)</td>
<td>4 (5.2)</td>
<td>8 (5.2)</td>
<td>0.51</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>54 (23.5)</td>
<td>6 (7.8)</td>
<td>48 (31.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SPO\textsubscript{2} at admission &lt;95%</td>
<td>24 (10.4)</td>
<td>2 (2.6)</td>
<td>22 (14.4)</td>
<td>0.003</td>
</tr>
<tr>
<td>Use of RASB</td>
<td>96 (41.7)</td>
<td>32 (41.6)</td>
<td>64 (41.8)</td>
<td>0.56</td>
</tr>
<tr>
<td>Hospital type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private clinic</td>
<td>75 (32.6)</td>
<td>23 (29.9)</td>
<td>52 (34.0)</td>
<td></td>
</tr>
<tr>
<td>Nursing hospital</td>
<td>43 (18.7)</td>
<td>18 (23.4)</td>
<td>25 (16.3)</td>
<td></td>
</tr>
<tr>
<td>University hospital</td>
<td>34 (14.8)</td>
<td>10 (13.0)</td>
<td>24 (15.7)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>38 (16.5)</td>
<td>14 (18.2)</td>
<td>24 (15.7)</td>
<td></td>
</tr>
<tr>
<td>Laboratory data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (*1,000/μL)</td>
<td>6.34 ± 3.32</td>
<td>5.91 ± 2.48</td>
<td>6.56 ± 3.66</td>
<td>0.16</td>
</tr>
<tr>
<td>Neutrophil (*1,000/μL)</td>
<td>4.71 ± 3.12</td>
<td>4.12 ± 2.19</td>
<td>4.99 ± 3.46</td>
<td>0.02</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.8 ± 1.4</td>
<td>10.8 ± 1.4</td>
<td>10.7 ± 1.4</td>
<td>0.59</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>65.8 ± 28.1</td>
<td>55.8 ± 18.7</td>
<td>70.8 ± 30.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>9.31 ± 3.79</td>
<td>8.76 ± 3.72</td>
<td>9.58 ± 3.81</td>
<td>0.12</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.8 ± 0.5</td>
<td>3.83 ± 0.59</td>
<td>3.72 ± 0.51</td>
<td>0.14</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>5.69 ± 6.04</td>
<td>3.56 ± 5.01</td>
<td>6.76 ± 6.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BNP (pg/mL)</td>
<td>590.7 ± 786.8</td>
<td>459.7 ± 524.3</td>
<td>656.7 ± 884.4</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Data are expressed as number only, mean ± standard deviation, or number (%). BMI, body mass index; BUN, blood urea nitrogen; BNP, brain natriuretic peptide; CAOD, coronary artery obstructive disease; CHF, congestive heart failure; CVA, cerebrovascular accident; DM, diabetes mellitus; PKD, polycystic kidney disease; RASB, renin-angiotensin system blockade; SPO\textsubscript{2}, saturation of partial pressure oxygen; WBC, white blood cell.
istics and the distribution of underlying diseases between the two groups. More patients complained of dyspnea in the no regdanvimab than in the regdanvimab group, whereas there were no differences in other symptoms such as fever, cough, sputum, sore throat, and rhinorrhea. The proportion of patients whose SPO$_2$ was <95% at the time of admission was higher in the no regdanvimab group than in the regdanvimab group. Laboratory data showed that neutrophil count and blood urea nitrogen, C-reactive protein, and brain natriuretic peptide levels were higher in the no regdanvimab group than those in the regdanvimab group.

**Information on treatment and mortality**

In addition to the administration of therapeutic agents, various supportive treatments were administered to the patients (Table 2). Remdesivir was administered in 79.1% of patients in the no regdanvimab group and 19.1% of patients in the regdanvimab group. Dexamethasone was administered in 53.2% of patients in the no regdanvimab group and 37.7% of patients in the regdanvimab group. The proportions of antibiotics use rate were 83% in the no regdanvimab group and 72.7% in the regdanvimab group, which were quite high in both groups, but there was no significant difference between the groups. The proportion of patients who received oxygen, high flow oxygen, and MV was higher in the no regdanvimab group than in the regdanvimab group. More patients in the no regdanvimab group needed ICU admission during hospitalization than did patients in the regdanvimab group. The proportion of patients receiving CRRT during hospitalization was similar in both groups.

**Mortality based on regdanvimab administration**

Fig. 2 shows the mortality of study patients. The mean length of hospital stay was 19.3 ± 12.3 days and was significantly longer in the regdanvimab group (22.6 ± 14.4 days) than in the no regdanvimab group (17.6 ± 10.7 days) (p = 0.009). Fifty-nine deaths (25.7%) occurred during hospitalization, 49 in the no regdanvimab group (32.0%) and 10 in the regdanvimab group (13.0%), and the mortality rate was significantly higher in the no regdanvimab group than that in the regdanvimab group (p = 0.001). According to the Kaplan-Meier curve, the regdanvimab group showed a significantly better prognosis with a higher survival rate compared with that in the no regdanvimab group (log-rank p = 0.001) (Fig. 3).

**Factors associated with mortality in COVID-19–infected patients on hemodialysis**

We investigated the factors associated with mortality among COVID-19–infected patients on HD (Table 3). Univariate Cox regression analysis showed that older age (p < 0.001), arrhythmia (p = 0.01), malignancy (p = 0.001), SPO$_2$ <95% at admission (p < 0.001), and low serum albumin and creatinine (both p < 0.001) were significant risk factors, while use of renin-angiotensin-system blockers (p = 0.04) and administration of antibiotics and regdanvimab (p = 0.001 and p = 0.002, respectively) were associated with low mortality in these patients. Factors related to disease severity, such as high flow oxygen, MV, CRRT, and ICU care, were all significantly associated with high mortality (p < 0.001). Multivariate Cox regression analysis with the

<table>
<thead>
<tr>
<th>Table 2. Comparison of pharmacological and supportive treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>--------------------</td>
</tr>
<tr>
<td>Dexamethasone</td>
</tr>
<tr>
<td>Remdesivir</td>
</tr>
<tr>
<td>Antibiotics</td>
</tr>
<tr>
<td>Oxygen apply</td>
</tr>
<tr>
<td>High flow O$_2$</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
</tr>
<tr>
<td>CRRT</td>
</tr>
<tr>
<td>ICU care</td>
</tr>
</tbody>
</table>

Data are expressed as number (%).

CRRT, continuous renal replacement therapy; ICU, intensive care unit.
significant variables in the univariate analysis showed that malignancy (hazard ratio [HR], 3.39; 95% confidence interval [CI], 1.62–7.11; p = 0.001), SPO₂ <95% at admission (HR, 2.83; 95% CI, 1.43–5.59; p = 0.003), MV (HR, 2.85; 95% CI, 1.21–6.75; p = 0.02), ICU care (HR, 3.03; 95% CI, 1.31–7.03; p = 0.01) and administration of antibiotics and regdanvimab (HR, 0.41; 95% CI, 0.22–0.79; p = 0.007 and HR, 0.282; 95% CI, 0.128–0.624; p = 0.002, respectively) were important factors in patient outcomes. These data suggest that regdanvimab had a beneficial effect on mortality in COVID-19–infected patients undergoing HD.

In the clinical setting, regdanvimab is generally considered in patients with mild-to-moderate disease with low oxygen demand; thus, we conducted additional analyses in patients with SPO₂ greater than 95%. The total number of included patients was 206, 131 in the no regdanvimab group and 75 in the regdanvimab group. Herein, the regdanvimab group showed a higher survival rate compared with that in the no regdanvimab group (log-rank p = 0.002) (Fig. 4). Furthermore, the use of regdanvimab was significantly associated with low mortality even after adjustment for several associated factors (HR, 0.233; 95% CI, 0.105–0.517; p < 0.001) (Table 4).

**Discussion**

This study found an association between mortality and regdanvimab use in hospitalized COVID-19 patients undergoing HD. The patients who received regdanvimab alone or in combination during hospitalization achieved better mortality compared with that in patients who did not. In addition, we elucidated the risk factors related to mortality, and regdanvimab showed an association with better survival even after adjusting for factors showing significant differences between groups. These findings suggest that the use of regdanvimab in COVID-19 patients on HD has a significantly favorable impact on mortality.

Along with diabetes mellitus, hypertension, and cardiovascular disease, chronic kidney disease has been reported to be associated with the severity and mortality of COVID-19 [11–13]. Specifically, patients with ESRD are immunosuppressed and usually have other chronic systemic diseases related to clinical outcomes of COVID-19 [14,15]; therefore, they have particularly higher morbidity and mortality than those of the general population [16]. Center-based HD is the main renal therapeutic modality in many countries including Korea [17]. For HD patients, the possibility of infection is high due to the confinement in an indoor environment for several hours and frequent contact with medical staff members or other patients. Therefore, during the outbreak of the COVID-19 pandemic, effective strategies were required to prevent disease transmission and improve prognosis in HD patients infected with...
COVID-19.

The COVID-19 pandemic has seen clinical development and the use of antiviral treatment at an unprecedented speed. Several potential antiviral agents have been identified that have been useful to inhibit the clinical progression and complication of COVID-19 [18]. However, many clinical trials are still required to prove the efficacy and safety of these agents; furthermore, even if some regimens are effective, most of them have not been recommended for use in HD patients. Thus, unfortunately, the development of treatment protocols and the use of potentially beneficial treatment have been delayed in HD patients infected with COVID-19, and there is still limited data for effective treatment methods in these patients.

Regdanvimab (Regkirona) is a recombinant human monoclonal immunoglobulin G1 antibody that neutralizes SARS-CoV-2 by binding to the receptor binding domain of the virus’ spike protein and was effective at reducing viral

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>1.05 (1.02–1.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥50</td>
<td>1.05 (0.38–2.91)</td>
<td>0.92</td>
</tr>
<tr>
<td>≥60</td>
<td>2.26 (1.07–4.76)</td>
<td>0.03</td>
</tr>
<tr>
<td>≥70</td>
<td>2.89 (1.66–5.04)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.89 (0.53–1.51)</td>
<td>0.67</td>
</tr>
<tr>
<td>BMI</td>
<td>1.01 (0.92–1.11)</td>
<td>0.80</td>
</tr>
<tr>
<td>RASB</td>
<td>0.57 (0.33–0.98)</td>
<td>0.04</td>
</tr>
<tr>
<td>DM</td>
<td>0.74 (0.44–1.23)</td>
<td>0.25</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.82 (0.52–1.31)</td>
<td>0.41</td>
</tr>
<tr>
<td>CAOD</td>
<td>1.25 (0.66–2.36)</td>
<td>0.49</td>
</tr>
<tr>
<td>CHF</td>
<td>1.34 (0.42–4.27)</td>
<td>0.62</td>
</tr>
<tr>
<td>CVA</td>
<td>1.54 (0.78–3.04)</td>
<td>0.21</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>2.75 (1.25–6.06)</td>
<td>0.01</td>
</tr>
<tr>
<td>Malignancy</td>
<td>3.22 (1.63–6.36)</td>
<td>0.001</td>
</tr>
<tr>
<td>SPO2 at admission (&lt;95%)</td>
<td>3.63 (1.93–6.84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High flow O2</td>
<td>2.97 (1.74–5.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>10.35 (6.10–17.58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRRT</td>
<td>7.66 (4.39–13.36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ICU care</td>
<td>9.80 (5.55–17.29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WBC</td>
<td>1.04 (0.97–1.11)</td>
<td>0.28</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>1.05 (0.98–1.12)</td>
<td>0.16</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.88 (0.73–1.06)</td>
<td>0.17</td>
</tr>
<tr>
<td>BUN</td>
<td>1.00 (0.99–1.01)</td>
<td>0.499</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.86 (0.80–0.92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.43 (0.27–0.69)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>1.03 (0.96–1.07)</td>
<td>0.09</td>
</tr>
<tr>
<td>BNP</td>
<td>1.00 (1.00–1.00)</td>
<td>0.12</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>0.40 (0.24–0.69)</td>
<td>0.001</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>1.58 (0.94–2.65)</td>
<td>0.09</td>
</tr>
<tr>
<td>Remdesivir</td>
<td>1.58 (0.91–2.72)</td>
<td>0.10</td>
</tr>
<tr>
<td>Regdanvimab</td>
<td>0.35 (0.18–0.68)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

BMI, body mass index; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; CAOD, coronary artery obstructive disease; CHF, congestive heart failure; CI, confidence interval; CRRT, continuous renal replacement therapy; CVA, cerebrovascular accident; DM, diabetes mellitus; HR, hazard ratio; ICU, intensive care unit; RASB, renin-angiotensin system blockade; SPO2, saturation of partial pressure oxygen; WBC, white blood cell.
Survival probability

Figure 4. Kaplan-Meier curve for in-hospital mortality according to the administration of regdanvimab in patients with SPO₂ >95% (n = 206). SPO₂, saturation of partial pressure oxygen.

Table 4. Factors associated with mortality of study patients with SPO₂ >95%

<table>
<thead>
<tr>
<th>Variable</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</td>
<td>1.04 (1.02–1.07)</td>
<td>0.002</td>
<td>1.02 (0.99–1.06)</td>
<td>0.19</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.04 (0.58–1.87)</td>
<td>0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>1.05 (0.94–1.16)</td>
<td>0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RASB</td>
<td>0.51 (0.27–0.97)</td>
<td>0.04</td>
<td>0.78 (0.38–1.62)</td>
<td>0.50</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.63 (0.36–1.12)</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.50 (0.45–1.48)</td>
<td>0.495</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAOD</td>
<td>1.01 (0.45–2.26)</td>
<td>0.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHF</td>
<td>1.09 (0.91–1.09)</td>
<td>0.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVA</td>
<td>1.59 (0.74–3.41)</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>2.42 (0.95–6.13)</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignancy</td>
<td>2.85 (1.27–6.37)</td>
<td>0.011</td>
<td>2.00 (0.82–4.85)</td>
<td>0.13</td>
</tr>
<tr>
<td>High flow O₂</td>
<td>3.84 (2.12–6.96)</td>
<td>&lt;0.001</td>
<td>0.92 (0.43–1.97)</td>
<td>0.82</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>10.64 (5.83–19.40)</td>
<td>&lt;0.001</td>
<td>3.15 (1.18–8.37)</td>
<td>0.02</td>
</tr>
<tr>
<td>CRRT</td>
<td>9.03 (4.69–17.38)</td>
<td>&lt;0.001</td>
<td>1.49 (0.55–4.01)</td>
<td>0.43</td>
</tr>
<tr>
<td>ICU care</td>
<td>9.10 (4.89–16.92)</td>
<td>&lt;0.001</td>
<td>3.12 (1.31–7.41)</td>
<td>0.01</td>
</tr>
<tr>
<td>WBC</td>
<td>0.87 (0.91–1.11)</td>
<td>0.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophil</td>
<td>1.02 (0.92–1.13)</td>
<td>0.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.93 (0.75–1.16)</td>
<td>0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN</td>
<td>1.00 (0.99–1.01)</td>
<td>0.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.85 (0.78–0.93)</td>
<td>&lt;0.001</td>
<td>0.92 (0.82–1.02)</td>
<td>0.12</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.44 (0.25–0.75)</td>
<td>0.003</td>
<td>0.74 (0.40–1.38)</td>
<td>0.34</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>1.03 (0.98–1.07)</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BNP</td>
<td>1.00 (1.00–1.100)</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotics</td>
<td>0.40 (0.22–0.73)</td>
<td>0.003</td>
<td>0.65 (0.30–1.42)</td>
<td>0.28</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>1.24 (0.70–2.22)</td>
<td>0.46</td>
<td>1.02 (0.47–2.23)</td>
<td>0.96</td>
</tr>
<tr>
<td>Remdesivir</td>
<td>1.55 (0.84–2.83)</td>
<td>0.16</td>
<td>0.97 (0.42–2.27)</td>
<td>0.95</td>
</tr>
<tr>
<td>Regdanvimab</td>
<td>0.32 (0.15–0.69)</td>
<td>0.003</td>
<td>0.27 (0.10–0.75)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

BMI, body mass index; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; CAOD, coronary artery obstructive disease; CHF, congestive heart failure; CI, confidence interval; CRRT, continuous renal replacement therapy; CVA, cerebrovascular accident; HR, hazard ratio; ICU, intensive care unit; RASB, renin-angiotensin system blockade; SPO₂, saturation of partial pressure oxygen; WBC, white blood cell.
regdanvimab administration (alone or in combination) for
the treatment of HD patients infected by COVID-19 would
be beneficial in improving prognosis.

We further evaluated the factors associated with the mor-
tality of HD patients infected with COVID-19. Higher age
is the main determinant of increased risk of infection and
mortality caused by COVID-19 \[23,24\]. Similar to previous
results, we also found a significant association between
age and patient outcomes, with a 2.3-fold increase in mor-
tality in patients aged ≥60 years and a 2.9-fold increase in
mortality in patients aged ≥70 years. In addition, the indi-
cation for regdanimab is the presence of mild disease, age
≥50 years, and the presence of at least one risk factor; in
our study, significant survival benefit effects were shown
in patients aged ≥60 years. Furthermore, several studies
showed that preexisting comorbidities, such as hyperten-
sion, diabetes mellitus, dyslipidemia, chronic kidney dis-
 ease, chronic obstructive pulmonary disease, and history
of cardiac diseases, were associated with an increased risk
of COVID-19-related mortality \[25–29\]. This study showed
that arrhythmia and malignancy in the study patients were
significantly associated with increased mortality. Patients
with HD are often elderly and have multiple comorbidities
identified as risk factors for COVID-19-related mortality;
thus, rapid interventions with effective treatments are
important to improve the prognosis. Despite the viral ori-
gin, antibiotics are frequently prescribed to patients with
COVID-19. The rationale for antibiotic treatment in these
patients seems to be based on the experience with bacte-
rial superinfection in hospitalized patients. In our study,
the proportion of patients receiving antibiotics with other
treatment agents was 79.6% without a significant difference
between the two groups; the use of antibiotics was sig-
nificantly associated with low mortality. Previous studies
showed that the prevalence of bacterial co-infection and
secondary infection in patients with COVID-19 is relatively
low (3.5% and 14.3%, respectively) \[30\], and over-prescrib-
ing of antibiotics in these patients could result in increased
 antimicrobial resistance \[31,32\]. Therefore, further studies
are needed to improve the appropriateness of antibiotics
use in these patients.

This study has some limitations. First, being a retrospec-
tive observational study, the study design has inherent
biases such as selection and confounding biases, and un-
measured confounders might have affected the observed
results. As an example, residual renal function is related
not only to the prognosis of ESRD patients but also to the
pharmacodynamics and pharmacokinetics, so it could be
an important factor in a study related to drug effect. How-
ever, data on the residual renal function of study patients
were not initially investigated, so we could not analyze
by including the data on them. Second, information on
the safety of regdanvimab in ESRD patients is still lacking
because we could not investigate the occurrence of side
effects and adverse events in study patients due to incom-
plete medical records. Third, we could not study the impact
of regdanvimab on the disease progression as assessed by
the need for MV, transfer to the ICU, and receipt of extra-
corporeal membrane oxygenation during hospital admis-
sion. Further studies with larger samples and prospective
designs are required to consider regdanvimab as a safe and
potential agent for ESRD patients infected with COVID-19.
Finally, we enrolled patients before the Delta (the 4th
variant of concern) and Omicron variant (the 5th variant
of concern) became dominant. In South Korea, the Delta
variant was first identified in the local community in May
2021 and became predominant in October 2021. This study
was conducted with patients who were admitted to the
institution from 1 December 2020 to 30 November 2021.
Therefore, it is assumed that Delta and Omicron were not
the predominant variants during the study period. Since
the effectiveness of antiviral agents may differ depending
on the variant, further studies on the individual subtypes
of SARS-CoV-2 are required. Despite these limitations, the
current findings have important clinical implications; this
study is the first one evaluating the effect of regdanvimab
on COVID-19 infection in patients on HD who were, thus
far, limited from using this potentially beneficial treatment
due to the lack of data.

In conclusion, our results showed that regdanvimab ad-
mistration was beneficial in improving prognosis in hos-
pitalized COVID-19 patients on HD. Considering the vul-
nerability to infection and high mortality of ESRD patients,
regdanvimab may be considered as a therapeutic option in
COVID-19 patients on HD.

Conflicts of interest

All authors have no conflicts of interest to declare.
Funding

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We would like to thank the Korean Society of Nephrology COVID-19 Task Force Team and the patients treated at Good Samaritan Bagae Hospital.

Data sharing statement

The data presented in this study are available on request from the corresponding author.

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Conceptualization: YKK, HCP, YKL
Data curation: YKK, SJY, SY, JK, AC
Formal analysis: EK, JK, AC
Funding acquisition: HCP
Investigation, Resources: SJY, SY
Methodology, Validation: EK, DHK, AC
Project administration, Supervision: HCP, YKL
Software: JK
Visualization: YKK
Writing—original draft: YKK
Writing—review & editing: All authors
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References


A 47-year-old man had had a long-standing asymptomatic neck lump for years. He assumed it to be goiter and did not seek medical advice. He reported suffering from recurrent episodes of nephrolithiasis, which had been treated with shock wave lithotripsy and ureteroscopy. Serum blood calcium levels were not measured until a recent admission for ureteroscopy and revealed hypercalcemia.

On physical examination, a moveable nontender elastic nodule was palpable in the right neck. There were no enlarged cervical lymph nodes. Laboratory investigations identified severe hypercalcemia at 13.8 mg/dL (reference range, 8.9–10.3 mg/dL). This was associated with hypophosphatemia at 1.9 mg/dL (reference range, 2.7–4.5 mg/dL) and a markedly raised intact parathyroid hormone level of 1,286.04 pg/mL (reference range, 6.87–64.87 pg/mL). His kidney function was moderately impaired with an estimated glomerular filtration rate of 59.2 mL/min per 1.73 m². Thyroid function test results were within normal range.

Computed tomography revealed an elongated right neck mass with intrathoracic extension (Fig. 1). The mass led to modest tracheal deviation, but there was no evidence of invasion into surrounding tissues. Tc-99m sestamibi scintigraphy showed persistent residual uptake in the right neck lesion. Dual-energy X-ray absorptiometry demonstrated low bone mineral density across all sites. The T-score was −3.4 and the Z-score was −3.4 at the lumbar spine.

On physical examination, a moveable nontender elastic nodule was palpable in the right neck. There were no enlarged cervical lymph nodes. Laboratory investigations identified severe hypercalcemia at 13.8 mg/dL (reference range, 8.9–10.3 mg/dL). This was associated with hypophosphatemia at 1.9 mg/dL (reference range, 2.7–4.5 mg/dL) and a markedly raised intact parathyroid hormone level of 1,286.04 pg/mL (reference range, 6.87–64.87 pg/mL). His kidney function was moderately impaired with an estimated glomerular filtration rate of 59.2 mL/min per 1.73 m². Thyroid function test results were within normal range.

Histopathology indicated a parathyroid chief cell adenoma, measuring 11.4 × 6.0 × 3.3 cm in size and 59 g in weight, without features of atypia or malignancy. At a 5-month follow-up visit, the patient remained normocalcemic and did not experience any symptoms of kidney stone recurrence.

The possibility of parathyroid origin, albeit rare, should be considered in patients with nephrolithiasis and the presence of a neck lump. Parathyroid adenomas are usually small and unpalpable. The term large or giant adenoma has been applied to describe oversized parathyroid tumors weighing more than 3.5 g. Giant parathyroid adenomas appear to be a distinct clinical entity with greater functionality and lower frequencies of multiglandular disease. Differ
Figure 1. Computed tomography (A) and Tc-99m sestamibi scintigraphy (B) of the right neck tumor with intrathoracic extension. Upon contrast enhancement, the mass was relatively hypodense compared to the thyroid parenchyma. Persistent, heterogeneous sestamibi uptake was evident during delayed imaging in scintigraphy.

Figure 2. Characterization of giant parathyroid adenoma: ultrasound, gross examination, and histology. (A) Neck ultrasound shows a hypoechoic complex nodule with solid and cystic components (arrow) situated behind the right thyroid. (B) Gross examination of the surgical specimen revealed a well-circumscribed lobular mass measuring 11.4 cm in length. (C) Histological examination of the giant parathyroid adenoma disclosed that the lesion consists of chief cells intermingled with dilated vessels (hematoxylin and eosin stain, 40×). (D) There were no signs of atypia or malignancy (hematoxylin and eosin stain, 200×). T, trachea.
Differentiation from parathyroid carcinoma can be challenging.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Acknowledgments**

This report was approved by the Institutional Review Board of MacKay Memorial Hospital (No. 23MMHIS278e).

**Data sharing statement**

The data presented in this study are available upon reasonable request from the corresponding author.

**Authors’ contributions**

Conceptualization, Data curation: All authors
Writing–original draft: YH, SPC
Writing–review & editing: MCT, SHD
All authors read and approved the final manuscript.

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Successful diagnosis and treatment of recurrent atypical hemolytic uremic syndrome posttransplantation caused by the heterozygous deletion of CFH in a patient with end-stage kidney disease of uncertain etiology

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Atypical hemolytic uremic syndrome (aHUS) is a rare disease caused by the dysregulation of the alternative pathway of the complement system, leading to microvascular damage. It is a type of thrombotic microangiopathy (TMA) characterized by non-immune hemolytic anemia, thrombocytopenia, and renal impairment. Half of the patients with aHUS develop end-stage kidney disease (ESKD) [1]. Genetic testing is crucial for the diagnosis of aHUS, as variants in complement regulatory protein gene significantly increase disease risk. Advances in genetic testing and its widespread use have revealed cases of aHUS recurring after kidney transplantation (KT) [2]. Herein, we present a case of recurrent aHUS after deceased-donor KT (DDKT) in a patient with ESKD of uncertain etiology. This study was approved by the Institutional Review Board of The Catholic University of Korea, Seoul St. Mary’s Hospital (No. KC22ZISI0823).

A 45-year-old man with ESKD of uncertain etiology pre-
sented with allograft dysfunction 1 month after DDKT. The patient was on triple immunosuppressive therapy, including tacrolimus (trough level, 11.5 ng/mL). On admission, serum creatinine level was 2.7 mg/dL (baseline level, 1.4 mg/dL). Laboratory tests revealed Coombs-negative hemolytic anemia and thrombocytopenia with decreased complement levels. Schistocytes on a peripheral blood smear test enabled a presumptive diagnosis of TMA. However, allograft biopsy (Fig. 1B–D) revealed focally proliferative glomerulonephritis without pathological findings of TMA on light microscopy and bright glomerular C3 staining on immunofluorescence microscopy. Meanwhile, electron microscopy revealed segmentally thickened capillary basement membranes with subendothelial widening, indicating possibly early TMA. A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) activity was 63.6%, and the Shiga toxin test result was negative. Despite seven sessions of plasma exchange, the patient’s allograft function did not recover, necessitating subsequent hemodialysis (Fig. 1A).

**Figure 1. Patient’s clinical timeline and biopsy results.** (A) Timeline of the patient’s clinical course and laboratory findings. (B) Light microscopy on allograft kidney (H&E, ×400). Focally proliferative glomerulonephritis with many neutrophils in capillary loops (arrow). (C) Positive immunofluorescence for C3 showing a diffuse, fine granular pattern of the glomerular basement membrane in allograft kidney. (D) Electron microscopy on allograft kidney. Segmentally thickened capillary basement membranes with endothelial damage and subendothelial widening (arrows). (E) Light microscopy on native kidney (trichrome, ×400). Occluded arteriolar lumen with thrombosis and intimal edema (arrows).

CMV, cytomegalovirus; HD, hemodialysis; HPF, high-power field; KT, kidney transplantation; LDH, lactate dehydrogenase; PLT, platelet; SCr, serum creatinine.
Figure 2. Results of the genetic evaluation. (A) The clinical exome sequencing result viewed in the Integrative Genome Viewer. A novel heterozygous missense variant, NM_000204.4: c.119A>C, p.His40Pro, is observed in CFI. (B) Results of the direct Sanger sequencing for the patient and his parents. The father is confirmed to carry the same CFI variant. (C) Multiplex ligation-dependent probe amplification shows a heterozygous deletion of exon 22 and its downstream in CFH.
In differentially diagnosing primary aHUS, we performed clinical exome sequencing and discovered a novel heterozygous missense variant (NM_000204.4: c.119A>C, p.His40Pro) of CFI gene (Fig. 2A). Subsequently, the variant was confirmed to originate from the father by direct sequencing of the trio samples (Fig. 2B) and was classified as a variant of uncertain significance (VUS), according to the guideline [3]. Additionally, we employed multiplex ligation-dependent probe amplification (MLPA) to examine copy number variations in CFH and CFH-related genes, and found a pathogenic heterozygous deletion in exon 22 and its downstream region in CFH (Fig. 2C). Furthermore, we found arteriolar thrombotic occlusion upon review of his native kidney biopsy (Fig. 1E). Finally, aHUS was identified as the cause of ESKD and allograft dysfunction. The patient underwent hemodialysis for 4 weeks before completing a 4-week induction with eculizumab (1,200 mg/week). After induction, the patient successfully terminated hemodialysis and, with continued eculizumab therapy (900 mg every 2 weeks), hemolysis resolved and allograft function steadily improved (Fig. 1A).

Recent registry data [4] have shown that up to 20% of KT recipients have ESKD of uncertain etiology. Groopman et al. [5] conducted whole-exome sequencing for 3,315 patients with chronic kidney disease and identified diagnostic variants in 307 patients (9.3%). Ten variants were associated with TMA; however, none were clinically diagnosed with TMA before genetic evaluation. This suggests that aHUS may be underdiagnosed or misdiagnosed in patients with ESKD.

The main pathological features of TMA are arteriolar and capillary thrombosis with characteristic abnormalities in the endothelium and vessel wall [1]. However, unlike typical hemolytic uremic syndrome and thrombotic thrombocytopenic purpura, aHUS biopsies rarely show thrombi [6]. Herein, no prominent vascular thrombosis was observed under light microscopy; however, electron microscopy revealed endothelial damage, indicating early-stage disease and the potential for allograft salvage.

We identified a novel missense CFI variant, c.119A>C. However, the patient’s clinical course was more severe than that expected for a CFI VUS carrier. Techniques such as MLPA allow the identification of CFH/CFHR-related 1 (CFH/CFHR1) hybrid alleles, which are undetectable by sequencing and present in approximately 3%-5% of all aHUS cases [7]. Using MLPA, we discovered a partial deletion of CFH, predisposing the patient to aHUS. However, the locations of other CFHR deletions could not be identified; therefore, it was difficult to determine which CFH/CFHR hybrid was generated, which requires further research.

Eculizumab, a humanized monoclonal anti-C5 antibody, is a well-documented therapeutic agent for aHUS. Eculizumab prophylaxis significantly reduces recurrence rates and its timely use improves allograft survival in patients with recurrent aHUS [8]. However, the optimal duration for its maintenance remains inconclusive [8]. As the prognosis of aHUS is highly dependent on genetic variants, individualized risk stratification based on the genetic background may be necessary to determine appropriate treatment durations. As our patient harbors a CFH variant belonging to the high-risk variant [8], treatment discontinuation should be approached with utmost caution.

In summary, we report a case of aHUS that was overlooked in a patient carrying a heterozygous deletion of CFH until recurrence occurred post-DDKT. This report emphasizes the importance of primary kidney disease evaluation and careful monitoring for TMA, especially in patients with ESKD of uncertain etiology. Genetic testing is crucial for aHUS diagnosis, regardless of typical TMA features in pathology. Prompt initiation of eculizumab can prevent allograft failure.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

The data presented in this study are available upon reasonable request from the corresponding author.

Authors’ contributions

Conceptualization: Haeun Lee, HSK, Hanbi Lee
Data curation: Haeun Lee, HSK, SHE, IOS, JS
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References

Correction to “Obesity-related hypertension and chronic kidney disease: from evaluation to management”

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There is a correction to the Funding section of the above-mentioned article, as follows:

Funding

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The original version has been rectified. We would like to apologize for any inconvenience caused.
INSTRUCTIONS FOR AUTHORS

1. Manuscript Submission

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Important information

Articles should be prepared in the simplest form and submitted in the format of Microsoft Word (*.doc or *.docx). Manuscripts must be typed in English and double-spaced. All pages must be numbered consecutively starting from the title page. You may use automatic page numbering, but do NOT use other kinds of automatic formatting such as footnotes. Place text, references, tables and legends in one file with each table on a new page.

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.

(2) A conflict of interest disclosure statement (see relevant section 4.2 below).

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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).

(7) Clinical trials should be registered at a primary national clinical trial registration site such as www.clinicaltrials.gov, https://cris.nih.go.kr/cris/index.jsp, or other sites accredited by the World Health Organization or the International Committee of Medical Journal Editors.

(8) Where material has been reproduced from other copyrighted sources, letter(s) of permission from the copyright holder(s) to use the copyrighted sources must be supplied.

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(10) All authors must register and update information about academic degree, affiliation, and position when they register or submit a journal online at https://www.editorialmanager.com/krcp.

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2. Types of Articles

2.1. Original Articles

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.

2.2. Review Articles
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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Articles in this section should provide insightful analysis and commentary about any important topic in medicine, research, ethics, or health policy. They may also address consensus statements, guidelines, statements from task forces, or recommendations. Most reviews are solicited by the editors, but unsolicited submissions may also be considered for publication. The text should be limited to 5,000 words (excluding tables, figures and references) and 50 references.

2.4. Correspondence
Correspondence generally takes one of the following forms: (1) Reader’s comment on an article previously published in KRCP and/or a reply from the authors; (2) An article that may not fit to the format of original or review article but suggest creative perspectives for medical issues; (3) A brief report of any kind that presents important research findings adequate for the journal’s scope and of particular interest to the readers. The submitted manuscript includes title page, main text, conflict of interest, acknowledgments (if applicable) and references. No abstract is included, and the text should be limited to 800 words (excluding tables, figures and references) and 8 references. A maximum of 2 figures or tables may be included.

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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.

2.6. Images in Practice
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.

3. Manuscript Preparation

3.1. Title Page
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
and the work should be stated here clearly and explicitly.

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://www.ncbi.nlm.nih.gov/books/NBK7256/). 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.

Journal articles:

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Entire Book:

Book chapter:

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3.6. Tables
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.

3.7. Figures
Figure legends should be submitted for all figures. They should be brief and specific, and placed on a separate sheet after the References section. Figures are numbered consecutively using Arabic numerals in the order of their citation in the text. Figures should be uploaded as separate files, not embedded in the manuscript file. Figures that are line drawing or photographs must be submitted separately in high-resolution EPS or TIF format (or alternatively in high-resolution JPEG format). Only high-resolution figure files (preferably 300 dpi for color figures and 1,200 dpi for line art and graphs) should be submitted. The files are to be named according to the figure number and format (e.g., Fig1.tif). Figures that are reproduced from other published sources require written permission from the authors and copyright holders.

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Authors can submit supplementary digital contents to supplement the information provided in the print version of the manuscript. Supplementary materials will be published online-only. When uploading supplementary files through the online system, please use the “supplemental” file designation. Supplementary materials must be cited consecutively in the main body of the submitted manuscript and include the type of material submitted (e.g., “Supplementary Table 1”; “Supplementary Fig. 1”).
3.9. Certificate of English editing
All submitted manuscripts should be written in clear, correct English. Non-native English-speaking authors are required to attach an English language editing certificate when submitting their manuscript in order to undergo further review. For authors who use English as their native language, please upload an empty file with the filename “Certificate of English Editing (empty).”

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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/policies-post/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.

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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.

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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.

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Keep in mind the following:
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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

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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.

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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 papers as follows:

- Original Article, Review Article, Special Article, and Study Protocol: KRW 1,000,000 (Korea) / USD 1,000 (rest of world)
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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.
Slow ADPKD. Preserve Hope.
Introducing Samsca — The first and only treatment proven to slow cyst progression

Samsca® Tablet ADPKD product information summary

[INDICATION] To slow the progression of cyst development and renal insufficiency of autosomal dominant polycystic kidney disease (ADPKD) in adults with CKD stage 1 ~ 4 at initiation of treatment with evidence of rapidly progressing disease.

[DOSAGE & ADMINISTRATION] Tolvaptan must only be prescribed by physicians who have registered in Risk Management Program to the patients who have agreed and signed on conditions specified in Risk Management Program. Patient should follow the program. And, to mitigate the risk of significant and/or irreversible liver injury, blood testing for hepatic transaminases and bilirubin is required prior to initiation of SAMSCA, continuing monthly for 18 months and at regular 3 monthly intervals thereafter. The initial dose is 60 mg tolvaptan per day as a split-dose regimen of 45 mg + 15 mg (45 mg taken upon waking and prior the morning meal and 15 mg taken 8 hours later). The initial dose is to be titrated upward to a split-dose regimen of 90 mg tolvaptan (60 mg + 30 mg) per day and then to a target split-dose regimen of 120 mg tolvaptan (90 mg + 30 mg) per day, if tolerated, with at least weekly intervals between titrations. Dose titration has to be performed cautiously to ensure that high doses are not poorly tolerated through overly rapid up-titration. Patients may down-titrate to lower doses based on tolerability. Patients have to be maintained on the highest tolerable tolvaptan dose.

Samsca® Tablet has an indication for hyponatremia as well. For further information, please refer to the latest prescribing information at www.otsuka.co.kr.
A Better Choice of Hyperphosphatemia Treatment


Nephoxil® Capsule 500mg (Ferric citrate hydrate) product information summary

**[DOSE FORMS AND STRENGTHS]** Capsule: Ferric citrate hydrate 500mg (equivalent to 105mg ferric iron)

**[INDICATION]** For the control of hyperphosphatemia in adult patients with chronic kidney disease undergoing hemodialysis.

**[DOSE AND ADMINISTRATION]** The recommended starting dose of Nephoxil is 4 g/day with a maximum dose of 6 g/day and should be taken three times daily with meals or immediately after meals. During the treatment, the dose should be adjusted based on the concentration of serum phosphorus. 1 g (2 capsules) daily per increment or decrement, until serum phosphorus concentration reaches the target range, and afterwards regular monitoring should be maintained and dose adjustments should be made at intervals of one week or more. **[WARNING]** Accidental overdose of iron-containing products in children under six years of age may lead to fatal poisoning. This drug should be stored in a place not accessible to children. In case of accidental overdose, please contact a doctor or medical organization immediately. **[CONTRAINDICATION]** 1) Patients with hypophosphatemia 2) Patients who are allergic to ferric citrate 3) Patients with abnormal iron metabolism or symptoms of excessive iron e.g. hemochromatosis. For further information, please refer to the latest prescribing information at [https://nedrug.mfds.go.kr](https://nedrug.mfds.go.kr)
파사톨주 (PACITOL Injection)

[제품명] 파사톨주 (PACITOL Injection) [분류번호] 311(비타민 A 및 D제) [성상] 무색 투명한 바이알에 든 무색 투명한 액상 주사제 [원료약품 및 분량] 1 mL 중, 유효성분(주성분) : 파리칼시톨(USP) 5 μg 기타 첨가제 : 에탄올, 프로필렌글리콜, 주사용수 [효능·효과] 만성신부전과 관련된 이차적 부갑상샘기능항진증의 치료 및 예방 [용법·용량] 이 약의 적절한 용량은 각 환자에 따라 주의 깊게 결정되어야 한다. 만성신부전 환자에서 현재 인정되는 완전한 부갑상샘호르몬(intact PTH) 수치의 목표 범위는 요독증이 없는 정상치 상한의 1.5~3 배보다 높지 않다. 이 약의 권장 초기 용량은 2일 1회 또는 이보다 반반하지 않은 병도로 투석 시 0.04~0.1 μg/kg(2.8~7 μg)을 일시 주사한다.(상세 내용은 제품 설명서 참조) [포장정보] 5바이알/상자[1밀리리터/바이알x5] [사용기간] 제조일로부터 24 개월

Ref.) 제품 허가사항. 식약처 의약품안전나라. accessed on 2022.06.20

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References
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In Asia Pacific, we draw on our decades of experience and expertise to deliver our vision – **Creating a future worth living. For patients. Worldwide. Every day.**
판매자

시판 후 안전성 조사결과, 총 1,182에 경구투여시 151명(12.8%)에서 159건의 이상반응이 보고되었다. 이 중 가장 많이 보고된 이상반응은 변비(109건, 9.2%), 식욕부진(18건, 1.5%), 구역(16건, 1.4%), 저칼륨혈증 (13건, 1.1%) 등이었다. 3. 적용상의 주의 1) 이 약의 소르비톨 현탁액 경구투여시 결장협착, 결장궤양 등이 보고되었다. 2) 이 약의 유사 약물(폴리스티렌설폰산나트륨)의 소르비톨 현탁액 경구투여시 소장내 천공, 장점막 괴사, 소장종양과 결장괴사 등이 보고되었다. 3) 이 약 경구투여시 소화관에 잔류되지 않도록 충분히 제거하여야 한다. 특히 정상적인 배설이 곤란한 환자인 경우 다른 적절한 방법을 이용하여 장관에서 배설시킨다. 

유통자


calci...
Patients with aHUS can be at continuous risk of the life-threatening consequences of unpredictable complement-mediated TMA\(^1,2\)

Chronic, uncontrolled complement activity in aHUS leads to ongoing endothelial injury, organ damage, and sudden death\(^2,3\)

Homechoice Claria enabled by Sharesource
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