HIGHLIGHTS

- Trends in clinical outcomes of older hemodialysis patients: data from the 2023 Korean Renal Data System (KORDS)
- Urinary podocyte markers in diabetic kidney disease
- School urinary screening program in Japan: history, outcomes, perspectives
- Genome-wide association study and fine-mapping on Korean biobank to discover renal trait-associated variants
- High water intake induces primary cilium elongation in renal tubular cells
Aims and Scope

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

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

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This journal was supported by the Korean Federation of Science and Technology Societies Grant funded by the Korean Government (Ministry of Education).

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Publisher The Korean Society of Nephrology
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Published on May 31, 2024

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The image on the front cover: Kong et al studied the length of the primary cilia on renal tubular cells after high water intake. The primary cilium length in proximal tubule, distal tubule and collecting duct was elongated after high water intake. Please see the text for more details (pp. 313-325).
Chronic kidney disease (CKD) poses a significant global health burden, affecting hundreds of millions of people worldwide. The current nephrology society consensus has announced that CKD is under-recognized and further global awareness and resources are necessary to solve the critical socioeconomic burden of kidney disease [1]. Its multifactorial nature, with roots in both environmental exposures and genetic predispositions, complicates its management and necessitates a deeper understanding of its etiology. Historically, the genetic exploration of CKD has predominantly focused on populations of European descent, with seminal works such as those by Köttgen et al. [2] and Wuttke et al. [3] identifying multiple genetic loci associated with renal function indicators and CKD. Furthermore, the knowledge of genetic architecture of kidney function expanded from the recent multiethnic meta-analysis of genome-wide association studies (GWAS) and epi-GWAS [4,5]. Those studies, mainly led by the CKDGen Consortium [6], have been instrumental in unraveling the genetic landscape of CKD.

However, the genetic structure of CKD exhibits considerable variation across different populations, underscoring the necessity for research within diverse ethnic groups. The study by Lee et al. [7] addresses this gap by analyzing over five million SNPs from 58,406 participants, employing sophisticated meta-GWAS and fine-mapping techniques. The article leverages the extensive data from the Korean Genome and Epidemiology Study (KoGES), to illuminate the association between SNPs and estimated glomerular filtration rate (eGFR), a vital measure of kidney function. This study not only enhances our understanding of the genetic factors influencing CKD in Koreans but also serves as a critical step toward personalizing medical interventions based on genetic predispositions.

The identification of 1,360 loci associated with eGFR at a genome-wide significant level, with 399 loci validated with additional biomarkers, significantly contributes to our comprehension of the intricate genetic framework governing kidney function. This achievement is particularly notable given the unique genetic background of the Korean population, which may harbor specific genetic variants influencing CKD susceptibility and progression which may be useful in our society.

The relevance of Lee et al.’s findings [7] is further magnified when considering the implications of these genetic insights for clinical practice and public health. Understanding the genetic determinants of CKD can facilitate the development of predictive models for disease risk, en-
abling early intervention strategies and tailored treatment plans. The genetic variants identified from the study may be used to construct polygenic risk scores, a useful method to quantify one’s genetic predisposition towards a disease in precision medicine [8].

Moreover, the identification of novel single nucleotide polymorphisms (SNPs) and their associated pathways offers new targets for therapeutic intervention, potentially leading to innovative treatments for CKD. The study’s integration of functional mapping and annotation tools, such as MAGMA (Multi-marker Analysis of GenoMic Annotation) gene analysis and FUMA (Functional Mapping and Annotation of Genome-Wide Association Studies), underscores the significance of genetic pathways and tissues in kidney function. The concordance with the work conducted by the GTEx Consortium [8], which detailed genetic regulatory effects across human tissues, and Pattaro et al. [9], who identified genetic associations highlighting relevant cell types and biological pathways, reinforces the knowledge that kidney function and disease are influenced by a complex interplay of genetic factors. Together with the previous literatures, the GWAS in the Korean population may facilitate future studies to validate the identified genetic loci in relation to their potential diagnostic or therapeutic values. The identified 20 putative genes may be prioritized in future mechanistic researches in the Korean population, additionally expanding our knowledge of pathways related to kidney function.

Lee et al. [7] also highlights the importance of inclusivity in genetic research. The genetic determinants of disease can vary significantly between populations, and studies focusing exclusively on certain ethnic groups may not fully capture the global genetic diversity associated with health and disease. By broadening the scope of genetic studies to include underrepresented populations, researchers can develop a more comprehensive understanding of the genetic basis of diseases like CKD. Moreover, the Korean population-specific findings may be used in future analytic researches (i.e. Mendelian randomization) in Koreans contributing to the expansion of knowledge for the causal pathways of CKD in the nation [10].

On the other hand, this study has a common limitation of the current GWAS literature analyzing kidney function traits; kidney function is determined by surrogate markers (e.g. creatinine or cystatin C-based parameters). In clinical practice, eGFR has remaining limitations as creatinine or even cystatin C levels are affected by non-kidney factors. Even considering that eGFR is the most commonly used clinical marker, there’s a lingering limitation that GWAS analyses targeting such markers may miss genetic variant information related to actual kidney function or include variants related to pleiotropic phenotypes. To overcome this, this study and others have conducted analyses on secondary markers like blood urea nitrogen. However, it’s necessary to remember that exploration into the genetic architecture of the measured but not eGFR needs additional investigation in the future.

In conclusion, the study by Lee et al. [7] is an important contribution to the genetic study of CKD in a Korean population. It exemplifies the critical need for incorporating diverse populations into genetic research to uncover the intricate tapestry of genetic factors influencing health and disease. The integration of the current findings with global genetic studies of CKD will be crucial for developing a holistic understanding of CKD’s genetic architecture and associated pathways. Such comprehensive insights hold the key to unlocking new frontiers in the prevention, diagnosis, and treatment of CKD, heralding an era of precision nephrology that benefits populations worldwide.

Conflicts of interest

The author has no conflicts of interest to declare.

Data sharing statement

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

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Does the primary cilium elongation play a role in urine concentration?

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See Article on Pages 313–325

Nearly every mammalian cell contains the primary cilium, a solitary, immotile cellular organelle, that operates as a mechanosensor and chemosensor [1]. Primary cilia are microtubule-based, hair-like organelles, projecting from the mammalian cells [2]. In the kidney, primary cilia are found largely in the epithelial cells of the parietal layer of Bowman’s capsule and throughout the renal tubular segments, including proximal and distal tubules and collecting ducts [2]. These primary cilia detect and convert extracellular signals from the tubular lumen into the cells, thereby activating intracellular signaling pathways. For example, Madin-Darby canine kidney cells with chloral hydrate-induced loss of primary cilia failed to exhibit fluid flow-induced intracellular Ca\textsuperscript{2+} increase [1]. Moreover, the appropriate functioning of primary cilia is essential for epithelial cell proliferation, differentiation, and kidney organogenesis [3]. Ciliopathies are a group of disorders caused by alterations in the structure and function of cilia [3]. Mutations in polycystin 1 (Pkd1), Pkd2, or polycystic kidney and hepatic disease 1 (Pkhd1) cause primary cilium dysfunction in the kidney, resulting in polycystic kidney disease, which leads to renal functional decline and end-stage renal disease [4]. However, how loss of cilia or cilia dysfunction leads to disease development remains elusive.

Interestingly, the length of the primary cilia in renal tubular cells was found to be dynamically altered under diverse conditions. A study done by Kong et al. [5] in this issue postulated that the alteration in primary cilium length in tubular epithelial cells is an important process for urine concentration. This may provide new insights into the relationship between urine concentration and cilia length alteration in renal tubular cells. Microtubules, the central elements of the primary cilium, undergo the processes of assembly and disassembly, which are linked to the lengthening and shortening of the primary cilium. Post-translational modifications regulate these processes. α-tubulin acetyltransferase 1 (αTAT1) adds acetyl groups to α-tubulins to make primary cilia longer, while histone deacetylase 6 (HDAC6) takes acetyl groups away from α-tubulins to make them shorter. Kong et al. [5] reported that high water intake (HWI) in mice induces elongation of the primary cilium in renal tubular cells through extracellular signal-regulated kinases 1 and 2 (ERK1/2) activation (phosphorylation at Thr\textsuperscript{202} and Tyr\textsuperscript{204}) and increases in αTAT1 and Exoc5 (exocyst complex components 5) expressions. The expression and activity of HDAC6 were not affected by HWI. In contrast, ERK inhibition through inhibition of mitogen-activated kinase kinase (MEK) with an inhibitor (U0126) in mice blocks these HWI-induced changes, including α-tubulin acetylation and...
elongation of the primary cilium. Moreover, MEK inhibitor (U0126) treatment in mice inhibited the HWI-induced downregulation of aquaporin-2 (AQP2) and the kidney’s ability to produce diluted urine in response to HWI. Based on the results, the authors concluded that elongation of the primary cilium length in tubular epithelial cells via ERK1/2 activation is a required response to produce diluted urine under HWI conditions.

However, there are several complexities in the interpretation of the results. Previous studies have suggested that mitogen-activated protein kinases (MAPKs) are involved as a downstream signaling pathway of the vasopressin V2 receptor (V2R) and play a role in the regulation of AQP2 [6–8]. MAPKs, which are serine/threonine (Thr) kinases, convert extracellular stimuli into various cellular responses, including gene expression, metabolism, mitosis, and apoptosis. So far, five different groups of MAPKs have been characterized: ERK1/2; c-Jun amino-terminal kinases (JNKs) 1, 2, and 3; p38 isoforms α, β, γ, and δ; ERK 3 and 4; and ERK5. Among them, ERK1/2, JNKs, and p38 kinases are the most extensively studied. Each group of MAPKs comprises a set of three sequentially acting kinases: a MAPK kinase (MAPKK) kinase (MAPKKK), a MAPKK, and a MAPK. MAPKKK activation induces the phosphorylation and activation of MAPKs [9], which in turn activate MAPK via phosphorylation on both Thr and tyrosine (Tyr) residues within the activation loop containing a conserved Thr-X-Tyr motif. MAPKs then phosphorylate their target substrate proteins on serine or threonine residues with proline at the +1 position [9].

Previous studies have demonstrated that vasopressin affects AQP2 phosphorylation at serine 261 (pS261-AQP2) through the regulation of MAPK activity. Nedvetsky et al. [6] demonstrated that the selective p38 MAPK inhibitor SB202190 decreases phosphorylation of p38 MAPK and pS261-AQP2 in primary cultured rat kidney inner medullary collecting duct (IMCD) cells. Pisitkun et al. [7] demonstrated that 1-deamino-8-D-arginine vasopressin (dDAVP), a selective V2R agonist, reduces ERK1/2 phosphorylation at Thr111 and Thr147 in IMCD cells. In contrast, Cheung et al. [8] revealed that vasopressin dephosphorylates pS261-AQP2, but significantly increases phosphorylation of ERK1/2 at Thr111 and Thr147 in LLC-PK1 cells stably expressing c-myc-tagged AQP2. These data indicated that the changes in MAPK expression after vasopressin stimulation and the roles of MAPKs in vasopressin signaling (i.e., the response to high or low water intake), including regulation of AQP2 and urine concentration, have not been clearly understood. A recent study demonstrated that tolvaptan, a V2R antagonist, increases ERK1/2 phosphorylation at Thr202 and Thr204 in mpkCCD cells, which was dependent on protein kinase A (PKA) [10].

Furthermore, the authors did not provide a detailed explanation of how changes in the length of the primary cilium in tubular epithelial cells affect the expression and subcellular localization of AQP2 in the kidney collecting duct principal cells. Specifically, it is unclear whether downregulation of AQP2 protein abundance and decreased AQP2 in the membrane fractions are the direct results of the elongation in primary cilium length in the collecting duct principal cells in response to HWI. A previous study, however, demonstrated that Gd³⁺ (inhibiting intracellular Ca²⁺ entry) and forskolin treatment (increasing intracellular cyclic adenosine monophosphate [cAMP] levels), respectively, increased cilia length in the immortalized kidney collecting duct line (IMCD), and both the embryonically derived kidney epithelia and primary bone mesenchymal cells also showed similar findings [11]. In contrast, activation of Ca²⁺ signaling by triptolide or thapsigargin, as well as inhibition of PKA signaling (Rp-cAMPS, KT-5270, H-89), resulted in a reduction in primary cilium length [11]. AQP2 protein abundance and apical targeting in the collecting duct principal cells are largely dependent on an increase in intracellular cAMP levels and PKA activation, both of which, however, cause the elongation of primary cilia in IMCD cells [11]. As a result, this study demonstrating that HWI lengthens primary cilia while decreasing AQP2 expression contradicts previous findings.

Table 1 in the Kong et al.’s study [5] shows that ERK inhibition with U0126 in mice prevented the HWI-induced increase in urine volume. However, it seems difficult to determine exactly the changes in body water and sodium balance since the daily sodium intake and changes in plasma sodium levels and body weight are missing. Moreover, mice treated with the MEK inhibitor U0126 showed a dramatic decrease in urine sodium concentration in response to HWI compared to normal water intake (NWI), despite unchanged urine output, presumably due to altered food intake and a perturbation in daily sodium balance. Although it is not accurate to predict the solute-free water clearance (\( C_{H,O} = V [1 - Uosm/Posm] \)), where \( V \) means urine volume, \( Uosm \) means urine osmolality, and \( Posm \) means
plasma osmolality) using the data presented in Table 1 [5], presumably the solute-free water clearance is different between NWI and HWI under the U0126 treatment, despite no difference in AQP2 expression levels. This issue needs to be clarified.

In addition, primary cilia have also been hypothesized to play a role in metabolic regulation, as they may be involved in detecting metabolic signals. Adipocyte development in adipose tissue is regulated by primary cilia through the transduction of the classical Hedgehog (HH) and Wnt signaling pathways. Moreover, noncanonical HH signaling controls glucose and energy metabolism in skeletal muscle and brown adipocytes [12]. The role of primary cilia in the metabolic processes of renal tubular epithelial cells remains unclear. In this study, mice were subjected to water containing 3% sucrose for a period of 2 days in order to induce HWI [5]. Metabolomics allows for the study of metabolic profiling of the kidney and urine [13]. Therefore, it is intriguing to investigate the alterations in the metabolism of the renal tubular epithelial cells, specifically in relation to the elongation of the primary cilia and the resulting modifications in signaling pathways. In summary, while this study is intriguing, it lacks mechanistic studies [5]. We should conduct more direct experiments to determine conclusively whether alterations in cilia length are associated with AQP2 and body water balance regulation.

Conflicts of interest

The author has no conflicts of interest to declare.

Funding

This work was supported by grants from the National Research Foundation of Korea (NRF) grant funded by the Korea Government (MIST) (2023R1A2C2005570).

Data sharing statement

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

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References

With an increasing aging population, the mean age of patients with end-stage kidney disease (ESKD) is globally increasing. However, the current clinical status of elderly patients undergoing hemodialysis (HD) is rarely reported in Korea. The current study analyzed the clinical features and trends of older patients undergoing HD from the Korean Renal Data System (KORDS) database. The patients were divided into three groups according to age: <65 years (the young group), n = 50,591 (35.9%); 65–74 years (the younger-old group), n = 37,525 (26.6%); and ≥75 years (the older-old group), n = 52,856 (37.5%). The proportion of older-old group undergoing HD significantly increased in incidence and decreased in prevalence from 2013 to 2022. The median levels of hemoglobin, serum creatinine, albumin, calcium, phosphorus, and intact parathyroid hormone significantly decreased in the older-old group. The proportions of arteriovenous fistula creation and left forearm placement showed decreased trends with age. Although the utilization of low surface area dialyzers increased with age, the dialysis adequacy, including urea reduction ratio and Kt/V was within acceptable range in the older-old group on HD. Over the past 20 years, the mortality rate in the older-old group has increased, with cardiovascular diseases decreasing and infectious diseases increasing. The incidence of elderly patients undergoing HD has increased over time, but the high mortality of the older-old group needs to be solved. Therefore, it is imperative to develop holistic strategies based on age and individual needs for patients with ESKD.

**Keywords:** Elderly, End-stage kidney disease, Geriatrics, Hemodialysis, Mortality

**Introduction**

As the global population of older individuals continues to grow, the prevalence and incidence of end-stage kidney disease (ESKD) among elderly patients are on the rise [1–4]. According to data from the United States Renal Data System (USRDS), patients aged ≥65 years occupied the highest incidence of ESKD. Other studies have shown that
the increase in ESKD incidence has been predominantly driven by patients aged ≥75 years [4]. In addition, the prevalence of ESKD is significantly higher in individuals aged 65 to 74 and those aged 75 years and older when compared to those aged <65 years [4]. According to the annual report of the Korean Renal Data System (KORDS), the number of patients aged ≥65 years exceeded 50% for the first time in 2018 and has been steadily increasing to date in Korea [2].

The elderly population with ESKD often contends with comorbidities, limited life expectancy, frailty, and reduced functional capacity, all of which significantly increase the burden of dialysis and present challenges to delivering optimal care [1,5]. Hence, a precise understanding of the current characteristics of elderly patients with ESKD is crucial for providing rationale for policy and planning of dialysis. However, previous studies focusing on dialysis prescriptions and clinical features among this demographic are limited. An extensive analysis comparing hemodialysis (HD) practices and clinical outcomes between elderly and young patients across 12 countries during the period of 2005–2007 was documented in the Dialysis Outcomes and Practice Patterns Study [6]. In Korea, the registry committee of the Korean Society of Nephrology (KSN) presented the clinical features of elderly patients aged ≥65 years receiving maintenance HD using data from 2015 KORDS [3]. Then, there has not been any updated information on the clinical characteristics of elderly patients undergoing HD.

The term “elderly” has traditionally encompassed individuals aged ≥65 years [7]. However, functional vulnerability is different by age even within the same category of “elderly” and this difference leads to an increase in mortality and complications with increased age [1]. Therefore, recent studies suggest that elderly patients were divided into a “younger-old” and an “older-old” group [8–12]. This study aims to explore the demographic traits, vascular access patterns, dialysis adequacy, and clinical outcomes of elderly patients with ESKD depending on age in Korea.

**Methods**

We retrospectively analyzed the data from patients undergoing HD using the KORDS data [13], a nationwide Korean ESKD patient registry updated on an annual basis, to investigate the demographic characteristics and clinical outcomes of elderly patients undergoing HD. A total of 140,972 patients from 2001 to 2022 were classified according to age into the following three groups: <65 years (the young group), n = 50,591 (35.9%); 65–74 years (the younger-old group), n = 37,525 (26.6%); and ≥75 years (the older-old group), n = 52,856 (37.5%). A flow diagram for patient selection is shown in Supplementary Fig. 1 (available online).

The trends in incidence and prevalence of elderly patients undergoing HD were analyzed from 2013 to 2022 due to the lack of information for the enrollment date in this study. Variables including demographic factors, laboratory data, vascular access, dialyzer surface area, dialysis adequacy, complications, and cause of death were obtained from the data collected in 2022. Blood samples were obtained in the fasting state. Body mass index (BMI, kg/m²) was divided into four groups according to the Asia-Pacific obesity classification: underweight, <18.5 kg/m²; normal, 18.5–22.9 kg/m²; overweight, 23.0–24.9 kg/m²; obese, ≥25.0 kg/m² [14]. The definitions of complications and the cause of death are followed by those in the KORDS data. Vascular diseases as complications included cerebrovascular accident, hypertension, and other vascular disease, and cardiac diseases included coronary artery disease, heart failure, pericardial effusion, and arrhythmia. On the other hand, vascular diseases as the cause of death included cerebrovascular accident, pulmonary embolism, gastrointestinal bleeding, gastrointestinal embolism, and other vascular diseases, and cardiac diseases included coronary artery disease, heart failure, pericardial effusion, and arrhythmia. The Kruskal-Wallis test was used to compare the differences between the three groups because data were not normally distributed, while the chi-square test was used for categorical variables.

Annual mortality rates and survival curves were analyzed for patients undergoing HD enrolled in KORDS from 2001 to 2022. Trends in mortality rates are presented for patients treated each year according to the number of patient-years at risk. Unadjusted survival rates were calculated using the Kaplan-Meier method, and absolute mortality rates are expressed per 1,000 person-years of follow-up. The log-rank test was used to compare the survival distributions across each group. All survival data were analyzed using R programming (version 4.2.1; R Foundation for Statistical Computing).
**Results**

**Trends in the incidence and prevalence of end-stage kidney disease according to age**

The age-related rates for the ESKD incidence were 40.2% for the young group, 26.0% for the younger-old group, and 33.8% for the older-old group in 2022. From 2013 to 2022, the proportion of older-old group in the incidence exhibited a gradual increase from 29.4% to 33.8%, but the proportion of the young group showed a decrease from 44.2% to 40.2% (p < 0.001) (Fig. 1A).

On the other hand, the prevalence showed a distinct aspect. The proportion of the older-old group in the prevalence decreased from 47.7% to 34.1%. Meanwhile, the prevalence in the young group increased from 29.3% to 39.3%, and that in the younger-old group exhibited a slight rise from 23.0% to 26.6% (p < 0.001) (Fig. 1B).

**Distribution of sex, cause of end-stage kidney disease, and body mass index according to age**

The prevalence of male patients undergoing HD displayed an age-related decline of 64.1% in the young group, 63.2% in the younger-old group, and 55.3% in the older-old group with a statistically significant difference (p < 0.001) (Fig. 1C). The major cause of ESKD were diabetes mellitus (DM), hypertension, and chronic glomerulonephritis. The proportion of patients with DM is higher in the older-old group (64.0%) and in the younger-old group (70.8%), compared with the young group (59.4%) (p < 0.001). Hypertension was most prevalent in the older-old group at 28.6%, followed by the young group at 23.6%, and the younger-old group at 20.6% (p < 0.001). In contrast, the proportion of patients with chronic glomerulonephritis is the lowest rate in the older-old group (7.4%), compared with the younger-old group (8.6%) and the young group (17.0%) (p < 0.001) (Fig. 1D). The percentage of underweight patients undergoing HD significantly higher in the older-old group (17.5%), compared to the younger-old group (12.7%) and the young group (20.0%). These results suggest that BMI in patients undergoing HD had lower trends over age (p < 0.001) (Fig. 1E).

**Figure 1.** Trends in incidence and prevalence, sex distribution, primary renal disease, and BMI according to age. (A) Incidence trends of end-stage kidney disease (ESKD) according to age. (B) Prevalence trends of ESKD according to age. (C) Sex distribution according to age. (D) Distribution of primary renal disease according to age. (E) Distribution of BMI according to age. BMI, body mass index; CGN, chronic glomerulonephritis; DM, diabetes mellitus; HTN, hypertension.
Distribution of the findings based on laboratory test according to age

The median value of various parameters, including hemoglobin, serum creatinine, albumin, calcium, phosphorus, and intact parathyroid hormone (PTH) levels exhibited a significant decrease over age (p < 0.001 for each parameter) (Fig. 2A). The percentage of patients with the lowest value among hemoglobin, serum creatinine, albumin, calcium, phosphorus, and intact PTH was significantly highest in older-old group, followed by the younger-old group and the young group (p < 0.001 for each parameter) (Fig. 2B).

Types of vascular access and dialysis adequacy according to age

In all age groups, the major type for vascular access in HD was the arteriovenous fistula (AVF), followed by arteriovenous graft (AVG), tunneled cuffed catheters, and temporary catheters (p < 0.001) (Fig. 3A). AVFs were predominantly located in the left forearm across all age groups, followed by the left upper arm, right forearm, and right upper arm (p < 0.001) (Fig. 3B). The proportion of patients using AVFs in the vascular access type decreased with increasing age (p < 0.001) (Fig. 3A). In contrast, the older-old group showed a significantly higher proportion of AVG (20.3%) compared to the younger-old (15.1%) and young (10.4%) groups (p < 0.001) (Fig. 3A). Furthermore, the highest proportion of tunneled catheter placements was observed in the older-old group (13.3%), compared to the younger-old group (8.6%) and the young group (9.8%) (p < 0.001) (Fig. 3A).

Dialyzers with a surface area of 1.5–2.0 m$^2$ were more frequently used in the young male group (53.3%), followed by the younger-old male group (49.8%) and the older-old group (42.4%). In contrast to the other male groups, the older-old male group showed a higher proportion of 1.0–1.5 m$^2$ dialyzers (52.8%) than 1.5–2.0 m$^2$ dialyzers (42.4%). Across all age groups, female used more low surface area dialyzers (1.0–1.5 m$^2$) compared to male (p < 0.001) (Fig. 3C). The urea reduction ratio (URR) and the single pooled Kt/V (spKt/V) exhibited an upward trajectory with advancing age (p < 0.001 for both), ultimately meeting the dialysis adequa-
Cy target in all the groups (Fig. 3D, E). Notably, female consistently showed higher URR and spKt/V levels compared to male across all age groups ($p < 0.001$) (Fig. 3D, E). On the other hand, the normalized protein catabolic rate (nPCR) showed a declining trend over age, with higher nPCR values in female compared to male ($p < 0.001$) (Fig. 3F).

**Complications and cause of death according to age**

Vascular diseases were the most prevalent complication across all age groups. However, their occurrence demonstrated a declining trend with age, with 57.3% in the young group, 53.2% in the younger-old group, and 49.0% in the older-old group, respectively ($p < 0.001$) (Fig. 4A). In con-

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**Figure 3.** Types and locations of vascular access, dialysis adequacy, and nPCR according to age and sex. (A) Distribution of types of vascular access according to age. (B) Location of arteriovenous fistula (AVF) according to age. (C) Distribution of dialyzer surface area according to age and sex. (D) Distribution of Kt/V according to age and sex. (E) Distribution of urea reduction ratio (URR) according to age and sex. (F) Distribution of nPCR according to age and sex. AVG, arteriovenous graft; nPCR, normalized protein catabolic rate.
cardiac diseases as complications showed an upward trend with increasing age, affecting 14.9% of the young group, 18.5% of the younger-old group, and 21.0% of the older-old group (p < 0.001). A similar tendency was observed for infectious diseases, with prevalence rates of 3.7%, 4.1%, and 5.7% in the young group, younger-old group, and older-old group, respectively (p < 0.001) (Fig. 4A).

While the incidence of cardiac diseases increased with age, the rates of death caused by cardiac diseases decreased, accounting for 36.9% in the young group, 31.9% in the younger-old group, and 29.2% in the older-old group (p = 0.002) (Fig. 4B). In contrast, the rates of death attributed to infectious diseases exhibited an increasing trend with age: 19.9% in the young group, 24.0% in the younger-old group, and 25.8% in the older-old group. The overall proportion of cause-specific death was significantly different among the three groups (p = 0.025) (Fig. 4B).

Trends in mortality rates stratified by sex and diabetic status

From 2001 to 2022, a consistent increase in mortality rates among patients undergoing HD was noted in the older-old group, surpassing other age groups starting in 2009. In contrast, the mortality rates in the younger-old group and the young group steadily decreased over the past two decades (Fig. 4C). Mortality rates of female patients undergoing HD were lower compared to their male counterparts across all age groups (Fig. 4D).

Mortality rates, defined as the number of deaths per 1,000 person-years, were consistently higher in patients with than in patients without DM across all age groups until 2017. However, the difference in mortality rates between patients with and without DM decreased over time, eventually becoming comparable in the younger-old group and the young group in 2022. Since 2017, the mortality rate of patients without DM was slightly higher than that of patients with DM in the older-old group (Fig. 4E).

Comparison of survival rates according to age stratified by sex and diabetic status

Over the past 20 years, the survival rate has consistently been the lowest in the older-old group, followed by the

Figure 4. Complications, cause of death, and trends in mortality rates according to age stratified by sex and diabetes. (A) Proportion of complications according to age. (B) Proportion of cause of death according to age. (C) Trends in mortality rates according to age. (D) Trends in mortality rates according to age stratified by sex. (E) Trends in mortality rates according to age stratified by diabetes. DM, diabetes mellitus.
younger-old group and the young group (p < 0.001) (Fig. 5A). Female patients undergoing HD consistently showed a superior survival rate compared to male across all age groups (p < 0.001 for all age groups) (Fig. 5B).

The survival rate was the highest in the young group without DM, followed by the younger-old group without DM. The older-old group with DM showed the lowest survival rate, followed by the younger-old group with DM. The young group with DM had a similar survival rate to the older-old group without DM. The groups without DM consistently showed a superior survival rate compared to the groups with DM across all age groups (p < 0.001 for all age groups) (Fig. 5C).

Discussion

This study investigated demographic characteristics, vascular access, dialysis adequacy, and clinical outcomes in elderly patients undergoing HD from recent KORDS data. The proportion of older-old group was increased in incidence, while was declined in prevalence over the past decade. In contrast, the young group has exhibited an opposite trend, with a decline in incidence and an increase in prevalence. DM is the most important cause of ESKD in both the older-old group and the younger-old group. Hemoglobin, serum creatinine, albumin, phosphorus, intact PTH, and nPCR levels were notably lower in the older-old group compared to other groups. Although AVF was the major vascular access type, the proportion of AVF gradually decreased with age. Despite the higher use of low surface area dialyzers, dialysis adequacy of the elderly patients undergoing HD was achieved within a target range. Mortality rates were significantly higher in the older-old group, with infection-related deaths increasing and cardiac-related deaths declining with age.

According to the 2022 USRDS report, ESKD has the highest incidence globally in patients aged 75 years and older, with a higher prevalence in both patients aged 75 years and older and those aged 65 to 74 years than in those younger than 65 years [4]. Recent studies from Europe and Japan also reported that the increase in ESKD prevalence was primarily owing to an increase in the number of patients over 70 years old [15,16]. In contrast, our study revealed a decreasing prevalence rate among patients in the older-old group, despite the high incidence rate observed in this age group. A possible explanation is that the elevated incidence of ESKD in the older-old group may be mitigated by a high mortality rate during early period after initiating dialysis. Currently, there are no previous studies presenting accurate early mortality rates for Korean elderly patients and comparing mortality between Korea and other countries. Therefore, additional research may be helpful to investigate the status and risk factors associated with early mortality following dialysis in elderly patients with ESKD.

The KORDS data showed a decrease in nutritional parameters, including BMI, hemoglobin, serum albumin, phosphorus, and nPCR, with increasing age. This trend is consistent with the USRDS 2022 Annual Report, which
demonstrated lower levels of nutritional parameters in the older-old group compared to the younger-old group and the young group [4]. The poor nutritional status among the elderly patients undergoing HD has been also observed in several studies [17–19] and is considered one of the critical risk factors contributing to mortality. Consequently, our results suggest the importance of comprehensive strategies for ameliorating malnutrition among Korean elderly patients undergoing HD.

To date, there have been some reports regarding the trends and outcomes of vascular access based on age group in Korea [20–23]. The recent KORDS data revealed comparable outcomes with significantly lower primary success and patency rates for AVF, increased proportions of AVG and tunneled cuffed catheters, and decreased use of AVF in elderly patients from previous studies. Kim et al. [23] found a diminished survival rate in patients with a central venous catheter in both patients aged 65 to 74 years and 75 years and older, while there was no noticeable decrease in patients younger than 65 years. In addition, the rate of conversion from central venous catheter to AVF declined with increasing age, reaching its lowest point in patients aged ≥75 years [23]. Trends in the type and location of vascular access in KORDS data seem to be in line with tailored strategies according to the life plan outlined in the 2019 KDOQI clinical practice guideline for vascular access.

This study also revealed that Korean elderly patients with ESKD achieved dialysis adequacy targets as outlined in clinical practice guidelines [24], despite the more frequent use of lower-surface-area dialyzers. Consistent with our study, the 2022 USRDS report showed that a higher percentage of elderly patients achieved the Kt/V target compared to younger patients, and the percentage of female achieving the dialysis adequacy target was greater than that of male [4]. Except for the USRDS annual analysis, detailed analysis on dialyzer type or dialysis adequacy in elderly patients is scarce. Although the guidelines from the KSN and European Renal Best Practice recommend high-flux dialysis membranes for adult patients [24,25], the actual use of dialyzer in elderly patients undergoing HD was different from the current guidelines. A recent study from the Japanese Society for Dialysis Therapy Renal Data Registry including elderly patients revealed that the use of a low-flux dialyzer was associated with a significantly higher all-cause mortality compared to that of a high-flux dialyzer after adjusting Kt/V [26]. The higher use of low-flux-area dialyzer may be one of the risk factors for increasing mortality in Korean elderly patients. Therefore, the use of high-flux dialyzer in elderly patients undergoing HD needs to be recommended in the real world.

Finally, the present study showed the older-old group displayed a constant increase in mortality rates since 2001. In contrast, the USRDS 2022 and European Renal Association–European Dialysis and Transplant Association registry indicated a decreasing trend in mortality rates for all age groups, with the most significant decline in patients aged ≥75 years [4,27]. In the present study, the increase in mortality may consequently lead to an increase in incidence but a decrease in prevalence in the older-old group. Although we cannot fully explain the differences in mortality between KORDS data and the registry data of other countries, high mortality rates among elderly patients undergoing HD may be associated with several factors, including low BMI, reduced serum albumin and phosphorus levels, as well as clinical conditions such as sarcopenia and frailty [28–33]. In particular, malnutrition and sarcopenia in elderly patients undergoing HD are associated with advanced age and low BMI, leading to an almost three-fold increase in mortality compared to those without these conditions [34]. Therefore, the assessment and management of malnutrition and sarcopenia should be emphasized to prevent the increase in mortality in Korean elderly patients with ESKD.

This study has some limitations. The KORDS data includes missing data because it is created through the voluntary participation of KSN members. This limitation can make it difficult to interpret some variables correctly. The KORDS data does not provide information on variables that can directly assess sarcopenia and malnutrition, which affect mortality in elderly patients. In addition, the KORDS data did not include the etiology of infectious diseases including coronavirus disease 2019 (COVID-19), which contributed to mortality during the COVID-19 pandemic. Future nationwide epidemiologic research needs to include additional information associated with age-specific risk factors in Korean elderly patients with ESKD.

In conclusion, the 2023 KORDS data reveal an increasing incidence coupled with declining prevalence within the older-old group. To mitigate the increasing mortality rate in the older-old group, it is crucial to prioritize a comprehen-
sive understanding of clinical features specific to this age group. Tailored strategies to provide optimal care to elderly patients with ESKD hold promise for improving survival rates, minimizing complications, and preserving their quality of life throughout dialysis treatment.

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Conflicts of interest

Tae Hyun Ban is the Deputy Editor of Kidney Research and Clinical Practice and was not involved in the review process of this article. All authors have no other conflicts of interest to declare.

Acknowledgments

The ESRD Registry Committee of the Korean Society of Nephrology thanks all the medical doctors and nurses of dialysis centers in Korea for participating in this registry.

Data sharing statement

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

Authors’ contributions

Conceptualization: SHA, YKK, THB, YAH
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Urinary podocyte markers in diabetic kidney disease

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Podocytes are involved in maintaining kidney function and are a major focus of research on diabetic kidney disease (DKD). Urinary biomarkers derived from podocyte fragments and molecules have been proposed for the diagnosis and monitoring of DKD. Various methods have been used to detect intact podocytes and podocyte-derived microvesicles in urine, including centrifugation, visualization, and molecular quantification. Quantification of podocyte-specific protein targets and messenger RNA levels can be performed by Western blotting or enzyme-linked immunosorbent assay and quantitative polymerase chain reaction, respectively. At present, many of these techniques are expensive and labor-intensive, all limiting their widespread use in routine clinical tests. While the potential of urinary podocyte markers for monitoring and risk stratification of DKD has been explored, systematic studies and external validation are lacking in the current literature. Standardization and automation of laboratory methods should be a priority for future research, and the added value of these methods to routine clinical tests should be defined.

Keywords: Biomarkers, Diabetes mellitus, Nephrin, NPHS2 protein, Podocalyxin, Podocytes

Introduction

Epidemiology of chronic kidney disease

Chronic kidney disease (CKD) affects over 10% of the world’s population worldwide, amounting to over 800 million individuals [1]. With the advancement in the pharmaceutical industry and the increasing availability of dialysis, the healthcare expenditure used for the treatment of CKD has risen drastically in the past decades [2]. In 2022, between 4.90 and 7.08 million patients worldwide had end-stage kidney disease and need kidney replacement therapy [3]. Of note, CKD represents an especially large burden in low- and middle-income countries, which are least equipped to deal with its consequences [4].

CKD is more common among older individuals, women, racial minorities, and people with diabetes mellitus and hypertension. Over the past two decades, CKD has become one of the leading causes of death worldwide. This disease is one of the few non-communicable diseases that has seen an increase in associated deaths during this time period [1,5]. Effective means for the diagnosis, risk stratification, and monitoring of kidney disease are much needed.
Podocyte as the focus of kidney disease

The primary function of the kidney is the excretion of metabolic wastes and excessive body water [6]. This objective is achieved by three processes: 1) filtration of plasma through the glomerulus; 2) reabsorption of useful substances via renal tubule; and 3) secretion of other metabolic wastes from peritubular capillary to the tubular fluid [6]. Although the kidney has a delicate three-dimensional (3D) structure with multiple cell types, the podocyte plays a central role in the maintenance of glomerular architecture and is the primary focus of many kidney diseases. Podocytes are terminally differentiated cells with a voluminous cell body that gives rise to primary processes, each of which has an extensive array of foot processes that affix to the glomerular capillary basement membrane (GCBM) and, together with the endothelial cells, from the glomerular filtration barrier [7]. The cell body of podocytes could be divided into three distinct compartments: the base, the top, and the slit diaphragm (SD) [8]. Specific cell membrane proteins are present in each compartment, and their interaction with the cytoplasmic cytoskeleton is responsible for the stability of podocyte structure and function [9].

Podocyte dysfunction plays a pivotal role in the pathogenesis of many kidney diseases. Germline mutations of podocyte-related genes result in kidney diseases, which typically present as steroid-resistant nephrotic syndrome and focal glomerulosclerosis (FGS). On the other hand, acquired podocyte dysfunctions, including a reduction in podocyte number or the density and fusion of podocyte foot processes, lead to the damage of glomerular filtration barrier and proteinuria [10]. Notably, many types of podocyte injury lead to a weakened interaction with GCBM, resulting in the detachment of podocyte and its cellular fragments to the urinary space, which may serve as biomarkers of kidney diseases (Fig. 1).

Podocyte damage and metabolic stress

A hot topic in podocyte research in recent years is the metabolic stress of podocyte during disease processes. Glycolysis and oxidative phosphorylation (OXPHOS) are the two major cellular pathways for energy production. Most cell types may switch between these two pathways in order to cope with any changes in energy demand, and podocyte is not an exception [11]. Podocytes are rich in mitochondria and heavily dependent on them for energy to maintain normal functions. A previous study found that mitochondrial OXPHOS contributes to over 50% of adenosine triphosphate production in mature podocytes [11]. Impairment of mitochondrial function can result in an energy crisis, oxidative stress, inflammation, and cell death. In diabetic kidney disease (DKD), mitochondrial pathways involved in podocyte injury include mitochondrial dynamics and mitophagy, mitochondrial biogenesis, mitochondrial OXPHOS and oxidative stress, and mitochondrial protein quality control. Mitochondria are dynamic organelles that respond to pathophysiologic cues by altering mitochondrial content, fusion, fission, mitophagy, and the unfolded protein response. Fission and fusion work together to maintain mitochondrial morphology, while mitophagy selectively removes damaged mitochondria from the network [12]. In DKD, excessive mitochondrial fission combined with decreased mitochondrial fusion is a typical feature of podocytes [13]. Additionally, the lack of proper mitochondrial turnover in the diabetic kidney is due to the inhibition of mitophagy [14,15]. Another key feature of mitochondrial dysfunction in podocytes is the reduced efficiency of mitochondrial biogenesis [15]. Under high glucose conditions, intracellular reactive oxygen species (ROS) production, mitochondrial DNA damage, and protein and lipid peroxidation are enhanced [16]. Furthermore, maintaining mitochondrial protein homeostasis is challenging due to the continuous exposure of mitochondrial proteins to mitochondrial ROS. Mitochondria cannot exist in isolation within a cell. They interact with the endoplasmic reticulum by forming mitochondrial-associated membranes (MAMs). The disruption of MAMs leads to abnormal intracellular Ca2+ influx, mitochondrial damage, and apoptosis [17]. The combination of the above factors results in podocyte injury and the progression of DKD.

However, mitochondria are mainly located around the podocyte nucleus and are scarce in the foot processes, probably because mitochondria are larger than the foot processes [18]. As a result, the energy expenditure of foot processes is largely supported by glycolysis, which probably explains the predominant expression of phosphofructokinase, the rate-limiting enzyme of glycolysis, in podocyte foot processes [11,18], and there were relatively low levels of tricarboxylic acid cycle intermediates in the
glomerulus [18]. Recent evidence suggests that anaerobic glycolysis and fermentation of glucose to lactate are the primary sources of energy for podocytes. Additionally, anaerobic glycolysis maintains the glomerular filtration barrier independently of mitochondrial metabolism [18]. In diabetic mice, compromised glycolysis results in increased amino acid catabolism in podocytes [19]. In DKD, ornithine catabolism contributes to activating mammalian target of rapamycin (mTOR) signaling and cytoskeletal remodeling in podocytes. Therefore, inhibiting ornithine catabolism or mTOR signaling may help mitigate podocyte damage in diabetic mice [19]. Further studies showed that the blockade of glycolysis reduces the migration ability of podocytes [11], while cell-specific knockout of mitochondrial genes in podocytes does not lead to any major changes in the structure and function of podocytes [18].

Available evidence showed that the metabolic pathway of podocytes alters in response to cell damage. Under high glucose conditions, the cellular levels of glycolytic enzymes significantly increased, while that of tricarboxylic acid cycle and mitochondrial respiratory chain proteins significantly decreased [20], and similar changes were observed in kidney tissue samples of diabetes [21]. In streptozotocin-induced diabetic mice, podocyte-specific knockout of pyruvate kinase M2 (PKM2), another key enzyme of the glycolysis pathway, resulted in significantly aggravated kidney injury [21], indicating that increased glycolysis is part of the protective mechanism in response to podocyte injury.

It follows that the impairment of podocyte glycolysis promotes the development of DKD. Notably, activation of the renin-angiotensin system leads to PKM2 inhibition [22]. When podocytes are treated with angiotensin II, PKM2 expression rapidly decreases [22]. Specific deletion of PKM2 in mouse podocytes further exacerbates angiotensin II-induced podocyte damage, which was followed by foot pro-
cess detachment and proteinuria [22].

Increased glycolysis leads to the formation of pyruvate and lactate, resulting in a certain degree of intracellular acidosis. Podocytes express the G protein-coupled receptor 81 (GPR81), the lactate receptor that is known to modulate the vascular endothelial growth factor A and N-methyl-D-aspartate receptor signaling cascades in podocytes, and regulate glomerular permeability and podocyte remodeling, respectively [23]. Lactate accumulation may therefore contribute to podocyte dysfunction in a diabetic milieu [24,25]. Since glomerular mesangial cells also express lactate transporters [26,27], it is possible that lactate accumulation in diabetic podocytes may also affect mesangial cell function.

Other metabolic disturbances in podocytes probably come secondary to the impaired glycolysis. Hyperglycemia leads to the accumulation of lipids in podocytes, which may accelerate the development of DKD, and this process may be aggravated in an environment with high angiotensin II level [28]. Adipose triglyceride lipase (ATGL), which is the first step in the hydrolysis of triglycerides, is important for maintaining the dynamic balance between lipid droplet storage and metabolism [29]. High-fat diet reduces ATGL expression and increases the accumulation of lipid droplets in podocytes, and the process can be reverted by promoting ATGL expression [30]. The depletion of ATGL in podocytes increases the intracellular level of ROS, promotes cytoskeleton redistribution, induces foot process fusion, increases the permeability of the glomerular filtration barrier, and promotes cell apoptosis [31]. The hyperglycemia-induced reduction in ATGL expression in podocytes is found to be the result of lactate accumulation, which activates the GPR81 receptor, with the downstream suppression of the cyclic adenosine monophosphate/protein kinase A pathway [31]. On the other hand, the facilitation of lipid beta-oxidation in human podocytes by the inhibition of acetyl-CoA carboxylase 2 alleviates glucose-induced insulin resistance via the sirtuin 1/peroxisome proliferator-activated receptor gamma coactivator-1 alpha pathway [32]. In addition, Luo et al. [19] showed that in a diabetic environment, impaired glycolysis of podocytes enhanced ornithine catabolism. Putrescine, the metabolite of ornithine catabolism, activated Rheb, thereby promoting the activation of mTOR signaling, which leads to cytoskeleton remodeling and fusion of podocyte foot processes [19].

**Podocyte-specific targets as biomarkers**

The major podocyte-derived molecules that are potential targets for biomarker development are summarized in Table 1.

### Nuclear targets

Wilms’ tumor suppressor gene-1 (WT1) is a zinc finger-like transcription factor in the podocyte nucleus and is the most commonly used podocyte marker for histological study [33].

### Slit-diaphragm protein complex

Components of the podocyte SD have been extensively studied as biomarkers of kidney diseases. The major targets in this group include nephrin, podocin, CD2-associated protein (CD2AP), and podoplanin [34]. Nephrin is a transmembrane protein with extracellular domains that form the core of the SD. It acts as a physical sieve barrier as well as a signaling scaffold [35]. Podocin connects nephrin to the actin cytoskeleton. CD2AP is a scaffolding molecule that interacts with nephrin and actin and is implicated in actin remodeling and membrane trafficking of podocytes [36]. Podoplanin is a small transmembrane glycoprotein in the SD compartment and has been implicated in the non-canonical signaling of mineralocorticoids [37].

### Glomerular capillary basement membrane targets

The major protein components of the podocyte basal region are the α3β1 integrin and α-dystroglycan (α-DG). These proteins anchor podocytes to the GCBM and are critical for maintaining the orientation of podocyte as well as the integrity of the filtration barrier [38]. The α3β1 integrin is a transmembrane protein that connects GCBM with the cytoskeleton via α-actinin-4. The α-DG is a glycosylated protein that is connected to the cytoskeleton via utropin, and to the podocyte and GCBM via its interaction with laminin.

### Targets at the top membrane

Podocalyxin is the major transmembrane protein on the
apical side of podocyte. It is a negatively charged sialic acid protein and contributes to the charge barrier of the glomerulus [39]. It is connected to the actin cytoskeleton by ezrin and sodium-proton exchanger regulatory factor 2 (NHERF2).

**Cytoskeleton targets**

Several podocyte-specific proteins are linked to the actin cytoskeleton and maintain the 3D structure of the podocyte. The best-studied ones are synaptopodin and α-actinin-4. Both of them bind to the actin skeleton via interaction with the membrane-associated guanylate kinase with inverted orientation-1 (MAGI-1) [40]. Synaptopodin is only expressed in differentiating podocytes after they develop the foot processes, and it is often considered as the marker of mature podocytes [41].

**Other cytoplasmic/mitochondrial targets**

Cytoplasmic proteins of podocytes are less well studied as biomarkers. The aarF domain containing kinase 4 (ADCK4) is specifically present in the mitochondria within the foot processes of rat podocytes [42]. Changes in ADCK4 level may represent metabolic disturbance of the podocyte [43].

**Methods of study**

**Urinary podocytes**

Intact podocytes are detectable in the urine of healthy people and patients with kidney diseases [44]. In fact, urinary loss is probably the major cause of podocyte depletion in CKD [45]. The traditional method for the identification of podocytes in urine involves cytospin and immunofluorescence study with specific antibodies (most commonly anti-podocalyxin antibody) [46]. The technique can be partly automated but has limited sensitivity and specificity because of cell debris contamination. With this technique, healthy people excrete less than 0.5 podocytes/mg creatinine, whereas patients with glomerular disease excrete up to 400 podocytes/mg creatinine [44].

In addition to the cytospin technique, podocytes could also be identified by the detection of podocyte-specific tryptic peptides with liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Although the equipment cost is high, this technique is not operator-dependent and therefore highly reproducible [47].

The majority of urinary podocytes are viable when tested with propidium iodide exclusion and TUNEL staining [44]. In theory, a culture of viable podocytes ex vivo would im-

| Table 1. Major podocyte-related molecules that are potential targets for biomarker development |
|---------------------------|------------------|---------------------|
| **Molecules**             | **Gene**         | **Associated genetic disease** |
| Nucleus                   |                  |                       |
| Wilms’ tumor suppressor gene-1 | WT1              | Deny-Drash syndrome, Frasier syndrome |
| Cytoskeleton              |                  |                       |
| Synaptopodin              | SYNPO            | Congenital nephrotic syndrome |
| Alpha-actinin-4           | ACTN4            | Early or late onset SRNS |
| Slit diaphragm             |                  |                       |
| Nephrin                   | NPHS1            | Early onset SRNS      |
| Podocin                   | NPHS2            | Early or late onset SRNS |
| CD2-associated protein    | CD2AP            | Early onset SRNS      |
| Podoplanin                | PDPN             | (None reported in human) |
| Foot process              |                  |                       |
| α3β1 Integrin             | ITGA3, ITGB1     | Kidney development defect (α3 integrin) |
| α-Dystroglycan            | DAG1             | Muscular dystrophy    |
| Top membrane              |                  |                       |
| Podocalyxin               | PODXL            | Congenital nephrotic syndrome |
| Other cytoplasmic/mitochondrial targets |                  |                       |
| AarF domain containing kinase-4 | ADCK4           | SRNS                  |

SRNS, steroid-resistant nephrotic syndrome.
prove the specificity of podocyte identification. However, podocytes do not normally proliferate in vivo, and special experimental conditions are required for the ex vivo culture [48]. This method has great value in translational study and therapeutic target identification but is too complicated for routine clinical use.

**Urinary podocyte-derived fragments**

Extracellular vesicles (EVs) are spherical membranous bodies released by various cell types [49]. In urine, EVs containing apical membrane and intracellular fluid are shed from all nephron segments, including podocytes, renal tubular epithelial cells, and uroepithelial cells. Urinary EVs may contain protein markers for kidney damage. In addition, EVs may contribute to intercellular communication within the nephron [50]. Previous studies showed that apical cell membrane fragments are shed from injured podocytes into the urine and could be identified as podocalyxin-positive granular structures by electron microscopy [51]. Urinary exosomes from podocytes could also be isolated by serial centrifugation, and microparticles quantitated by flow cytometry with annexin V antibody (which detects all microparticles), followed by antibody to podocalyxin or podoplanin (which specifically identifies podocyte-derived microparticles) [52,53]. Additional light-scattering analyses have been applied to determine the size of the microparticles [54]. With these sophisticated techniques, it has been shown that podocyte-derived microparticles are increased in mice upon exposure to high glucose [53], as well as in diabetic mice before the onset of albuminuria [54]. However, the application of these techniques to human kidney disease has not been explored.

In addition to measuring the number and size of podocyte-derived EVs and microparticles in the urine, there is a growing interest in measuring podocyte-specific molecules in urinary exosomes. For example, podocyte-derived signal transduction factors in urinary EVs have been found to be a promising candidate for the assessment of podocyte injuries [54]. However, their clinical applications remain under development.

**Urinary podocyte-specific protein targets**

The urinary levels of several podocyte-specific protein targets can be measured with ease, and their role as biomarkers of kidney disease has been studied extensively. In healthy adults, urinary podocalyxin and nephrin levels are generally detectable by enzyme-linked immunosorbent assay but not by traditional Western blotting [55]. Urinary nephrin level correlates with the severity of proteinuria [56] and kidney function [57]. Urinary podocalyxin level can also be measured by indirect immunofluorescence or flow cytometry [58], and it has been proposed as a marker for urinary podocyte count. Notably, urinary podocalyxin originated from the apical membrane of injured podocytes, and its level is increased in early kidney injury [51]. The major problem of using a urinary level of podocyte-specific protein targets as a biomarker is the confounding effect of urine concentration dilution. Urinary creatinine concentration is commonly used for adjustment, but this is only appropriate for free proteins in urinary supernatant but not for the levels in the cellular sediment.

**Urinary podocyte-specific messenger RNAs**

Measurement of urinary podocyte-specific messenger RNA (mRNA) level has been explored as the marker of intrarenal podocyte injury. Podocyte-specific mRNA level in urine is readily measured by real-time quantitative polymerase chain reaction (RT-QPCR). Two previous studies showed that urinary nephrin mRNA levels had a close correlation with urinary podocyte count [56,59]. In an animal study, urinary podocyte-specific mRNA levels also correlated with the rate of glomerular podocyte loss as determined by kidney biopsy [54].

Recent studies, however, have shifted the focus to derived urinary mRNA indices. In a rat model, the urinary podocin-to-nephrin mRNA ratio had a better correlation with the severity of histological progression than the absolute mRNA levels [60]. In healthy adults, urinary podocin mRNA-to-creatinine ratio (a marker of podocyte detachment), podocin-to-nephrin mRNA ratio (a marker of podocyte stress), and urinary podocin-to-aquaporin-2 mRNA ratio (a marker of relative podocyte injury versus tubular injury) correlated with the arterial blood pressure [61]. Glomerular injury is specifically associated with increased urinary podocin-to-aquaporin-2 and nephrin-to-aquaporin-2 mRNA ratios [62].
Urinary podocyte-specific micro-RNAs

Micro-RNAs (miRNAs) are short noncoding RNAs that regulate many biological pathways by targeting specific mRNAs [63]. Similar to mRNA, urinary miRNA level is easily measured by RT-QPCR [64]. Unlike mRNA, miRNAs are resistant to degradation, which makes them promising biomarkers for clinical use [64]. A number of specific miRNA changes have been reported in kidney diseases [65]. For example, podocyte-specific loss of miR-23b, miR-24, and miR-26a was associated with rapidly progressive glomerular injury [66], and urinary miR-21, miR-124, and miR-192 levels correlated urinary nephrin, synaptopodin, and podocalyxin levels in diabetic patients [67]. In addition, there are studies that delineated the pathophysiologic mechanism of miRNA-mediated podocyte dysfunction. Notably, miR-193a suppresses the expression of WT-1 and affects podocyte differentiation [68]. Similarly, miR-26a was the most abundantly expressed miRNA in the glomerulus of normal C57BL/6 mouse, and miR-26a was responsible for the regulation of podocyte differentiation and cytoskeletal integrity [69]. In human studies, urinary exosomal miR-26a levels were significantly higher in lupus nephritis than in healthy controls [69].

However, although many miRNA targets may be significantly altered in podocyte dysfunction, their urinary levels would unlikely to serve as podocyte markers. This is because podocytes contribute to only a small proportion of urinary miRNA. Any specific miRNA alterations observed in the podocytes may not be readily discernable in the urine, while any alteration in the urinary miRNA levels may reflect the pathological change in other renal cell types [70,71]. For example, miR-21 inhibits the expression of tissue inhibitor of metalloproteinase 3 (TIMP3) [72], miR-26a inhibits the expression of transforming growth factor beta 1 [73], miR-23b targets Ras GTPase-activating protein SH3 domain-binding protein 2 (G3BP2) [74], and miR-29c targets Sprouty homolog 1 (Spry1) [75]. Although these miRNA targets and their corresponding pathways are relevant for the pathogenesis of podocyte dysfunction, they may also be affected in other cell types in the kidney. Similarly, although urinary levels of miR-155, miR-663, and miR-1915 are significantly different between patients with FGS or minimal change nephropathy and healthy controls [76], it has not been shown that miR-155, miR-663, or miR-1915 specifically originates from podocytes.

Adjusting for urinary concentration

The key advantages and disadvantages of the above-mentioned methodologies are summarized in Table 2. An important methodological consideration for studying any urinary biomarker is how to adjust for the concentration-dilution effect of the urine. For the study of urinary electrolytes, the traditional methods are via the calculation of fractional excretion or the trans-tubular gradient [77]. Both of these methods require the concomitant measurement of plasma electrolyte level. For urinary biomarkers, a common method is to adjust for urinary creatinine concentration, which originates from the practice of measuring urinary protein- or albumin-creatinine ratio in chronic kidney disease [78]. However, this method is actually only suitable for molecules that are freely soluble in the urine, and therefore the concentration of which is truly affected by urinary concentration-dilution and could be adjusted appropriately by the urinary creatinine concentration. For molecular targets present in urinary sediment, the quantity in a centrifuged sample is not affected by any concentration-dilution effect of the urine, but by the duration of urine collection (i.e., if the rate of excretion is constant, an 8-hour urine sample should contain twice the amount of the molecule as compared to a 4-hour urine sample). A timed urine sample or expressing the result as a rate of excretion per hour would seem appropriate for this kind of molecular target—akin to the reporting of albuminuria [79]. For molecular targets in urinary microvesicles, the optimal method of adjustment is less well-defined, and it probably depends on the centrifugation protocol.

Urinary podocyte markers in diabetic kidney disease

There is a wealth of literature on the use of various urinary podocyte markers for the diagnosis and monitoring of DKD. Amongst all podocyte-specific protein targets, the most promising ones are podocalyxin and glycogen synthase kinase 3β (GSK3β). Podocalyxin level in urine was increased in diabetic patients before the onset of microalbuminuria, and therefore likely valuable for the early detection of DKD [80]. In patients with overt DKD, urine
podocalyxin levels correlated with albumin-to-creatinine ratio [81,82]. On the other hand, GSK3β levels in urinary exfoliated cells were more accurate than albuminuria in discriminating diabetic patients with and without progressive renal impairment [83], this assay is cumbersome and may not be applicable to routine clinical practice. Our recent study showed that urinary phosphorylated GSK3β (p-GSK3β) level correlated with kidney function, but urinary total GSK3β, p-GSK3β level, its mRNA level, or the p-GSK3β/GSK3β ratio did not predict dialysis-free survival or the rate of kidney function decline [84].

As to other podocyte-specific mRNA targets, urinary mRNA levels of nephrin, podocin, synaptopodin, WT-1, and α-actin-4 were elevated in DKD [85], and they also correlated with urinary podocyte count, urinary nephrin level, albuminuria, and the severity of renal impairment [86]. More importantly, urinary podocyte-specific mRNA levels preceded the onset of microalbuminuria in diabetic patients [86], and the urinary podocin mRNA-to-creatine ratio (a marker of podocyte detachment) predicted the subsequent rate of kidney function decline [87]. However, all of these studies have small sample sizes and the inclusion criteria are prone to selection bias. Further large-scale studies are necessary to validate their results before routine clinical application can be considered.

In addition to biomarkers specific for DKD, the urinary level of several podocyte-specific targets has been explored as biomarkers for CKD in general. For example, urine synaptopodin level correlated with kidney function in both diabetic and non-diabetic CKD, regardless of the degree of albuminuria [88]. However, not all kidney diseases have podocyte injury [89], and the association between podocyturia and proteinuria varied markedly in different diseases, indicating that urinary podocyte markers should be explored as disease-specific biomarkers [89].

### Challenges and future studies

Although podocytes play a crucial role in the development and progression of DKD, the use of podocyte-associated molecules in urine as biomarkers of DKD is still in its early stages of development [90,91]. Before this technology can be applied in clinical practice, much work is needed to define the molecular target (or combination of targets) to

<table>
<thead>
<tr>
<th>Methods</th>
<th>Advantage</th>
<th>Disadvantage and limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Podocyte quantification</td>
<td>Possibility of automation</td>
<td>Limited sensitivity and specificity; long turn-around time</td>
</tr>
<tr>
<td>Cytospin and immunofluorescence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex vivo culture</td>
<td>Specific</td>
<td>Technically demanding, time consuming; unlikely feasible for routine clinical practice</td>
</tr>
<tr>
<td>Podocyte-derived extracellular vesicles</td>
<td>Standardized method</td>
<td>Result difficult to interpret and bd quantified; long turn-around time</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td></td>
<td>Protocol not standardized; expensive and labor intensive</td>
</tr>
<tr>
<td>Serial centrifugation/flow cytometry</td>
<td>Able to identify sublethal podocyte injury</td>
<td></td>
</tr>
<tr>
<td>Podocyte-specific proteins</td>
<td>ELISA-based method is simple and often commercially available</td>
<td>Uncertain pathophysiological relevance and clinical utility; variable degradation of the protein target after sample collection; method to adjust for urinary concentration effect controversial; choice of target (or combination of targets) to be defined</td>
</tr>
<tr>
<td>Podocyte-specific mRNA</td>
<td>PCR-based method is standardized, new targets readily accommodated</td>
<td>Handling of urine sample is tedious and often not feasible for archive materials; method to adjust for urinary concentration effect controversial; choice of target (or combination of targets) to be defined</td>
</tr>
<tr>
<td>MiRNA markers</td>
<td>PCR-based method is method standardized, new targets readily accommodated, handling of archive sample is simple</td>
<td>Targets unlikely to be podocyte-specific, uncertain pathophysiological relevance; method to adjust for urinary concentration effect controversial; choice of target (or combination of targets) to be defined</td>
</tr>
</tbody>
</table>

ELISA, enzyme-linked immunosorbent assay; mRNA, messenger RNA; miRNA, microRNA; PCR, polymerase chain reaction.
be tested. Since the amount of mRNA and miRNA in urine is small, there is no reliable method to determine the gene expression library in urine [92,93], and identifying suitable targets is often a fishing exercise. Moreover, a urine sample contains separate compartments (i.e., conventional sediment, microvesicles, and truly cell-free supernatant), and podocyte-associated molecules (proteins, mRNA, and miRNA) are present in them at different concentrations [94,95]. Since the compartment to be tested determines the centrifugation protocol [50,96], it is a necessary but arduous task to define the suitable molecular target in each specific urinary compartment for clinical use.

In developing biomarkers for DKD, there is another theoretical aspect that is often overlooked. Specifically, two types of biomarkers can be identified: those that are specific to DKD, and others that are generic for any type of kidney damage. The type of biomarker identified depends on the study protocol and the selection of the control group [97]. It can be difficult to identify markers that are specific to podocyte injury or dysfunction, as opposed to markers of kidney inflammation or other pathological processes. However, if a reliable generic marker of kidney disease (a predictor of kidney function loss regardless of the underlying cause of kidney damage) were identified, it would have great clinical potential. Since podocyte loss is a central factor in the progression of many kidney diseases, we believe it would be beneficial to focus on the search for urinary biomarkers related to podocytes.

Taken together, although available studies provide preliminary ideas on the potential of podocyte-specific targets as biomarkers, there is much to do before they can be applied to clinical practice. While the published data focused on the potential of these biomarkers, they did not clearly define their clinical relevance or practical implications. It would be crucial to delineate the prospect of translation of these biomarkers into clinical practice, including the potential challenges and opportunities that may arise. Additionally, the validation of these biomarkers in large-scale studies is necessary to ensure their accuracy and reliability. Finally, it is important to consider the prospective impact of these biomarkers on patient management, including potential changes in treatment plans. By addressing these important considerations, we can gain a deeper understanding of the potential of podocyte-specific targets as biomarkers and their impact on clinical practice.

**Conclusion**

Podocyte injury plays an important role in the pathogenesis and progression of many kidney diseases. Urinary levels of podocyte-derived cellular fragments and podocyte-specific molecules may serve as biomarkers for the diagnosis and monitoring of kidney diseases. With the advance in our understanding of podocyte biology, the development of new technologies, and the increasing availability of existing ones, urinary podocyte markers are expected to have an expanding application. On the other hand, the identification of specific urinary podocyte markers may shed light on the pathophysiology of kidney diseases. Further research should focus on the standardization and automation of laboratory methods, as well as defining their added value to the available tests.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

This work was supported by the Richard Yu Chinese University of Hong Kong (CUHK) PD Research Fund, and CUHK research accounts 6905134 and 8601286. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Data sharing statement**

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

**Authors’ contributions**

Conceptualization: CCS
Methodology: CL
Writing—original draft: CL
Writing—review & editing: CCS
All authors read and approved the final manuscript.
Comparative health care at cultured me...

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School urinary screening program in Japan: history, outcomes, perspectives

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In Japan, pediatric urinary screening in schools for asymptomatic hematuria and proteinuria began in 1974 and has been very successful in detecting asymptomatic kidney diseases at an early stage. While the American Academy of Pediatrics recommended discontinuing urinalysis as a public health service in 2007, urinary screening in Japan has proven extremely successful in reducing the incidence of kidney failure with replacement therapy in children and young adults, especially through the early treatment of glomerulonephritis, such as immunoglobulin A nephropathy. Furthermore, the positivity rate on urinary screening in Japan is significantly lower than in the United States where the rate of false positive results is typically very high. Japan’s seamless and efficient pediatric urinary screening may be a helpful example for other countries as well. However, the present investigation revealed several, unresolved problems with the system. For example, the methods used varied in terms of their cutoff point, additional examinations, and types of detailed testing. In Japan, various urinary screening methods are being tested to optimize the system for national use. Recently, the authors also recommended a system of detailed examinations, including beta-2 microglobulin testing and ultrasonography, to detect congenital anomalies of the kidney and urinary tract, the most common, underlying disease in kidney failure with replacement therapy, which is often overlooked until the symptoms have become grave. While school urinary screening has been ongoing for about 50 years and should be continued, improvements should also be made to it as needed.

Keywords: Child, Chronic kidney diseases, Mass screening, Proteinuria, School health services

Introduction

In Japan, a program of school urinary screening (SUS) for asymptomatic hematuria and proteinuria, begun in 1974 by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), has proved immensely successful in detecting asymptomatic kidney diseases at an early stage [1]. On the other hand, the American Academy of Pediatrics (AAP) removed urinary screening from its health supervision recommendations in 2007 [2] because of the high rate of false positive results, which led to expensive and sometimes invasive procedures with a very low, positive yield [3].

In contrast to the American experience, SUS in Japan has...
produced excellent results for kidney failure with replacement therapy (KFRT) and has evolved into an extremely efficient and seamless system. The present review will first discuss the history and protocol of SUS in Japan, which may be helpful in other countries, before discussing how it has contributed to reducing KFRT and which current problems need to be resolved to improve the detection of congenital anomalies of the kidney and urinary tract (CAKUT), the most frequent underlying disease in KFRT.

**History of school urinary screening and comparable programs in Japan**

SUS was begun in 1974 with the passage of the School Health Law in 1973. At the time, kidney disease topped the list of health-related causes of long-term absence (>50 days/year) from school, accounting for 15% of absences [4]. Early diagnosis and management of diseases in school children, including exercise programs, were considered very important for reducing absenteeism at the time. As a result, the severity of kidney disease dictated the degree of exercise restriction, with even hematuria alone considered to require exercise restriction.

In recent years, the prognosis of kidney diseases in school children has improved [5–7] thanks to progress in their treatment, leading pediatric nephrologists to recommend a relaxation of the traditional exercise restrictions. Thus, the health services involved in administering SUS transferred the focus from managing school life to a more thorough-going implementation of screening. In 2010, the Japanese Society for Pediatric Nephrology (JSPN) decided to help revise the SUS guidebook, which had previously been prepared solely by school health professionals, as a first step towards relaxing exercise management. SUS was recommended by the Japanese Society for School Health (JSSH) under the auspices of the MEXT. The Japanese guidebook was revised in 1990, 2003, 2011, and 2021 and is currently available in its fourth edition. The JSPN worked closely with the JSSH in revising the 2011 edition of the guidebook, which has been distributed to schools and municipal boards of education throughout Japan.

**Fig. 1** shows the SUS implemented in Japan. Municipal boards of education and school health officials in regional medical associations make the final decision as to the specific methods to be employed at each school within their jurisdiction. In SUS, urinalysis is performed at each school

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**Figure 1. School urinary screening system in Japan.**
annually by nongovernment testing services with the assistance of school physicians, nurses, and teachers. The JSSH guidebook recommends adopting certain detailed tests for use in SUS, but each municipality is able to choose its own methods.

Fig. 2 shows the specific methods recommended by the JSSH guidebook for use in schools. The first-morning urine is to be tested by dipstick twice, and a cutoff of (+) is to be used for both proteinuria and occult blood (OB). An emergency notification is issued to the examinee’s parents via telephone call or post if the proteinuria value exceeds (3+). If a urine test returns positive, the student receives printed recommendations in the form of an instruction sheet for further, detailed testing. This testing is done by population mass screening or the primary physician (school physician, family physician, or health center) and includes history-taking, urinalysis, a physical examination, measurements of blood pressure, urinary protein-to-creatinine ratio (PCR), and beta-2 microglobulin-to-creatinine ratio (BMCR), and blood tests for albumin, creatinine, complement 3, etc. (Fig. 3).

After the detailed testing is done, each physician or an evaluation committee issues a provisional diagnosis (Table 1, Fig. 3). The 2011 version includes postural proteinuria, which is generally considered to have a good prognosis based on a comparison of the first-morning urine with a sample obtained at the time of the visit. However, despite first-morning urine samples being compared twice, detailed testing still detected postural proteinuria (Table 2). Although PCR is recommended for detailed testing for proteinuria, the present review recommends the dipstick test as an alternative if PCR is unavailable. Nonetheless, a PCR value of 0.15 g/gCr or higher should be used instead of positive findings on a dipstick test whenever possible. The 2012 chronic kidney disease (CKD) guidelines published by KDIGO (Kidney Disease: Improving Global Outcomes) [8] and The Nelson textbook of pediatrics [9] suggest that the PCR reference value should be <0.2 g/gCr for the first-morning urine. However, in a previous study of Japanese subjects, the PCR value for healthy children was 0.12 g/gCr, which is the 97.5th percentile value [10]. The Japanese CKD guidelines for adults give 0.15 g/gCr as the cutoff [11], which was adopted in this study to avoid confusion.

The instruction sheet is also important as means of compiling epidemiological data because it includes the provisional diagnosis. Every school nurse using the instruction sheet is required to write down the relevant data for submission to the local board of education. The instruction sheet includes any restrictions on exercise. The school nurse plans the student’s exercise regimen based

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**Figure 2. Urinary screening being performed at a school.**
on the results. For this reason, all children who have received detailed testing are required to submit the results to their school. Although exercise restrictions have been relaxed as mentioned above, and aerobic exercise is recommended even for patients with severe proteinuria and severe kidney dysfunction, physicians may restrict strenuous activities, such as long-distance running, if they deem it necessary to do so.

Criteria for a referral to pediatric nephrologists were included in the revised 2011 edition for use in remote areas to minimize the need for consultations with specialists for only hematuria or mild urinary abnormalities, which can be dealt with by primary care physicians (Table 3). PCR findings and the proteinuria value obtained by dipstick testing were adopted as the major criteria for referring a patient to a pediatric nephrologist. In addition, if a student has gross hematuria, hypoalbuminemia, decreased serum complement, hypertension, or decreased kidney function, the primary care physician is to refer the student to a designated facility immediately. These facilities are designated by JSPN to perform kidney biopsies and diagnose and treat glomerulonephritis (GN) and other CKDs and have a pediatric nephrologist on staff.

**Significance and effect of school urinary screening**

Epidemiological data should be the starting point for assessing the need for screening. Recently, the number of patients with KFRT as well as KFRT caused by chronic GN (CGN) has fallen significantly. Before 1980, about 50% of

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**Table 1. Provisional diagnosis after detailed testing**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>PCR (g/g Cr)</th>
<th>Protein (dipstick)</th>
<th>Occult blood</th>
<th>Urinary sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;0.15</td>
<td>– to ±</td>
<td>– to ±</td>
<td>RBC &lt;5/HPF</td>
</tr>
<tr>
<td>Asymptomatic P</td>
<td>≥0.15</td>
<td>≥+</td>
<td>– to ±</td>
<td>RBC &lt;5/HPF</td>
</tr>
<tr>
<td>Postural P</td>
<td>First morning &lt; 0.15</td>
<td>First morning – to ±</td>
<td>– to ±</td>
<td>RBC &lt;5/HPF</td>
</tr>
<tr>
<td></td>
<td>At visit ≥ 0.15</td>
<td>At visit ≥+</td>
<td>– to ±</td>
<td>RBC &lt;5/HPF</td>
</tr>
<tr>
<td>Asymptomatic H</td>
<td>&lt;0.15</td>
<td>– to ±</td>
<td>≥+</td>
<td>RBC ≥5/HPF</td>
</tr>
<tr>
<td>Asymptomatic P and H, suspected GN</td>
<td>≥0.15</td>
<td>≥+</td>
<td>≥+</td>
<td>RBC ≥5/HPF</td>
</tr>
<tr>
<td>WBCs in urine, suspected UTI</td>
<td>&lt;0.15</td>
<td>– to ±</td>
<td>– to +</td>
<td>WBC ≥5/HPF</td>
</tr>
<tr>
<td>High β2MG urine, suspected CAKUT</td>
<td>β2MG/Cr (μg/mgCr): &lt;6 yr old, &lt;0.50; 6–12 yr old, &lt;0.35; &gt;12 yr old, &lt;0.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>Hypertension, nephrotic syndrome, IgA nephropathy, etc.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

β2MG, beta-2 microglobulin; CAKUT, congenital anomaly of kidney and urinary tract; Cr, creatinine; GN, glomerulonephritis; IgAN, immunoglobulin A nephropathy; NS, nephrotic syndrome; UTI, urinary tract infection; β2MG, beta-2 microglobulin; WBC, white blood cell.
cases of a primary disease associated with KFRT were of CGN, but this figure fell to 13.3% in 1998 and 5.9% in 2006 to 2011 in patients younger than 20 years. However, the frequency of CAKUT increased to about 27.6% in 1998 and 39.8% in 2006 to 2011[5–7]. Ishikura et al.[12] surveyed primary diseases in patients younger than 15 years with stage 3 to 5 CKD. Only 1.8% of CGN cases progressed to CKD in Japan while 62% of CAKUT cases progressed to CKD, making it the main cause of the latter condition[12]. In their report on the incidence of CGN leading to KFRT in both children and adults, Yamagata et al.[13] found that only patients younger than 45 years who received SUS had a lower incidence of CKD between 1983 and 1999 (Fig. 4). However, no decrease was observed in the same period in the United States[13]. In their global report on the incidence of KFRT, Harambat et al.[14] found that in Japan the incidence of KFRT was significantly lower than elsewhere. The KFRT incidence in Japanese children aged <20 years was four per million age-related population (pmarp), which is much lower than in other, high-income countries. Eleven Western European countries and Australia on the one hand and the United States on the other hand had a pmarp of 9.5 and 15.5, respectively. The incidence of KFRT caused by GN, excluding focal segmental glomerulosclerosis, was 13.3% in Japan in 1998 and 19.6% in the United States in 1993 to 1997[7]. The incidence fell to 5.9% in Japan according to a 2006 to 2011 survey[5] and to 10.1% in the United States in 2022. These data demonstrated that SUS was clearly successful in reducing KFRT caused by CGN in children and young adults.

The AAP removed screening urinalysis from its 2007 health supervision guidelines[15] on the grounds that many false positive results occurring in routine urinalysis led to increases in testing costs and invasive procedures with a very low positive yield[3]. Dodge et al.[16] reported that the positive rate for urinary protein was very high in the United States at 11.7% and that transient or orthostatic proteinuria accounted for a considerable number of these cases. Proteinuria (>10 mg/dL) was found in 1,440 children (11.7% of all children) in a single specimen whereas only 736 of 1,440 children (51.1%) were positive on testing of a second or third specimen collected within 2 to 7 days of the first test. Furthermore, only 18% to 27% of the 1,440 children (2.1% to 3.2%) were positive on testing of all three specimens[16]. They also stated that 210 of 340 children (61.8%) had orthostatic proteinuria. Hogg[2] reported the absence of a global consensus as to whether screening for CKD should be done in children and adolescents. How-

### Table 2. Difference in provisional diagnosis by proteinuria cutoff level (instruction sheet)

<table>
<thead>
<tr>
<th>School</th>
<th>Cutoff level of protein</th>
<th>Asymptomatic proteinuria</th>
<th>Postural proteinuria</th>
<th>Asymptomatic hematuria and proteinuria or suspected GN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elementary school</td>
<td>±</td>
<td>1,355 (0.05)</td>
<td>797 (0.03)</td>
<td>931 (0.03)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>550 (0.03)</td>
<td>293 (0.01)</td>
<td>597 (0.03)</td>
</tr>
<tr>
<td>Junior high school</td>
<td>±</td>
<td>1,573 (0.12)</td>
<td>821 (0.06)</td>
<td>750 (0.05)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>746 (0.07)</td>
<td>338 (0.03)</td>
<td>464 (0.04)</td>
</tr>
<tr>
<td>Senior high school</td>
<td>±</td>
<td>776 (0.08)</td>
<td>321 (0.03)</td>
<td>584 (0.06)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>347 (0.05)</td>
<td>152 (0.02)</td>
<td>291 (0.04)</td>
</tr>
<tr>
<td>Total</td>
<td>±</td>
<td>3,714 (0.07)</td>
<td>1,952 (0.04)</td>
<td>2,270 (0.04)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1,645 (0.04)</td>
<td>784 (0.02)</td>
<td>1,356 (0.04)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>5,461 (0.06)</td>
<td>2,782 (0.03)</td>
<td>3,716 (0.04)</td>
</tr>
</tbody>
</table>

Data are expressed as number (%).
GN, glomerulonephritis.

### Table 3. Criteria for referral to a pediatric nephrologist

1. When proteinuria (PCR in first-morning urine) has the following duration:
   
   PCR: 0.15–0.4 (or by dipstick 1+): continuance of 6–12 months
   
   PCR: 0.5–0.9 (or by dipstick 2+): continuance of 3–6 months
   
   PCR: 1.0–1.9 (or by dipstick 3+): continuance of 1–3 months

As soon as one of the following (with or without proteinuria) is detected:

2. Gross hematuria (including gross hematuria in sediment)
3. Hypoalbuminemia (serum albumin < 3.0 g/dL)
4. Hypocomplementemia (C₃ < 75 mg/dL)
5. Hypertension (see reference table)
6. Decreased kidney function (see reference table)

C₃, complement 3; PCR, protein-to-creatinine ratio.

Where the KFRT incidence in Japanese children aged <20 years was four per million age-related population (pmarp), which is much lower than in other, high-income countries. Eleven Western European countries and Australia on the one hand and the United States on the other hand had a pmarp of 9.5 and 15.5, respectively. The incidence of KFRT caused by GN, excluding focal segmental glomerulosclerosis, was 13.3% in Japan in 1998 and 19.6% in the United States in 1993 to 1997 [7]. The incidence fell to 5.9% in Japan according to a 2006 to 2011 survey [5] and to 10.1% in the United States in 2022. These data demonstrated that SUS was clearly successful in reducing KFRT caused by CGN in children and young adults.

The AAP removed screening urinalysis from its 2007 health supervision guidelines [15] on the grounds that many false positive results occurring in routine urinalysis led to increases in testing costs and invasive procedures with a very low positive yield [3]. Dodge et al. [16] reported that the positive rate for urinary protein was very high in the United States at 11.7% and that transient or orthostatic proteinuria accounted for a considerable number of these cases. Proteinuria (>10 mg/dL) was found in 1,440 children (11.7% of all children) in a single specimen whereas only 736 of 1,440 children (51.1%) were positive on testing of a second or third specimen collected within 2 to 7 days of the first test. Furthermore, only 18% to 27% of the 1,440 children (2.1% to 3.2%) were positive on testing of all three specimens [16]. They also stated that 210 of 340 children (61.8%) had orthostatic proteinuria. Hogg [2] reported the absence of a global consensus as to whether screening for CKD should be done in children and adolescents. How-
ever, in Japan, Korea, and Taiwan, a value of 0.4% based on two tests of early morning urine qualifies as positivity for proteinuria. These data clearly demonstrated a lower positivity rate than the figures reported by Dodge et al. [16], as mentioned above [1,17,18]. Although mass screening programs are now well established in Japan, Taiwan, and Korea, there appears to be a movement away from mass screening for CKD in children and adolescents in North America and Europe because of issues related to cost [2].

A 2013 nationwide Japanese survey [19] demonstrated that the positivity rate for protein on a dipstick test using the first urine was 0.4%, 1.5%, and 1.7% for elementary school-aged (E), junior high school-aged (J), and senior high school-aged (S) children, respectively. The positivity rate for protein on a second urine test, however, decreased to 0.1%, 0.2%, and 0.2% in the respective group. The positivity rate for OB on a secondary urine test also decreased from 0.6%–1.9% to 0.2% in all three groups. The positivity rate on a secondary urine test for both protein and OB was only 0.07, 0.08, and 0.10% in E, J, and H, respectively. These findings, which differed significantly from the United States data, indicated that only 0.3% to 0.5% of students needed further testing.

In a study conducted in Chiba City [20], the positivity rate in all students (75,000 to 129,000 per year) was 0.35% to 0.68% on urinary screening and 0.22% to 0.43% on detailed testing per year. In total, 9,544 students from 1975 to 2011

---

**Table 4. Detection of kidney disease via SUS in Chiba City (1975 to 2011)**

<table>
<thead>
<tr>
<th>Kidney disease</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA nephropathy</td>
<td>204</td>
</tr>
<tr>
<td>Non-IgA proliferative GN</td>
<td>54</td>
</tr>
<tr>
<td>MPGN</td>
<td>22</td>
</tr>
<tr>
<td>Membranous GN</td>
<td>15</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>7</td>
</tr>
<tr>
<td>FSGS</td>
<td>4</td>
</tr>
<tr>
<td>Lupus GN</td>
<td>3</td>
</tr>
<tr>
<td>Sjögren syndrome</td>
<td>1</td>
</tr>
<tr>
<td>GN total</td>
<td>310</td>
</tr>
<tr>
<td>CAKUT</td>
<td>18</td>
</tr>
<tr>
<td>Tumor</td>
<td>2</td>
</tr>
<tr>
<td>Others</td>
<td>4</td>
</tr>
</tbody>
</table>

CAKUT, congenital anomaly of the kidney and urinary tract; FSGS, focal segmental glomerulosclerosis; GN, glomerulonephritis; IgA, immunoglobulin A; MPGN, membranoproliferative glomerulonephritis; SUS, school urinary screening.

---

**Figure 4. Incidence rate of chronic glomerulonephritis causing end-stage kidney disease.** Modified from the article of Yamagata et al. (Am J Kidney Dis 2004;43:433–443) [13] with the original author’s permission. SUS, school urinary screening.
had an abnormal result after detailed testing. Of these students, 334 (3.5%) were found to have a kidney disease, and 310 (3.2%) had CGN, including 204 (65.8%) with immunoglobulin A (IgA) nephropathy (Table 4). The kidney disease detection rate was 0.6/10,000 students. Murakami et al. [1] reported about 450 students with a positive result on detailed testing; 30 of 49 of students (61.2%) who were positive for proteinuria and hematuria had GN whereas only 4.7% with hematuria alone had GN, indicating that 60% to 70% of GN cases in children were caused by IgA nephropathy. Similar results were described in Korea in students who were positive for proteinuria and hematuria [17].

Kamei et al. [21] reported on long-term survival in patients with IgA nephropathy (Fig. 5). The long-term prognosis was significantly better with combination therapy, including steroids and immunosuppressive drugs than with anticoagulation therapy for the first 2 years, suggesting that early detection and treatment are important. Of these patients, 82% were children with an average age of 12 years who were positive for protein and hematuria detected incidentally, most likely via SUS [22]. The use of combination therapy in Japan, begun in 1990, reduced the incidence of GN with KFRT and may be responsible for the dramatic drop in KFRT caused by GN in children and young adults, as previously mentioned.

A simplified, cost-benefit study found that the cost of urine dipstick testing was 300 Japanese yen (JPY) per person per examination center (personal communication to one examination center). With approximately 15 million students in Japan, the total cost of dipstick testing would run to 4.5 billion yen. The effectiveness of this method of testing was assessed on the basis of the expected reduction in the KFRT incidence. Hemodialysis costs approximately 5.5 million JPY per patient per year in Japan. If the need for dialysis could be eliminated in 82 patients over 10 years, the total cost would fall to 4.5 billion JPY. The duration of dialysis is assumed to be 10 years, but if the patient is younger than 40 years, the duration may be longer, given that the average life expectancy of dialysis patients is greater than 20 years. The incidence of IgA nephropathy in Japanese children is 4.5 to 9.9 per 100,000, with approximately 700 to 1,500 cases being diagnosed yearly [23]. KFRT is prevented in more than 10% of patients, or 70 to 150 patients with IgA nephropathy, through early diagnosis, resulting in less outlay than the cost of urinalysis. End-stage kidney disease also involves social welfare costs, such as disability payments while the costs associated with CKD are not known. These data indicate that SUS is relatively cost-effective.

**Current issues in school urinary screening in Japan**

In 2013, the boards of education in all municipalities and public schools throughout Japan were surveyed concern-
ing their views on SUS and screening results. Data were obtained from 1,330 of 1,741 of municipalities (76.4%), 16,904 of 20,677 of elementary schools (81.3%), 7,885 of 9,707 of junior high schools (81.2%), and 2,959 of 3,481 of high schools (85.0%). The results revealed problems with the SUS that varied considerably by municipality [19,24].

First, each school had a different cutoff value, (±) and (+), for the dipstick test for protein and OB. Despite recommendations to use (+), the frequency of (+) vs. (±) was expressed as 40.4% vs. 56.9% for OB and 38.9% vs. 58.2% for protein. Data on provisional diagnoses were obtained from all 27,748 schools using the previously mentioned instruction sheet. Asymptomatic proteinuria and postural proteinuria, which were assessed using different cutoff levels, yielded a positivity rate of (±) twice that of (+) whereas the rate for asymptomatic protein and hematuria was identical (Table 2) [15]. These data suggested that for GN detection, (+) is better than (±) as a cutoff because it reduces the false positive rate.

The next problem was that the method of the second urine test for students with an abnormal result on dipstick testing of the first urine sample varied by school. Thirteen percent of all 27,748 schools did not perform a second urine test, increasing the rate of positive results for detailed testing. The urinary white blood cell (WBC) count via dipstick test for the second urine test was performed by 27% of all the schools, possibly contributing to the high, false positive rate. In addition, 32% of all the schools tested for urine sediment, which should be done at the detailed testing stage because of the high costs involved. As already mentioned, only testing for protein and OB by dipstick is recommended for secondary urinalysis. However, this is problematic because further testing may increase the number of cases requiring more detailed testing as well as the cost of the second urine test.

The frequency of use of the instruction sheet also varied among schools. The instruction sheet is used not only to determine the need for exercise restrictions in each student but also to provide a provisional diagnosis for each student and to inform the board of education in each municipality about the number of abnormalities found. On average, 53% of elementary schools and 60% of junior high schools used the instruction sheet. These data indicated that about half of all municipalities were unaware of the data on students with an abnormality. Moreover, the schools were unaware of whether the students had undergone detailed testing. When each municipal educational board was asked for data on the number of students with abnormal findings and the diagnosis they received after screening, only 38% of elementary schools had information about kidney diseases in their students, compared to the figure of 49% for heart disease and 55% for allergic diseases. A similar trend was seen in junior high schools.

Detailed testing was also conducted by the municipalities on various SUS systems. For students with abnormal urinalysis findings, detailed testing was recommended via mass screening or at a designated clinic or hospital. However, only 19% of the schools surveyed conducted mass screening, and only 13% were able to provide a referral to a designated clinic or hospital for detailed testing, with 66% recommending that families find a facility for detailed testing on their own. It is unlikely that all primary care physicians are aware of the necessary tests and criteria for referral to a pediatric nephrologist. Also, not all students with abnormal SUS findings will receive detailed testing. Even in Shiga Prefecture, which has a relatively well-developed system, 5.9% and 23.6% of elementary and junior high school students, respectively, who were positive for proteinuria, did not receive detailed testing [25]. It is extremely important that children with abnormal test findings receive further, detailed testing for CKD.

Since 2011, the JSPN contributed to preparing certain sections of the JSSH guidebook but in 2021 became fully involved in all its aspects. The JSPN discussed revamping the SUS system; at present, each municipality performs its own version of SUS, and the pertinent issues were unable to be discussed with all 1,741 municipalities. Any newly developed system would ideally be adopted nationally. To this end, the JSPN is currently recommending the formation of a kidney disease committee in each prefecture (Fig. 1), with a JSPN-appointed nephrologist to work with the municipalities in each prefecture. The JSPN sent a written request and list of candidate nephrologists to each prefectural government and prefectural medical association as a first step towards forming this committee and also requested that each prefecture perform an epidemiological survey.

Because the 2011 guidebook on SUS lacked some important details, many municipalities and prefectures made their own version. JSPN decided to publish the 2015 Manual on Urinary Screening for use by family physicians,
school nurses, and the relevant personnel on municipal educational boards. The specifics of the SUS methodology were illustrated in a flow chart, and 41 possible questions or issues were addressed in a question-and-answer section [26]. About 6,000 copies have already been sold. The latest edition was prepared in April 2022 [27] (Supplementary Fig. 1, available online). JSPN has also distributed a flow chart of the SUS system (Supplementary Fig. 1, available online) to each prefectural government and medical association.

**Discovery of the congenital anomalies of the kidney and urinary tract and the 2022 guidebook**

The current prevalence of CAKUT in Japan is 62% of CKD patients and 40% of KFRT patients as mentioned previously, but many patients with CAKUT still elude detection via SUS. The new edition of the guidebook released in 2021 contains information aimed at closing this gap in the detection rate by examining the reasons for CAKUT detection.

In patients with stage 3 to 5 CKD, only 10 of 278 of cases (3.6%) were diagnosed via urinary screening at age 3 years, and 28 of 278 (10.0%) were diagnosed on the basis of SUS results. Only 88 patients (31.7%) were found to have CKD by prenatal or perinatal ultrasound examination [12]. Wühl et al. [28] reported the rate of KFRT in patients with CAKUT with a median age of 35.1 years. Therefore, CAKUT with milder CKD must be diagnosed earlier before it progresses.

Fig. 6 shows the sensitivity of various urinary markers in patients with CAKUT [29]. In patients with CAKUT, 82% of stage 3 CKD cases and 50% of stage 2 CKD cases had a positive BMCR. When the cutoff level of protein (±) on a dipstick test was used, the positivity rate was 44% for stage 3 CKD and 30% for stage 2 CKD on a dipstick test. When the cutoff level of protein (+) was used, the positivity rate decreased to 24% for stage 3 CKD and 11% for stage 2 CKD. The urine of patients with CAKUT was diluted, with urinary creatinine of <100 mg seen in 92% of the patients. Therefore, false negative results were likely to occur when only dipstick testing was used, and BMCR was the best urine marker for detecting CAKUT. The present study found that BMCR was more useful than the dipstick test for urinary protein in detecting CAKUT with kidney dysfunction. Of course, CAKUT without kidney dysfunction can also be detected by screening [30,31], but the incidence of abnormal values in patients with CAKUT without kidney dysfunction was not examined. BMCR is also useful for detecting Dent’s disease and other tubular diseases [8].

---

**Figure 6. Urinary markers in patients with CAKUT. CKD stage 2–4 in 77 patients with CAKUT in Japan.** Created based on the data from the article by Hamada et al. (Pediatr Nephrol 2023;38:479–487) [29].

CAKUT, congenital anomaly of the kidney and urinary tract; CKD, chronic kidney disease; PCR, protein-to-creatinine ratio; BMCR: beta-2 microglobulin-to-creatinine ratio; AMCR, alpha-1 microglobulin-to-creatinine ratio; ACR, albumin-to-creatinine ratio; Cr, creatinine.
An analysis of the first-morning urine, randomly collected from 1,712 pupils aged ≥3 years to <18 years at school and kindergarten urinary screenings, found that the median urinary creatinine level was about 100 mg/dL for elementary school pupils but 180 mg/dL for high school students (Table 5). Because a PCR value for creatine of >0.15 g/gCr is abnormal for both age groups children, the analysis revealed that the positivity rate for high school-aged students as assessed by (+) on a dipstick test was higher than for the elementary school pupils [10].

The relationship between creatinine concentration and dipstick test results bears closer scrutiny (Table 6). If (+) on the dipstick test was used and the creatinine level was found to be 100 mg, the PCR value should be within the range of 0.15 to 0.3. However, if the creatinine concentration were 50 mg, a false negative result might occur. The mean creatinine level in older children is about 200 mg. Use of dipstick (+) would produce a false positive result despite normal PCR findings. These data suggest that the cutoff of (+) on a dipstick test in children younger than 7 years old is most appropriate. However, the problem of detecting CAKUT using only a dipstick test remains.

The 2022 guidebook reflects changes made to the SUS system. Among these changes is the adoption of ultrasonographic criteria for CAKUT detection. WBCs of >50/high-power field (HPF), red blood cells of >50/HPF in urinary sediment, and the reference BMCR value (0.5 μg/mgCr for 3 to 5-year-olds, 0.35 μg/mgCr for 6 to 12-year-olds, and 0.30 μg/mgCr for 12 to 18-year-olds) were also adopted as diagnostic criteria. Furthermore, criteria for referral to a specialist after ultrasonography, such as the Society for Fetal Urology grade, kidney size, presence of stones, tumors, etc. (Table 7), for detailed testing were also listed.

Conclusion

Japan’s SUS program has been in existence for almost 50 years and has achieved exemplary results. On the other hand, the AAP has removed urinary screening from health care despite its proven usefulness and cost-effectiveness in reducing KFRT.

However, despite the merits of urinary screening, the present review found various problems with the system. The SUS methods used in the municipalities of Japan varied, and the low detection rate of CAKUT was also a problem. The JSPN has developed various strategies to address these issues in the hope that they will contribute to improving the efficiency and effectiveness of the nationwide system.

### Table 5. Urinary creatinine level in children (n = 1,712) (mg/dL)

<table>
<thead>
<tr>
<th>Generation</th>
<th>Age (yr)</th>
<th>Urinary creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>97.5th percentile</td>
<td>50th percentile</td>
</tr>
<tr>
<td>Young children</td>
<td>3–5</td>
<td>171.10</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>171.46</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>162.34</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>165.08</td>
</tr>
<tr>
<td>Elementary school-age</td>
<td>6–11</td>
<td>195.80</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>183.46</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>190.18</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>171.66</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>164.08</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>198.23</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>224.06</td>
</tr>
<tr>
<td>High school-age</td>
<td>12–17</td>
<td>343.00</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>288.48</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>317.67</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>315.79</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>337.73</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>358.73</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>404.14</td>
</tr>
</tbody>
</table>

### Table 6. Relationship between urinary creatinine concentration and dipstick test results

<table>
<thead>
<tr>
<th>Dipstick (protein, mg)</th>
<th>Protein/creatinine ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCr 50 mg/dL (diluted)</td>
<td>UCr 100 mg/dL (normal)</td>
</tr>
<tr>
<td>+ (30–100)</td>
<td>0.60–2.00</td>
</tr>
<tr>
<td>± (15–30)</td>
<td>0.30–0.60</td>
</tr>
<tr>
<td>– (8–15)</td>
<td>0.16–0.30</td>
</tr>
</tbody>
</table>

*False positive; †false negative.

### Table 7. Criteria for referral to a specialist for ultrasonography

- SFU grade ≥ 3
- Kidney size (major axis), <−2 SD; left-right difference, >1 cm
- Increased echogenicity of kidney parenchyma
- Suspected stone, tumor
- Abnormal kidney or ureter structure
- Bladder wall thickening

SFU, Society for Fetal Urology; SD, standard deviation.
Conflicts of interest

All authors have no conflicts of interest to declare.

Acknowledgments

We deeply appreciate the opportunity to present this review at KSN2021 (the virtual meeting of the Korean Society of Nephrology) held in September 2021. We thank the Board of Directors of the Japanese Society for Pediatric Nephrology and the members of the committee for CKD Task Force for their cooperation and James Robert Valera for his assistance with editing this manuscript.

Data sharing statement

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

Authors’ contributions

Conceptualization, Data curation, Formal analysis, Investigation, Methodology: All authors
Project administration, Resources: MH
Supervision: TY, YG
Writing–original draft: MH
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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Genome-wide association study and fine-mapping on Korean biobank to discover renal trait-associated variants

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Background: Chronic kidney disease is a significant health burden worldwide, with increasing incidence. Although several genome-wide association studies (GWAS) have investigated single nucleotide polymorphisms (SNP) associated with kidney trait, most studies were focused on European ancestry.

Methods: We utilized clinical and genetic information collected from the Korean Genome and Epidemiology Study (KoGES).

Results: More than five million SNPs from 58,406 participants were analyzed. After meta-GWAS, 1,360 loci associated with estimated glomerular filtration rate (eGFR) at a genome-wide significant level (p = 5 × 10⁻⁸) were identified. Among them, 399 loci were validated with at least one other biomarker (blood urea nitrogen [BUN] or eGFRcysC) and 149 loci were validated using both markers. Among them, 18 SNPs (nine known ones and nine novel ones) with 20 putative genes were found. The aggregated effect of genes estimated by MAGMA gene analysis showed that these significant genes were enriched in kidney-associated pathways, with the kidney and liver being the most enriched tissues.

Conclusion: In this study, we conducted GWAS for more than 50,000 Korean individuals and identified several variants associated with kidney traits, including eGFR, BUN, and eGFRcysC. We also investigated functions of relevant genes using computational methods to define putative causal variants.

Keywords: Chronic kidney disease, Estimated glomerular filtration rate, Genetics, Genome-wide association study, Korean Genome and Epidemiology

Introduction

Chronic kidney disease (CKD) is a significant health issue with a globally increasing incidence, affecting over 850 million individuals with kidney diseases worldwide [1]. To uncover the pathogenesis of CKD, global consensus initiatives have conducted large-scale genome-wide association studies (GWAS) and meta-analyses of GWAS (meta-GWAS),
During the earlier stage of the GWAS era, several loci associated with renal function and kidney disease have been identified [2,3]. These studies have demonstrated the vast potential of GWAS in CKD research. Recently, several fine-mapping studies have been conducted in conjunction with GWAS to perform functional annotations of identified variants, shedding light on the pathogenesis of CKD [4–8]. However, most GWAS were focused on European ancestry.

The Korean Genome and Epidemiology Study (KoGES) is a nationwide cohort that has collected clinical and genetic information since 2001 [9]. Using this cohort, GWAS have been conducted on various traits, including alcoholic liver disease [10], serum uric acid [11], and muscle mass [12]. Estimated glomerular filtration rate (eGFR) is a widely accepted kidney trait for GWAS. A few studies have been conducted to identify single nuclear polymorphisms (SNP) associated with eGFR in the Korean population [13,14]. However, these studies have reported associated SNPs without functional fine-mapping or functional annotations. Given that SNPs discovered by GWAS are regulatory variants associated with complex traits and diseases, uncovering functional annotation and fine-mapping are important [15]. Therefore, we conducted a GWAS to identify loci associated with eGFR and a fine-mapping study to reveal putative causal SNPs in the Korean populations.

**Methods**

**Participants and genotyping**

This study protocol was reviewed and approved by the Institutional Review Board (IRB) of Soonchunhyang University Cheonan Hospital (Cheonan, Korea) and the need for informed consent was waived by IRB (No. SCHCA 2021-11-035). This study complied with the principles of the Declaration of Helsinki.

All genotype data were obtained from the Korea Biobank Array Project managed by the Korea National Institute of Health. All samples and clinical data were collected as part of the KoGES [9]. The cohort comprised three sub-cohorts, namely the Korean Association Resource (KARE), Health Examinee (HEXA), and Cardiovascular Disease Association Study (CAVAS). The methodology for genotyping across all cohorts has been detailed elsewhere [16]. Shortly, these cohorts comprised community-dwellers aged ≥40 years at baseline. The KARE cohort, also known as the Ansan and Anseong cohort, included participants from the Ansan and Anseong regions representing urban and rural areas, respectively. The HEXA study recruited participants who attended regular health check-ups in urban areas. The CAVAS cohort had participants from rural areas to investigate cardiovascular diseases.

The three sub-cohorts consisted of 10,030, 177,357, and 28,338 participants, respectively. Among them, 8,840, 58,694, and 8,105 subjects were genotyped using Affymetrix Genome-Wide Human SNP array version 5.0 (Affymetrix) [17] for KARE and Korea Biobank Array [16] for HEXA and CAVAS. Although the quality control process of genotyping was initially conducted before distributing cohort data to researchers, as previously reported [16–18], we additionally performed rigorous quality control processes [19]. These processes involved quality controls based on predetermined criteria, such as SNPs including low genotype calls (<0.01), individuals with high rates of genotype missingness (<0.05), sex discrepancy-based X-chromosome homozygosity, low minor allele frequency (MAF, <0.05), Hardy-Weinberg equilibrium (p < 1 × 10^-6), individuals with heterozygosity rate deviated ±3 standard deviation from the mean, cryptic relatedness based on pi-hat threshold of 0.2, and population stratification. Population stratification was analyzed using a multidimensional scaling (MDS) approach for KARE and CAVAS cohorts. However, principal component analysis was used to calculate population stratification in the HEXA cohort due to the large sample size that made MDS analysis unfeasible.

**Genotype imputation**

After completing quality control, the imputation of all cohorts was carried out using the 1000 Genome Project Phase 3 reference panel which comprised an Asian population [20]. The imputation was performed using Beagle 5.4 and involved haplotype phasing [21] and imputation [22]. Following the imputation process, additional quality control was executed based on imputation information quality score of >0.8 and MAF of >0.05.

**Phenotype and covariates**

In the HEXA cohort, hemoglobin A1c (HbA1c) was avail-
able for a subset (only 54%) of participants at baseline. Due to the limited availability of laboratories in the HEXA cohort at baseline, we utilized data from the first follow-up where almost all individuals had HbA1c results. Hence, the first follow-up data from the HEXA cohort were utilized for this analysis. For the KARE and CAVAS cohorts, baseline data were used for analysis.

The primary phenotype in all cohorts was a quantitative trait, which was defined by eGFR using the Chronic Kidney Disease Epidemiology Collaboration equation \[23\]. Creatinine levels were measured using a Hitachi Analyzer 7600 (Hitachi) in KARE and an ADVIA 1650 (Siemens Healthcare) in the CAVAS cohort. Serum cystatin C was measured using a Cobas c702 (Roche). All KARE and CAVAS cohort samples were measured at Seoul Clinical Laboratories. It should be noted that the HEXA cohort was based on data from a nationwide health examination where each laboratory result was measured at the institution where the health examination was performed. Thus, specific methods used by each institution were unavailable.

To estimate an unbiased effect of genotype on renal function (i.e., eGFR), subjects with diabetes mellitus (DM) or albuminuria were excluded. As covariates, age, sex, body mass index, systolic blood pressure (SBP), and past medical history of hypertension were used. Past medical history of hypertension was defined as SBP of \(\geq 140\) mmHg, diastolic blood pressure of \(\geq 90\) mmHg, and self-report by participants. History of DM was defined by HbA1c of \(\geq 6.5\)%, fasting glucose of \(\geq 126\) mg/dL, and self-report by participants. Albuminuria was defined by \(\geq 1+\) in the urine dipstick test. Individuals with missing values in covariates were excluded from further analysis. Population structure was visually inspected and found to be homogenous, with all participants having Korean ancestry (Supplementary Fig. 1, available online). Nonetheless, to adjust for population stratification, 10 principal components calculated based on genotypes were included as covariates.

In addition to eGFR, blood urea nitrogen (BUN) and eGFR calculated using serum cystatin C (eGFR_{cysC}) [24] were used for validation, although serum cystatin C was available for the CAVAS cohort. All phenotypes were quantitative traits since the prevalence of CKD based on eGFR criteria was too low to perform a case-control analysis. When the histogram of each variable was normally distributed, original values were used as phenotype. Otherwise, naturally transformed values were used.

**Genome-wide association study and meta-analysis**

Most GWAS were performed using plink version 1.9 [25]. Quantitative trait was analyzed using linear regression with the plink command “--linear” and prespecified covariates with the assumption of an additive genetic model. An association study was performed for the three cohorts individually, then a meta-GWAS analysis was performed using METAL (Meta-Analysis Tool for Genome-wide Association Scans) with genomic control correction [26]. Genome-wide significant (GWS) level was defined as \(p < 5 \times 10^{-8}\). Distribution of observed p-values and estimated p-values of given SNPs were depicted using quantile-quantile (QQ) plots. QQ plots and Manhattan plots were drawn using qqman R packages of R software (R Foundation for Statistical Computing).

**Functional mapping and annotation**

Following meta-GWAS, variants associated with eGFR with GWS level were selected for validation analysis. To confirm associations of these variants with other biomarkers, results from meta-GWAS of log-transformed BUN (log-BUN) and eGFR_{cysC} were utilized. Variants that had both a directionally opposite, nominally significant association \((p < 0.05)\) with log-BUN and a directionally concordant, nominally significant association with eGFR_{cysC} were defined as validated loci.

We used FUMA (Functional Mapping and Annotation of Genome-Wide Association Studies) to perform functional mapping and annotation with specific purposes: (1) to specify genomic risk loci that were independently significant with other SNPs (i.e., clumping); (2) to annotate genes; and (3) to validate enrichment in tissue expression data [27]. At first, FUMA selected independent significant SNPs that exhibited a GWS association \((p < 5 \times 10^{-8})\) and were independent \((r^2 < 0.6)\). Thus, independent significant SNPs were equivalent to SNPs that remained after clumping GWAS-tagged SNPs with the same p-value and \(r^2\) threshold. Based on these SNPs, lead SNPs were identified when they were independent of other SNPs with a level of \(r^2 < 0.1\). If the linkage disequilibrium (LD) blocks of those independent significant SNPs were closely positioned.
(within ±250 kb from the first and last of the LD block), they were integrated into a genomic risk region. FUMA defined the lead SNP of the genomic risk region as the genomic risk loci. FUMA used ANNOVAR to annotate and map identified variants [28]. Additionally, we performed MAGMA (Multi-marker Analysis of Genomic Annotation) gene analysis [29] and conditional and interaction gene-set analysis (i.e., gene-property analysis) [30] using FUMA. The gene-property analysis was based on tissue expression data from Genotype-Tissue Expression (GTEx) v8 [31]. Of all 54 GTEx tissues, 49 tissues with a sample size of ≥70 were used.

Gene-set and tissue enrichment analysis

Additional gene-set enrichment analysis was performed using the DOSE R package [32]. To investigate tissue type-specific enrichment based on GWAS summary statistics, stratified LD score regression applying to specifically expressed genes (LDSC-SEG) was performed [33]. Two gene expression datasets from the GTEx project [31] and Franke lab [34], of which annotation data had previously been curated specifically for East Asians by LDSC-SEG builders, were used for LDSC-SEG [35].

When more than two independent significant SNPs were identified and remained after validation with other biomarkers (i.e., log-BUN and eGFRcysC) within a genomic risk region, conditional analysis was performed using a Genome-wide Complex Analysis (GCTA) tool [36]. Loci with p of <5 × 10^-8 were retained after conditional analysis for the most significant SNPs (lowest p-value) within a genomic risk region. Genotype data from the HEXA cohort, which had the majority of data, were used as the LD reference for conditional analysis.

Results

Baseline characteristics and phenotypes

After genotype quality control, 8,384, 58,079, and 7,966 subjects remained in the KARE, HEXA, and CAVAS cohorts, respectively. Among the 58,079 subjects in the HEXA cohort, 51,902 visited at the first follow-up schedule. Individuals with missing values and those with DM or albuminuria were then removed. Finally, 6,848, 44,787, and 6,771 individuals remained in the KARE, HEXA, and CAVAS cohorts, respectively (Supplementary Fig. 2, available online).

Table 1 shows the baseline characteristics of the KoGES cohort. Median serum creatinine levels in KARE, HEXA, and CAVAS cohorts were 0.80 (interquartile range [IQR], 0.70–1.00), 0.75 (IQR, 0.66–0.89), and 0.91 (IQR, 0.83–1.03), respectively. The median age of the KARE cohort was lower than that of the HEXA cohort or the CAVAS cohort. As all cohorts targeted the general population, the proportion of CKD patients was low. The highest proportion of CKD, defined by an eGFR of <60 mL/min/1.73 m^2, was observed in the CAVAS cohort.

Histograms indicated that eGFR and eGFRcysC of each cohort were normally distributed, while BUN was more likely to be normally distributed when it was naturally log-transformed (Supplementary Fig. 3, available online). Hence, eGFR and natural log-BUN were used as quantitative traits.

Genotypes

After quality control and genotype imputation processes, 4,864,729, 5,741,581, and 5,746,961 variants remained in the KARE, HEXA, and CAVAS cohorts, respectively. Following meta-GWAS analysis, 1,360 loci were found to be associated with eGFR at the GWS level. Manhattan plots of meta-GWAS for eGFR revealed several regions with significant association (outer circle of Fig. 1). Results from the meta-GWAS on log-BUN demonstrated that 2,454 loci were associated at the GWS level (Supplementary Fig. 4, available online). In addition, 143 SNPs were associated with eGFRcysC at the GWS level. Of 1,360 variants associated with eGFR, 399 were validated by at least one biomarker (i.e., log-BUN or eGFRcysC) and 149 were validated by both biomarkers (Supplementary Table 1, available online).

Validated loci (yellow points in Fig. 1) showed a similar pattern to that of a previously reported large GWAS study (inner circle of Fig. 1) [37]. The inner plot in Fig. 1 illustrates 399 validated loci for at least one biomarker. It was worth noting that the effect (beta) of validated variants was more prominent in cases where the mean allele frequency was lower.

Functional mapping and annotation

FUMA identified 87 independent significant SNPs, consist-
Table 1. Baseline characteristics of HEXA, KARE, and CAVAS cohorts

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>KARE</th>
<th>HEXA</th>
<th>CAVAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>6,848</td>
<td>44,787</td>
<td>6,771</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>49 (44–59)</td>
<td>58 (52–64)</td>
<td>59 (51–65)</td>
</tr>
<tr>
<td>40–65</td>
<td>6,281 (91.7)</td>
<td>35,414 (79.1)</td>
<td>5,137 (75.9)</td>
</tr>
<tr>
<td>&gt;65</td>
<td>567 (8.3)</td>
<td>9,373 (20.9)</td>
<td>1,634 (24.1)</td>
</tr>
<tr>
<td>Male sex</td>
<td>3,163 (46.2)</td>
<td>14,796 (33.0)</td>
<td>2,518 (37.2)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1,949 (28.5)</td>
<td>8,252 (18.4)</td>
<td>2,039 (30.1)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>118 (107–130)</td>
<td>121 (112–132)</td>
<td>122 (111–134)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79 (71–87)</td>
<td>74 (68–80)</td>
<td>78 (70–85)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3 (22.4–26.3)</td>
<td>23.5 (21.8–25.4)</td>
<td>24.3 (22.4–26.3)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>82.0 (75.7–87.9)</td>
<td>80.5 (74.8–86.2)</td>
<td>84.0 (78.0–89.8)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.5 (12.5–14.7)</td>
<td>13.8 (13.0–14.8)</td>
<td>13.7 (12.8–14.7)</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.5 (4.3–4.7)</td>
<td>4.6 (4.5–4.8)</td>
<td>4.5 (4.3–4.6)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>87 (82–92)</td>
<td>95 (89–101)</td>
<td>92 (87–99)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.5 (5.3–5.8)</td>
<td>5.5 (5.2–5.7)</td>
<td>5.5 (5.2–5.7)</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>22.0 (19.0–28.0)</td>
<td>23.0 (20.0–27.0)</td>
<td>24.0 (21.0–28.0)</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>19.0 (14.0–26.0)</td>
<td>19.0 (15.0–24.0)</td>
<td>20.0 (16.0–27.0)</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>13.4 (11.2–16.0)</td>
<td>14.5 (12.2–17.1)</td>
<td>15.2 (12.7–18.0)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.80 (0.70–1.00)</td>
<td>0.75 (0.66–0.89)</td>
<td>0.91 (0.83–1.03)</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>95.5 (81.8–105.4)</td>
<td>92.7 (83.4–99.6)</td>
<td>76.1 (68.9–83.4)</td>
</tr>
<tr>
<td>eGFRcys (mL/min/1.73 m²)</td>
<td>125 (1.8)</td>
<td>865 (1.9)</td>
<td>491 (7.3)</td>
</tr>
</tbody>
</table>

Data are expressed as number only, median (interquartile range), or number (%). There were some missing values: 1) 6 for waist circumference, 24 for glucose level, 1 for HbA1c, and 3 for ALT in KARE cohort; 2) 2 for DBP, 7 for waist circumference, 5 for hemoglobin, 5 for HbA1c, 1 for AST, 29 for ALT in HEXA cohort; 3) 14 for waist circumference, 475 for HbA1c, 5 for ALT, and 80 for cystatin C in CAVAS cohort.

ALT, alanine transferase; AST, aspartate transferase; BUN, blood urine nitrogen; BMI, body mass index; CAVAS, Cardiovascular Disease Association Study; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate calculated using serum creatinine by CKD-EPI equation; eGFRcys, estimated glomerular filtration rate calculated using serum cystatin C by CKD-EPI equation; HbA1c, glycated hemoglobin; HEXA, Health Examinee; KARE, Korean Association Resource; SBP, systolic blood pressure.
Figure 1. Circos plot for GWAS meta-analysis. The outer circle depicts the loci associated with eGFR in KoGES, while the inner circle represents those in a previously published GWAS by Stanzick et al. [37]. Yellow points indicate validated loci by at least one biomarker (log-BUN or eGFR<sub>cysC</sub>). The Y-axis denotes –log<sub>10</sub>(p) for association with eGFR. In the inner circle, the Y-axis was truncated at 40. Red dotted lines indicate a genome-wide significant level (p = 5 × 10<sup>-8</sup>). The inner plot illustrates a correlation between beta and mean allele frequency of validated loci (red points, lead SNPs; blue points, independent significant SNPs; gray points, validated by both log-BUN and eGFR<sub>cysC</sub>).

BUN, blood urine nitrogen; eGFR, estimated glomerular filtration rate calculated using serum creatinine by CKD-EPI equation; eGFR<sub>cysC</sub>, eGFR calculated using serum cystatin C by CKD-EPI equation; GWAS, genome-wide association study; KoGES, the Korean Genome and Epidemiology Study; SNP, single nucleotide polymorphism.

shown in Table 2, 12 loci were noted.

Gene-set and tissue enrichment study

MAGMA gene analysis was also performed using FUMA, resulting in 65 genes that remained statistically significant after Bonferroni correction (p<sub>bon</sub> < 0.05). Among these genes, 33 were located within the genomic risk region (Fig. 4A). Gene-property analysis based on GTEx v8 revealed that the kidney cortex was the most enriched tissue (Fig.)
Figure 2. Independent significant SNPs validated by at least one biomarker, log-BUN or eGFR<sub>cysC</sub>. FUMA identified 87 independent significant SNPs. Among them, 14 SNPs were validated by both log-BUN and eGFR<sub>cysC</sub>, whereas 56 were validated by none of them. The concordance between eGFR and log-BUN (B) and that between eGFR and eGFR<sub>cysC</sub> (C) are represented. Red dots correspond to independent significant SNPs validated by both log-BUN and eGFR<sub>cysC</sub>, while blue dots represent loci validated by log-BUN (B) and green dots represent those validated by eGFR<sub>cysC</sub> (C).

BUN, blood urine nitrogen; eGFR, estimated glomerular filtration rate calculated using serum creatinine by CKD-EPI equation; eGFR<sub>cysC</sub>, eGFR calculated using serum cystatin C by CKD-EPI equation; SNP, single nucleotide polymorphism.

4B), out of the 49 GTEx tissues (nominal p = 0.002).

To further investigate the significance of these genes, overrepresentation analysis was performed using the disease gene network (DieGeNet) [38] for genes that remained significant after Bonferroni correction from the MAGMA gene analysis (Fig. 4C, D). Overrepresented genes were enriched in kidney-associated terms, such as glomerular filtration rate, creatinine measurement, uric acid measurement, and BUN measurements (Fig. 4C). A network plot of enriched terms revealed several genes shown in Table 2 as
Table 2. Validated SNPs within each genomic risk region and mapped gene list

<table>
<thead>
<tr>
<th>CHR</th>
<th>Genomic risk region</th>
<th>rsID</th>
<th>Position (b37)</th>
<th>EA/NEA (EAF)</th>
<th>BETA</th>
<th>p-value</th>
<th>Cond P</th>
<th>Mapped gene list</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:10,702,266–10,718,377</td>
<td>rs3790638</td>
<td>10,707,812</td>
<td>A/G (0.05)</td>
<td>0.84</td>
<td>3.6 × 10^{-8}</td>
<td>-</td>
<td>RP4-734G22.3</td>
</tr>
<tr>
<td>2</td>
<td>2:27,598,097–27,844,601</td>
<td>rs1260326^b</td>
<td>27,707,812</td>
<td>A/G (0.45)</td>
<td>0.56</td>
<td>9.6 × 10^{-17}</td>
<td>-</td>
<td>GCKR</td>
</tr>
<tr>
<td>2</td>
<td>2:27,598,097–27,844,601</td>
<td>rs4665985^c</td>
<td>27,730,940</td>
<td>C/T (0.45)</td>
<td>0.42</td>
<td>2.9 × 10^{-19}</td>
<td>-</td>
<td>MANBA, UBE2D3</td>
</tr>
<tr>
<td>5</td>
<td>5:176,757,841–176,842,474</td>
<td>rs33921462^b</td>
<td>176,814,656</td>
<td>A/G (0.31)</td>
<td>-0.70</td>
<td>8.0 × 10^{-21}</td>
<td>-</td>
<td>SLC34A1</td>
</tr>
<tr>
<td>6</td>
<td>6:43,804,571–43,829,941</td>
<td>rs744103^b</td>
<td>43,805,362</td>
<td>T/A (0.14)</td>
<td>-0.63</td>
<td>2.3 × 10^{-10}</td>
<td>-</td>
<td>VEGFA, RP11-344J7.2</td>
</tr>
<tr>
<td>7</td>
<td>7:1,243,525–1,299,800</td>
<td>rs35578578</td>
<td>1,270,738</td>
<td>G/GC (0.10)</td>
<td>0.65</td>
<td>4.5 × 10^{-9}</td>
<td>AC091729.9, UNCX</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10:847,688–1,081,293</td>
<td>rs17159964</td>
<td>913,064</td>
<td>T/G (0.09)</td>
<td>-0.88</td>
<td>3.2 × 10^{-14}</td>
<td>-</td>
<td>LARP4B</td>
</tr>
<tr>
<td>11</td>
<td>11:30,749,090–30,777,790</td>
<td>rs56870952</td>
<td>30,750,092</td>
<td>T/TACAAA-CAAA (0.33)</td>
<td>-0.58</td>
<td>2.9 × 10^{-16}</td>
<td>-</td>
<td>RP5-1024C24.1, DCDC1</td>
</tr>
<tr>
<td>12</td>
<td>12:111,301,027–113,187,897</td>
<td>rs11066132^b</td>
<td>112,468,206</td>
<td>T/C (0.16)</td>
<td>-0.84</td>
<td>9.0 × 10^{-20}</td>
<td>-</td>
<td>NAA25</td>
</tr>
<tr>
<td>15</td>
<td>15:53,882,419–54,006,275</td>
<td>rs572528</td>
<td>53,972,484</td>
<td>A/G (0.38)</td>
<td>0.50</td>
<td>2.9 × 10^{-15}</td>
<td>-</td>
<td>WDR72</td>
</tr>
<tr>
<td>16</td>
<td>16:20,383,049–20,407,196</td>
<td>rs35449439</td>
<td>20,385,182</td>
<td>C/G (0.18)</td>
<td>0.51</td>
<td>3.0 × 10^{-9}</td>
<td>-</td>
<td>PDILT</td>
</tr>
<tr>
<td>17</td>
<td>17:59,239,149–59,498,250</td>
<td>rs2240736^b</td>
<td>59,485,393</td>
<td>T/C (0.45)</td>
<td>0.57</td>
<td>2.8 × 10^{-17}</td>
<td>-</td>
<td>RP11-332H18.4</td>
</tr>
<tr>
<td>18</td>
<td>18:77,156,103–77,160,235</td>
<td>rs549752^b</td>
<td>77,158,225</td>
<td>A/G (0.32)</td>
<td>-0.52</td>
<td>3.8 × 10^{-12}</td>
<td>-</td>
<td>NFATC1</td>
</tr>
</tbody>
</table>

CHR, chromosome; EA, effect allele; EAF, effect allele frequency; NEA, non-effect allele; rsID, reference SNP cluster ID; SNP, single nucleotide polymorphism.

*a* The mapped gene was annotated by ANNOVAR when performing FUMA. *b* These data denote an SNP previously linked to kidney trait (e.g., estimated glomerular filtration rate, uric acid, blood pressure, or serum creatinine). *c* These data indicate an SNP that has been searched for in the genome-wide association study catalog without exhibiting an association with kidney traits (rs4665985 for alcohol consumption, triglyceride, and liver fat; rs58063923 for height).

Discussion

In this study, we conducted a GWAS for the trait of eGFR using a Korea Biobank Array on the Korean population. There were some previous GWAS studies for Koreans. However, one study has only involved the KARE cohort of KoGES [13]. Another study has reported GWAS results only for selected patients [14]. In addition, previous Korean GWAS studies did not perform fine-mapping analysis. We attempted to define functional annotation and fine-map-
Figure 3. Scatter plot for concordance between studies. Of 399 loci validated by log-BUN or eGFR_{cysC}, 333 variants were also identified in results from a meta-GWAS by Stanzick et al. [37]. The x- and y-axis shows beta and 95% confidence interval from our study (KoGES) and a previous large meta-GWAS (by Stanzick et al.), respectively. Gray dots represent validated only loci. Blue dots indicate validated independent significant single nucleotide polymorphisms. Red dots represent validated genomic significant loci. BUN, blood urine nitrogen; eGFR, estimated glomerular filtration rate calculated using serum creatinine by CKD-EPI equation; eGFR_{cysC}, eGFR calculated using serum cystatin C by CKD-EPI equation; GWAS, genome-wide association study; KoGES, the Korean Genome and Epidemiology Study.

Among genes found in this study, some were concordant with previous reports and their functional roles were also demonstrated. For example, LRP2, also known as megalin, has been reported to be a target molecule associated with anti-brush border antibodies and renal failure (ABBA disease) [39]. SHROOM3 is well known as a GFR-associated gene [2,37]. It is associated with the development of kidneys in an animal model [40]. A recent study has shown the role of lysosomal beta-mannosidase (MANBA) expression in kidney disease. Manba and Ubed2d were expressed in kidney tubule cells and fibroblasts, respectively [41]. The association of SLC34A1 with kidney disease has already been reported [3]. Given that mutations in SLC34A1 are associated with nephrolithiasis, the association between genotype and phenotype might be due to renal stone-relat-
Figure 4. MAGMA gene analysis and pathway analysis based on significant genes. MAGMA gene analysis identified significant genes based on GWAS summary statistics. (A) Manhattan plots showing 33 genes located within the genomic risk region that were significant after Bonferroni correction ($p_{\text{bon}} < 0.05$). (B) To investigate enrichment in tissues, gene-property analysis was performed. The top 20 enrichment tissues (out of 54 GTEx tissues, with 49 tissues having sample sizes above 70) are depicted to be ordered according to their significance. The dashed line indicates $p_{\text{bon}} < 0.05$ and the solid line indicates nominal $p < 0.05$. (C, D) Overrepresentation analysis based on disease gene network (DieGeNet).

GTEx, Genotype-Tissue Expression; GWAS, genome-wide association study.
ed damage to the kidney [42]. PDILT is known to be located near UMOD and to regulate uromodulin expression [6]. NFATC1 is associated with tumor necrosis factor-associated podocyte injury by NFATC1/ABCA1-dependent mechanism [43]. The role of WDR72 remains unclear, although it has been reported in previous a meta-GWAS study [4]. Recent work showed WDR72 might have a role associated with uromodulin along with the UMOD-PDILT locus [44].

We searched whether 18 SNPs discovered in this study were available in the GWAS catalog [45]. Only nine SNPs (rs1260326, rs4665985, rs223471, rs33921462, rs744103, rs58063923, rs11066132, rs2240736, and rs549752) could be found in the GWAS catalog (searched in February 2023). This was quite different from what was expected, while previous GWAS discovered many loci associated with kidney traits. This might be attributed to the fact that previous studies were almost based on European ancestry. Indeed, the population structure in the KoGES cohort was quite different from those of other populations (Supplementary Fig. 1, available online). Hence, we examined the possibility of novel SNPs being in LD with previously reported SNPs, excluding rs62141288 and rs35578578, which were determined to be conditional on rs1260326 and rs744103, respectively (both rs1260326 and rs744103 were previously identified as kidney-associated SNPs). Of the remaining SNPs (n = 7), all were found to be in nearly complete LD with SNPs that were previously known to be associated with kidneys (Supplementary Fig. 6, available online). In addition, we conducted an investigation on the associations between nine SNPs that were not previously identified in the GWAS catalog and kidney-related traits using the BioBank Japan database [46]. Among them, six SNPs (rs3790638, rs62141288, rs77366165, rs7677847, rs572528, and rs35449439) exhibited a significant association (p < 5 × 10⁻⁸) with kidney-related traits (Supplementary Table 2, available online). Given the similarity in ethnicity between Koreans and Japanese, the novel SNPs discovered in this study may be extrapolated as specific to the northeastern Asian population.

Our study has a limitation. It only involved Koreans. Thus, significant loci were different from previous studies and the GWAS catalog. Ethnic differences in population structure and genetic architecture might have influenced our results as described above. Most loci discovered by GWAS were known to be non-coding variants that might exert regulatory functions [47]. Gene expression varies

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**Figure 5. Heritability enrichment in tissues from GTEx and Franke using LDSC-SEG.** Tissue-specific enrichment was performed using gene expression dataset from GTEx (A) and from Franke lab (B). The red dashed line indicates significance after Bonferroni correction (p<sub>bon</sub> < 0.05). The blue line represents nominal significance (p < 0.05). LDSC-SEG, linkage disequilibrium score regression applied to specifically expressed genes; GTEx, Genotype-Tissue Expression.
according to their ancestry [48]. Given that regulatory variants might be different between ancestries, lead SNPs associated with the trait (i.e., eGFR) were different from other studies based on most of the European population. However, enrichment analysis showed that tissue-specific expression was not significantly different from the previous report. Target genes were expressed in a kidney-specific manner (Fig. 4, 5), although the concrete pattern of expression might differ slightly from results from European ancestry. Therefore, Korean eQTL data for other diseases should be curated in the future. In addition, the sample size in our study was not sufficient to draw robust conclusions. Particularly, the SNPs we discovered did not demonstrate significance in the KARE or CAVAS cohorts, which increases the risk of false positives. The number of participants needs to be increased in future studies.

In conclusion, we discovered several SNPs associated with kidney traits in the Korean population based on KoGES, the largest cohort in Korea. We also discovered that variants were validated in other enrichment analyses. Although a detailed causality and associated mechanisms should be elucidated in the future, we found not only concordant results with previous GWAS but also novel loci that might be specific to the Korean population.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

This work was supported by the Basic Science Research Program (NRF-2022R1F1A1071128 to Samel Park and 2021R1C1C1007810 to Jong-Seok Moon) from the National Research Foundation of Korea.

Acknowledgments

This study was conducted with bioresources from the National Biobank of Korea, the Korea Disease Control and Prevention Agency, Republic of Korea (NBK-21120702-01-01).

Data sharing statement

The data presented in this study was obtained from the Korean Genome-wide Epidemiology Study (KoGES). It could be requested on the website (https://biobank.nih.go.kr/).

Authors’ contributions

Conceptualization, Formal analysis, Visualization: SP
Data curation: DSK, NJC
Funding acquisition, Project administration: JSM, SP
Investigation: DJL, SP
Methodology: DKS, YSL
Resources: DJL, NJC
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High water intake induces primary cilium elongation in renal tubular cells

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Background: The primary cilium protrudes from the cell surface and functions as a mechanosensor. Recently, we found that water intake restriction shortens the primary cilia of renal tubular cells, and a blockage of the shortening disturbs the ability of the kidneys to concentrate urine. Here, we investigate whether high water intake (HWI) alters primary cilia length, and if so, what is its underlying mechanism and its role on kidney urine production.

Methods: Experimental mice were given free access to normal water (normal water intake) or 3% sucrose-containing water for HWI for 2 days. Some mice were administered with U0126 (10 mg/kg body weight), an inhibitor of MEK kinase, from 2 days before HWI, daily. The primary cilium length and urine amount and osmolality were investigated.

Results: HWI-induced diluted urine production and primary cilium elongation in renal tubular cells. HWI increased the expression of α-tubulin acetyltransferase 1 (αTAT1), leading to the acetylation of α-tubulins, a core protein of the primary cilia. HWI also increased phosphorylated ERK1/2 (p-ERK1/2) and exocyst complex component 5 (Exoc5) expression in the kidneys. U0126 blocked HWI-induced increases in αTAT1, p-ERK1/2, and Exoc5 expression. U0126 inhibited HWI-induced α-tubulin acetylation, primary cilium elongation, urine amount increase, and urine osmolality decrease.

Conclusion: These results show that increased water intake elongates the primary cilia via ERK1/2 activation and that ERK inhibition prevents primary cilium elongation and diluted urine production. These data suggest that the elongation of primary cilium length is associated with the production of diluted urine.

Keywords: α-tubulin acetyltransferase, Aquaporin 2, ERK, Hydration, Primary cilia

Introduction

The primary cilium is a solitary, immotile cellular organelle and is observed in nearly every mammalian cell. This primary cilium functions as a mechanosensor and chemosensor in cells [1,2]. The length of the primary cilium is altered dynamically by cellular conditions. Recent studies have shown that the abnormal structure and function of the pri-
primary cilium are associated with several diseases [3–5]. In the kidneys, the primary cilia protrude from the tubular lumens of the tubular epithelial cells, directly contacting the prourine. Previously, we found that unilateral nephrectomy induces primary cilium elongation in the remaining renal tubular cells, which are exposed to increased prourine flow, and that renal tubular cells suffering from fibrosis and stress contain primary cilia of diverse lengths, compared with normal renal tubular cells [5–7]. Furthermore, we found that water intake restriction by discontinuing water supply in mice causes the shortening of the primary cilium in renal tubular cells and that the inhibition of this shortening impedes the kidney’s ability to concentrate urine [8]. These findings suggest that the alteration of primary cilium length is associated with the urine concentration process in the kidneys and kidney diseases. Despite these interesting findings, the role of primary cilium length alteration on urine production and its underlying mechanisms are largely unknown.

The microtubule is a central core component of the primary cilium, and its assembly and disassembly are associated with the elongation and shortening of the primary cilia, respectively [5,9,10]. The assembly and disassembly of microtubules are controlled by α-tubulins posttranslational modifications, such as acetylation and deacetylation [11]. Recent studies have demonstrated that α-tubulin acetyltransferase 1 (αTAT1), which catalyzes the acetylation of α-tubulins, elongates primary cilia in various ciliated cells [12]. By contrast, histone deacetylase 6 (HDAC6) shortens primary cilia by the deacetylation of α-tubulins [11,13]. Recently, we also reported that water intake restriction activates HDAC6 and shortens the primary cilium length in renal tubular epithelial cells and that the inhibition of water restriction-induced activation of HDAC6 using an HDAC6 inhibitor disrupts the kidney’s ability to concentrate urine [8].

Mitogen-activated protein kinases (MAPKs) regulate various cellular functions, including proliferation, differentiation, and cytoskeleton remodeling. Furthermore, studies have reported that ERK is associated with the expression and cellular localization of aquaporin-2 (AQP-2) in renal tubular cells and urine production [14,15]. Trepiccione et al. [14] reported that MAPK inhibitors prevent lithium-induced basolateral localization and downregulation of AQP-2 in the tubular cells. Previously, we found that ERK inhibition prevents primary cilium elongation in renal tubular cells, together with the inhibition of exocyst complex component 5 (Exoc5, which is also called sec10), which serves as an important regulatory protein in primary ciliogenesis [16]. These suggest that primary ciliogenesis is associated with urine production.

Here, we investigate whether high water intake (HWI) alters primary cilium length of kidney tubular epithelial cells, if so, what its underlying mechanism and its role on urine production are. In this study, we show for the first time that water intake increase induces primary cilium elongation along with increased αTAT1 expression, ERK1/2 activation, and Exoc5 expression and that ERK inhibition blocks HWI-induced primary cilium elongation, disturbing the kidney’s urine-producing ability. These findings indicate that the alteration in primary cilium length is involved in the regulation of body water balance.

Methods

Animals

The study analyzed 10- to 12-week-old C57BL/6 male mice (Koatech). All experiments were approved and performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Kyungpook National University (No. KNU-2022-0335). Mice were given free access to normal water (normal water intake, NWI) or 3% sucrose-containing water for HWI for 2 days. Some mice were administered with U0126 (10 mg/kg body weight [BW]; Selleckchem), an inhibitor of MEK kinase, or saline (vehicle) from 2 days before HWI daily. Mice were sacrificed 48 hours after HWI supply without fasting. For biochemical and histological experiments, the kidneys were either frozen in liquid nitrogen or perfusion-fixed with periodate-lysine-paraformaldehyde (4% paraformaldehyde, 75-mM l-lysine, 10-mM sodium periodate; Sigma-Aldrich) immediately following retrieval.

Blood and urine biochemistry

To evaluate plasma glucose level, about 30 µL of blood were obtained from the retro-orbital vein plexus using heparinized glass capillary tubes and then plasma was obtained by centrifugation. Concentrations of glucose in plas-
Kidney paraffin sections were stained with anti–acetylated-α-tubulin (Cat. No. T745; Sigma-Aldrich), anti–Na/K-ATPase (Cat. No. ab76020; Abcam), anti–AQP-1 (Cat. No. AQP-001; Alomone Laboratories), and anti–AQP-2 (Cat. No. AQP-002; Alomone Laboratories) antibodies. To detect the cell nuclei, DAPI was applied to the sections. Images were captured using a Leica microscope (DM2500). Fixed cells were stained with anti–acetylated-α-tubulin for measurement of primary cilium length.

Measurement of the primary cilium length

In this study, 5 to 10 fields per kidney (n = 5) were randomly captured (400×) using a Leica microscope (DM2500), and the primary cilium length was measured in >100 cells using i-Solution software (IMT i-Solution). Cilium length was measured by tracing the cilium curvilinear line with several straight lines as instructed by the user’s guide for i-Solution. Cilium length was measured blindly by a person unaware of the grouping of the samples.

Western blot analysis

Western blot analyses were performed as described previously [17]. The following antibodies were used: anti–αTAT1 (Cat. No. NBP1-57650; NOVUS), anti–HDAC6 (Cat. No. abs134070; Absin), anti–acetylated-α-tubulin (Cat. No. T7451; Sigma-Aldrich), anti–α-tubulin (Cat. No. T7451; Sigma-Aldrich), anti–phosphorylated ERK1/2 (p-ERK; Cat. No. 9101; Cell Signaling), anti–total ERK (t-ERK; Cat. No. sc271269; Santa Cruz Biotechnology), anti–Exoc5 (Cat. No. 17593-1-AP; Proteintech), anti–AQP-2 (Cat. No. ab3274; Merck Millipore), anti–E-cadherin (Cat. No. 610181; BD Bioscience), anti–GAPDH (Cat. No. NB300-221; NOVUS), and anti–β-actin (Cat. No. A2228; Sigma-Aldrich) antibodies.
ences among the groups were assessed using the Student t test for comparison between two groups for Fig. 1, 2 and a two-way analysis of variance with repeated measures followed by Tukey multiple comparisons post hoc test for more than three groups for Fig. 3–6 and Table 1. Differences were considered statistically significant at p < 0.05.

**Results**

High water intake elongates primary cilia on renal tubular cells

Mice were given free access to either normal water (NWI) or 3% sucrose-containing normal water (HWI). In this study, a 3% sucrose-containing water significantly increased the amount of water intake in mice compared with NWI (0.53 mL/g BW/day in HWI and 0.28 mL/g BW/day in NWI, p < 0.001). HWI increased the urine output (Fig. 1A). Furthermore, HWI significantly decreased urine osmolality (Fig. 1B). However, plasma glucose concentration (227.3 ± 9.1 in NWI and 249.3 ± 16.5 in HWI, p = 0.15), BWs (22.2 ± 0.4 g in NWI and 22.3 ± 0.3 g in HWI, p = 0.42), and food intake (3.0 ± 0.17 g in NWI and 2.9 ± 0.12 g in HWI, p = 0.19) did not differ between the groups. Also, all mice survived the entire experimental period.

When primary cilia were visualized by immunofluorescence staining using acetylated α-tubulin (a marker of the primary cilia), primary cilia were observed in the lumen of tubules and in most of the tubular epithelial cells in both HWI and NWI groups, except in intercalated cells (Fig. 1C).

![Figure 1](image)

**Figure 1. Length of the primary cilia on renal tubular cells after high water intake (HWI).** Mice can freely access normal water (normal water intake, NWI) or 3% sucrose-containing water (HWI) for 2 days. Urine samples were collected using metabolic cages for 4 hours before the mice were sacrificed. Urine volume (A) and osmolality (B) were determined. (C) Kidney sections were costained with anti-acetylated-α-tubulin (ac-α-tubulin, a marker of primary cilia, green), anti-aquaporin-1 (AQP-1; a marker of principal cells of the collecting duct, red), or anti–Na⁺-K⁺-ATPase (a basolateral protein and a marker of distal tubule cells, red) antibodies. DAPI (blue) was used to visualize the nuclei. (D–F) The average values of the primary cilium length were determined in the proximal tubule (PT), distal tubule (DT), and collecting duct (CD) in the cortex. The arrowheads indicate the primary cilia. Results expressed as mean ± standard error of the mean (n = 5–8).

*p < 0.05.
Among tubular epithelial cells, the primary cilium length in proximal tubular cells was the longest (Fig. 1C). HWI-induced primary cilium elongation in tubular epithelial cells (Fig. 1C–1F).

High water intake increases αTAT1, p-ERK, and Exoc5 expressions but not HDAC6 expression and activity in the kidneys

The elongation and shortening of primary cilia are regulated by the assembly and disassembly of the microtubules through the acetylation and deacetylation of α-tubulins, respectively [19]. αTAT1 acetylated α-tubulin and is required for cilogenesis [12], whereas HDAC6 catalyzes the deacetylation of α-tubulins [20]. Therefore, we investigated αTAT1 and HDAC6 expressions and HDAC6 activity in the kidneys. The expression of αTAT1 in the kidneys was significantly increased after HWI compared with NWI (Fig. 2A, B), whereas the expression and activity of HDAC6 were not changed by HWI (Fig. 2C, D, E). Acetylated α-tubulin, but not total α-tubulin, expression in the kidney was greater in HWI than in NWI (Fig. 2E, F). Furthermore, HWI elevated p-ERK1/2 and Exoc5 expressions in the kidneys compared with NWI (Fig. 2G–I). These results indicate that

**Figure 2.** Changes in αTAT1, HDAC6, ac-α-tubulin, p-ERK, and Exoc5 expressions in the kidney after high water intake (HWI). Mice were given free access to normal water (normal water intake, NWI) or 3% sucrose-containing water (HWI) for 2 days. Kidney samples were subjected to Western blotting (A–C, E–J) or HDAC6 activity (D) assay. Antibodies were anti-αTAT1 (A), anti-HDAC6 (A), anti-ac-α-tubulin (E), anti-α-tubulin (E), anti-p-ERK (G), anti-total-ERK (t-ERK, G), and anti-Exoc5 (I) antibodies. (A, E, G, I) β-actin and GAPDH were used as the loading controls. (B, C, F, H, J) Densities of blots were determined using ImageJ. Results are expressed as mean ± standard error of the mean (n = 4).

α-α-tubulin, acetylated-α-tubulin; αTAT1, α-tubulin acetyltransferase 1; Exoc5, exocyst complex component 5; HDAC6, histone deacetylase 6; NS, no significant difference; p-ERK, phosphorylated-ERK.

*p < 0.05.
Figure 3. Blockage of high water intake (HWI)-induced p-ERK, αTAT1, Exoc5, ac-α-tubulin expressions and HDAC6 activity by U0126. Mice were given free access to water (normal water intake, NWI) or HWI with 3% sucrose buffer for 2 days. Some mice were administered either U0126 (10 mg/kg body weight) or saline (vehicle) intraperitoneally daily, starting from 48 hours before HWI until the end of the experiment. (A, C, H) Kidneys were subjected to Western blotting (A–F, H–I) and HDAC6 activity (G) assay. The following antibodies were used; anti–p-ERK (A), anti–total-ERK (t-ERK, A), anti–αTAT1 (A), anti-Exoc5 (C), anti-HDAC6 (C), anti–ac-α-tubulin (H), and anti–α-actin (H) antibodies. GAPDH and β-actin were used as the loading control. (G) HDAC6 activity was determined in the whole kidneys. (B, D–F, I) Densities of blots were determined using ImageJ. Results are expressed as mean ± standard error of the mean (n = 4–8). α-actin, acetylated-α-tubulin; αTAT1, α-tubulin acetyltransferase 1; Exoc5, exocyst complex component 5; HDAC6, histone deacetylase 6; NS, no significant difference; p-ERK, phosphorylated-ERK.

* p < 0.05.

HWI-induced primary cilia elongation is associated with increased αTAT1, p-ERK1/2, and Exoc5 expressions, thereby increasing α-tubulin acetylation and assembly of the microtubule.

U0126 blocks the high water intake-induced primary cilia elongation

Then, we investigated whether U0126, an inhibitor of MEK kinase, blocked HWI-induced primary cilia elongation. U0126 nearly completely blocked HWI-induced increases in p-ERK, αTAT1, and Exoc5 expression (Fig. 3A–E).

However, U0126 did not affect the expression and activity of HDAC6 in both NWI and HWI conditions (Fig. 3C, F, G). U0126 blocked HWI-induced increases in α-tubulin acetylation, without a significant change in the total α-tubulin amount (Fig. 3H, I). Furthermore, U0126 blocked the HWI-induced primary cilia elongation without significant cell feature changes (Fig. 4). However, there were no significant morphological changes such as tubule cell expansion and damage (data not shown).

To define the role of ERK on the elongation, we determined that U0126 prevents primary cilia elongation of MDCK cells. U0126 treatment to confluent-grown MDCK
cells for 2 days inhibited the elongation of primary cilia (Fig. 5A, B). However, U0126 treatment did not affect Exoc5 expression (Fig. 5C, D). As expected, U0126 inhibited ERK phosphorylation (Fig. 5E, F). These results indicate that the HWI-induced primary cilium elongation is associated with ERK1/2 activation.

High water intake-induced diluted urine production is impeded by U0126

Finally, we assessed the effect of HWI-induced ERK activation on AQP-2 expression and diluted urine production. First, we determined the AQP-2 expression after HWI. Compared with NWI, HWI decreased AQP-2 expression in the kidneys (Fig. 6A, B). In addition, HWI decreased AQP-2 expression in the membrane fraction of the kidneys, leading to an increase in AQP-2 expression in the cytosolic fraction of the kidneys (Fig. 6C–F). U0126 treatment inhibited the HWI-induced decreases in AQP-2 expression in both whole-kidney lysates and membrane fraction of the kidneys (Fig. 6A–D). Furthermore, U0126 prevented the HWI-induced increase in AQP-2 expression in the cytosolic fraction of the kidneys (Fig. 6E, F). In NWI mice, U0126 did not affect the AQP-2 expression in the whole-kidney lysates and fractioned kidney samples compared with vehicles (Fig. 6A–6F). Consistent with the reduction in the membrane fraction, HWI led to decreased AQP-2 expression at the apical plasma membrane with increased cytosolic expression in principal cells (Fig. 6G). U0126 blocked this HWI-induced membrane localization of AQP-2 (Fig. 6G).

Next, we determined the effect of U0126 on HWI-induced urine production. U0126 administration significantly in-

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**Figure 4.** Blockage of high water intake (HWI)-induced primary cilia shortening by U0126 administration. Mice were given free access to water (normal water intake, NWI) or HWI with 3% sucrose buffer for 2 days. Some mice were administered either U0126 (10 mg/kg body weight) or saline (vehicle) intraperitoneally daily starting from 48 hours before HWI until the end of the experiment. (A) Kidney sections were co-stained with anti–acetylated-α-tubulin (ac-α-tubulin, green), anti–aquaporin-1 (AQP-1, red), and anti–AQP-2 (red), or anti–Na⁺-K⁺-ATPase (red) antibodies. DAPI (blue) was used to visualize the nuclei. (B–D) The average values of primary cilium length were determined in the proximal tubule (PT), distal tubule (DT), and collecting duct (CD) in the cortex. Arrowheads indicate primary cilia. Results are expressed as mean ± standard error of the mean (n = 5–6). NS, no significant difference.

*p < 0.05.
Figure 5. Blockage of primary cilia elongation by U0126 in MDCK cells. MDCK cells were treated with either 10-μM U0126 or vehicle (saline) for 2 days. (A, B) Cells were fixed and processed for immunofluorescence staining using anti–acetylated-α-tubulin antibody (green). DAPI (blue) indicates nuclear staining. (B) The length of primary cilia was measured. The results are expressed as the mean ± standard error of the mean (n = 5). (C–F) Cell lysates were subjected to Western blot analysis using anti–exocyst-complex-component-5 (Exoc5), –GAPDH, –phospho-ERK (p-ERK), and –total-ERK (t-ERK) antibodies. (D, F) Band densities were measured using ImageJ software.

MDCK, Madin-Darby canine kidney; NS, no significant difference.

*p < 0.05 vs. vehicle.

Hibited HWI-induced increases in urine volume, urine Na+ concentration, and glomerular filtration rate (GFR), and decreases in urine osmolality when compared to those in vehicle administration (Table 1). However, U0126 administration in NWI did not induce significant changes in urine volume, urine Na+ concentration, urine osmolality, and GFR when compared with vehicle administration (Table 1).

There were no significant changes in water intake amount, food intake amount, and BW in both NWI and HWI mice after U0126 administration. These results indicate that U0126 disturbs the HWI-induced ability of the kidneys to dilute urine, suggesting that ERK activation is a critical process for diluted urine production of the kidneys under HWI conditions.

Discussion

In this study, we report for the first time that HWI induces primary cilia elongation in renal tubular cells through ERK activation and increases αTAT1 and Exoc5 expressions and that ERK inhibition blocks these HWI-induced changes, thereby impeding the kidney’s ability to diluted urine production in response to water intake increase. These data indicate that the alteration in primary cilia length in tubular epithelial cells is an essential process for urine production and provides new insights into how cilia length alteration in renal tubular cells is associated with urine concentration.

The lengths of the primary cilia in renal tubular cells are dynamically altered under diverse pathological and physiological conditions [21–23]. Studies have reported that fluid flow stimulates the change in primary cilia length and that primary cilium length alteration is also required for this loading-induced cellular response to fluid flow changes [24,25]. Espinha et al. [24] reported that fluid flow stimulates the microtubule attachments around the primary cilia anchoring area, which can result in extension of primary cilia. In a recent study, we found that total water intake restriction for 48 hours shortens the primary cilia in the renal tubular cells of mice, producing concentrated urine [8]. By contrast, an increase in urine flow by unilateral nephrectomy elongates the primary cilia in the tubular
cells of the remaining kidney [6]. Ichii et al. [26] reported that primary cilium elongation increases the sensitivity of cells to urine flow. In this study, we found that HWI, which increases kidney fluid flow and GFR and produces diluted urine [27], elongates the primary cilia in renal tubular epithelial cells. In the present study, 3% sucrose water intake for 2 days did not induce any significant changes in plasma glucose concentration compared to NWI, suggesting that the cilia elongation in HWI mice may not, or minimally, be associated with blood glucose. Based on previous and present studies, we speculate that increased fluid flow in the kidneys due to HWI stimulates primary cilium elongation and that primary cilium elongation is associated with diluted urine production.

Evidence showed that ciliogenesis is involved in the regulation of microtubule organization in various cells [28]. The assembly and disassembly of microtubules are major processes in the elongation and shortening of the primary cilia, respectively [6,25]. The assembly of microtubules is stimulated by the acetylation of α-tubulins, whereas the
Table 1. Effect of U0126 on HWI-induced changes in water intake, urine volume, osmolality and sodium concentration, plasma osmolality, and GFR

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vehicle (NWI, n = 5)</th>
<th>HWI (NWI, n = 10)</th>
<th>U0126 (NWI, n = 5)</th>
<th>HWI (NWI, n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water intake (mL/g BW/day)</td>
<td>0.28 ± 0.02</td>
<td>0.53 ± 0.04 a</td>
<td>0.25 ± 0.03</td>
<td>0.46 ± 0.04 a</td>
</tr>
<tr>
<td>Urine volume (mL/day)</td>
<td>2.17 ± 0.24</td>
<td>5.02 ± 0.88 a</td>
<td>2.52 ± 0.23</td>
<td>2.63 ± 0.44 b</td>
</tr>
<tr>
<td>Urine osmolality (mosmol/kg H₂O)</td>
<td>1,895.0 ± 145.0</td>
<td>633.3 ± 98.7 a</td>
<td>2,223.3 ± 109.7</td>
<td>1,167.5 ± 53.9 ab</td>
</tr>
<tr>
<td>Urine Na⁺ (mmol/L)</td>
<td>139.5 ± 13.5</td>
<td>113.5 ± 3.51 a</td>
<td>179.0 ± 5.7</td>
<td>86.0 ± 0.6 b</td>
</tr>
<tr>
<td>Plasma osmolality (mosmol/kg H₂O)</td>
<td>284.0 ± 5.0</td>
<td>283.4 ± 2.2</td>
<td>286.3 ± 3.4</td>
<td>284.0 ± 0.9</td>
</tr>
<tr>
<td>GFR (μL/g BW/min)</td>
<td>4.6 ± 1.5</td>
<td>11.3 ± 2.0 a</td>
<td>3.8 ± 2.3</td>
<td>6.2 ± 2.1 b</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard error of the mean.

Mice were given free access to water (NWI) or HWI with 3% sucrose buffer for 2 days. Some mice were administered either U0126 (10 mg/kg BW) or saline (vehicle) intraperitoneally daily starting from 2 days before HWI until the end of the experiment.

*p < 0.05 vs. respective NWI, *p < 0.05 vs. vehicle-HWI.

disassembly is stimulated by the deacetylation of α-tubulins [5,9,10]. Recent studies have shown that αTAT1, a regulator of α-tubulin acetylation, elongates the primary cilia by increased α-tubulin assembly in microtubules [6,29], whereas the activation and overexpression of HDAC6, an inducer of α-tubulin deacetylation, shorten the primary cilia [30,31]. We previously reported that HDAC6 inhibition prevents the shortening of the primary cilia due to water intake restriction [8]. In this study, we found that HWI increases αTAT1 expression and that HWI increased the acetylated form of α-tubulins. Interestingly, unlike our expectation based on our previous study showing that cilia shortening is associated with lowered HDAC6 activity [8], HWI did not induce significant changes of HDAC6 expression and activity. This phenomenon may be explained by the deferent responses of kidney tubule cells against water restriction and water intake increase and the involvement of variety of factors such as αTAT1. Further studies are required for the clear explanation of this phenomenon. However, our data suggest that increased αTAT1 expression is associated with HWI-induced primary cilia elongation, by increased α-tubulin acetylation, thereby increasing the assembly of microtubules.

Recent studies have demonstrated that the ERK pathway is associated with ciliogenesis [5,28,32]. In the present study, HWI activated ERK, and an ERK inhibitor prevented HWI-induced primary cilia elongation, along with increased α-tubulin deacetylation, and the ERK inhibitor blocked HWI-induced αTAT1 increase, but not HDAC6 inactivation. However, Dougherty et al. [28] reported that ERK inhibition blocked cilia growth by the defect of cilia assembly in hTERT RPE-1 cells and hTERT-immortalized retinal pigment epithelial cells. In the present and previous studies, we found that ERK inhibition by U0126 inhibited primary cilia elongation in the MDCK tubular epithelial cells [5]. In contrast, Wang et al. [32] reported that ERK suppressed ciliogenesis in renal tubular epithelial cells after cisplatin-induced acute kidney injury. This discrepancy (in Wang et al.’s report, ERK suppresses ciliogenesis, whereas in our study, ERK activates ciliogenesis) may be due to experiments. Wang et al. [32] investigated cilia length in renal tubular epithelial cells recovered from severe cisplatin injury, whereas we determined cilia length under hydration conditions that do not induce renal tubule cell damage. ERK is involved in cell differentiation, which affects cillum length [16,33]. Moreover, cillum length is closely related to cell differentiation [25,34]. In the present study, we did not find significant changes in PCNA expression and cell proliferation changes under hydration conditions (data not shown).

We recently found that the overexpression of Exoc5, a critical protein for ciliogenesis, activates ERK in MDCK cells [35], whereas Exoc5 gene deletion in mice and the mutation of the Exoc5 ciliary targeting sequence in MDCK cells shorten the primary cilia in renal tubular epithelial cells [36]. In the present study, we found that U0126 prevented HWI-induced Exoc5 increase. However, U0126 did not affect Exoc5 expression in MDCK cells. These data indicate that ERK activation stimulates HWI-induced primary cilia elongation. However, it is not clear that this ERK-associated cilia elongation is associated with Exoc5 expression. Therefore, to define the precise mechanism of
ERK pathways on ciliogenesis, further work is needed to fully control ERK activation and Exoc5 expression.

Recent studies have demonstrated that the ERK pathway is associated with urine concentration in the kidneys by the regulation of AQP-2 expression \([37,38]\). HWI stimulates diluted urine production by the kidneys by regulating water channel movement from the apical plasma membrane to the cytosol and decrease in tubular cells. AQP-2 expression and apical localization in the kidneys decrease during overhydration and increase after dehydration \([39,40]\). Consistent with these, in the present study, HWI decreased AQP-2 expression and apical localization in the kidneys, producing diluted urine, and these HWI-induced effects were blocked by pretreatment with an ERK inhibitor. In present study, ERK inhibition prevented HWI-induced increases in urine volume, urine Na\(^+\) concentration, and GFR and decreases in urine osmolality. However, HWI and U0126 administration into NWI and HWI did not induce significant changes in plasma osmolality compared to NWI and vehicle-administered NWI or HWI, respectively. Therefore, we speculate that either those amount of changes of urine volume and urine osmolality induced by HWI and U0126 may not influence the plasma osmolality, or that this 2 days of HWI and U0126 administration may not exceed body fluid maintaining range. These data indicates that ERK activation is linked to diluted urine production, suggesting that ERK inhibition disturbs kidney compensatory response to HWI conditions.

Taken together, although our studies have not provided a precise mechanism for how primary cilium elongation regulates urine concentration, our data clearly show that changes in the primary cilium length are a required response to produce diluted urine under HWI conditions, suggesting that control of primary cilia length could be a tool for the various diseases which are related with abnormal body water and electrolyte balances.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

This study was supported by the National Research Foundation of Korea (NRF) grant (NRF-2020R1A2C2006903 to KMP), funded by the Korean government.

**Data sharing statement**

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

**Authors’ contributions**

Conceptualization, Methodology: MJK, KHH, JHL, KMP
Data curation, Formal analysis, Investigation: MJK, SJH, SYS
Funding acquisition: KMP
Writing–original draft: MJK, KHH, JHL, KMP
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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Anti-SARS-CoV-2 spike antibody response to the third dose of BNT162b2 mRNA COVID-19 vaccine and associated factors in Japanese hemodialysis patients

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Background: We assessed the anti-SARS-CoV-2 spike antibody response to the third dose of BNT162b2 mRNA COVID-19 vaccine in Japanese hemodialysis patients and determined factors associated with the anti-SARS-CoV-2 spike antibody titer after the third dose of COVID-19 vaccine.

Methods: Overall, 64 patients were enrolled in this single-center, prospective, longitudinal study. Anti-SARS-CoV-2 spike antibody titers were compared between hemodialysis patients and 18 healthcare workers. Multiple linear regression analysis was used to identify factors associated with the anti-SARS-CoV-2 spike antibody titer after the third vaccination.

Results: There was no significant difference in anti-SARS-CoV-2 spike antibody titer 4 weeks after the third vaccination between hemodialysis patients and healthcare workers (18,500 [interquartile range, 11,000–34,500] vs. 11,500 [interquartile range, 7,918–19,500], all values in AU/mL; p = 0.17). Uric acid (standard coefficient [β] = −0.203, p = 0.02), transferrin saturation (β = −0.269, p = 0.003), and log–anti-SARS-CoV-2 spike antibody titer 1 week before the third vaccination (β = 0.440, p < 0.001) correlated with the log–anti-SARS-CoV-2 spike antibody titer 4 weeks after the third vaccination. In contrast, only the log–anti-SARS-CoV-2 spike antibody titer 1 week before the third vaccination (β = 0.410, p < 0.001) correlated with the log–anti-SARS-CoV-2 spike antibody titer 12 weeks after the third vaccination.

Conclusion: The anti-SARS-CoV-2 spike antibody titer after the third dose of COVID-19 vaccine was comparable between hemodialysis patients and healthcare workers. Uric acid concentration, transferrin saturation, and anti-SARS-CoV-2 spike antibody titer before the third dose were associated with the anti-SARS-CoV-2 spike antibody titer after the third dose in Japanese hemodialysis patients.

Keywords: BNT162 vaccine, COVID-19, Hemodialysis, SARS-CoV-2

Introduction

Hemodialysis patients are one of the most vulnerable populations at risk for severe and fatal coronavirus disease 2019 (COVID-19) [1]. COVID-19 vaccination was reported to reduce the risk of hospitalization and death associated with COVID-19 in hemodialysis patients [2], and its preventable effects were associated with the anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike antibody titer after COVID-19 vaccination [3]. Therefore,
maintaining an adequate anti-SARS-CoV-2 spike antibody titer is important to prevent severe COVID-19 and COVID-19-related deaths in hemodialysis patients.

Recently, several studies have investigated the anti-SARS-CoV-2 spike antibody response to the third dose of COVID-19 vaccine in patients undergoing hemodialysis [4,5]. An observational study reported that the anti-SARS-CoV-2 spike antibody titer after the third dose of COVID-19 vaccine was lower in hemodialysis patients compared with that in healthcare workers [4]. Another observational study reported that the anti-SARS-CoV-2 spike antibody titer in hemodialysis patients after the third dose of COVID-19 vaccine was similar to that of healthcare workers [5]. Recent studies also revealed that the anti-SARS-CoV-2 spike antibody level before the third COVID-19 vaccination, immunosuppressive medication, and hypoalbuminemia were associated with the anti-SARS-CoV-2 spike antibody response after the third COVID-19 vaccination in patients undergoing hemodialysis [4,6,7]. However, factors associated with the anti-SARS-CoV-2 spike antibody response to the third COVID-19 vaccination have not been investigated in Asian individuals undergoing hemodialysis. Therefore, in the present study, we determined which clinical factors were associated with the anti-SARS-CoV-2 spike antibody titer after the third dose of COVID-19 vaccine in Japanese patients undergoing hemodialysis. We also assessed the anti-SARS-CoV-2 spike antibody titers in hemodialysis patients before and after the third dose of COVID-19 vaccine and compared it with that in healthcare workers.

Methods

Ethical approval

The Ethical Committee of Mizue Yuai Clinic approved this study (No. MYC 2021-01), which was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all study participants.

Patients

The study inclusion criteria were: 1) age, ≥20 years; 2) currently receiving maintenance hemodialysis; and 3) vaccinated with two doses of BNT162b2 messenger RNA (mRNA) COVID-19 vaccine (Pfizer Inc. and BioNTech) with a dose interval of 3 weeks between the first and second doses and scheduled to receive the third dose with a dose interval of 24 weeks between the second and third doses. The following exclusion criteria were applied: 1) unable or unwilling to give consent and 2) any history of COVID-19 infection. Healthcare workers who were vaccinated three times with the BNT162b2 mRNA COVID-19 vaccine and consented to participate were used as the control group.

Study design

This was a single-center, prospective, longitudinal study conducted between April 1, 2021 and June 30, 2022 at the Mizue Yuai Clinic in Tokyo. Fig. 1 illustrates the study flow chart. Each participant’s anti-SARS-CoV-2 spike antibody titer was measured 1 week before and 4 and 12 weeks after the third dose of the BNT162b2 mRNA COVID-19 vaccine. Clinical and demographic parameters were collected during the week when the third BNT162b2 mRNA COVID-19 vaccine was administered. We assessed the change in anti-SARS-CoV-2 spike antibody titers in hemodialysis patients between 1 week before and 12 weeks after the third BNT162b2 mRNA COVID-19 vaccination. We compared the anti-SARS-CoV-2 spike antibody titers between healthcare workers (control group) and hemodialysis patients (hemodialysis patient group) 1 week before and 4 weeks after the third dose of the BNT162b2 mRNA COVID-19 vaccine. We also conducted a multiple linear regression analysis to identify factors associated with the anti-SARS-CoV-2 spike antibody titers in hemodialysis patients 4 weeks and 12 weeks after the third BNT162b2 mRNA COVID-19 vaccination. Based on a previous report [5], we divided hemodialysis patients into three categories according to their anti-SARS-CoV-2 spike antibody response status 4 weeks after the third BNT162b2 mRNA COVID-19 vaccination (no, low, and high responder) and compared their clinical and demographic parameters.

Laboratory methods

Hemodialysis patients’ blood samples were obtained using an arteriovenous fistula immediately before the start of their first hemodialysis session of the week. A commercial laboratory (BML) measured the patients’ anti-SARS-CoV-2 spike antibody titers and blood parameters. The SARS-
CoV-2 IgG II Quant immunoassay (Abbott) was used to determine anti-SARS-CoV-2 spike antibody titers. Shinzato et al.’s formula [8] was used to calculate the single-pool urea clearance and normalized protein catabolism rate.

**Statistical analyses**

Data of continuous variables are shown as the mean ± standard deviation when they are normally distributed. Data that were not normally distributed are shown as the median (interquartile range [IQR]). Data of categorical variables are presented as numbers and percentages. The hemodialysis vintage, C-reactive protein, ferritin, and anti-SARS-CoV-2 spike antibody titers including that 1 week before the third BNT162b2 mRNA COVID-19 vaccination did not show normal distributions; therefore, these variables were transformed using the natural logarithm. The Friedman test and the Steel-Dwass test were conducted to compare the anti-SARS-CoV-2 spike antibody titers within each group. Comparisons of anti-SARS-CoV-2 spike antibody titers between healthcare workers and hemodialysis patients were conducted using the Mann-Whitney U test. Comparisons of clinical and demographic parameters between the two groups were conducted using the Student t test for continuous variables and Fisher’s exact test for categorical variables. Comparisons of clinical and demographic parameters among the three groups were conducted using the Kruskal-Wallis test with the Steel-Dwass test for continuous variables and the Fisher exact test with Bonferroni correction for categorical variables. Simple linear regression analyses were performed with the anti-SARS-CoV-2 spike antibody titers 4 weeks and 12 weeks after the third BNT162b2 mRNA COVID-19 vaccination as dependent variables and with clinical and demographic parameters including the anti-SARS-CoV-2 spike antibody titer 1 week before the third BNT162b2 mRNA COVID-19 vaccination as independent variables.

**Figure 1. Study flow chart.**

mRNA COVID-19 vaccination as independent variables. In a multiple linear regression analysis, we included the parameters that appeared to be correlated significantly with the anti-SARS-CoV-2 spike antibody titers 4 weeks and 12 weeks after the third BNT162b2 mRNA COVID-19 vaccination in the simple linear regression analyses (p < 0.10), to identify which variables were independently correlated with the anti-SARS-CoV-2 spike antibody titers 4 weeks and 12 weeks after the third BNT162b2 mRNA COVID-19 vaccination. The p-values of <0.05 were considered statistically significant. All statistical analyses were conducted using JMP version 11 (SAS Institute).

Results

Patient characteristics

All the patients undergoing hemodialysis in our center during the study period were screened for study entry. Of these, eight were unwilling or unable to give consent, and the remaining 75 hemodialysis patients were enrolled in this study. A total of 22 healthcare workers were enrolled as controls. Of the hemodialysis patients, 10 developed COVID-19 infection and one changed to another hospital. Therefore, the hemodialysis patient group was comprised of 64 patients. Blood samples were not available for four healthcare workers and the control group was comprised of 18 healthcare workers. Therefore, we analyzed the data of 64 hemodialysis patients and 18 healthcare workers (Fig. 1). Table 1 summarizes the patient characteristics and medications of both groups. Hemodialysis patients’ laboratory data were obtained at the start of their first hemodialysis session of the week when the third BNT162b2 mRNA COVID-19 vaccine was administered. Healthcare workers’ laboratory data were obtained at the time of the third BNT162b2 mRNA COVID-19 vaccination.

The hemodialysis patient group consisted of 37 male and 27 female patients, with a mean age of 71.4 ± 11.7 years, body mass index (BMI) of 21.9 ± 3.9 kg/m², and hemodialysis vintage of 4.5 years (IQR, 2.0–9.0 years). Twelve patients (18.8%) had a history of past or current smoking and 11 patients (17.2%) had a habit of alcohol consumption. The proportions of patients with hypertension, diabetes mellitus, autoimmune diseases, and allergic diseases were 60.9%, 43.8%, 9.4%, and 29.7%, respectively. The percent-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hemodialysis patients</th>
<th>Healthcare workers</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>No. of subjects</td>
<td>64</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>71.4 ± 11.7</td>
<td>45.9 ± 12.2</td>
<td>&lt;0.001*</td>
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<tr>
<td>Male sex</td>
<td>37 (57.8)</td>
<td>8 (44.4)</td>
<td>0.42</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.9 ± 3.9</td>
<td>24.8 ± 5.8</td>
<td>0.02*</td>
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<td>Hemodialysis vintage (yr)</td>
<td>4.5 (2.0–9.0)</td>
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<tr>
<td>Dialysis frequency</td>
<td></td>
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<tr>
<td>Twice weekly</td>
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<tr>
<td>Thrice weekly</td>
<td>64 (100)</td>
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<td>NA</td>
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<td>Hemodiafiltration</td>
<td>41 (64.1)</td>
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<td>Past or current smoking</td>
<td>12 (18.8)</td>
<td>7 (38.9)</td>
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<td>Alcohol drinking</td>
<td>11 (17.2)</td>
<td>11 (61.1)</td>
<td>&lt;0.001*</td>
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<td>Hypertension</td>
<td>39 (60.9)</td>
<td>3 (16.7)</td>
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<tr>
<td>Diabetes mellitus</td>
<td>28 (43.8)</td>
<td>1 (5.6)</td>
<td>0.002*</td>
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<td>Autoimmune disease</td>
<td>6 (9.4)</td>
<td>0 (0)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Allergic disease</td>
<td>19 (29.7)</td>
<td>5 (27.8)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Previous HBV infection</td>
<td>11 (17.2)</td>
<td>0 (0)</td>
<td>0.11</td>
</tr>
<tr>
<td>Previous HCV infection</td>
<td>2 (3.1)</td>
<td>0 (0)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Previous syphilis infection</td>
<td>4 (6.3)</td>
<td>0 (0)</td>
<td>0.57</td>
</tr>
<tr>
<td>RAS inhibitor</td>
<td>24 (37.5)</td>
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<td>Antihyperuricemic drug</td>
<td>14 (21.9)</td>
<td>4 (22.2)</td>
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<td>Statin</td>
<td>20 (31.3)</td>
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<td>Erythropoiesis-stimulating agent</td>
<td>56 (87.5)</td>
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<tr>
<td>HIF-PH inhibitor</td>
<td>4 (6.3)</td>
<td>0 (0)</td>
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<tr>
<td>Iron supplement</td>
<td>40 (63.5)</td>
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<tr>
<td>Zinc supplement</td>
<td>7 (10.9)</td>
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<td>Phosphate binder</td>
<td>51 (79.7)</td>
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<tr>
<td>Vitamin D analog</td>
<td>54 (84.4)</td>
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<td>&lt;0.001*</td>
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<tr>
<td>Calcimimetic</td>
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<td>0 (0)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Corticosteroid</td>
<td>3 (4.7)</td>
<td>0 (0)</td>
<td>&gt;0.99</td>
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<td>Albunim (g/dL)</td>
<td>3.6 ± 0.5</td>
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<td>NA</td>
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<td>7,136 ± 4,846</td>
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<tr>
<td>Lymphocyte count (/μL)</td>
<td>1147 ± 415</td>
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<td>NA</td>
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<td>Hemoglobin (g/dL)</td>
<td>11.0 ± 1.2</td>
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<td>NA</td>
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<td>Platelet count (×10^4/μL)</td>
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<td>NA</td>
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<td>Blood urea nitrogen (mg/dL)</td>
<td>61.0 ± 15.9</td>
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<td>NA</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>9.6 ± 2.8</td>
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<td>NA</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>138.8 ± 2.6</td>
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<tr>
<td>Potassium (mEq/L)</td>
<td>4.7 ± 0.7</td>
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<td>NA</td>
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<td>Chloride (mEq/L)</td>
<td>101.8 ± 3.3</td>
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<td>NA</td>
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<td>Total calcium (mg/dL)</td>
<td>8.3 ± 0.6</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Phosphate (mg/dL)</td>
<td>5.2 ± 1.3</td>
<td>NA</td>
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</tbody>
</table>

(Continued to the next page)
Table 1. Continued

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hemodialysis patients</th>
<th>Healthcare workers</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Magnesium (mg/dL)</td>
<td>2.5 ± 0.4</td>
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<tr>
<td>Uric acid (mg/dL)</td>
<td>6.9 ± 1.2</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Total cholesterol (mg/dL)</td>
<td>160.0 ± 38.6</td>
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</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>0.11 (0.05–0.44)</td>
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</tr>
<tr>
<td>iPTH (pg/mL)</td>
<td>178.6 ± 77.0</td>
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<td>NA</td>
</tr>
<tr>
<td>β2 microglobulin (mg/L)</td>
<td>28.4 ± 6.9</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Ferritin (ng/mL)</td>
<td>183.5 (111.5–314.8)</td>
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<td>Transferrin saturation (%)</td>
<td>30.0 ± 16.7</td>
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<td>NA</td>
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<tr>
<td>Zinc (μg/dL)</td>
<td>61.2 ± 13.4</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>5.8 ± 1.0</td>
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<td>NA</td>
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<tr>
<td>Glycoalbumin (%)</td>
<td>19.6 ± 4.3</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>nPCR (g/kg/day)</td>
<td>0.78 ± 0.23</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Single-pool Kt/V</td>
<td>1.57 ± 0.43</td>
<td>NA</td>
<td>NA</td>
</tr>
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</table>

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

HBV, hepatitis B virus; HCV, hepatitis C virus; HIF-PH, hypoxia-inducible factor prolyl hydroxylase; iPTH, intact parathyroid hormone; Kt/V, urea clearance; NA, not available; nPCR, normalized protein catabolic rate; RAS, renin-angiotensin system.

*p < 0.05.

Change in anti-SARS-CoV-2 spike antibody titers in hemodialysis patients

As shown in Fig. 2, the anti-SARS-CoV-2 spike antibody titer was significantly increased from 220 AU/mL (IQR, 134–419 AU/mL) 1 week before the third BNT162b2 mRNA COVID-19 vaccination to 18,500 AU/mL (IQR, 11,000–34,500 AU/mL) 4 weeks after the third BNT162b2 mRNA COVID-19 vaccination (p < 0.001). Thereafter, it decreased significantly to 10,355 AU/mL (IQR, 4,584–22,250 AU/mL) 12 weeks after the third BNT162b2 mRNA COVID-19 vaccination (p < 0.001); however, it was still significantly higher than at 1 week before the third BNT162b2 mRNA COVID-19 vaccination (p < 0.001).

Comparison of anti-SARS-CoV-2 spike antibody titers between the control and hemodialysis patient groups

The anti-SARS-CoV-2 spike antibody titer 1 week before the third BNT162b2 mRNA COVID-19 vaccination was significantly lower in hemodialysis patients than in healthcare workers (220 AU/mL [IQR, 134–419 AU/mL] vs. 2,626 AU/mL [IQR, 1,869–5,730 AU/mL], p < 0.001) (Fig. 2). However, there was no significant difference between the two groups 4 weeks after the third BNT162b2 mRNA COVID-19 vaccination (18,500 AU/mL [IQR, 11,000–34,500 AU/mL] vs. 11,500 AU/mL [IQR, 7,918–19,500 AU/mL], p = 0.17).

Factors associated with the anti-SARS-CoV-2 spike antibody titer after the third BNT162b2 mRNA COVID-19 vaccination

According to the simple linear regression analyses, the log-anti-SARS-CoV-2 spike antibody titer 4 weeks after the third BNT162b2 mRNA COVID-19 vaccination correlated significantly with the BMI, presence of diabetes mellitus, blood urea nitrogen, total calcium, uric acid, transferrin saturation (TSAT), normalized protein catabolism rate, and log-anti-SARS-CoV-2 spike antibody titer 1 week before the third BNT162b2 mRNA COVID-19 vaccination (Table 2). In contrast, the log-anti-SARS-CoV-2 spike antibody titer 12 weeks after the third BNT162b2 mRNA COVID-19 vaccination correlated significantly with the presence of diabetes mellitus, blood urea nitrogen, uric acid, total cholesterol, TSAT, normalized protein catabolism rate, and log-anti-SARS-CoV-2 spike antibody titer 4 weeks after the third BNT162b2 mRNA COVID-19 vaccination.
SARS-CoV-2 antibody titer 1 week before the third BNT162b2 mRNA COVID-19 vaccination (Table 3). We then conducted multiple linear regression analyses using the variables that showed significant or marginal correlations (p < 0.10) with the log-anti-SARS-CoV-2 spike antibody titers 4 and 12 weeks after the third BNT162b2 mRNA COVID-19 vaccination in the simple linear regression analyses. These analyses revealed that uric acid (standard coefficient $\beta = -0.203$, $p = 0.02$), TSAT ($\beta = -0.269$, $p = 0.003$), and log-anti-SARS-CoV-2 spike antibody titer 1 week before the third BNT162b2 mRNA COVID-19 vaccination ($\beta = 0.440$, $p < 0.001$) were correlated independently with the log-anti-SARS-CoV-2 spike antibody titer 12 weeks after the third BNT162b2 mRNA COVID-19 vaccination (Table 3).

Patient characteristics categorized by the anti-SARS-CoV-2 spike antibody response status 4 weeks after the third BNT162b2 mRNA COVID-19 vaccination

Patient characteristics categorized by the anti-SARS-CoV-2 spike antibody response status 4 weeks after the third BNT162b2 mRNA COVID-19 vaccination are shown in Table 4. The number of hemodialysis patients for each antibody response status was one for no responder (<50 AU/mL), nine for low responder (50–7,021 AU/mL), and 54 for high responder (≥7,021 AU/mL). The rate of high responders was 84% (54 of 64). Only uric acid was significantly different among these three categories ($p = 0.01$).

Figure 2. Comparison of anti-SARS-CoV-2 spike antibody titers between hemodialysis patients (n = 64) and healthcare workers (n = 18). Values are presented as the median (interquartile range). *p < 0.001, **p < 0.05. NS, not significant; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
Table 2. Simple and multiple linear regression analyses of the variables correlated with log–anti-SARS-CoV-2 spike antibody titer 4 weeks after the third BNT162b2 mRNA COVID-19 vaccination

<table>
<thead>
<tr>
<th>Variable</th>
<th>Simple linear regression analysis</th>
<th>Multiple linear regression analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard coefficient</td>
<td>p-value</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.307</td>
<td>0.01*</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.324</td>
<td>0.009*</td>
</tr>
<tr>
<td>Blood urea nitrogen</td>
<td>-0.303</td>
<td>0.02*</td>
</tr>
<tr>
<td>Total calcium</td>
<td>0.301</td>
<td>0.02*</td>
</tr>
<tr>
<td>Uric acid</td>
<td>-0.350</td>
<td>0.005*</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.227</td>
<td>0.07</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>-0.380</td>
<td>0.002*</td>
</tr>
<tr>
<td>nPCR</td>
<td>-0.301</td>
<td>0.02*</td>
</tr>
<tr>
<td>Log–anti-SARS-CoV-2 spike antibody titer 1 week before the third BNT162b2 mRNA COVID-19 vaccination</td>
<td>0.639</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

COVID-19, coronavirus disease 2019; log, logarithm; mRNA, messenger RNA; nPCR, normalized protein catabolic rate; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
*Using variables with p < 0.10 in univariate analyses.
*p < 0.05.

Table 3. Simple and multiple linear regression analyses of the variables correlated with log–anti-SARS-CoV-2 spike antibody titer 12 weeks after the third BNT162b2 mRNA COVID-19 vaccination

<table>
<thead>
<tr>
<th>Variable</th>
<th>Simple linear regression analysis</th>
<th>Multiple linear regression analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard coefficient</td>
<td>p-value</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.312</td>
<td>0.01*</td>
</tr>
<tr>
<td>Blood urea nitrogen</td>
<td>-0.295</td>
<td>0.02*</td>
</tr>
<tr>
<td>Sodium</td>
<td>-0.234</td>
<td>0.06</td>
</tr>
<tr>
<td>Total calcium</td>
<td>0.211</td>
<td>0.09</td>
</tr>
<tr>
<td>Uric acid</td>
<td>-0.332</td>
<td>0.007*</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.249</td>
<td>0.048*</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>-0.259</td>
<td>0.04*</td>
</tr>
<tr>
<td>nPCR</td>
<td>-0.267</td>
<td>0.04*</td>
</tr>
<tr>
<td>Log–anti-SARS-CoV-2 spike antibody titer 1 week before the third BNT162b2 mRNA COVID-19 vaccination</td>
<td>0.574</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

COVID-19, coronavirus disease 2019; log, logarithm; mRNA, messenger RNA; nPCR, normalized protein catabolic rate; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
*Using variables with p < 0.10 in univariate analyses.
*p < 0.05.

Discussion

In the present study, we found that the anti-SARS-CoV-2 spike antibody titer 4 weeks after the third dose of COVID-19 vaccine was comparable between hemodialysis patients and healthcare workers. We also found that the uric acid concentration, TSAT, and anti-SARS-CoV-2 spike antibody titer 1 week before the third dose of COVID-19 vaccine were correlated with the anti-SARS-CoV-2 spike antibody titer 4 weeks after the third dose of COVID-19 vaccine. In contrast, only the anti-SARS-CoV-2 spike antibody titer 1 week before the third dose of COVID-19 vaccine was correlated with the anti-SARS-CoV-2 spike antibody titer 12 weeks after the third dose of COVID-19 vaccine.

Several recent studies have reported the anti-SARS-CoV-2 spike antibody response against the third COVID-19 vaccination in hemodialysis patients [4,5]. One observational study involving 80 hemodialysis patients and 56 healthcare
### Table 4. Patient characteristics categorized by the anti-SARS-CoV-2 spike antibody response status 4 weeks after the third BNT162b2 mRNA COVID-19 vaccination

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No responder</th>
<th>Low responder</th>
<th>High responder</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>1</td>
<td>9</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>73.0</td>
<td>78.7 ± 12.1</td>
<td>70.1 ± 11.4</td>
<td>0.10</td>
</tr>
<tr>
<td>Male sex</td>
<td>1 (100)</td>
<td>4 (44.4)</td>
<td>32 (59.3)</td>
<td>0.70</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>20.9</td>
<td>19.5 ± 2.5</td>
<td>22.3 ± 4.0</td>
<td>0.14</td>
</tr>
<tr>
<td>Hemodialysis vintage (yr)</td>
<td>26.0</td>
<td>3.0 (3.0–6.0)</td>
<td>4.5 (2.0–8.8)</td>
<td>0.22</td>
</tr>
<tr>
<td>Dialysis frequency</td>
<td></td>
<td></td>
<td></td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Twice weekly</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Thrice weekly</td>
<td>1 (100)</td>
<td>9 (100)</td>
<td>54 (100)</td>
<td></td>
</tr>
<tr>
<td>Dialysis type</td>
<td></td>
<td></td>
<td></td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>0 (0)</td>
<td>3 (33.3)</td>
<td>20 (37.0)</td>
<td></td>
</tr>
<tr>
<td>Hemodiafiltration</td>
<td>1 (100)</td>
<td>6 (66.7)</td>
<td>34 (63.0)</td>
<td></td>
</tr>
<tr>
<td>Past or current smoking</td>
<td>0 (0)</td>
<td>1 (11.1)</td>
<td>11 (20.4)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>11 (20.4)</td>
<td>0.45</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1 (100)</td>
<td>4 (44.4)</td>
<td>34 (63.0)</td>
<td>0.56</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0 (0)</td>
<td>2 (22.2)</td>
<td>26 (48.1)</td>
<td>0.28</td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td>0 (0)</td>
<td>2 (22.2)</td>
<td>4 (7.4)</td>
<td>0.28</td>
</tr>
<tr>
<td>Allergic disease</td>
<td>0 (0)</td>
<td>1 (11.1)</td>
<td>18 (33.3)</td>
<td>0.48</td>
</tr>
<tr>
<td>Previous HBV infection</td>
<td>0 (0)</td>
<td>2 (22.2)</td>
<td>9 (16.7)</td>
<td>0.71</td>
</tr>
<tr>
<td>Previous HCV infection</td>
<td>0 (0)</td>
<td>1 (11.1)</td>
<td>1 (1.9)</td>
<td>0.29</td>
</tr>
<tr>
<td>Previous syphilis infection</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (7.4)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>RAS inhibitor</td>
<td>1 (100)</td>
<td>1 (11.1)</td>
<td>22 (40.7)</td>
<td>0.06</td>
</tr>
<tr>
<td>Antihyperuricemic drug</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>14 (25.9)</td>
<td>0.29</td>
</tr>
<tr>
<td>Statin</td>
<td>0 (0)</td>
<td>4 (44.4)</td>
<td>16 (29.6)</td>
<td>0.62</td>
</tr>
<tr>
<td>Erythropoiesis-stimulating agent</td>
<td>1 (100)</td>
<td>9 (100)</td>
<td>46 (85.2)</td>
<td>0.64</td>
</tr>
<tr>
<td>HIF-PH inhibitor</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (7.4)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Iron supplement</td>
<td>0 (0)</td>
<td>7 (77.8)</td>
<td>33 (62.3)</td>
<td>0.37</td>
</tr>
<tr>
<td>Zinc supplement</td>
<td>0 (0)</td>
<td>2 (22.2)</td>
<td>5 (9.3)</td>
<td>0.34</td>
</tr>
<tr>
<td>Phosphate binder</td>
<td>1 (100)</td>
<td>5 (55.6)</td>
<td>45 (83.3)</td>
<td>0.13</td>
</tr>
<tr>
<td>Vitamin D analog</td>
<td>0 (0)</td>
<td>9 (100)</td>
<td>45 (83.3)</td>
<td>0.08</td>
</tr>
<tr>
<td>Calcimimetic</td>
<td>0 (0)</td>
<td>4 (44.4)</td>
<td>20 (37.0)</td>
<td>0.83</td>
</tr>
<tr>
<td>Corticosteroid</td>
<td>0 (0)</td>
<td>1 (11.1)</td>
<td>2 (3.7)</td>
<td>0.40</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.7</td>
<td>3.4 ± 0.3</td>
<td>3.6 ± 0.5</td>
<td>0.18</td>
</tr>
<tr>
<td>White blood cell count (/μL)</td>
<td>4,780</td>
<td>5,926 ± 2,045</td>
<td>7,386 ± 5,189</td>
<td>0.34</td>
</tr>
<tr>
<td>Lymphocyte count (/μL)</td>
<td>808</td>
<td>1,052 ± 325</td>
<td>1,169 ± 430</td>
<td>0.35</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.6</td>
<td>11.3 ± 1.2</td>
<td>10.9 ± 1.3</td>
<td>0.80</td>
</tr>
<tr>
<td>Platelet count (×10⁴/μL)</td>
<td>17.9</td>
<td>20.5 ± 5.7</td>
<td>21.4 ± 6.7</td>
<td>0.75</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>84.8</td>
<td>65.8 ± 12.4</td>
<td>59.8 ± 16.1</td>
<td>0.11</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>12.3</td>
<td>9.0 ± 1.7</td>
<td>9.7 ± 2.9</td>
<td>0.35</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>141</td>
<td>139.7 ± 1.3</td>
<td>138.6 ± 2.7</td>
<td>0.30</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.8</td>
<td>4.8 ± 0.5</td>
<td>4.7 ± 0.8</td>
<td>0.99</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>103</td>
<td>103.2 ± 2.2</td>
<td>101.6 ± 3.4</td>
<td>0.29</td>
</tr>
<tr>
<td>Total calcium (mg/dL)</td>
<td>6.5</td>
<td>8.1 ± 0.5</td>
<td>8.4 ± 0.6</td>
<td>0.10</td>
</tr>
<tr>
<td>Phosphate (mg/dL)</td>
<td>5.0</td>
<td>5.4 ± 1.6</td>
<td>5.2 ± 1.3</td>
<td>0.86</td>
</tr>
<tr>
<td>Magnesium (mg/dL)</td>
<td>2.4</td>
<td>2.5 ± 0.3</td>
<td>2.5 ± 0.4</td>
<td>0.96</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>8.3</td>
<td>7.7 ± 0.7</td>
<td>6.7 ± 1.2</td>
<td>0.01*</td>
</tr>
</tbody>
</table>

(Continued to the next page)
workers in Israel showed that the anti-SARS-CoV-2 spike antibody level after the third COVID-19 vaccination was lower in hemodialysis patients than in healthcare workers [4]. Another observational study involving 350 hemodialysis patients and 130 healthcare workers in Japan showed that the anti-SARS-CoV-2 spike antibody level after the third COVID-19 vaccination was comparable between hemodialysis patients and healthcare workers [5]. In the present study, the anti-SARS-CoV-2 spike antibody titer after the third COVID-19 vaccination was comparable between 64 Japanese hemodialysis patients and 18 healthcare workers. This inconsistency among study results might be explained by a difference in the ethnicity of patients because humoral immune responses to COVID-19 vaccination were reported to vary depending on ethnicity [9]. Further studies incorporating different ethnicities are necessary to assess the efficacy of the third COVID-19 vaccination in hemodialysis patients. These recent studies have also reported the anti-SARS-CoV-2 spike antibody response status after the third dose of COVID-19 vaccine in patients undergoing hemodialysis [4,5]. The first study reported that 88% of hemodialysis patients became high responders (>1,000 AU/mL) [4]. The second study reported that 87% of hemodialysis patients became high responders (≥7,021 AU/mL) [5]. In the present study, 84% of hemodialysis patients became high responders (≥7,021 AU/mL). These results suggest that the third dose of COVID-19 vaccine substantially improved the anti-SARS-CoV-2 spike antibody response in hemodialysis patients. It was shown that the anti-SARS-CoV-2 spike antibody level peaks 3 to 4 weeks after the third COVID-19 vaccination and then declines linearly with time [10]. In the present study, the anti-SARS-CoV-2 spike antibody titer decreased by approximately 50% between 4 weeks and 12 weeks after the third COVID-19 vaccination. Further research is necessary to determine the optimal timing of anti-SARS-CoV-2 spike antibody titer measurements in hemodialysis patients for subsequent COVID-19 vaccination.

Several studies reported that the anti-SARS-CoV-2 spike antibody level before the third COVID-19 vaccination was positively associated with the anti-SARS-CoV-2 spike antibody level in hemodialysis patients after the third COVID-19 vaccination [4,6,7]. In the present study, the anti-SARS-CoV-2 spike antibody titer 1 week before the third COVID-19 vaccination was positively correlated with that at 4 and 12 weeks after the third COVID-19 vaccination, which was consistent with the results of previous reports [4,6,7]. These results indicate that the degree of humoral immunity against SARS-CoV-2 before the third COVID-19 vaccination may influence the anti-SARS-CoV-2 spike antibody response in hemodialysis patients after the third COVID-19 vaccination.

<table>
<thead>
<tr>
<th>Table 4. Continued</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
</tr>
<tr>
<td>iPTH (pg/mL)</td>
</tr>
<tr>
<td>β2 microglobulin (mg/L)</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
</tr>
<tr>
<td>Zinc (μg/dL)</td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
</tr>
<tr>
<td>Glycoalbumin (%)</td>
</tr>
<tr>
<td>nPCR (g/kg/day)</td>
</tr>
<tr>
<td>Single-pool Kt/V</td>
</tr>
</tbody>
</table>

Data are expressed as number only, mean ± standard deviation, number (%), or median (interquartile range). The anti-SARS-CoV-2 spike antibody response status was defined as follows: no responder (anti-SARS-CoV-2 spike antibody titer < 50 AU/mL), low responder (50–7,021 AU/mL), and high responder (≥7,021 AU/mL).

COVID-19, coronavirus disease 2019; HBV, hepatitis B virus; HCV, hepatitis C virus; HIF-PH, hypoxia-inducible factor prolyl hydroxylase; iPTH, intact parathyroid hormone; Kt/V, urea clearance; mRNA, messenger RNA; nPCR, normalized protein catabolic rate; RAS, renin-angiotensin system; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

*p < 0.05.
Uric acid is a poor water-soluble molecule and for those with hyperuricemia, excess uric acid can precipitate as urate crystals in the blood and tissues [11]. Urate crystals stimulate granulocytes and monocytes through the assembly and activation of the NLRP3 inflammasome complex, thereby enhancing innate immunity [12]. However, the influence of uric acid on immune responses after vaccination remains unclear [13]. To the best of our knowledge, there are no reports on the relationship between serum uric acid concentration and antibody titer after vaccination. In our study, the serum uric acid concentration correlated negatively with the anti-SARS-CoV-2 spike antibody titer and antibody response status in hemodialysis patients after the third COVID-19 vaccination. Further research is necessary to investigate the relationship between serum uric acid concentration and anti-SARS-CoV-2 spike antibody titer in hemodialysis patients after the COVID-19 vaccination.

Ferritin and TSAT are widely and commonly used indicators of iron metabolism [14]. The serum ferritin concentration reflects the amount of iron stored in the body whereas TSAT indicates the availability of iron in the body. An observational study reported that a serum ferritin level greater than 600 ng/mL was associated with higher anti-SARS-CoV-2 spike antibody levels in hemodialysis patients [15]. Another observational study reported that a higher serum ferritin level was associated with lower anti-SARS-CoV-2 spike antibody levels in hemodialysis patients [16]. In the present study, no significant association was observed between the serum ferritin concentration and anti-SARS-CoV-2 spike antibody titer whereas TSAT was correlated negatively with the anti-SARS-CoV-2 spike antibody titer in hemodialysis patients after the third COVID-19 vaccination. Therefore, the relationship between iron metabolism and anti-SARS-CoV-2 spike antibody response is still controversial. Further research is necessary to elucidate the influence of iron metabolism on the anti-SARS-CoV-2 spike antibody response in hemodialysis patients after the COVID-19 vaccination.

This study has several advantages compared with previous studies [4–7]. First, we assessed the anti-SARS-CoV-2 spike antibody titers 1 week before and 4 and 12 weeks after the third COVID-19 vaccination. Second, we analyzed various clinical and demographic parameters associated with the anti-SARS-CoV-2 spike antibody titer after the third dose of COVID-19 vaccine in Asian individuals undergoing hemodialysis. The results of the present study might be useful for further studies to investigate the factors associated with the anti-SARS-CoV-2 spike antibody response to the third COVID-19 vaccination.

Several study limitations should be addressed. First, the patients were recruited from a single institution, which limits the external validity of the results. Second, the number of participants was low and this decreased the statistical power for detecting between-group differences. Third, patients with asymptomatic COVID-19 might have been included in this study although we excluded patients who developed COVID-19. Fourth, the anti-SARS-CoV-2 spike antibody titers were not compared between control and hemodialysis patient groups 12 weeks after the third COVID-19 vaccination, because we could not obtain the anti-SARS-CoV-2 spike antibody titer in healthcare workers 12 weeks after the third COVID-19 vaccination. Fifth, we did not assess clinical outcomes such as COVID-19 infection, hospitalization, and death, although there are several reports focusing on clinical outcomes as well as serology [17,18]. Therefore, large-scale multicenter studies incorporating an appropriate control group are necessary to validate the present study findings and the clinical effectiveness of COVID-19 vaccine in hemodialysis patients.

In conclusion, the anti-SARS-CoV-2 spike antibody titer after the third dose of COVID-19 vaccine was comparable between hemodialysis patients and healthcare workers. The uric acid concentration, TSAT, and anti-SARS-CoV-2 spike antibody titer before the third dose of COVID-19 vaccine were associated with the anti-SARS-CoV-2 spike antibody titer in hemodialysis patients after the third dose of COVID-19 vaccine.

Conflicts of interest

All authors have no conflicts of interest to declare.

Acknowledgments

We thank all medical staff members of the Mizue Yuai Clinic for their wonderful medical care and support. We thank J. Ludovic Croxford, PhD and Charles Allan, PhD, from Edanz for editing a draft of this manuscript.
Data sharing statement

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

Authors’ contributions

Conceptualization, Methodology: KH, MS
Data curation, Formal analysis, Investigation: MS, TO
Writing—original draft: KH
Writing—review & editing: SO, YM
All authors read and approved the final manuscript.

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References

Mortality associated with the neutrophil-lymphocyte ratio in septic acute kidney injury requiring continuous renal replacement therapy

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Introduction

Acute kidney injury (AKI) is a pivotal factor of increased mortality among critically ill patients admitted to the intensive care unit (ICU) [1–3]. Continuous renal replacement therapy (CRRT) is a rescue treatment option for patients with unstable vital signs and severe AKI. The number of severe AKI cases requiring CRRT has increased to more than 150,000 over several decades in the United States [4]. Despite advances in CRRT, its clinical outcomes owing to...
AKI remain negative [3,5,6]. Even though guidelines exist for CRRT implementation [7–9], CRRT-associated complications can occur, and performing CRRT does not always guarantee a survival benefit [10–12].

Sepsis is one of the most important causes of AKI in ICU patients, accounting for 15% to 20% of renal replacement therapy prescriptions [13,14]. Septic AKI has been associated with short-term mortality, subsequent progression to chronic kidney disease, end-stage renal disease, and increased long-term mortality [15,16]. Unfortunately, most patients with septic AKI are accompanied by many other risk factors [13,14], which make accurate and timely diagnosis difficult. Therefore, the ability to assess the severity of sepsis and initiate CRRT at an optimal time is important for improving survival in patients with septic AKI.

The neutrophil-lymphocyte ratio (NLR), a maker of systemic inflammation and immune response, has been actively studied worldwide to elucidate its diagnostic and prognostic efficacy in various disease conditions including malignancy, cardiovascular disease, diabetes, and autoimmune disease [17–20]. Also, NLR has been suggested as a useful marker of AKI and adverse clinical outcomes in AKI patients [21,22]. However, the association between NLR and clinical outcomes in septic AKI, particularly in those who require CRRT, is quite limited. Herein, we aimed to examine how NLR is associated with mortality outcomes in patients with septic AKI requiring CRRT.

**Methods**

**Patients and data collection**

This research was designed as a retrospective, observational, and single-center study. It was approved by the Institutional Review Board of Seoul National University Hospital (No. H-2110-085-1262) and complied with the Declaration of Helsinki. The requirement for informed consent was waived under approval.

A total of 2,397 patients undergoing CRRT because of severe AKI were retrospectively reviewed at the Seoul National University Hospital from June 2010 to December 2020. In this study, we excluded patients aged <18 years (n = 24) and with a diagnosis of end-stage kidney disease (n = 91) or without a diagnosis of sepsis (n = 1,378) at the time of starting CRRT. Although 904 patients were eligible for the primary outcome analysis, patients without sufficient information about the neutrophil and lymphocyte count (n = 106) were excluded.

Baseline data, such as age, sex, weight, ICU division, use of inotropes, application of mechanical ventilator, type of central catheter, setting of CRRT (e.g., blood flow rate, target dose, and ultrafiltration), presence of anuria, and blood cell counts, were collected. Illness severity was assessed using the Charlson Comorbidity Index (CCI) [23], Sequential Organ Failure Assessment (SOFA) [24], and Acute Physiology Assessment and Chronic Health Evaluation (APACHE) II [25]. We retrieved the results of blood cell counts measured within 48 hours before starting CRRT; the result measured at the point closest to the start of CRRT was used for NLR calculation.

Since steroid use can affect not only the blood counts of neutrophils and lymphocytes but also their ratios [26,27], information on steroid use was collected based on the equivalent dose of intravenous (IV) hydrocortisone [28]. The primary outcome of this study was in-hospital mortality after starting CRRT.

**Diagnostic criteria for infections**

Septic AKI was defined as the occurrence of AKI within 7 days of sepsis diagnosis. AKI and sepsis were diagnosed according to Kidney Disease ImprovingGlobal Outcome (KIDIGO) criteria and Sepsis-3 criteria, respectively. The primary care physicians clinically diagnosed the infections, and then the definition of infection was referenced in the International Sepsis Forum Consensus Conference guidelines since 2005 [29]. The diagnosis of pneumonia required high clinical suspicion, including radiographic infiltration, fever or hypothermia, leukocytosis or leukopenia, and purulent respiratory secretions. Urinary tract infection was diagnosed based on typical symptoms and signs such as fever, dysuria, urgency, frequency, suprapubic tenderness, pyuria, bacteriuria, and suggestive imaging. Urine cultures were considered positive with the isolation of >10^5 colony-forming units (CFU)/mL of microorganisms (or 10^5 CFU/mL in catheterized patients). Intraabdominal infections included intraabdominal abscesses, peritonitis, biliary tract infections, pancreatic infections, enteritis, and colitis. Skin and soft tissue infections included cellulitis, necrotizing fasciitis, cutaneous gangrene, and surgical site...
infections. Infective endocarditis was diagnosed adherent to the revised Duke criteria. Patients were regarded as culture-positive if etiologic pathogens were obtained from blood or pleural fluid. In other cases, patients were considered culture-positive if semiquantitative cultures of sputum, blind endotracheal aspirates, or bronchoalveolar lavage detected moderate to heavy growths of bacteria with few epithelial cells on Gram stain examination (≤10 per high-power field). In diagnosing bacteremia, common contaminating organisms such as coagulase-negative staphylococci, *Bacillus* species, *Corynebacterium* species, micrococci, and *Propionibacterium* species were ignored unless considered clinically important by the primary care physicians or cultured from two or more blood sets. In this study, the diagnosis of culture-positive catheter-related bacteremia required a positive peripheral blood culture, while culture-negative catheter-related bacteremia was diagnosed clinically in the presence of purulent discharge, exit site, or tunnel tract infection.

**Clinical management**

Treatment of patients in the ICU was at the physician’s discretion, and physicians were recommended to follow the guidelines of the up-latest Survival Sepsis Campaign [30,31]. Although the treatments were not protocolized, they included active fluid resuscitation and vasopressors with hemodynamic data obtained via lactate and N-terminal B-type natriuretic peptide measurements, transthoracic echocardiography, and arterial pressure waveform analyses when indicated. An early intubation strategy was encouraged in impending respiratory failure. Blood cultures were drawn early with 20 mL of blood equally injected for each set of aerobic and anaerobic media, while cultures of other sites were performed based on the focus of infection. Empirical broad-spectrum antibiotics were chosen considering the suspected pathogen and were optimized and/or de-escalated according to the culture results.

The implementation of CRRT because of severe AKI was adherent to the KDIGO guideline [8]. To deliver 20 to 25 mL/kg/hour, we conducted CRRT with a target dose of 30 to 35 mL/kg/hour in accordance with the KDIGO guideline [8], and for some patients with acute respiratory distress syndrome, the target dose was raised up to 40 mL/kg/hour.

**Statistical analysis**

Baseline characteristics are described as proportions and means ± standard deviations when categorical and continuous variables are normally distributed and as medians with interquartile ranges when they are not normally distributed. The normality of the distribution was analyzed using the Kolmogorov-Smirnov test. The chi-square test or Fisher exact test was employed to compare categorical variables. The Student t test or Mann-Whitney U test was used for continuous variables with or without a normal distribution, respectively.

We determined NLRs 6 and 18 as the criteria for dividing NLRs into three groups based on the tertiles of the NLR values observed within our study cohort. All statistical analyses were performed on these three NLR groups. The criteria of 6 and 18 were set slightly higher than those in previous studies, considering that steroids are commonly used in sepsis and reactive neutrophilia accompanied by lymphopenia is also common. We tested the proportional hazard assumption using the Schoenfeld test. The Kaplan-Meier survival curves were drawn, and differences in the curves were determined using a log-rank test. Because many baseline parameters differed between groups, propensity score-based matching with inverse probability treatment weighting was additionally performed. All baseline variables, such as age, sex, weight, ICU division, use of inotropes, application of mechanical ventilator, type of central catheter, blood flow rate, target dose, target ultrafiltration, presence of anuria, CCI, SOFA score, APACHE II score, and IV hydrocortisone equivalent dose, were included for calculating propensity scores. Finally, a two-way analysis of variance was performed to evaluate the effect modification of NLR on the all-cause mortality in each subgroup. A two-tailed p-value of <0.05 was considered statistically significant. All statistical analyses were performed using R software (version 4.1.2; R Foundation for Statistical Computing).

**Results**

**Data collection and baseline characteristics**

A total of 798 patients with septic AKI requiring CRRT were included for survival analysis (Fig. 1), and they were classi-
fied into three NLR groups: NLR of <6, ≥6 and <18, and ≥18 (277, 115, and 406 patients, respectively) (Table 1). Among the 798 participants, the mean age was 64.2 ± 14.5 years, and 63.0% of the patients were male. Moreover, 58.4% of the patients were hospitalized in the medical ICU. Half of the patients used inotropes, and approximately 80% were supported by mechanical ventilation. The mean SOFA and APACHE II scores were 12.8 ± 3.7 and 27.4 ± 7.7, respectively. Both SOFA and APACHE II scores were highest in the group with NLR ≥18, with 13.7 ± 3.3 and 27.8 ± 7.4, respectively. Among all participants, 67.2% used steroids at least once. The group with NLR <6 had the highest percentage of steroid-using patients and the highest daily dose of IV hydrocortisone equivalent, with 81.9% and 200 mg, respectively, whereas the group with NLR ≥6 and <18 had the lowest percentage. While the percentage of patients with bacteremia confirmed by positive blood cultures did not differ significantly amongst the three NLR groups, it is interesting to note that there were some distinct patterns in the infection sources across each NLR group. In the group with NLR <6, there was a higher incidence of infections linked to features of hematologic, oncologic, and immunocompromised conditions, including instances of neutropenic septic shock. On the other hand, the group with NLR >18 demonstrated the highest prevalence of pneumonia and intraabdominal infections. Significant differences were noted between the three groups regarding the ICU division, SOFA score, underlying diabetes mellitus, steroid use, and more. Figure 1. Flow diagram of study subject selection. AKI, acute kidney injury.

**Table 1.** Baseline characteristics of the patients according to the neutrophil-lymphocyte ratios

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>&lt;6</th>
<th>≥6, &lt;18</th>
<th>≥18</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>798</td>
<td>277</td>
<td>115</td>
<td>406</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>64.2 ± 14.5</td>
<td>64.0 ± 14.9</td>
<td>65.7 ± 13.1</td>
<td>65.1 ± 11.6</td>
<td>0.62</td>
</tr>
<tr>
<td>Male sex</td>
<td>503 (63.0)</td>
<td>177 (63.9)</td>
<td>73 (63.5)</td>
<td>228 (56.2)</td>
<td>0.37</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.0 ± 4.4</td>
<td>23.0 ± 4.3</td>
<td>23.2 ± 4.7</td>
<td>22.9 ± 4.4</td>
<td>0.79</td>
</tr>
<tr>
<td>ICU division</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.02*</td>
</tr>
<tr>
<td>MICU</td>
<td>466 (58.4)</td>
<td>166 (59.9)</td>
<td>48 (41.7)</td>
<td>210 (51.7)</td>
<td></td>
</tr>
<tr>
<td>SICU</td>
<td>187 (20.9)</td>
<td>53 (19.1)</td>
<td>31 (27.0)</td>
<td>132 (32.5)</td>
<td></td>
</tr>
<tr>
<td>CPICU</td>
<td>140 (17.5)</td>
<td>50 (18.1)</td>
<td>28 (24.3)</td>
<td>51 (12.6)</td>
<td></td>
</tr>
<tr>
<td>EICU</td>
<td>21 (2.6)</td>
<td>7 (2.5)</td>
<td>8 (7.0)</td>
<td>9 (2.2)</td>
<td></td>
</tr>
<tr>
<td>DICU</td>
<td>4 (0.5)</td>
<td>1 (0.4)</td>
<td>0 (0.0)</td>
<td>4 (1.0)</td>
<td></td>
</tr>
</tbody>
</table>

(Continued to the next page)
<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>&lt;6</th>
<th>≥6, &lt;18</th>
<th>≥18</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inotropics use</td>
<td>388 (48.6)</td>
<td>135 (48.7)</td>
<td>56 (48.7)</td>
<td>192 (47.3)</td>
<td>0.96</td>
</tr>
<tr>
<td>Mechanical ventilator</td>
<td>622 (77.9)</td>
<td>219 (79.1)</td>
<td>81 (70.4)</td>
<td>292 (71.9)</td>
<td>0.15</td>
</tr>
<tr>
<td>Catheter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.85</td>
</tr>
<tr>
<td>Intrajugular</td>
<td>424 (53.1)</td>
<td>145 (52.3)</td>
<td>62 (53.9)</td>
<td>237 (58.4)</td>
<td></td>
</tr>
<tr>
<td>Femoral</td>
<td>280 (35.1)</td>
<td>98 (35.4)</td>
<td>42 (36.5)</td>
<td>128 (31.5)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>94 (11.8)</td>
<td>34 (12.3)</td>
<td>11 (9.6)</td>
<td>41 (10.1)</td>
<td></td>
</tr>
<tr>
<td>Blood flow rate (mL/min)</td>
<td>113.9 ± 24.8</td>
<td>113.9 ± 25.1</td>
<td>110.5 ± 22.8</td>
<td>115.4 ± 23.6</td>
<td>0.58</td>
</tr>
<tr>
<td>Target dose (mL/kg/hr)</td>
<td>46.7 ± 18.8</td>
<td>46.5 ± 18.7</td>
<td>48.5 ± 18.9</td>
<td>47.6 ± 19.3</td>
<td>0.71</td>
</tr>
<tr>
<td>Target ultrafiltration (mL/day)</td>
<td>0 (0–500)</td>
<td>0 (0–500)</td>
<td>0 (0–700)</td>
<td>0 (0–500)</td>
<td>0.43</td>
</tr>
<tr>
<td>CCI score</td>
<td>3 (2–4)</td>
<td>3 (2–4)</td>
<td>3 (1–3)</td>
<td>3 (2–4)</td>
<td>0.32</td>
</tr>
<tr>
<td>SOFA score</td>
<td>12.8 ± 3.7</td>
<td>12.7 ± 3.7</td>
<td>12.7 ± 3.8</td>
<td>13.7 ± 3.3</td>
<td>0.03*</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>27.4 ± 7.7</td>
<td>27.3 ± 7.8</td>
<td>26.5 ± 7.2</td>
<td>27.8 ± 7.4</td>
<td>0.64</td>
</tr>
<tr>
<td>Co-morbidities (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>284 (35.6)</td>
<td>84 (30.3)</td>
<td>44 (38.3)</td>
<td>156 (38.4)</td>
<td>0.08</td>
</tr>
<tr>
<td>Hypertension</td>
<td>246 (30.8)</td>
<td>73 (26.4)</td>
<td>45 (39.1)</td>
<td>127 (31.3)</td>
<td>0.04*</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>93 (11.7)</td>
<td>22 (7.9)</td>
<td>16 (13.9)</td>
<td>55 (13.5)</td>
<td>0.06</td>
</tr>
<tr>
<td>Steroid use</td>
<td>536 (67.2)</td>
<td>227 (81.9)</td>
<td>53 (46.1)</td>
<td>256 (63.1)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>IV hydrocortisone equivalent (mg/day)</td>
<td>200 (0–100)</td>
<td>200 (0–100)</td>
<td>25 (0–200)</td>
<td>150 (0–200)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Infection source</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Respiratory</td>
<td>247 (31.0)</td>
<td>68 (24.5)</td>
<td>34 (29.6)</td>
<td>145 (35.7)</td>
<td></td>
</tr>
<tr>
<td>Intraabdominal</td>
<td>242 (30.3)</td>
<td>60 (21.7)</td>
<td>40 (34.8)</td>
<td>142 (35.0)</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>184 (23.1)</td>
<td>118 (42.6)</td>
<td>18 (15.7)</td>
<td>48 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Skin/soft tissue/bone</td>
<td>47 (5.9)</td>
<td>8 (2.9)</td>
<td>7 (6.1)</td>
<td>32 (7.9)</td>
<td></td>
</tr>
<tr>
<td>Hematologic/oncologic/immunocompromised</td>
<td>38 (4.8)</td>
<td>13 (4.7)</td>
<td>5 (4.3)</td>
<td>20 (4.9)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>28 (3.5)</td>
<td>6 (2.2)</td>
<td>8 (7.0)</td>
<td>14 (3.4)</td>
<td></td>
</tr>
<tr>
<td>Source unknown/not specified</td>
<td>12 (1.5)</td>
<td>4 (1.4)</td>
<td>3 (2.6)</td>
<td>5 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Total WBC (&gt;10^3/μL)</td>
<td>14.8 ± 23.2</td>
<td>10.9 ± 30.5</td>
<td>15.5 ± 19.1</td>
<td>17.1 ± 17.6</td>
<td>0.003*</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>9.5 ± 2.3</td>
<td>9.4 ± 2.4</td>
<td>9.5 ± 2.6</td>
<td>9.6 ± 2.2</td>
<td>0.55</td>
</tr>
<tr>
<td>Platelet (&gt;10^9/μL)</td>
<td>1078 ± 98.7</td>
<td>82.7 ± 76.4</td>
<td>135.2 ± 117.6</td>
<td>117.1 ± 102.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>72.1 ± 27.4</td>
<td>46.3 ± 29.5</td>
<td>78.0 ± 14.2</td>
<td>87.9 ± 10.5</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>14.0 ± 19.7</td>
<td>29.9 ± 25.9</td>
<td>9.3 ± 4.8</td>
<td>4.5 ± 6.0</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>5.4 ± 6.5</td>
<td>6.6 ± 8.7</td>
<td>6.5 ± 6.7</td>
<td>4.2 ± 4.0</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Absolute neutrophil count (/μL)</td>
<td>12,284.6 ± 15,376.1</td>
<td>4,591.9 ± 9,935.6</td>
<td>12,291.1 ± 15,959.8</td>
<td>15,546.8 ± 16,640.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.5 ± 0.6</td>
<td>2.4 ± 0.6</td>
<td>2.6 ± 0.6</td>
<td>2.6 ± 0.6</td>
<td>0.02*</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>16.6 ± 11.3</td>
<td>18.1 ± 12.1</td>
<td>15.4 ± 10.9</td>
<td>16.0 ± 10.9</td>
<td>0.027*</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>2.8 ± 2.0</td>
<td>2.4 ± 1.3</td>
<td>3.1 ± 1.9</td>
<td>3.1 ± 2.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Estimated GFR (mL/min/1.73 m^2)</td>
<td>30.9 ± 22.9</td>
<td>35.9 ± 25.0</td>
<td>29.0 ± 21.9</td>
<td>28.0 ± 21.1</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>136.3 ± 7.8</td>
<td>138.0 ± 7.6</td>
<td>136.4 ± 8.1</td>
<td>135.2 ± 7.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.5 ± 1.0</td>
<td>4.4 ± 1.0</td>
<td>4.6 ± 1.0</td>
<td>4.6 ± 1.0</td>
<td>0.03*</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>102.3 ± 8.4</td>
<td>103.5 ± 8.3</td>
<td>102.3 ± 8.5</td>
<td>101.5 ± 8.3</td>
<td>0.009*</td>
</tr>
<tr>
<td>HCO_3– (mmol/L)</td>
<td>16.9 ± 5.3</td>
<td>16.0 ± 4.9</td>
<td>17.2 ± 5.7</td>
<td>17.5 ± 5.3</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

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

APACHE, acute physiologic and chronic health evaluation; CCI, Charlson Comorbidity Index; CPICU, cardio-pulmonary intensive care unit; DICU, disaster intensive care unit for coronavirus disease 2019 infection; EICU, emergency intensive care unit; GFR, glomerular filtration rate; ICU, intensive care unit; IV, intravenous; MICU, medical intensive care unit; SICU, surgical intensive care unit; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell.

*p < 0.05, statistically significant.
IV hydrocortisone equivalent dose, and infection source. The baseline characteristics of each group after propensity score-based matching with all variables are shown in Supplementary Table 1 (available online).

**Association between neutrophil-lymphocyte ratio and survival**

During a median follow-up period of 10 days (interquartile range, 3–28 days), 603 patients (75.6%) died. The incidence of mortality was 29.4 deaths per 1,000 person-days (Table 2). The in-hospital mortality rates of the groups with NLR <6, ≥6 and <18, and ≥18 were 83.4%, 74.8%, and 70.4%, respectively (p < 0.001). All kinds of mortality rates were better in the group with NLR ≥18 than in the other two groups. The survival curves of the three groups revealed significantly different curve trends (p < 0.001) and the survival probability was higher in the group with NLR ≥18 than in the other two groups (Fig. 2A). When all variables were adjusted in model 4, the group with NLR ≥18 was associated with a 26.4% reduction in mortality based on hazard ratio values, compared with the group with NLR <6 (Table 3). However, there was no significant difference in mortality rates between the group with NLR <6 and the group with NLR ≥6 and <18 in the fully adjusted model 4. In the univariable Cox survival analysis, performed after propensity

**Table 2. Mortality outcomes according to the neutrophil-lymphocyte ratios**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (n = 798)</th>
<th>Neutrophil-lymphocyte ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;6 (n = 277)</td>
<td>≥6, &lt;18 (n = 115)</td>
<td>≥18 (n = 406)</td>
</tr>
<tr>
<td>On CRRT mortality</td>
<td>490 (61.4)</td>
<td>195 (70.4)</td>
<td>70 (60.9)</td>
</tr>
<tr>
<td>ICU mortality</td>
<td>533 (66.8)</td>
<td>207 (74.7)</td>
<td>75 (65.2)</td>
</tr>
<tr>
<td>In-hospital mortality</td>
<td>604 (75.7)</td>
<td>231 (83.4)</td>
<td>86 (74.8)</td>
</tr>
</tbody>
</table>

Data are expressed as number (%).

*p < 0.05, statistically significant.

**Figure 2. Kaplan-Meier survival curves.** (A) Kaplan-Meier survival curves of three different neutrophil-lymphocyte ratio (NLR) tertile groups before propensity score matching. (B) Kaplan-Meier survival curves of three different NLR tertial groups after propensity score matching.

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In this study, we identified that a higher NLR was associated with a lower mortality rate, even after propensity score matching and adjustment for the IV hydrocortisone equivalent dose in the multivariable Cox survival analysis. This is inconsistent with many previous studies dealing with various diseases such as cancer, cardiovascular disease, autoimmune disease, and infection. In those studies, a high NLR was associated with poor prognosis and showed predictive power in diagnosing diseases [17–20]. For instance, Sarraf et al. [17] reported that increasing preoperative NLRs were associated with a more advanced cancer stage but remained an independent predictor of survival after complete resection for primary lung cancer. Moreover, Huang et al. [18] stated that an increased NLR was significantly associated with diabetic nephropathy, and high NLR values might be a reliable predictive marker of early-stage diabetic nephropathy. In a meta-analysis performed on systemic lupus erythematosus (SLE), NLR was significantly higher in patients with SLE compared with healthy controls and was positively correlated with the SLE disease activity index, suggesting that NLRs can be useful biomarkers in the management of SLE [19]. Even in the context of infections like sepsis, elevated NLRs were observed in the early phase of sepsis, and NLRs could be helpful biomarker in predicting the prognosis and be employed to discontinue antimicro-

### Table 3: Relationship between all-cause mortality and NLRs

<table>
<thead>
<tr>
<th>Model</th>
<th>NLR</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>&lt;6</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥6, &lt;18</td>
<td>0.71 (0.54–0.92)</td>
<td>0.01*</td>
</tr>
<tr>
<td></td>
<td>≥18</td>
<td>0.63 (0.52–0.76)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Model 2</td>
<td>&lt;6</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥6, &lt;18</td>
<td>0.71 (0.55–0.93)</td>
<td>0.01*</td>
</tr>
<tr>
<td></td>
<td>≥18</td>
<td>0.62 (0.52–0.75)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Model 3</td>
<td>&lt;6</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥6, &lt;18</td>
<td>0.86 (0.62–1.19)</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>≥18</td>
<td>0.66 (0.51–0.85)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Model 4</td>
<td>&lt;6</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥6, &lt;18</td>
<td>0.92 (0.70–1.20)</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>≥18</td>
<td>0.74 (0.61–0.89)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

CI, confidence interval; HR, hazard ratio; NLR, neutrophil-lymphocyte ratio.

*Unadjusted. #Adjusted for age and sex. *Model 2 plus weight, intensive care unit division, presence of anuria, Charlson Comorbidity Index, Sequential Organ Failure Assessment score, and Acute Physiology Assessment and Chronic Health Evaluation II. #Model 3 plus use of inotropes and mechanical ventilation, catheter type, blood flow rate, target dose, target ultrafiltration, and intravenous hydrocortisone equivalent.

*p < 0.05, statistically significant.

Among these factors, changes in circulating white blood cells, particularly neutrophilia with relative lymphocytopenia, are known to be associated with the degree of systemic inflammatory response [17–20]. Moreover, because routine hematological tests, such as complete blood count, are performed regularly for patients admitted to ICU, NLR is easy to calculate and apply in real-world practice.

In this study, we identified that a higher NLR was associated with a lower mortality rate, even after propensity score matching and adjustment for the IV hydrocortisone equivalent dose in the multivariable Cox survival analysis. This is inconsistent with many previous studies dealing with various diseases such as cancer, cardiovascular disease, autoimmune disease, and infection. In those studies, a high NLR was associated with poor prognosis and showed predictive power in diagnosing diseases [17–20]. For instance, Sarraf et al. [17] reported that increasing preoperative NLRs were associated with a more advanced cancer stage but remained an independent predictor of survival after complete resection for primary lung cancer. Moreover, Huang et al. [18] stated that an increased NLR was significantly associated with diabetic nephropathy, and high NLR values might be a reliable predictive marker of early-stage diabetic nephropathy. In a meta-analysis performed on systemic lupus erythematosus (SLE), NLR was significantly higher in patients with SLE compared with healthy controls and was positively correlated with the SLE disease activity index, suggesting that NLRs can be useful biomarkers in the management of SLE [19]. Even in the context of infections like sepsis, elevated NLRs were observed in the early phase of sepsis, and NLRs could be helpful biomarker in predicting the prognosis and be employed to discontinue antimicro-

### Table 4: Comparison of the mortality risk according to the NLRs after propensity score matching

<table>
<thead>
<tr>
<th>Matching method</th>
<th>NLR</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPTW-LR</td>
<td>&lt;6</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥6, &lt;18</td>
<td>0.95 (0.72–1.25)</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>≥18</td>
<td>0.75 (0.61–0.93)</td>
<td>0.007*</td>
</tr>
<tr>
<td>IPTW-XGboost</td>
<td>&lt;6</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥6, &lt;18</td>
<td>0.95 (0.71–1.26)</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>≥18</td>
<td>0.77 (0.62–0.95)</td>
<td>0.01*</td>
</tr>
</tbody>
</table>

CI, confidence interval; HR, hazard ratio; IPTW, inverse probability treatment weighting; LR, logistic regression; NLR, neutrophil-lymphocyte ratio; XGboost, extreme gradient boosting.

*p < 0.05, statistically significant.

Discussion

Because patients with septic AKI who require CRRT are in a critical condition [13,14], clinicians should consider the patient’s status comprehensively, including vital signs, biochemical results, imaging tests, and medical history.
Several previous studies that explored the relationship between NLR and AKI showed a prevailing trend that higher NLRs with unfavorable outcomes in AKI patients [21,22,32]. Chen et al. [21] presented a J-shaped relationship between NLR and a composite outcome including stage 3 AKI, the need for dialysis, or 14-day in-hospital mortality with the lowest odds ratio observed for an NLR between 7 and 38. Further, Wei et al. [22] highlighted that an NLR exceeding 8.69 upon hospital admission corresponded to increased mortality and disease severity in septic AKI patients. Similarly, Gameiro et al. [32] revealed that an elevated neutrophils to lymphocytes and platelets (N/LP) ratio upon ICU admission was an independent risk factor for in-hospital mortality among septic AKI patients. This study included patients with septic AKI admitted to the ICU with or without dialysis and showed that mortality was particularly high in patients with N/LP >14, but the area under the curve for predicting mortality in septic AKI was relatively low at 0.565 [32]. These results suggest that

![Figure 3. Forest plot for subgroup analysis](image-url)

**Figure 3. Forest plot for subgroup analysis.** Event is all-cause mortality and 1st + 2nd NLR tertiles are reference (vs. 3rd NLR tertile). APACHE, acute physiologic and chronic health evaluation; CI, confidence interval; CPICU, cardio-pulmonary intensive care unit; EICU, emergency intensive care unit; HR, hazard ratio; ICU, intensive care unit; MICU, medical intensive care unit; NLR, neutrophil-lymphocyte ratio; SICU, surgical intensive care unit; SOFA, Sequential Organ Failure Assessment.

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>HR (95% CI)</th>
<th>p for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;65</td>
<td>0.63 (0.48–0.82)</td>
<td>0.71</td>
</tr>
<tr>
<td>≥65</td>
<td>0.63 (0.49–0.81)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.63 (0.49–0.81)</td>
<td>0.08</td>
</tr>
<tr>
<td>Female</td>
<td>0.74 (0.58–0.94)</td>
<td></td>
</tr>
<tr>
<td>ICU division</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MICU</td>
<td>0.59 (0.47–0.74)</td>
<td>0.29</td>
</tr>
<tr>
<td>SICU</td>
<td>0.89 (0.55–1.45)</td>
<td></td>
</tr>
<tr>
<td>CPICU</td>
<td>2.99 (0.36–24.7)</td>
<td></td>
</tr>
<tr>
<td>EICU</td>
<td>0.49 (0.32–0.75)</td>
<td></td>
</tr>
<tr>
<td>SOFA Score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st tertile</td>
<td>0.87 (0.52–1.43)</td>
<td></td>
</tr>
<tr>
<td>2nd tertile</td>
<td>0.66 (0.48–0.91)</td>
<td>0.29</td>
</tr>
<tr>
<td>3rd tertile</td>
<td>0.71 (0.55–0.92)</td>
<td></td>
</tr>
<tr>
<td>APACHE II score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st tertile</td>
<td>0.77 (0.51–1.18)</td>
<td>0.21</td>
</tr>
<tr>
<td>2nd tertile</td>
<td>0.51 (0.36–0.72)</td>
<td></td>
</tr>
<tr>
<td>3rd tertile</td>
<td>0.83 (0.64–1.09)</td>
<td></td>
</tr>
<tr>
<td>Steroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0.71 (0.46–1.07)</td>
<td>0.53</td>
</tr>
<tr>
<td>Yes</td>
<td>0.67 (0.55–0.83)</td>
<td></td>
</tr>
</tbody>
</table>
higher NLR is primarily associated with poorer prognosis in AKI patients \([21,22,32]\). In this cohort of patients with septic AKI who received CRRT, a higher NLR before CRRT initiation was associated with a higher survival rate, which is contrary to the previous study results \([17–22,32]\). These conflicting findings are likely due to the different time points for measuring NLR in each study, different inclusion criteria of the study population, and institutional-specific factors like clinical practice and patient features.

There have been several experimental rationales that CRRT has an advantage in removing cytokines and endotoxins \([33,34]\). In addition, it is well established that endotoxins and cytokines such as interleukin (IL) 1, IL-6, and tumor necrosis factor alpha are associated with biochemical markers such as C-reactive protein and procalcitonin \([35]\), as well as clinical features such as mortality, hospitalization, and hospital length of stay \([36]\). Although all subjects enrolled in this study were classified as septic AKI, the actual etiology of AKI was not solely due to sepsis. Therefore, in the high-NLR status, the burden of cytokines and endotoxins would be relatively higher, and CRRT might have a more beneficial effect on clinical outcomes by removing larger amounts of cytokines and endotoxins.

To date, there have been several observational retrospective trials showing that early CRRT results in increased survival of patients with severe septic AKI \([37,38]\). The beneficial effect of early CRRT initiation was inconsistently found in randomized controlled trials \([35,39]\). In most of those studies, serum creatinine elevation and oliguria were used as the criteria for early initiation of CRRT. However, in early-stage AKI, a nearly 50% reduction in creatinine clearance is required for a significant increase in the serum creatinine concentration \([40]\). Therefore, the serum creatinine concentration would be inappropriate as a sensitive indicator for diagnosing early-stage AKI. We suggest that monitoring the NLR alongside oliguria and azotemia could be helpful in predicting the prognosis of patients with severe septic AKI when contemplating the implementation of CRRT.

The strengths of the present study include robust statistical analysis without missing values. Additionally, although baseline SOFA and APACHE II scores were higher in the group with a higher NLR, they showed better survival outcomes, strongly supporting the hypothesis of this study. However, there are several limitations to be discussed. Since the study design was retrospective, the results could not imply causality between high NLRs and survival outcomes. Even though we used matching techniques to overcome selection bias and other problems, they might still have persisted. Specifically, the association between changing patterns of NLRs and survival outcomes remains unknown, as the NLR was not continuously measured after CRRT initiation. Because we did not measure the amount of cytokines or endotoxins removed before and after CRRT, it remains unclear whether the high survival rate associated with a high NLR is a consequence of more cytokine and endotoxin removal. Also, even though steroid use was adjusted with an IV hydrocortisone equivalent dose, the effect of steroids on the NLR could not be completely controlled. Finally, there is an important consideration that the lowest NLR group had a significantly higher proportion of patients on immunosuppressants or chemotherapy, which may have affected our results.

In conclusion, a high NLR \((\geq 18)\) is associated with better clinical outcomes, such as low mortality, particularly in septic AKI patients requiring CRRT. Although we do not directly advocate for initiating CRRT based solely on NLR values from our findings, we believe that these associations offer valuable insights into the potential clinical utility of NLR. We eagerly anticipate further studies on NLR to explore potential causal relationships and determine the best strategies for employing NLR to enhance patient outcomes.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Data sharing statement**

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

**Authors’ contributions**

Conceptualization, Methodology: JL, JS, YCK
Formal analysis: JL, JS, SGK, DY, MWK
Investigation: DKK, KHO, KWJ, YSK, SSH, YCK
Writing–original draft: JL, JS, YCK
Writing–review & editing: SSH, YCK
All authors read and approved the final manuscript.
References


Clinical relevance of blood urea nitrogen to serum albumin ratio for predicting bacteremia in very young children with febrile urinary tract infection

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Background: Urinary tract infections (UTIs) are one of the most common bacterial infections in febrile children and a common cause of hospitalization, especially in very young children. We examined the clinical characteristics and predictive factors of concomitant bacteremia in pediatric patients with febrile UTI aged ≤24 months.

Methods: This retrospective multicenter study reviewed medical data from 2,141 patients from three centers from January 2000 to December 2019. Enrolled cases were classified into the bacteremic UTI and non-bacteremic UTI groups according to the presence of blood culture pathogens.

Results: Among 2,141 patients with febrile UTI, 40 (1.9%) had concomitant bacteremia. All patients in the bacterial group were aged ≤6 months. Multivariate analysis revealed that younger age, lower blood lymphocyte counts and serum albumin levels, higher C-reactive protein (CRP) levels, blood urea nitrogen (BUN) levels, and BUN/serum albumin ratio were independent risk factors of concomitant bacteremia. The area under the receiver-operating characteristic curves predicting bacteremia were 0.668 for CRP, 0.673 for lymphocytes, and 0.759 for the BUN/albumin ratio.

Conclusion: The present study identified the BUN/albumin ratio and lower blood lymphocyte counts as novel predictive factors for bacteremia in young infants with febrile UTI in addition to the previously identified factors of younger age and higher CRP levels. Our findings could help to identify patients at high risk of bacteremia and benefit decision-making in the management of infants with febrile UTI.

Keywords: Bacteremia, Blood urea nitrogen, Lymphocyte count, Serum albumin, Urinary tract infections

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections in febrile children and a common cause of hospitalization, especially in very young children [1–3]. Most patients with febrile UTI have an uncomplicated clinical course [4]; however, some experience complications, including bacteremia, during or as a result of febrile
UTI. UTI with bacteremia is associated with poor outcomes, such as longer hospitalization, intensive care unit (ICU) admission, severe sepsis, and meningitis [5,6]. Since younger age is a well-known risk factor for bacteremia [7–9], clinicians routinely hospitalize very young infants with UTI for intravenous antibiotic therapy due to concerns about bacteremia. However, recent studies reported that outpatient treatment with oral antibiotics is safe and cost-saving for young infants with febrile UTIs unless the patient is at high risk of bacteremia [10,11]. Therefore, it is helpful to predict concurrent bacteremia in patients with febrile UTI prior to culture test to avoid unnecessary hospitalization of low-risk patients and initiate the management of high-risk patients. Previous studies have reported predictive factors for bacteremia in febrile children with UTI in terms of clinical presentation (e.g., ill appearance and poor feeding), laboratory findings (e.g., increased serum creatinine levels or inflammatory markers, such as C-reactive protein [CRP], and procalcitonin), and abnormalities in imaging studies [5,7,12,13]. However, the characteristics of the patient cohorts, including age, severity of patients’ illness, and number of participants, varied, and related findings for bacteremia were not consistent between studies. The present study examined the clinical and laboratory risk factors for concomitant bacteremia among 2,141 children aged ≤24 months with febrile UTI at initial presentation to confirm previously established factors and identify novel findings.

**Methods**

This study received ethical approval from the Institutional Review Board (IRB) of The Catholic University of Korea, Bucheon St. Mary’s Hospital (No. XC20RIDI0046). As the study subjects were deidentified, the IRB waived the need for written consent from the patients.

**Data collection**

This multicenter retrospective study was performed at three university hospitals and included very young children aged ≤24 months with UTI who were initially admitted presenting with fever (≥38.0 °C) from January 2000 to December 2019. Medical records were reviewed to obtain information on the patients’ clinical characteristics, including sex, age at admission, history of previous hospitalization and use of antibiotics, initial laboratory findings, and results of urologic images, including kidney ultrasonography (USG), 99mTc dimercaptosuccinic acid (DMSA) renal scan, and voiding cystourethrography (VCUG).

**Laboratory findings**

UTI was defined as a single pathogen titer of >100,000 colony-forming units/mL from a sterile bag-collected specimen or >50,000 colonies from a catheterized specimen from a patient with fever [14,15]. Blood cultures were obtained for all patients. Bacteremic UTI was defined as a UTI of a single pathogen in both blood and urine cultures. Patients were excluded if: their medical records or data were insufficient; their fever was caused by another condition, such as pneumonia, neutropenic fever, or malignancy; the same bacteria were not detected in blood and urine cultures; or they received ICU care. Degree of pyuria (≥5 white blood cells [WBC] per high power field [HPF]) in urine microscopy was categorized as: <5 WBC/HPF (no pyuria); 5 to 49 WBC/HPF; and ≥50 WBC/HPF. Since the reference ranges for the erythrocyte sedimentation rate (ESR) and CRP levels differed at each center, they were expressed as a multiple of the upper limit of the normal value for each institution. The neutrophil-to-lymphocyte (NLR) and platelet-to-lymphocyte (PLR) ratios have been reported as systemic inflammatory markers and predictive factors for bacteremia [16–18] and are calculated using a complete blood count. Blood urea nitrogen (BUN) and serum albumin levels were measured and expressed as a ratio (BUN/albumin) [19]. Abnormal kidney USG included hydronephrosis, obstructive uropathy, duplex kidney, and cystic kidney disease. A focal reduction or absence of uptake in more than one area of the kidney was considered abnormal in DMSA renal scan.

**Statistical analysis**

Enrolled cases were classified as bacteremic UTI and non-bacteremic UTI groups according to the presence of blood culture pathogens, and clinical and laboratory data were compared between the two groups. The chi-square test or Fisher exact test was used for categorical variables and t test or Mann-Whitney test was used for continuous variables. Univariate and multivariate analyses were performed using logistic regression to identify significant risk factors.
Factors for bacteremic UTI. Variables significantly associated with bacteremia in the univariate analysis were included in the multivariate analysis. Receiver-operating characteristic (ROC) curve analysis was performed by estimating the area under the curve (AUC) to identify the optimum cutoff values of variables that were significantly associated with bacteremia in multivariate analysis for the prediction of bacteremia. The maximum value of the Youden index was selected as the optimal cutoff value. The p-values of <0.05 were considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics version 22.0 (IBM Corp.).

Results

Clinical characteristics

A total of 2,141 patients aged ≤24 months with febrile UTI were identified during the study period. Table 1 describes the demographics of the patients. Among these patients, 40 (1.9%) were in the bacteremic group and 2,101 (98.1%) were in the non-bacteremic group. The mean age of all the patients was 4.94 months and 43.8% were aged 0 to 3 months, 34.5% were 4 to 6 months, 17.1% were 7 to 12 months, and 4.7% were 13 to 24 months. The mean age of the bacteremic group was younger than that of the non-bacteremic group (3.05 ± 1.68 months vs. 4.98 ± 3.76 months, p < 0.001). None of the patients in the bacteremic UTI group were aged ≥7 months. Among the patients in the bacteremic group, 29 (72.5%) were aged 0 to 3 months and 11 (27.5%) were 4 to 6 months. In contrast, among patients in the non-bacteremic group, 908 (43.2%) were aged 0 to 3 months and 727 (34.6%) were 4 to 6 months. Among all patients, 68.7% were male and the mean duration of fever before admission was 1.6 days. Sex distribution and duration of preceding fever before admission did not differ between the bacteremic and non-bacteremic groups. Among them, 117 (5.47%) and 128 (5.98%) had a history of hospitalization and received antibiotics therapy in the previous 3 months, respectively. Three patients in the bacteremic group had been hospitalized within 3 months before UTI admission due to feeding problems with laryngomalacia, respiratory viral infection, and rotaviral enteritis, respectively. In addition, one patient with a respiratory viral infection received empirical antibiotics during hospitalization. The proportions of patients with a history of hospitalization or antibiotics therapy before admission also did not differ between the two groups.

Table 1. Clinical characteristics of patients with bacteremic and non-bacteremic urinary tract infections

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients</th>
<th>Bacteremic group</th>
<th>Non-bacteremic group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>2,141</td>
<td>40</td>
<td>2,101</td>
<td></td>
</tr>
<tr>
<td>Age (mo)</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>All</td>
<td>4.94 ± 3.74</td>
<td>3.05 ± 1.68</td>
<td>4.98 ± 3.76</td>
<td></td>
</tr>
<tr>
<td>0–3</td>
<td>937 (43.8)</td>
<td>29 (72.5)</td>
<td>908 (43.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4–6</td>
<td>738 (34.5)</td>
<td>11 (27.5)</td>
<td>727 (34.6)</td>
<td></td>
</tr>
<tr>
<td>7–12</td>
<td>366 (17.1)</td>
<td>0 (0)</td>
<td>366 (17.4)</td>
<td></td>
</tr>
<tr>
<td>13–24</td>
<td>100 (4.7)</td>
<td>0 (0)</td>
<td>100 (4.8)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>0.492</td>
</tr>
<tr>
<td>Male</td>
<td>1,470 (68.7)</td>
<td>30 (75.0)</td>
<td>1,440 (68.5)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>671 (31.3)</td>
<td>10 (25.0)</td>
<td>661 (31.5)</td>
<td></td>
</tr>
<tr>
<td>Duration of fever before admission (day)</td>
<td>1.6 ± 1.36</td>
<td>1.32 ± 1.02</td>
<td>1.60 ± 1.36</td>
<td>0.097</td>
</tr>
<tr>
<td>Hospitalization in previous 3 mo</td>
<td></td>
<td></td>
<td></td>
<td>0.479</td>
</tr>
<tr>
<td>Yes</td>
<td>117 (5.5)</td>
<td>3 (7.5)</td>
<td>114 (5.4)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2,024 (94.5)</td>
<td>37 (92.5)</td>
<td>1,987 (94.6)</td>
<td></td>
</tr>
<tr>
<td>History of antibiotics therapy in previous 3 mo</td>
<td></td>
<td></td>
<td></td>
<td>0.512</td>
</tr>
<tr>
<td>Yes</td>
<td>128 (6.0)</td>
<td>1 (2.5)</td>
<td>127 (6.0)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2,013 (94.0)</td>
<td>39 (97.5)</td>
<td>1,974 (94.0)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or number (%).
Laboratory and imaging findings

Table 2 shows comparisons of the laboratory and imaging findings between the two groups. Pyuria (≥5 WBC/HPF) was present in 2,002 patients (93.5%) and absent in 139 patients (6.5%). *Escherichia coli* was the most frequently isolated bacterium in both the bacteremic and non-bacteremic UTI groups (97.5% and 87.7%, respectively). Among the patients who had *E. coli* or *Klebsiella* spp. in their urine culture, extended-spectrum β-lactamase (ESBL)-positive bacteria were found in six patients (15.0%) in the bacteremic UTI group and 326 (15.5%) in the non-bacteremic UTI group, respectively. There were no differences between the bacteremic and non-bacteremic UTI groups in terms of the degree of pyuria, types of isolated bacteria, or proportion of ESBL positivity.

### Table 2. Laboratory and imaging findings of patients with bacteremic and non-bacteremic urinary tract infection

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bacteremic group (n = 40)</th>
<th>Non-bacteremic group (n = 2,101)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine WBC (/HPF)</td>
<td></td>
<td></td>
<td>0.51</td>
</tr>
<tr>
<td>≥50</td>
<td>32 (80.0)</td>
<td>1,438 (68.4)</td>
<td></td>
</tr>
<tr>
<td>5–49</td>
<td>7 (17.5)</td>
<td>525 (25.0)</td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>1 (2.5)</td>
<td>138 (6.6)</td>
<td></td>
</tr>
<tr>
<td>Pathogen, urine</td>
<td></td>
<td></td>
<td>0.53</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>39 (97.5)</td>
<td>1,843 (87.7)</td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>0 (0)</td>
<td>98 (4.7)</td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus</em> spp.</td>
<td>0 (0)</td>
<td>69 (3.3)</td>
<td></td>
</tr>
<tr>
<td><em>Enterobacter</em> spp.</td>
<td>1 (2.5)</td>
<td>52 (2.5)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>0 (0)</td>
<td>39 (1.9)</td>
<td></td>
</tr>
<tr>
<td>Resistance</td>
<td></td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>ESBL</td>
<td>6 (15.0)</td>
<td>326 (15.5)</td>
<td></td>
</tr>
<tr>
<td>Non-ESBL</td>
<td>33 (82.5)</td>
<td>1,667 (79.3)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (2.5)</td>
<td>108 (5.1)</td>
<td></td>
</tr>
<tr>
<td>Serum, initial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (/μL)</td>
<td>14,175.8 ± 6,512.5</td>
<td>15,360.8 ± 5,880.1</td>
<td>0.26</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>7,997.5 ± 4,842.4</td>
<td>7,540.5 ± 4,161.7</td>
<td>0.56</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>4,480.2 ± 2,001.3</td>
<td>5,855.2 ± 2,425.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.61 ± 1.24</td>
<td>10.94 ± 1.09</td>
<td>0.10</td>
</tr>
<tr>
<td>Platelet (K/μL)</td>
<td>388.7 ± 141.24</td>
<td>400.71 ± 115.25</td>
<td>0.60</td>
</tr>
<tr>
<td>NLR</td>
<td>1.90 ± 1.09</td>
<td>1.47 ± 1.02</td>
<td>0.02</td>
</tr>
<tr>
<td>PLR</td>
<td>101.49 ± 54.46</td>
<td>81.49 ± 60.15</td>
<td>0.03</td>
</tr>
<tr>
<td>ESRa</td>
<td>2.2 ± 1.55</td>
<td>1.48 ± 1.51</td>
<td>0.01</td>
</tr>
<tr>
<td>CRP</td>
<td>21.32 ± 30.51</td>
<td>9.96 ± 11.35</td>
<td>0.02</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>11.06 ± 3.46</td>
<td>8.74 ± 3.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.26 ± 0.07</td>
<td>0.25 ± 0.07</td>
<td>0.46</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.81 ± 0.28</td>
<td>4.11 ± 0.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BUN/albumin (mg/g)</td>
<td>2.97 ± 0.86</td>
<td>2.14 ± 0.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>30.50 (24.0–36.5)</td>
<td>32.0 (26.0–41.0)</td>
<td>0.17</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>22.0 (17.75–29.25)</td>
<td>23.0 (17.0–32.5)</td>
<td>0.78</td>
</tr>
<tr>
<td>Abnormal kidney USG</td>
<td>24/40 (60.0)</td>
<td>882/2,084 (42.3)</td>
<td>0.04</td>
</tr>
<tr>
<td>Abnormal DMSA scan</td>
<td>20/30 (66.7)</td>
<td>786/1,287 (61.1)</td>
<td>0.58</td>
</tr>
<tr>
<td>VUR on VCUG</td>
<td>4/21 (19.1)</td>
<td>182/847 (21.5)</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

Data are expressed as number (%), mean ± standard deviation, or median (interquartile range). ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRP, C-reactive protein; DMSA, 99m-Tc dimercaptosuccinic acid; ESBL, extended-spectrum β-lactamase; ESR, erythrocyte sedimentation rate; HPF, high power field; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; USG, ultrasonography; VCUG, voiding cystourethrography; VUR, vesicoureteral reflux; WBC, white blood cell count.

*aESR and CRP were expressed as a multiple of the upper limit of the normal value of each institution.*
On admission, there were no significant differences in WBC, neutrophil, hemoglobin, or platelet counts between the bacteremic and non-bacteremic UTI groups. However, NLR (p = 0.02), PLR (p = 0.03), ESR (p = 0.01), and CRP levels (p = 0.02) were significantly elevated and lymphocyte levels (p < 0.001) were decreased in patients in the bacteremic group. Serum creatinine, aspartate transaminase, and alanine transaminase levels did not differ between the two groups. However, BUN levels were elevated and albumin levels decreased (p < 0.001 for both), subsequently the BUN/albumin ratio was higher in the bacteremic group (p < 0.001).

Kidney USG was available in 40 patients (100%) in the bacteremic group and 2,084 (99.2%) in the non-bacteremic group. Abnormal USG findings, such as hydronephrosis or pyelonephritis, were more frequent in the bacteremic group than in the non-bacteremic group (60.0% in the bacteremic group vs. 42.3% in the non-bacteremic UTI group; p = 0.04). However, the proportions of abnormal findings on DMSA scan (66.7% in the bacteremic group vs. 61.07% in the non-bacteremic group) and VCUG (19.1% in the bacteremic group vs. 21.49% in the non-bacteremic group) did not significantly differ between two groups.

Factors associated with bacteremic urinary tract infection

Univariate analysis revealed that younger age, lower lymphocyte and serum albumin levels, higher NRL, ESR, CRP, BUN levels and BUN/albumin ratio, and abnormal kidney USG findings were significantly associated with increased risk of bacteremic UTI (Table 3). Multivariate analysis showed that younger age (odds ratio [OR], 1.31; 95% confidence interval [CI], 1.12–1.70; p < 0.001), lower lymphocyte levels (OR, 1.00; 95% CI, 1.00–1.00; p = 0.005), higher CRP levels (OR, 1.03; 95% CI, 1.01–1.05; p = 0.005), lower albumin levels (OR, 0.22; 95% CI, 0.07–0.71; p = 0.01), and higher BUN levels (OR, 1.07; 95% CI, 1.00–1.18; p = 0.04) were independent risk factors associated with the development of bacteremia in patients with febrile UTI. Multivariate analysis revealed that younger age was an independent risk factor for the development of bacteremia.

Moreover, a significant age difference was observed between the bacteremic and non-bacteremic groups. In addition, univariate and multivariate logistic regression analyses were performed on participants aged 6 months or younger (n = 40 for the bacteremic group and n = 1,635 for the non-bacteremic group) to confirm whether the four laboratory variables (lower lymphocyte levels, higher CRP levels, lower albumin levels, and higher BUN levels) were independent risk factors associated with the development of bacteremia regardless of age (Table 4). In the multivariate analysis, lower lymphocyte (OR, 1.00; 95% CI, 1.00–1.00; p = 0.001), higher CRP levels (OR, 1.03; 95% CI, 1.01–1.05; p = 0.003), lower albumin levels (OR, 0.10; 95% CI, 0.03–0.33; p < 0.001) and higher BUN levels (OR, 1.08; 95% CI, 1.01–1.22; p = 0.04) were independent risk factors for bacteremia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted OR (95% CI)</th>
<th>p-value</th>
<th>Adjusted OR(^a) (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger age</td>
<td>1.33 (1.13–1.59)</td>
<td>&lt;0.001</td>
<td>1.31 (1.12–1.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum, initial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>1.00 (1.00–1.00)</td>
<td>&lt;0.001</td>
<td>1.00 (1.00–1.00)</td>
<td>0.005</td>
</tr>
<tr>
<td>NRL</td>
<td>1.34 (1.07–1.61)</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLR</td>
<td>1.00 (1.00–1.01)</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR</td>
<td>1.30 (1.08–1.56)</td>
<td>0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>1.04 (1.02–1.05)</td>
<td>&lt;0.001</td>
<td>1.03 (1.01–1.05)</td>
<td>0.005</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.75 (0.57–1.01)</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>0.08 (0.03–0.21)</td>
<td>&lt;0.001</td>
<td>0.22 (0.07–0.71)</td>
<td>0.01</td>
</tr>
<tr>
<td>BUN</td>
<td>1.11 (1.05–1.20)</td>
<td>0.001</td>
<td>1.07 (1.00–1.18)</td>
<td>0.04</td>
</tr>
<tr>
<td>BUN/albumin ratio</td>
<td>1.94 (1.40–2.74)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal kidney USG</td>
<td>2.02 (1.09–3.87)</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BUN, blood urea nitrogen; CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NRL, neutrophil-to-lymphocyte ratio; OR, odds ratio; PLR, platelet-to-lymphocyte ratio; USG, ultrasonography; UTI, urinary tract infection.

\(^a\)The model was adjusted for age, lymphocyte count, CRP, serum albumin, and BUN using multivariable logistic regression analysis.
in febrile UTI patients aged 6 months or younger.

Cutoff values for laboratory variables predicting bacteremic urinary tract infection

ROC analysis was performed for laboratory variables that were found to be independent risk factors for bacteremia in the multivariate analysis (Fig. 1). The AUCs were 0.67 (95% CI, 0.59–0.76; p < 0.001) for lymphocytes, 0.67 (95% CI, 0.59–0.75; p < 0.001) for CRP, and 0.76 (95% CI, 0.69–0.83; p < 0.001) for the BUN/albumin ratio. The optimal cutoff value for the BUN/albumin ratio for predicting bacteremia was 2.39, with 74.4% sensitivity and 64.2% specificity. The optimal values of lymphocyte count and serum CRP level were 4,772.25/μL with 62.5% sensitivity and 65.4% specificity, and 5.32 (5.32 times the upper limit of the normal value) with 82.5% sensitivity and 45.1% specificity, respectively.

Discussion

The present multicenter study revealed the clinical characteristics and risk factors for febrile UTI with concomitant bacteremia in very young children. Our study identified 40 patients (1.9%) aged ≤24 months with febrile UTIs with concomitant bacteremia. The incidence of bacteremia was 3.11% (29 of 937) in children aged 0 to 3 months and 1.5% (11 of 738) in those aged 4 to 6 months. There were no differences based on sex. Previous studies have reported various incidence rates of bacteremic UTI between 0% and

Table 4. Univariate and multivariate analyses of risk factors associated with bacteremic urinary tract infection in patients aged ≤6 months

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted OR (95% CI)</th>
<th>p-value</th>
<th>Adjusted OR* (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger age</td>
<td>1.18 (0.95–1.46)</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum, initial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>1.00 (1.00–1.00)</td>
<td>&lt;0.001</td>
<td>1.00 (0.99–1.00)</td>
<td>0.001</td>
</tr>
<tr>
<td>NRL</td>
<td>1.41 (1.12–1.73)</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLR</td>
<td>1.00 (1.00–1.01)</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR</td>
<td>1.40 (1.15–1.70)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>1.04 (1.02–1.06)</td>
<td>&lt;0.001</td>
<td>1.03 (1.01–1.05)</td>
<td>0.003</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>1.00 (1.00–1.00)</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>0.09 (0.03–0.23)</td>
<td>&lt;0.001</td>
<td>0.10 (0.03–0.33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BUN</td>
<td>1.15 (1.06–1.28)</td>
<td>0.001</td>
<td>1.08 (1.01–1.22)</td>
<td>0.04</td>
</tr>
<tr>
<td>BUN/albumin ratio</td>
<td>2.28 (1.55–3.33)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal kidney USG</td>
<td>1.86 (1.00–3.56)</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BUN, blood urea nitrogen; CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NLR, neutrophil-to-lymphocyte ratio; OR, odds ratio; PLR, platelet-to-lymphocyte ratio; USG, ultrasonography.

*The model was adjusted for lymphocyte count, CRP, serum albumin, and BUN using multivariable logistic regression analysis.
21%, depending on the age group studied and study design, and most studies have reported younger age as a risk factor [6–8,20]. This finding was also consistent with the findings of our study. Bacteremic UTI only developed in children aged ≤6 months and was inversely related to age.

As previously reported, higher CRP levels were also associated with bacteremic UTI in the present study [7,12,20]. While initial WBC or neutrophil counts and degree of urine pyuria are unable to distinguish patients with bacteremic UTI from those with non-bacteremic UTI, the mean CRP level in patients in the bacteremic UTI group was around twofold higher than that of those in the non-bacteremic UTI group and 21 times higher than the upper normal value. However, the CRP levels observed in the bacteremic and non-bacteremic groups overlapped considerably, as previously reported [12]. This was expressed as a relatively low specificity of CRP level in ROC analysis for predicting bacteremia. In addition, the cutoff value predicting bacteremia varied between studies [7,20,21]. Therefore, high CRP levels could be useful to predict bacteremic UTI, although CRP levels alone may not be sufficient and other clinical and laboratory factors should also be considered.

This study revealed that lower lymphocyte level was also an independent risk factor for bacteremia. Previous studies have shown that low lymphocyte counts are associated with poor prognosis in acute and chronic inflammatory conditions such as sepsis, cancer, and cardiovascular and pulmonary diseases [22–24]. However, it has not yet fully understood how low lymphocyte causes immune system abnormalities in patients with pediatric UTI and increases bacteremia [25,26]. Therefore, further research is needed on these mechanisms and cutoff values of lymphocyte levels to predict bacteremia in young infants with febrile UTI.

We found that higher levels of BUN and lower serum albumin levels were also independent risk factors for bacteremia. Based on the findings that patients with bacteremia had elevated levels of BUN but not serum creatinine, these patients appeared to be more dehydrated than non-bacteremic patients during severe illness due to reduced kidney function compared with non-bacteremic patients [27]. Lower levels of albumin result from the combined effects of decreased synthesis during the acute phase of inflammation and capillary leakage of albumin due to endotoxins released from Gram-negative bacteria, cytokines (e.g., interleukin 6), and chemokines [28]. In a study of adult patients with UTI, Leibovici et al. [29] reported lower albumin levels were an independent predictor of bacteremia. In addition, a recent study on febrile infants with UTI reported that serum albumin levels were lower in bacteremic UTI than in non-bacteremic UTI, although serum albumin levels were not associated with bacteremia in the multivariate analysis [7].

In the present study, the combined use of two parameters, elevated BUN and lower serum albumin (expressed as a ratio), showed the highest AUC (0.76 [95% CI, 0.74–0.78]; p < 0.001) in the ROC analysis, with an optimum cutoff value of 2.39 (mg/g). Several recent studies have highlighted the serum BUN/albumin ratio as a novel prognostic parameter predicting disease severity or mortality in adult patients with various inflammatory conditions [28,30,31]. Ryu et al. [27] reported that a BUN/albumin ratio of >7 mg/g at the initial visit to the emergency room was associated with increased mortality within 28 days in patients with aspiration pneumonia. In a study on patients with coronavirus disease 2019, elevated BUN/albumin ratio at admission was found to be an independent risk factor for critical outcomes, including admission to ICU, requirement for mechanical ventilation, or death [30]. In addition, Zou et al. [25] reported that an increased BUN to albumin ratio was a significant predictor of higher mortality rate and ICU requirement at the onset of E. coli bacteremia. To the best of our knowledge, the association between serum BUN/albumin ratio and UTI has not been previously reported in pediatric patients. However, based on the present study, BUN/albumin ratio could be a useful parameter for predicting bacteremia in young children with febrile UTI, though further validation is required to clarify its association with bacteremia in febrile UTI.

Genitourinary (GU) tract anomaly is associated with bacteremic UTI in children [7,12]. Yoon et al. [7] reported that VUR was found nearly twofold in bacteremic UTI infants than in those with non-bacteremic UTI (59.3% vs. 30.6%). Moreover, the presence of VUR was associated with the development of bacteremia in multivariate logistic regression analysis (OR, 3.66; 95% CI, 1.33–10.05; p = 0.012). Similarly, a study in Finnish children with bacteremic UTI showed that urinary tract obstruction (9% vs. 1%, p < 0.01) and grade 3 to 5 VUR (30% vs. 16%, p = 0.02) were detected more frequently in bacteremic patients with UTI than those without bacteremia [12]. In this study, abnormal USG
findings such as hydronephrosis or pyelonephritis were associated with bacteremic UTI in univariate regression analysis. However, such an association was not observed in multivariate analysis. Although the relationship between GU tract anomaly and bacteremic UTI remains to be determined entirely, we suggest clinicians should consider imaging studies to evaluate the presence of GU tract anomaly in pediatric patients with bacteremic UTI, as suggested in previous studies [7,12].

UTI is the most common primary source of Gram-negative infection in the blood of children. Infants aged <1 year were found to have the highest incidence of Gram-negative bloodstream infection among children and younger age was associated with mortality [32]. Therefore, early identification of bacteremia in infants with febrile UTI may reduce complications via prompt management, close monitoring of hemodynamic state, and early transfer to the ICU when indicated [13]. In contrast, outpatient and oral management with appropriate follow-up is safe, has lower medical costs, and prevents potential iatrogenic complications associated with hospitalization for young infants with UTI at low risk for bacteremia [10]. Ill appearance at initial presentation and/or elevated inflammatory markers, such as WBC count and CRP levels, can help predict patients’ risk of adverse events. However, many young infants with bacteremia show nonspecific symptoms at initial presentation and clinical judgment depends on the personal experience of clinicians [33]. The present study revealed that elevated BUN/albumin ratio, which is simple and easy to calculate and is not affected by the treating physician’s subjectivity, as well as higher CRP levels and lower blood lymphocyte counts may help to predict the risk of bacteremia in very young infants with febrile UTI [25].

The present study has some limitations. This was a retrospective cross-sectional study; therefore, some data, such as recurrence of UTI or follow-up laboratory data, were unavailable. Furthermore, only 40.5% (868 of 2,141) of the children in the present study underwent VCUG. As a result, there may be a statistical bias on the results of VUR and bacteremia in the present study. However, this was because we followed the recommendations of the American Academy of pediatrics guidelines, which do not recommend performing VCUG routinely after the first febrile UTI in children aged <24 months due to its risk of exposure to radiation and invasiveness [34]. Nevertheless, a strength of our study was that it was a multicenter study and the population size was relatively large considering the pediatric focus of the study.

In conclusion, the present study examined clinical and laboratory parameters to identify risk factors for bacteremia in pediatric UTI patients aged ≤24 months. Younger age, higher CRP levels, lower blood lymphocytes, and elevated BUN/albumin ratio were significant risk factors for bacteremia in febrile UTI. Our findings could be helpful to identify patients at high risk of bacteremia and enable appropriate decision-making in the management of febrile infants with UTI. Further prospective studies are required to validate the diagnostic value and establish reliable cutoff values for these parameters.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

This study was supported by the Institute of Clinical Medicine Research of The Catholic University of Korea, Bucheon St. Mary’s Hospital Research Fund (BCMC20BD04).

**Acknowledgments**

Statistical analysis was supported by biostatisticians employed by CC&I Research Co., Ltd.

**Data sharing statement**

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

**Authors’ contributions**

Conceptualization, Data curation: HH, YL, JSS
Formal analysis: NYK
Methodology: YL, NYK
Project administration: JSS
Writing—original draft: HH, YL, JSS
Writing—review & editing: YL, JSS
All authors read and approved the final manuscript.
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Association between hearing loss and physical performance in patients on maintenance hemodialysis

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Background: The correlation between hearing loss (HL) and physical performance in patients receiving maintenance hemodialysis (MHD) remains poorly investigated. This study explored the association between HL and physical performance in patients on MHD.

Methods: This multicenter cross-sectional study was conducted between July 2020 and April 2021 in seven hemodialysis centers in Shanghai and Suzhou, China. The hearing assessment was performed using pure-tone average (PTA). Physical performance was assessed using the Timed Up and Go Test (TUGT), handgrip strength, and gait speed.

Results: Finally, 838 adult patients (male, 516 [61.6%]; 61.2 ± 2.6 years) were enrolled. Among them, 423 (50.5%) had mild to profound HL (male, 48.6% and female, 53.4%). Patients with HL had poorer physical performance than patients without HL (p < 0.001). TUGT was positively correlated with PTA (r = 0.265, p < 0.001), while handgrip strength and gait speed were negatively correlated with PTA (r = –0.356, p < 0.001 and r = –0.342, p < 0.001, respectively). Physical performance in patients aged <60 years showed significant dose-response relationships with HL. After adjusting for confounders, the odds ratios (95% confidence intervals) for HL across the TUGT quartiles (lowest to highest) were 1.00 (reference), 1.15 (0.73–1.81), 1.69 (1.07–2.70), and 2.87 (1.69–4.88) (p for trend = 0.005).

Conclusion: Lower prevalence of HL was associated with a faster TUGT and a stronger handgrip strength in patients on MHD.

Keywords: Gait speed, Handgrip strength, Hearing loss, Hemodialysis, Physical performance
Introduction

Chronic kidney disease (CKD) is characterized by kidney structure or function abnormalities that are present for >3 months. The prevalence of CKD is 14.8% in adults in the United States [1] and 6% to 26% in Europe [2]. About 280 patients with end-stage renal disease (ESRD) in one million people need hemodialysis or peritoneal dialysis [3]. As short-term mortality in most patients with ESRD on hemodialysis continues to decrease due to improvements in dialysis technology, the increase in life expectancy has been accompanied by a burden of morbidity in later life [3]. Older patients on maintenance hemodialysis (MHD), in the same way as in the general population of older people, suffer from greater impairments of sensory and motor functions relating to aging, which reduces the quality of life and increases dependency [4].

Over 5% of the world’s population suffers from hearing loss (HL), one of the most common sensory disabilities with normal aging [5]. Hearing difficulties in older adults are undertreated, resulting in several adverse consequences such as decreased communication and reduced quality of life [6,7], including social withdrawal, anxiety, depression, and loss of confidence [8]. These adverse consequences and HL have been significantly related to cognitive impairment and independence in the older population [9]. Still, HL is manageable with hearing aids and even surgery [10].

Physical performance is an important health factor for patients undergoing hemodialysis and in the general population of older adults. With age, physical performance (such as balance, walking speed, and muscle strength) gradually decreases, leading to a high risk of falls [11] and subsequent fractures [12], hospitalizations, loss of independence, and even increased mortality [13,14]. Poor physical performance is also related to other serious problems, including cognitive deterioration and the inability to maintain health-related quality of life [11]. Good physical function and performance are essential to prevent various health conditions and immobility in the elderly [15], and exercise could improve frailty and quality of life among hemodialysis patients [16]. Therefore, it is vital to identify modifiable risk factors for diminished physical performance in patients on MHD because targeted interventions may ameliorate these outcomes.

HL is an overlooked potentially modifiable risk factor for poor physical performance in patients with MHD. A few previous studies examined the associations between HL and physical function and performance in other populations, but the results were inconsistent [17–20]. Whether there is a correlation between HL and physical performance in patients on MHD has not been investigated. Therefore, the present study aimed to explore the association between HL and physical performance.

Methods

Study design and population

This multicenter cross-sectional study was conducted between July 2020 and April 2021 in seven outpatient hemodialysis centers in Shanghai and Suzhou, China. The study was approved by the Ethics Committee of Shanghai Fifth People’s Hospital (No. 2020-182). The methods were carried out in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment in the study.

The inclusion criteria were 1) ≥18 years of age, 2) received MHD for at least 3 months, and 3) able to provide informed consent. The exclusion criteria were 1) unable to communicate with interviewers due to dementia, mental illness, or other neurodegenerative diseases, 2) ear infection, cochlear implant, or hearing aids, 3) assessed by the investigator as unable to complete all performance-based tests due to severe disability, 4) acute infection, pulmonary edema, acute cardiovascular disease (acute coronary syndrome or arrhythmia), acute cerebrovascular disease (infarct, hemorrhage), amputated limb, or malignancy, or 5) refused to provide informed consent.

Hearing assessment

The hearing assessment was performed using pure-tone audiometry (BTJ09; Jiangsu Better Life Medical Co., Ltd, China) by trained examiners in a sound-isolated room. Air conduction hearing thresholds were recorded, which were measured for each ear at different frequencies (0.125, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, and 8 kHz) and across an intensity range of 0 to 100 decibels (dB). The speech frequency pure-tone average (PTA) was calculated as the mean thresholds across 0.5, 1, 2, and 4 kHz [19]. HL was defined
as a PTA ≥ 25 dB, reflecting mild to profound HL according to the American Speech-Language-Hearing Association [21], which was used as a cutoff point in either ear in the present study [19,22].

**Physical performance**

The physical performance included balance function, muscle strength, and mobility measured using the Timed Up and Go Test (TUGT), handgrip strength, and gait speed, respectively. The TUGT involved getting up from a chair, walking for 3 m, turning around, walking back to the chair, and then sitting down again with back against the chair [23]. Handgrip strength assessment was performed on the non-fistula hand or the dominant hand of the patients with an indwelling dialysis catheter, using a dynamometer (GRIP-D; Takei Ltd.). The participants were asked to exert maximum effort twice, and the best of two measurements was registered [24,25]. Gait speed was assessed with the 4-m walk test. Walking aids were allowed during the test [23]. These tests were performed before a hemodialysis session.

**Data collection**

The demographic and clinical data were collected, including age, sex, body mass index (BMI), dialysis vintage, cause of ESRD, education, drinking, smoking, Charlson comorbidity index (CCI), living alone, fall, and laboratory parameters such as hemoglobin, serum albumin, and fractional urea removal rate (Kt/V).

**Statistical analysis**

Data were presented as means ± standard deviations or medians (interquartile range) for continuous variables and as number (%) for categorical variables. Comparisons were performed using a t test for the continuous variables and a chi-square test for the categorical variables. The Spearman correlation coefficient was used to assess the correlations of the PTA with physical performance. A trend test was used to detect the increased prevalence of HL using the Cochran-Armitage test among three groups (aged <60, 60–74, and ≥75 years). Logistic regression analysis was used to investigate the relationship between HL and TUGT, handgrip strength, and gait speed in all enrols and across different age groups. We then calculated odds ratios (ORs) and 95% confidence intervals (CIs) according to the quartiles of TUGT, handgrip strength, and gait speed when covariates were added sequentially to the logistic model. Crude was the unadjusted model. Model 1 was adjusted for age and sex. Model 2 was adjusted for Model 1 variables and BMI, CCI, education, smoking, drinking, fall, and serum albumin. The interaction effect between the component of physical performance and age was tested by adding three interacted items (TUGT × age; handgrip strength × age; and gait speed × age) in the logistic regression analysis. All statistical analyses were performed using IBM SPSS version 26.0 (IBM Corp.). A two-sided p-value of <0.05 was considered to be statistically significant.

**Results**

**Clinical characteristics**

This study included 880 patients on MHD, but the final sample consisted of 838 patients (male, 516) after excluding 20 patients who refused to participate in the hearing assessment, two who did not complete the handgrip strength test, and 20 who did not complete the TUGT or gait speed test (Fig. 1). Of the 838 participants (mean age, 61.2 ± 12.6
years), 516 (61.6%) were male, and 423 (50.5%) had mild to profound HL in at least one ear (male, 48.6% and female, 53.4%). The mean age of the individuals with and without HL were 67.0 ± 10.3 and 55.3 ± 11.9 years, respectively (p < 0.001). The patients’ socioeconomic and health-related characteristics according to their hearing status are presented in Table 1. Compared with those without HL, participants with HL were less educated (p < 0.001) and had a higher CCI (p < 0.001), lower serum albumin levels (p = 0.003), and a history of more frequent falls in the previous year (p = 0.011).

### Association between hearing thresholds and physical performance

Patients with HL had a significantly weaker handgrip strength, slower TUGT, and gait speed than subjects without HL (all p < 0.001) (Table 1). TUGT was positively correlated with PTA (r = 0.256, p < 0.001). Handgrip strength and gait speed were negatively correlated with PTA (r = –0.356, p < 0.001 and r = –0.342, p < 0.001, respectively) (Fig. 2).

Table 2 presents the ORs of HL for physical performance measures when the participants were divided into four groups by quartiles based on TUGT, handgrip, and gait speed. In the unadjusted model and model 1 (adjusted for age and sex), HL was associated with a slower TUGT (p

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**Table 1. Baseline characteristics of the study participants according to the presence of hearing loss**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No hearing loss group</th>
<th>Hearing loss group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>415</td>
<td>423</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>55.3 ± 11.9</td>
<td>67.0 ± 10.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.18</td>
</tr>
<tr>
<td>Male</td>
<td>265 (63.9)</td>
<td>251 (59.3)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>150 (36.1)</td>
<td>172 (40.7)</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.3 ± 3.9</td>
<td>23.4 ± 3.6</td>
<td>0.70</td>
</tr>
<tr>
<td>Dialysis vintage (mo)</td>
<td>47.4 (22.8–101.9)</td>
<td>48.9 (25.0–84.5)</td>
<td>0.17</td>
</tr>
<tr>
<td>Cause of ESRD</td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>54 (13.0)</td>
<td>72 (17.0)</td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>81 (19.5)</td>
<td>102 (24.1)</td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>138 (33.3)</td>
<td>117 (27.7)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>142 (34.2)</td>
<td>132 (31.2)</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Less than high school</td>
<td>195 (47.0)</td>
<td>288 (68.1)</td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>126 (30.4)</td>
<td>90 (21.3)</td>
<td></td>
</tr>
<tr>
<td>Higher education</td>
<td>94 (22.7)</td>
<td>45 (10.6)</td>
<td></td>
</tr>
<tr>
<td>Drinking</td>
<td>191 (46.0)</td>
<td>173 (40.9)</td>
<td>0.10</td>
</tr>
<tr>
<td>Smoking</td>
<td>203 (48.9)</td>
<td>192 (45.4)</td>
<td>0.31</td>
</tr>
<tr>
<td>Charlson comorbidity index</td>
<td>3.6 ± 1.6</td>
<td>4.1 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Living alone</td>
<td>22 (5.3)</td>
<td>21 (5.0)</td>
<td>0.84</td>
</tr>
<tr>
<td>Fall</td>
<td>104 (25.1)</td>
<td>138 (32.6)</td>
<td>0.01</td>
</tr>
<tr>
<td>Laboratory parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>111.1 ± 15.9</td>
<td>110.0 ± 17.8</td>
<td>0.35</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>40.0 ± 3.7</td>
<td>39.2 ± 3.3</td>
<td>0.003</td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.48 ± 1.76</td>
<td>1.47 ± 1.31</td>
<td>0.95</td>
</tr>
<tr>
<td>Handgrip strength (kg)</td>
<td>27.1 ± 8.7</td>
<td>21.8 ± 7.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Timed Up and Go Test (sec)</td>
<td>8.1 ± 4.0</td>
<td>11.4 ± 7.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gait speed (m/sec)</td>
<td>1.1 ± 0.3</td>
<td>0.9 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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

ESRD, end-stage renal disease; Kt/V, fractional clearance index for urea.
Figure 2. Correlations between pure-tone averages (PTA) and each test. (A) Timed Up and Go Test (TUGT), (B) handgrip strength, and (C) gait speed.

Table 2. Logistic regression analyses of the association of handgrip strength, TUGT, and gait speed with hearing loss

<table>
<thead>
<tr>
<th>Physical performance</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUGT(s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>216 (25.8)</td>
<td>204 (24.3)</td>
<td>209 (24.9)</td>
<td>209 (24.9)</td>
<td></td>
</tr>
<tr>
<td>Crudea</td>
<td>1.00</td>
<td>1.82 (1.21–2.74)</td>
<td>3.44 (2.29–5.15)</td>
<td>8.49 (5.48–13.14)</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 1b</td>
<td>1.00</td>
<td>1.24 (0.80–1.93)</td>
<td>1.78 (1.14–2.80)</td>
<td>2.97 (1.81–4.88)</td>
<td>0.004</td>
</tr>
<tr>
<td>Model 2c</td>
<td>1.00</td>
<td>1.15 (0.73–1.81)</td>
<td>1.69 (1.07–2.70)</td>
<td>2.87 (1.69–4.88)</td>
<td>0.005</td>
</tr>
<tr>
<td>Handgrip strength (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>209 (24.9)</td>
<td>211 (25.2)</td>
<td>209 (24.9)</td>
<td>209 (24.9)</td>
<td></td>
</tr>
<tr>
<td>Crudea</td>
<td>1.00</td>
<td>0.53 (0.35–0.79)</td>
<td>0.36 (0.24–0.54)</td>
<td>0.18 (0.12–0.28)</td>
<td>0.048</td>
</tr>
<tr>
<td>Model 1b</td>
<td>1.00</td>
<td>0.62 (0.39–0.97)</td>
<td>0.49 (0.30–0.81)</td>
<td>0.33 (0.19–0.58)</td>
<td>0.04</td>
</tr>
<tr>
<td>Model 2c</td>
<td>1.00</td>
<td>0.61 (0.39–0.97)</td>
<td>0.46 (0.28–0.76)</td>
<td>0.31 (0.17–0.56)</td>
<td>0.04</td>
</tr>
<tr>
<td>Gait speed (m/sec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>209 (24.9)</td>
<td>209 (24.9)</td>
<td>211 (25.2)</td>
<td>209 (24.9)</td>
<td></td>
</tr>
<tr>
<td>Crudea</td>
<td>1.00</td>
<td>0.38 (0.25–0.57)</td>
<td>0.25 (0.17–0.38)</td>
<td>0.16 (0.11–0.25)</td>
<td>0.06</td>
</tr>
<tr>
<td>Model 1b</td>
<td>1.00</td>
<td>0.55 (0.35–0.86)</td>
<td>0.51 (0.32–0.80)</td>
<td>0.36 (0.22–0.58)</td>
<td>0.04</td>
</tr>
<tr>
<td>Model 2c</td>
<td>1.00</td>
<td>0.53 (0.33–0.84)</td>
<td>0.52 (0.32–0.83)</td>
<td>0.35 (0.21–0.58)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Data are expressed as number (%) or odds ratio (95% confidence interval).
Q, quartile; TUGT, Timed Up and Go Test.
aNo adjustment. bAdjusted for age and sex. cAdjusted for Model 1 variables in addition to other variables (body mass index, Charlson comorbidity index, education, smoking, drinking, falling, and serum albumin).

for trend = 0.001 and p for trend = 0.004, respectively). The result still held true after adjusting for all covariates (p for trend = 0.005). In this final model, the OR for HL was 1.15 (95% CI, 0.73–1.81) for the second fastest quartile of TUGT, 1.69 (95% CI, 1.07–2.70) for the second slowest quartile of TUGT, and 2.87 (95% CI, 1.69–4.88) for the slowest TUGT quartile, compared with the fastest TUGT quartile. A similar trend was also observed for handgrip strength in the unadjusted and adjusted groups (models 1 and 2) (p for trend = 0.048, p for trend = 0.043, and p for trend = 0.045, respectively). In the final model, the multivariable-adjusted ORs for HL across handgrip strength (lowest to highest) were 1.00 (reference), 0.61 (95% CI, 0.39–0.97), 0.46 (95% CI, 0.28–0.76), and 0.31 (95% CI, 0.17–0.56). There were no associations between HL and gait speed in the unadjusted model and after adjustments for all covariates; however, a trend persisted (p for trend = 0.06 and p for trend = 0.05, respectively).

Fig. 3 shows that the prevalence of HL in patients aged ≥75, 60 to 74, and <60 years was 86.1%, 60.9%, and 26.7%, respectively. In the <60 years group, TUGT exhibited a significant dose-response relationship with HL (p = 0.004). This trend was also detected in the 60 to 74 years group (p = 0.082), while similar relationships were not observed
in the aged ≥75 years group (p > 0.05). This outcome was supported by our findings that there was a significant interactive effect of age on the relationships between HL and TUGT (p for interaction < 0.001). Additionally, handgrip strength showed a noteworthy dose-response relationship with HL only among individuals aged <60 years group (p = 0.02). A similar relationship between HL and gait speed was detected only among those aged 60 to 74 years (p = 0.02) (Table 3–5, Fig. 3).

Discussion

This study indicates that mild to profound HL at speech frequency PTA in either ear was associated with a slower TUGT and a weaker handgrip strength after adjustment for sociodemographic and lifestyle characteristics, comorbidities, falls, and serum albumin. The findings provide basic epidemiological data on HL in patients on MHD in China, as well as data comparing the physical performance according to the hearing status.

This population-based study of patients on MHD showed that 423 of the 838 participants (50.5%) had a certain degree of HL in at least one ear. Moreover, it is well known that patients on MHD often have comorbidities, including diabetes, hypertension, and cardiovascular diseases [26]. Previous studies argued that these chronic diseases were related to HL [27,28]. Our findings demonstrated a level of statistical significance in the association between PTA and physical performance. Despite relatively modest correlations, the results provide important clues for early evaluations that emphasize hearing and performance.

Regarding the relationship between HL and TUGT, the present study was consistent with Yévenes-Briones et al. [29] using data from the Seniors-ENRICA (Study on Nutrition and Cardiovascular Risk in Spain)-2 study, who observed that difficulty in rising from a chair and balance impairment were associated with HL. Although the definition of HL was different from the present study, it did not affect the consistency of the conclusions between the two studies. The results were also consistent with Bang et al.’s [30]. Their cross-sectional study indicated that postural instability was associated with HL. Furthermore, a prospective study revealed that both mild and moderate or greater hearing impairment were correlated with a lower Short-Physical Performance Battery (SPPB) [31]. The longitudinal analyses by Martinez-Amezcua et al. [19] demonstrated that compared with participants with normal hearing, those with HL had a faster decline in physical function over time. In another study, Martinez-Amezcua et al. [20] concluded that balance was independently related to hearing impair-
Table 3. Logistic regression analyses of the association of handgrip strength, TUGT, and gait speed with hearing loss in patients aged <60 years

<table>
<thead>
<tr>
<th>Physical performance</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUGT(s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>83 (25.2)</td>
<td>83 (25.2)</td>
<td>82 (24.9)</td>
<td>82 (24.9)</td>
<td></td>
</tr>
<tr>
<td>Crude*</td>
<td>1.00</td>
<td>1.087 (0.49–2.42)</td>
<td>2.419 (1.16–5.05)</td>
<td>3.154 (1.53–6.52)</td>
<td>0.04</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>1.088 (0.48–2.48)</td>
<td>1.975 (0.92–4.23)</td>
<td>2.459 (1.16–5.21)</td>
<td>0.04</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>1.063 (0.46–2.48)</td>
<td>1.900 (0.86–4.19)</td>
<td>2.398 (1.04–5.56)</td>
<td>0.004</td>
</tr>
<tr>
<td>Handgrip strength (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>82 (24.9)</td>
<td>84 (25.5)</td>
<td>82 (24.9)</td>
<td>82 (24.9)</td>
<td></td>
</tr>
<tr>
<td>Crude*</td>
<td>1.00</td>
<td>0.666 (0.35–1.26)</td>
<td>0.418 (0.21–0.83)</td>
<td>0.230 (0.11–0.50)</td>
<td>0.01</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>0.705 (0.35–1.41)</td>
<td>0.408 (0.18–0.93)</td>
<td>0.230 (0.09–0.58)</td>
<td>0.01</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>0.704 (0.35–1.44)</td>
<td>0.353 (0.15–0.83)</td>
<td>0.224 (0.08–0.59)</td>
<td>0.02</td>
</tr>
<tr>
<td>Gait speed (m/sec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>82 (24.9)</td>
<td>83 (25.2)</td>
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<td>82 (24.9)</td>
<td></td>
</tr>
<tr>
<td>Crude*</td>
<td>1.00</td>
<td>0.829 (0.43–1.61)</td>
<td>0.929 (0.48–1.79)</td>
<td>0.316 (0.14–0.69)</td>
<td>0.20</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>0.919 (0.46–1.83)</td>
<td>0.962 (0.49–1.89)</td>
<td>0.349 (0.16–1.78)</td>
<td>0.23</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>1.004 (0.49–2.07)</td>
<td>1.029 (0.51–2.09)</td>
<td>0.336 (0.14–0.79)</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Data are expressed as number (%) or odds ratio (95% confidence interval).
Q: quartile; TUGT, Timed Up and Go Test.
*No adjustment. Adjusted for age and sex. Adjusted for Model 1 variables in addition to other variables (body mass index, Charlson comorbidity index, education, smoking, drinking, falling, and serum albumin).

Table 4. Logistic regression analyses of the association of handgrip strength, TUGT, and gait speed with hearing loss in patients aged 60 to 74 years

<table>
<thead>
<tr>
<th>Physical performance</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUGT(s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>104 (25.6)</td>
<td>101 (24.8)</td>
<td>101 (24.8)</td>
<td>101 (24.8)</td>
<td></td>
</tr>
<tr>
<td>Crude*</td>
<td>1.00</td>
<td>0.663 (0.38–1.15)</td>
<td>1.261 (0.72–2.20)</td>
<td>3.423 (1.82–6.44)</td>
<td>0.037</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>0.637 (0.36–1.12)</td>
<td>1.006 (0.56–1.80)</td>
<td>2.726 (1.42–5.22)</td>
<td>0.061</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>0.542 (0.30–0.98)</td>
<td>0.929 (0.51–1.70)</td>
<td>2.464 (1.23–4.95)</td>
<td>0.082</td>
</tr>
<tr>
<td>Handgrip strength (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>104 (25.6)</td>
<td>100 (24.6)</td>
<td>102 (25.1)</td>
<td>101 (24.8)</td>
<td></td>
</tr>
<tr>
<td>Crude*</td>
<td>1.00</td>
<td>0.583 (0.33–1.04)</td>
<td>0.579 (0.33–1.03)</td>
<td>0.485 (0.27–0.86)</td>
<td>0.127</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>0.610 (0.33–1.12)</td>
<td>0.632 (0.32–1.25)</td>
<td>0.523 (0.25–1.08)</td>
<td>0.133</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>0.569 (0.30–1.06)</td>
<td>0.586 (0.29–1.19)</td>
<td>0.464 (0.22–0.99)</td>
<td>0.126</td>
</tr>
<tr>
<td>Gait speed (m/sec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>101 (24.8)</td>
<td>102 (25.1)</td>
<td>103 (25.3)</td>
<td>101 (24.8)</td>
<td></td>
</tr>
<tr>
<td>Crude*</td>
<td>1.00</td>
<td>0.623 (0.34–1.15)</td>
<td>0.357 (0.20–0.65)</td>
<td>0.282 (0.16–0.52)</td>
<td>0.028</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>0.693 (0.37–1.30)</td>
<td>0.432 (0.23–0.80)</td>
<td>0.332 (0.18–0.62)</td>
<td>0.017</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>0.647 (0.34–1.24)</td>
<td>0.450 (0.24–0.85)</td>
<td>0.338 (0.18–0.66)</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Data are expressed as number (%) or odds ratio (95% confidence interval).
Q: quartile; TUGT, Timed Up and Go Test.
*No adjustment. Adjusted for age and sex. Adjusted for Model 1 variables in addition to other variables (body mass index, Charlson comorbidity index, education, smoking, drinking, falling, and serum albumin).

Furthermore, our research uncovered that age acted as an effect modifier for the correlations between HL and TUGT, then age-specific evaluations and interventions focusing on the physical performance and auditory function ought to be conducted. On the other hand, Goins et al. [17] found that all individual SPPB component scores had no
Table 5. Logistic regression analyses of the association of handgrip strength, TUGT, and gait speed with hearing loss in patients aged ≥ 75 years

<table>
<thead>
<tr>
<th>Physical performance</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TUGT(s)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>25 (24.8)</td>
<td>26 (25.8)</td>
<td>25 (24.8)</td>
<td>25 (24.8)</td>
<td></td>
</tr>
<tr>
<td>Crude&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00</td>
<td>0.573 (0.12–2.70)</td>
<td>0.545 (0.12–2.58)</td>
<td>3.273 (0.32–33.84)</td>
<td>0.184</td>
</tr>
<tr>
<td>Model 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00</td>
<td>0.308 (0.05–1.77)</td>
<td>0.210 (0.04–1.22)</td>
<td>0.605 (0.05–7.99)</td>
<td>0.748</td>
</tr>
<tr>
<td>Model 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.00</td>
<td>0.299 (0.04–2.19)</td>
<td>0.190 (0.02–1.71)</td>
<td>0.806 (0.04–15.73)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td><strong>Handgrip strength (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>25 (24.8)</td>
<td>27 (26.7)</td>
<td>24 (23.8)</td>
<td>25 (24.8)</td>
<td></td>
</tr>
<tr>
<td>Crude&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.087 (0.14–8.36)</td>
<td>0.435 (0.07–2.63)</td>
<td>0.275 (0.05–1.53)</td>
<td>0.098</td>
</tr>
<tr>
<td>Model 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00</td>
<td>0.638 (0.10–4.19)</td>
<td>0.478 (0.08–2.88)</td>
<td>0.348 (0.06–1.99)</td>
<td>0.03</td>
</tr>
<tr>
<td>Model 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.00</td>
<td>0.345 (0.03–3.49)</td>
<td>1.774 (0.22–14.62)</td>
<td>1.076 (0.13–9.01)</td>
<td>0.66</td>
</tr>
<tr>
<td><strong>Gait speed (m/sec)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>25 (24.8)</td>
<td>25 (24.8)</td>
<td>26 (25.8)</td>
<td>25 (24.8)</td>
<td></td>
</tr>
<tr>
<td>Crude&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00</td>
<td>0.355 (0.10–11.89)</td>
<td>0.248 (0.01–12.67)</td>
<td>0.192 (0.01–9.63)</td>
<td>0.10</td>
</tr>
<tr>
<td>Model 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.076 (0.08–11.89)</td>
<td>0.177 (0.01–2.32)</td>
<td>1.704 (0.15–19.39)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Data are expressed as number (%) or odds ratio (95% confidence interval). Q, quartile; TUGT, Timed Up and Go Test.
<sup>a</sup>No adjustment. <sup>b</sup>Adjusted for age and sex. <sup>c</sup>Adjusted for Model 1 variables in addition to other variables (body mass index, Charlson comorbidity index, education, smoking, drinking, falling, and serum albumin).

Relation to HL, where audiometry was based on self-reported measurements. Mueller-Schotte et al. [18] showed that the number of instrumental activities of daily living limitations was not higher in self-reported HL compared with no sensory loss. This heterogeneity may be explained by the difference in audiometry (subjective self-report vs. objective clinical audiometry).

The present study showed that the gait speed was 1.0 ± 0.3 m/sec, which was lower than that of older adults in communities and similar to the data of 277 patients on MHD obtained by Lee et al. [32]. In older Japanese people, the gait speed was 1.2 ± 0.2 m/sec [33]. In Chinese community-dwelling older adults, the gait speed was 1.1 ± 0.2 m/sec [23]. Ozawa et al. [34] confirmed that the gait speed was significantly declined in elderly patients with heart failure. Another study showed that poor glycemic control was significantly correlated with the decrease in gait speed in Japanese patients with type 2 diabetes [35]. The high prevalence of sarcopenia in patients on MHD (40%) can have a negative impact on handgrip strength and gait speed [24].

Yévenes-Briones et al. [29] indicated that the chair stand test was significantly associated with hearing impairment but not gait speed, while other studies showed that gait speed was independently associated with hearing impairment [20,36]. Chen et al. [31] also reported that during a 10-year long-term follow-up, the participants with greater hearing impairment had slower gait speeds. The present study revealed that the correlation between HL and a slower gait speed nearly reached statistical significance, but further study is needed to explore the mechanisms of this relation.

HL was associated with worse physical activity [37] and was a risk factor for disability in older individuals [38]. Previous studies examined the associations between HL and frailty syndrome and disability [9,18,29,31,38], and their results were consistent. Interestingly, the present study revealed an association between HL and handgrip strength, consistent with Kim et al. [25], who concluded that both moderate and impaired self-reported hearing acuity of the 3,075 participants were significantly associated with weak handgrip strength. In contrast, Tomioka et al. [36] reported no significant association between self-perceived hearing handicap and handgrip strength in high-functioning older adults. Another prospective cohort study showed no significant negative correlation between handgrip strength and unilateral and bilateral HL development [39]. Therefore, whether grip strength can be used as an early warning signal of HL or hearing is beneficial to grip strength requires
confirmation in other settings and additional investigation. This study had some limitations. First, the cross-sectional design made it impossible to assess causal relationships between HL and physical performance, but only associations. Second, the hemodialysis centers of the seven hospitals did not randomly select the participants. Therefore, the samples might not be representative of the entire hemodialysis population. Third, not all patients were consecutively included but voluntarily participated. Thus, the patients not in the study might have more severe physical performance decline and/or HL or different socioeconomic characteristics, which might lead to bias. Fourth, our study did not analysis those dialysis related factors such as dialysis type (high flux, low flux, and hemodiafiltration), dialysis time, and frequency of dialysis, and the impact of these differences may have been overlooked. Despite these limitations, to the best of the authors’ knowledge, this work addressed the knowledge gap about the relationship between HL and physical performance in patients with MHD. In addition, multicenter prospective studies with larger sample sizes, such as randomized controlled trial, remain to be needed in the future.

In conclusion, the present study suggests that a faster TUGT and a stronger handgrip strength are associated with a lower prevalence of HL in patients with MHD. Recognizing these associations could lead to earlier hearing- and performance-based assessments with appropriate interventions.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

This work was supported by the Health Commission of Minhang District, Shanghai (No. 2021MW04), the Minhang District medical characteristic specialty construction project (No. 2020MWTZA01), and the Cooperation Program of Fudan University-Minhang District Joint Health Center (No. 2021FM21).

Acknowledgments

The authors thank all staff and patients who participated in this study.

Data sharing statement

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

Authors’ contributions

Conceptualization: YG, CY, JN
Data curation: WF, XZ, XC
Formal analysis: WF, XZ, QW
Investigation: WF, Lihong Zhang, ZY, XC, KZ, WD, HQ, JZ, Liming Zhang, SZ
Methodology: WF, XZ, JN
Project administration: QG, CY, HQ, SZ, JN
Supervision: QG, CY, WD, JZ, Liming Zhang, JN
Validation: CY
Writing – original draft: WF, XZ
Writing – review & editing: WF, XZ, JN
All authors read and approved the final manuscript.

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Efficacy and cost-effectiveness of darbepoetin alfa once every 4 weeks versus continuous erythropoietin receptor activator once every 4 weeks for anemia correction in patients with chronic kidney disease not on dialysis

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²Department of Biostatistics, Clinical Trial Center, Soonchunhyang University Bucheon Hospital, Bucheon, Republic of Korea

Background: For anemia management in patients with chronic kidney disease not on dialysis, darbepoetin alfa (DA), which has a shorter half-life but is more inexpensive than continuous erythropoietin receptor activator (CERA), is preferred in Korea. This study evaluated the efficacy, safety, and cost-effectiveness of once-in-4-weeks DA compared with once-in-4-weeks CERA in patients with chronic kidney disease not on dialysis.

Methods: In this randomized, prospective, non-inferiority study, 40 erythropoiesis-stimulating agent–naïve patients with chronic kidney disease not on dialysis were randomized 1:1 to the DA group and CERA group. They received the study drug once in 4 weeks during 10- or 12-week correction period and 24-week efficacy evaluation period. The primary outcomes were the mean difference in the changes in hemoglobin levels between baseline and efficacy evaluation period and hemoglobin response rates during the correction period. The secondary outcomes included differences in adverse events and costs.

Results: DA was non-inferior to CERA for anemia correction; the mean difference in the change in hemoglobin levels between the groups was –0.070 g/dL (95% confidence interval, –0.730 to 0.590 g/dL). Hemoglobin response rates were 100% with DA and 94.1% with CERA. Adverse events were comparable. The mean cost of DA was approximately one-third that of CERA (34,100 ± 7,600 Korean won/4 weeks vs. 115,500 ± 23,600 Korean won/4 weeks; p < 0.001).

Conclusion: Once-in-4-weeks DA safely corrects anemia in erythropoiesis-stimulating agent–naïve patients with chronic kidney disease not on dialysis and is more cost-effective than once-in-4-weeks CERA.

Keywords: Anemia, Chronic kidney disease, Darbepoetin alfa, Erythropoiesis stimulating agent
**Introduction**

Anemia is a common complication of chronic kidney disease (CKD). The most common cause of anemia in patients with CKD is decreased erythropoietin production owing to kidney dysfunction, and the prevalence of anemia increases when the disease progresses to end-stage kidney disease [1]. Anemia in patients with CKD is a known risk factor for the development of cardiovascular events and the progression of CKD [2–5]. It also lowers the quality of life and increases the economic burden on healthcare services [6,7].

Since the introduction of recombinant human erythropoietin in the 1980s, erythropoiesis-stimulating agents (ESAs) have become the primary treatment for anemia in CKD [8–10]. There are four types of ESA: epoetin alfa, epoetin beta, darbepoetin alfa (DA), and methoxy polyethylene glycol-epoetin beta, also known as continuous erythropoietin receptor activator (CERA) [9]. These agents have similar anemia correction effects but different pharmacokinetic profiles owing to differences in their molecular structures. The different half-lives result in different administration intervals [11,12]. ESAs with short half-lives require frequent injections, which create inconvenience for both patients and healthcare providers, and lead to poor treatment compliance, waste of healthcare resources, and high healthcare costs [13,14]. Therefore, recently developed ESAs have longer half-lives because of several molecular modifications [15]. DA and CERA are long-acting ESAs that are generally administered once in 2 weeks (Q2W) and once in 4 weeks (Q4W), respectively.

In the Korean National Health Insurance Service (NHIS), DA costs 40% less than CERA for the same dose, considering the dose conversion ratio (DCR). As the difference in the efficacy for anemia correction between the two ESAs is unclear, the cost aspect makes it difficult for clinicians to select ESAs for patients with monthly outpatient follow-ups. Patients with CKD who have economic problems often want to receive DA Q4W rather than CERA Q4W, and clinicians respect the patients’ wishes and often prescribe DA Q4W in Korea. In terms of half-life, DA Q4W is expected to be less effective for anemia management than CERA Q4W. However, in clinical practice, patients treated with DA Q4W often exhibit equally well-controlled anemia as those treated with CERA Q4W, contrary to expectations. Previous studies have also reported that DA Q4W is effective in patients whose hemoglobin (Hb) levels are well-maintained by Q2W administration [16]. Although the CORDATUS [17] and ACTOS studies [18] showed the non-inferiority of CERA Q4W to DA once in a week (QW) or Q2W, and CERA Q2W to DA QW, a randomized controlled trial (RCT) proving the non-inferiority of DA Q4W compared with CERA Q4W has not yet been performed.

The current study was conducted to test the hypothesis that subcutaneous DA Q4W is non-inferior in terms of anemia correction and superior in terms of cost-effectiveness to subcutaneous CERA Q4W in ESA-naive patients with CKD not undergoing dialysis (ND).

**Methods**

**Patients and clinical data**

This single-center, randomized, open-label, non-inferiority study was conducted following the principles outlined in the Declaration of Helsinki and was approved by the Institutional Review Board of Soonchunhyang University Bucheon Hospital (No. 2018-05-008; CRiS No. KCT0003999). Informed consent was obtained from all participants.

We prospectively screened 19- to 99-year-old patients with stage 4 or 5 CKD ND at Soonchunhyang University Bucheon Hospital from April 2020 to April 2021. We enrolled patients with Hb levels of 8 to 10 g/dL, serum ferritin levels of ≥100 ng/mL, and transferrin saturation (TSAT) of ≥20%.

Patients with active cancer; decompensated liver cirrhosis; decompensated heart failure; or a history of arrhythmia, asthma, or chronic obstructive pulmonary disease were excluded from the study. Patients who were pregnant or planned to become pregnant and those previously treated with ESAs were excluded. Patients with bleeding events, including gastrointestinal bleeding, trauma, and menorrhagia, which can cause anemia in the past 3 months, were excluded. Esophagogastroduodenoscopy and colonoscopy were performed on all participants during the screening period with no evidence of gastrointestinal bleeding. Although we did not explicitly mention acute infection in the exclusion criteria, the infection status that may affect ESA responsiveness [19] of all participants was confirmed during the screening period, and we confirmed that all patients were free of acute infection.
We obtained and analyzed the clinical information and laboratory data of the enrolled patients every 4 weeks during the study period. The estimated glomerular filtration rate (eGFR) was calculated using serum creatinine levels and the Chronic Kidney Disease Epidemiology Collaboration equation.

**Erythropoiesis-stimulating agent administration**

The study drugs were subcutaneously administered to each group. The first dose of each drug was defined as the starting dose. For the DA group, the initial dose was determined 2 weeks after the administration of the starting dose (0.45 μg/kg), and the dosing frequency was Q4W. Therefore, in the DA group, the initial dose differed from the starting dose. When the Hb level increased after 2 weeks, twice the starting dose (0.9 μg/kg/mo) was administered; however, when the Hb level did not increase, the doubled dose was increased by a further 25% (1.125 μg/kg/mo) (Fig. 1). The DA group underwent a 10-week correction period followed by a 24-week efficacy evaluation period (EEP). By contrast, CERA was administered Q4W at the same starting and initial doses of 1.2 μg/kg/mo. The CERA group underwent a 12-week correction period followed by a 24-week EEP (Fig. 1).

The target Hb level range was defined as 10 to 11 g/dL. Both drug doses were adjusted by 25% during the correction period until the target Hb level was reached. Dose adjustments were made during scheduled visits, no more than once every 4 weeks.

**Iron supplementation**

The protocol for iron supplementation was as follows: if the ferritin level was below 100 ng/mL or TSAT was below 20% during the study period, iron supplementation was initiated. If the ferritin level was 100 ng/mL or above and TSAT was 20% or above, supplementation was discontinued. Only oral iron supplements (ferrous sulfate, 512 mg/day) were used for iron supplementation.

**Clinical outcomes**

Two primary efficacy endpoints were analyzed: 1) the mean difference in the changes in Hb levels, defined as the difference between the mean Hb levels at the baseline and the EEP; and 2) the Hb response rates, defined as the proportion of patients who reached the target Hb level range during the correction period. The secondary efficacy endpoints included differences in the mean time to reach the target Hb level, the proportion of the duration within the target Hb range, and changes in Hb levels over time.

Safety profiles of the study drugs were monitored and analyzed during the study period. The following parameters were assessed: 1) changes in blood pressure and laboratory safety parameters, including Hb, creatinine, sodium, po-

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**Figure 1. Study design.**

Q4W, once in 4 weeks; CERA, continuous erythropoietin receptor activator.
tassium, ferritin, and TSAT; 2) the nature and frequency of all adverse events (AEs) reported by the enrolled patients or observed by the investigator; and 3) the severity and relevance of the study drugs for all AEs determined based on the judgment of the investigator. Red blood cell transfusions and iron replacement therapy were recorded.

The doses and costs of the drugs in each group were compared over the duration of the study. The DCR was calculated and defined as the dose ratio of DA to CERA. Because the mean dose and cost were calculated as the average value of only the doses administered Q4W, the starting dose and cost in the DA group were excluded and analyzed separately. The cost of the study drugs was calculated based on the price of the pre-filled syringe administered, not the price per milligram, to reflect actual clinical practice.

Statistical analysis

The study's sample size was calculated by analyzing the expected difference in the mean changes in Hb levels between the two groups. The participants were treated to achieve and maintain Hb levels between 10 and 11 g/dL. The expected difference in the changes in mean Hb levels from baseline to the EEP between the arms was 0 g/dL, and the anticipated standard deviation (SD) was 0.75 g/dL based on the historical DA and CERA clinical trial experience to date [16,20–23]. Because the SD was extremely small in previous studies, the sample size was considerably small when calculated based on it. Therefore, we set the SD at 0.75 g/dL more conservatively. For obtaining 80% power to test the primary non-inferiority Hb hypothesis with a two-sided 95% confidence interval (CI), a pre-specified margin of 0.75 g/dL, and a Student t test, a total of 16 evaluable participants per treatment group were required. Assuming a 20% non-evaluable efficacy rate, the sample size was calculated to be 20 participants per treatment group.

Descriptive characteristics of the study population are reported as mean ± SD for continuous variables and as frequency counts with percentages for categorical and binary variables. Comparisons between the groups were performed using Student t test for continuous variables and either Pearson chi-square test or Fisher exact test for categorical variables, as appropriate.

If the lower limit of the 95% CI was >–0.75 g/dL for the mean difference in the changes in Hb levels and >60% for the Hb response rate, we concluded that DA Q4W corrected anemia with non-inferiority to CERA Q4W. Differences between the two groups in terms of changes in variables over time were analyzed using a linear mixed model, which can minimize data omission due to missing values [24,25].

All statistical tests were two-sided, and p-values of less than 0.05 were considered to indicate statistical significance. All analyses were performed using IBM SPSS version 25 for Windows (IBM Corp.) or GraphPad Prism 5 (GraphPad Software, Inc.).

Results

Study population

Forty patients were enrolled in the study and randomized 1:1 to receive subcutaneous DA or CERA Q4W (n = 20 each) (Fig. 1). We used simple randomization for randomizing patients to each group. Table 1 shows the baseline characteristics and demographic data of patients in the DA and CERA groups. Demographic data, including sex, age, body mass index, blood pressure, and CKD etiologies; baseline serum creatinine levels and eGFR; and baseline anemia profiles, including the mean Hb and TSAT, did not differ between the two groups. Baseline ferritin levels tended to be higher in the CERA group than in the DA group (383.7 ± 404.1 and 235.0 ± 116.4 ng/mL, respectively; p = 0.13). The CERA group showed higher mean serum ferritin levels than the DA group (p < 0.001); however, the mean TSAT did not differ between the groups during the entire study period (Supplementary Fig. 1, available online). The type of antihypertensive agent administered did not differ between the groups. Nine patients dropped out of the study. The causes of discontinuation were loss to follow-up (five patients; three in the DA group and two in the CERA group) and starting hemodialysis (four patients; one in the DA group and three in the CERA group).
Table 1. Baseline characteristics and demographic data (full analysis population)

<table>
<thead>
<tr>
<th>Variable</th>
<th>DA</th>
<th>CERA</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>20</td>
<td>20</td>
<td>0.80</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>68.9 ± 12.0</td>
<td>68.0 ± 12.0</td>
<td>0.74</td>
</tr>
<tr>
<td>Male sex</td>
<td>7 (35.0)</td>
<td>8 (40.0)</td>
<td>0.99</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.0 ± 5.6</td>
<td>23.9 ± 2.9</td>
<td>0.32</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>135.7 ± 15.4</td>
<td>129.3 ± 24.0</td>
<td>0.58</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>67.7 ± 12.2</td>
<td>65.3 ± 15.0</td>
<td>0.52</td>
</tr>
<tr>
<td>Diabetes</td>
<td>13 (65.0)</td>
<td>11 (55.0)</td>
<td>0.15</td>
</tr>
<tr>
<td>Hypertension</td>
<td>20 (100.0)</td>
<td>18 (90.0)</td>
<td>0.72</td>
</tr>
<tr>
<td>Stroke</td>
<td>5 (25.0)</td>
<td>6 (30.0)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>3 (15.0)</td>
<td>2 (10.0)</td>
<td>0.70</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>5 (25.0)</td>
<td>3 (15.0)</td>
<td>0.70</td>
</tr>
<tr>
<td>Serum creatinine levels (mg/dL)</td>
<td>2.80 ± 1.09</td>
<td>3.22 ± 1.16</td>
<td>0.25</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>20.9 ± 6.5</td>
<td>18.1 ± 7.0</td>
<td>0.21</td>
</tr>
<tr>
<td>Chronic kidney disease stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 4</td>
<td>15 (75.0)</td>
<td>14 (70.0)</td>
<td>0.72</td>
</tr>
<tr>
<td>Stage 5</td>
<td>5 (25.0)</td>
<td>6 (30.0)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>9.19 ± 0.58</td>
<td>9.29 ± 0.46</td>
<td>0.55</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>235.0 ± 116.4</td>
<td>383.7 ± 404.1</td>
<td>0.13</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>33.2 ± 16.5</td>
<td>32.2 ± 13.3</td>
<td>0.82</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>0.77 ± 1.40</td>
<td>0.52 ± 1.07</td>
<td>0.52</td>
</tr>
<tr>
<td>Cause of chronic kidney disease</td>
<td></td>
<td></td>
<td>0.69</td>
</tr>
<tr>
<td>Diabetes</td>
<td>12 (60.0)</td>
<td>11 (55.0)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>7 (35.0)</td>
<td>6 (30.0)</td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>1 (5.0)</td>
<td>2 (10.0)</td>
<td></td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>0 (0)</td>
<td>1 (5.0)</td>
<td></td>
</tr>
<tr>
<td>Concomitant antihypertensive treatments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin II receptor blockers</td>
<td>14 (70.0)</td>
<td>14 (70.0)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>β-adrenoceptor antagonists</td>
<td>13 (65.0)</td>
<td>9 (45.0)</td>
<td>0.20</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>18 (90.0)</td>
<td>15 (75.0)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

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

CERA, continuous erythropoietin receptor activator; DA, darbepoetin alfa; eGFR, estimated glomerular filtration rate.

respectively (Fig. 2). The lower limit of the 95% CI for the group difference was above the protocol-specified non-inferiority limit of −0.75 in both the ITT and PP populations, signifying that DA Q4W is non-inferior to CERA Q4W for anemia correction.

The Hb response rates during the correction period were comparable between the DA and CERA groups: 100% (95% CI, 81.4–100) vs. 94.1% (95% CI, 71.3–99.8) and 100% (95% CI, 79.4–100) vs. 100% (95% CI, 88.2–100) in the ITT and PP populations, respectively (Table 2).

The mean time to reach target Hb levels did not differ between the DA and CERA groups in the ITT or PP population (Table 2). Furthermore, the mean percentage frequencies within, exceeding, and less than the target Hb range did not differ between the two groups in both the ITT and PP populations during the EEP (Supplementary Table 1, available online) and the total study period (Table 2). The mean Hb level increased in both treatment groups during the study period, and there was no significant difference in the mean change of iron supplementation between the groups (104,913 ± 40,832 and 91,787 ± 55,343 mg, respectively; p = 0.534). During the study period, there was no patient who received red blood cell transfusion.
Safety

The mean eGFR, systolic and diastolic blood pressure, and sodium and potassium levels over time did not differ between the two groups during the entire study period (p = 0.264, p = 0.999, p = 0.823, p = 0.941, and p = 0.978, respectively) (Supplementary Fig. 2, 3; available online).

All AEs in both groups were mild to moderate in intensity.
Table 2. Comparison of the time to the target Hb level and percentage of patients within the target Hb range between the two groups during the total study period

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intention-to-treat population</th>
<th>Per-protocol population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to target Hb level (wk)</td>
<td>DA (n = 20)</td>
<td>CERA (n = 20)</td>
</tr>
<tr>
<td>Mean % frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within target Hb range</td>
<td>47.08 ± 23.13</td>
<td>41.06 ± 18.63</td>
</tr>
<tr>
<td>Exceed target Hb range</td>
<td>18.38 ± 20.10</td>
<td>25.00 ± 26.70</td>
</tr>
<tr>
<td>Less than target Hb range</td>
<td>34.54 ± 27.01</td>
<td>33.94 ± 32.10</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation.
CERA, continuous erythropoietin receptor activator; DA, darbepoetin alfa; Hb, hemoglobin.

Dose and cost

The doses and costs of the drugs administered in each group are presented in Table 4 and Fig. 5. The DCRs of the mean initial and total doses were 1.178 and 0.716, respectively. Changes in the mean ESA dose administered over time did not differ between the groups (p = 0.79). The mean total cost of DA was nearly one-third of that of CERA (34,100 ± 7,600 vs. 115,500 ± 23,600 Korean won/4 weeks, p < 0.001).

Discussion

This is the first RCT to compare DA Q4W with CERA Q4W in ESA-naïve patients with CKD ND. DA Q4W was non-inferior to CERA Q4W for anemia correction, including the mean difference in the changes in Hb levels and Hb response rates. The safety profiles were comparable between the two groups, and no serious AEs occurred in either group. The mean cost of ESAs administered every 4 weeks was nearly one-third in the DA group compared with that in the CERA group. These results suggest that DA Q4W for these patients not only leads to proper management of anemia but is also cost-effective compared with CERA Q4W in the real-world setting of Korea.

To compare the anemia correction efficacies of DA and CERA under the same conditions, the administration interval of the two study drugs was set to Q4W. Less frequent administration of ESAs has various clinical benefits, including increasing patient compliance and lowering the medical burden [26–28]. Over the past 30 years, ESAs with longer half-lives and less frequent administration have been developed through molecular modifications. DA is a...
synthetic analog of erythropoietin with an increased carbohydrate content and a long serum half-life of approximately 72 hours, and its circulating levels are retained above the erythropoiesis threshold 168 hours after subcutaneous administration [29]. Based on these pharmacokinetic advantages, pharmacodynamic studies on extending the dosing interval of DA to Q4W in patients with CKD ND have been conducted [16,20–23]. Most of these studies were performed in patients whose Hb levels were stably maintained within the target range by DA Q2W [16,20,21]. For these patients, the initial DA dose was calculated by summing the DA doses administered for 4 weeks before switching to Q4W administration; subsequently, the dosing frequency was adjusted to Q4W according to the Hb levels. The clinical outcomes were compared between the post- and pre-switching groups [16,20,21] and the CERA Q4W group [22]. Another study compared DA Q2W and Q4W in ESA-naïve patients with CKD ND [23]. Although interpretation and comparison of the results of each study are needed, all these studies reported that DA, not only Q2W but also Q4W is effective in managing anemia in patients with CKD ND. Although the current study design differed from that of previous studies, the current study also showed that DA Q4W is comparable in anemia correction and similar in safety profile to CERA Q4W. This result provides compelling evidence that DA Q4W can be as effective as CERA Q4W in patients with CKD ND, even though its half-life is shorter than that of CERA.

In the Korean NHIS, the cost of DA is 40% lower than that of CERA for the same dose, considering the DCR. Because the DCR of DA to CERA during the study period was slightly lower than expected, the mean cost of ESAs administered every 4 weeks was nearly one-third for DA compared with that of CERA. The 2012 Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline for Anemia in Chronic Kidney Disease recommends selecting the type of ESA by considering the balance of pharmacodynamics, safety information, clinical outcome data, availability, and costs [30]. In this respect, the results of the current study suggest that DA Q4W is preferable over CERA Q4W in the real-world setting in Korea.

To reflect actual clinical practice in Korea, the current

### Table 3. Overall adverse events during the study period

<table>
<thead>
<tr>
<th>Variable</th>
<th>DA (n = 20)</th>
<th>CERA (n = 20)</th>
<th>Study drug association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild adverse events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>1</td>
<td>0</td>
<td>Probably not</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>0</td>
<td>1</td>
<td>Definitely not</td>
</tr>
<tr>
<td>Neck pain</td>
<td>1</td>
<td>0</td>
<td>Definitely not</td>
</tr>
<tr>
<td>Moderate adverse events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herpes zoster</td>
<td>1</td>
<td>0</td>
<td>Definitely not</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>1</td>
<td>0</td>
<td>Definitely not</td>
</tr>
<tr>
<td>Pulmonary edema</td>
<td>0</td>
<td>1</td>
<td>Probably not</td>
</tr>
<tr>
<td>Femoral neck fracture due to a car accident</td>
<td>0</td>
<td>1</td>
<td>Definitely not</td>
</tr>
</tbody>
</table>

CERA, continuous erythropoietin receptor activator; DA, darbepoetin alfa.

### Table 4. Mean administered study drug dose and cost in both groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>DA (n = 20)</th>
<th>CERA (n = 20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting dose (μg/kg)</td>
<td>0.44 ± 0.02</td>
<td>1.24 ± 0.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Initial dose (μg/kg)</td>
<td>0.95 ± 0.11</td>
<td>1.24 ± 0.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total dose (μg/kg)*</td>
<td>1.11 ± 0.44</td>
<td>1.55 ± 0.68</td>
<td>0.02</td>
</tr>
<tr>
<td>Total cost (Korean won/4 wk)*</td>
<td>34,100 ± 7,600</td>
<td>115,500 ± 23,600</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation.

CERA, continuous erythropoietin receptor activator; DA, darbepoetin alfa.

*The total dose and cost after the initial dose were calculated and analyzed as the average value of the doses administered once in 4 weeks during the study period. The starting dose and costs of the darbepoetin alfa group were excluded.
The study was designed according to the Korean NHIS’s reimbursement acceptance criteria for subject registration, initial ESA dose, ESA dose adjustment method, target Hb level range, and indications for iron replacement therapy. Therefore, there are several differences in current anemia management guidelines for patients with CKD ND. The 2012 KDIGO and the 2017 National Institute for Health and Care Excellence (NICE) anemia guidelines for CKD suggest that ESAs should not be used to maintain Hb levels above 11.5 g/dL [30] and ESA therapy should achieve Hb levels between 10 and 12 g/dL, respectively [31]. These guidelines indicate that decreasing the ESA dose is preferable to withholding ESA when Hb levels exceed the upper target limit [30,31]. In the Korean NHIS, physicians can start ESA treatment at Hb levels below 10 g/dL in patients with an eGFR of <30 mL/min/1.73 m², prescribe ESAs only when the Hb level is ≤11 g/dL, and should be withholding ESA treatment when the Hb level is >11 g/dL in patients with CKD receiving ESAs. Taken together, the target Hb level in Korea is actually 10 to 11 g/dL; therefore, it appears that Korean patients with CKD ND are being treated for anemia at a lower and narrower target Hb range. The treatment strategy of withholding the ESA administration rather than reducing the ESA dose may cause large fluctuations in the administered ESA dose. The large fluctuations in the administered ESA dose and the consequent fluctuations in the DCR in the current study appear to be the result of this phenomenon. The inclusion criteria of the current study, according to the iron profile and indication for iron replacement, were set to the lower limit (serum ferritin, <100 ng/mL; TSAT, <20%) for intravenous iron administration under the Korean NHIS. This is consistent with the 2006 Kidney Disease Outcomes Quality Initiative [32] and the 2017 NICE guideline, which recommend iron therapy to maintain ferritin levels at >100 ng/mL and TSAT at >20% and defined iron repletion as ferritin levels >100 ng/mL and TSAT >20%, respectively. However, owing to the lack of evidence for the specific ferritin and TSAT levels at which iron therapy should be initiated, the 2012 KDIGO guidelines suggested only the upper limits of ferritin and TSAT levels at which iron therapy should be initiated, the 2012 KDIGO guidelines suggested only the upper limits of ferritin and TSAT levels for iron therapy. As there is no clear definition of iron deficiency for initiating iron therapy in patients who have started ESA therapy, each center’s and physician’s strategy for iron therapy may differ from the design of this study. The results of this study fully reflect Korea’s actual clinical practice; however, there are limitations to applying these findings to patients treated using other anemia treatment strategies or guidelines.

We attempted to compare the DA and CERA groups under the same conditions as much as possible, but there were two major differences. First, the correction duration, frequency, and method used during the correction period...
differed between the two groups. Regarding the approval of the DA dosing interval in Korea, to administer DA as Q4W, the Hb level should be evaluated 2 weeks after the initial administration. Because the study design followed this guideline, the correction period in the DA group was 2 weeks shorter and the dose correction was less than once compared with the CERA group. Second, the CERA group tended to have higher baseline serum ferritin levels and showed higher mean serum ferritin levels than the DA group during the study period. One participant in the CERA group had significantly high ferritin values (1,824–3,569 ng/mL). We could not identify any clinical factors that could elevate ferritin levels in this participant, who had no infection event or C-reactive protein elevation for 3 years from the time of study enrollment to the present. As this participant was properly registered in accordance with the inclusion and exclusion criteria, we believed that it would be inappropriate to exclude this participant’s data. Despite these two major differences between the two groups, these factors may disadvantage the DA group in terms of efficacy endpoints; therefore, it would not affect the finding that DA was non-inferior to CERA.

The current study has several limitations. First, the small sample size is a critical issue that can be considered a major limitation of this study. We calculated the sample size based on previous studies [16,20–23]. Unfortunately, in the CERA group, one more participant dropped out, and the target PP population included 15 participants. The dropout rate was higher than expected because more patients dropped out due to the initiation of dialysis. Although the sample was one less than the target number of participants, the sample size may be sufficient to compare the efficacy outcomes because the SD was set conservatively when calculating the sample number. Second, as mentioned above, the current study was conducted as an RCT; however, the interventions during the correction period and serum ferritin levels during the study period differed between the two groups. Third, the protocol for iron supplementation in this study, which incorporated the reimbursement criteria of the Korean NHIS, did not reflect the recent trend recommending active iron replacement to reduce ESA use in patients with CKD [33,34]. Therefore, additional study reflecting the recent recommendations for iron replacement in patients with CKD is needed. Fourth, because the price of each ESA is different in different countries, the results of this study cannot be generalized. However, when calculated arithmetically, DA may be more cost-effective than CERA in countries where the price of the same dose, considering the DCR, is similar. Finally, the current study could not explain how DA, which has a shorter half-life, was comparable to CERA for anemia correction under Q4W administration. The administration interval of ESA depends both on the duration of the circulating ESA level above the erythropoiesis threshold, and the duration of the biological cascade resulting from the interaction between ESA and its receptors [11,29]. As the former is determined by half-life, it can be confirmed through pharmacokinetic studies and is generally used as an indicator of the ESA administration interval. However, the exact ESA administration interval remains unclear in that the latter is not yet known precisely [11,29]. It can be assumed that the unknown pharmacodynamic properties of each ESA agent may induce erythropoietic effects that offset the differences in the half-lives. We hypothesized that, when the level of circulating ESA surpasses the threshold required for erythropoiesis activation, erythropoiesis may be initiated, which may persist to some extent even when the concentration of circulating ESA falls below the threshold necessary for erythropoiesis. However, as this study did not intend to reveal causality, additional experimental studies are required to confirm this hypothesis.

In conclusion, our findings verified the hypothesis that DA Q4W is non-inferior to CERA Q4W for anemia correction. DA Q4W successfully and safely corrected anemia in ESA-naïve patients with CKD ND and is more cost-effective than CERA Q4W in a real-world setting in Korea.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

This work was supported by Kyowa Kirin Korea Co., Ltd. and the Soonchunhyang University Research Fund.

Data sharing statement

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

Conceptualization: GNP, JK BCY
Data curation: All authors
Formal analysis: GNP, JEM, JKK, BCY
Funding acquisition: BCY
Writing-original draft: GNP, BCY
Writing-review & editing: SJC, MYP, JKK, BCY
All authors read and approved the final manuscript.

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References


Triglyceride-glucose index is an independent predictor of coronary artery calcification progression in patients with chronic kidney disease

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Background: Coronary artery calcification (CAC) is highly prevalent in patients with chronic kidney disease (CKD) and is associated with major adverse cardiovascular events and metabolic disturbances. The triglyceride-glucose index (TyGI), a novel surrogate marker of metabolic syndrome and insulin resistance, is associated with CAC in the general population and in patients with diabetes. This study investigated the association between the TyGI and CAC progression in patients with CKD, which is unknown.

Methods: A total of 1,154 patients with CKD (grades 1–5; age, 52.8 ± 11.9 years; male, 688 [59.6%]) were enrolled from the KNOW-CKD (KoreaN Cohort Study for Outcomes in Patients With Chronic Kidney Disease). The TyGI was calculated as follows: In (fasting triglycerides × fasting glucose/2). Patients were classified into tertiles (low, intermediate, high) based on the TyGI. The primary outcome was annualized percentage change in CAC score [(percent change in CAC score + 1)12/follow-up months – 1] of ≥15%, defined as CAC progression.

Results: During the 4-year follow-up, the percentage of patients with CAC progression increased across TyGI groups (28.6%, 37.5%, and 46.2% in low, intermediate, and high groups, respectively; p < 0.001). A high TyGI was associated with an increased risk of CAC progression (odds ratio [OR], 2.11; 95% confidence interval [CI], 1.14–3.88; p = 0.02) compared to the low group. Moreover, a 1-point increase in the TyGI was related to increased risk of CAC progression (OR, 1.55; 95% CI, 1.06–1.76; p = 0.02) after adjustment.

Conclusion: A high TyGI may be a useful predictor of CAC progression in CKD.

Keywords: Cardiovascular disease, Chronic, Coronary artery calcification, Renal insufficiency, Insulin resistance, Triglyceride-glucose index

Received: October 24, 2023; Revised: November 14, 2023; Accepted: November 17, 2023

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Introduction

Coronary artery calcification (CAC) and its progression are known to be related to cardiovascular events \[1-3\]. Many studies have reported the association between CAC and metabolic disturbances such as central obesity, insulin resistance, and dyslipidemia \[4-8\]. The triglyceride-glucose index (TyGI) is a reliable surrogate marker of insulin resistance \[9\], and it is considered even more sensitive and specific than the Homeostatic Model Assessment for Insulin Resistance \[10\]. There have been several studies on insulin resistance and its relationship with cardiovascular events in the general population and in patients with diabetes mellitus (DM) \[11-14\]. For patients with chronic kidney disease (CKD), cardiovascular disease (CVD) and cerebrovascular disease are major causes of mortality \[15\]. While CKD itself is a potential risk factor for CVD, the previous CRIC (Chronic Renal Insufficiency Cohort) study consisting of patients with estimated glomerular filtration rate (eGFR) ranging from 20 to 70 mL/min/1.73 m\(^2\) showed that CAC was significantly independently related to increased risk of CVD \[16\]. In addition to this, CAC is associated with both a higher risk of CVD and an increased risk of CKD progression \[17\]. Nevertheless, few studies have investigated the connection between the TyGI and CAC progression in patients with CKD. Therefore, this study aimed to investigate the association between the TyGI and CAC progression in patients with CKD.

Methods

Patients

The participants were enrolled from the nationwide multicenter prospective observational cohort of the KNOW-CKD (KoreaN Cohort Study for Outcomes in Patients With Chronic Kidney Disease) which consists of 2,238 Korean predialysis patients with grade 1 to 5 CKD aged between 20–75 years. This cohort study included patients with grade 1 to 2 CKD who had albuminuria or different early kidney damage markers even with a normal or slightly decreased glomerular filtration rate. Patients with previous chronic dialysis, kidney transplantation, or unavailable demographic information, lab data, or CAC score were excluded. Finally, 1,154 patients with grade 1 to 5 CKD were enrolled and classified into one of three groups based on the baseline TyGI: low (n = 382), intermediate (n = 387), or high (n = 385) (Fig. 1).

The study rationale, design, and methods are described in detail elsewhere \[18\]. The Institutional Review Board of each participating center approved the study protocol. All patients gave written informed consent at enrollment.

Data collection

Demographic and clinical data including age, sex, alcohol consumption, smoking history, comorbidities, and med-

---

**Figure 1. Flow diagram for the patients enrolled in this study.**

CAC, coronary artery calcification.
ications were collected at the time of enrollment. Patients stood barefoot to measure their height to the nearest 0.1 cm using a digital stadiometer. Bodyweight was measured to the nearest 0.1 kg in light clothes without shoes, using standard methods. Body mass index (BMI) was calculated as weight/height². Blood pressure (BP) was measured after 5 minutes of resting in a sitting position at the office by a trained nurse using a mercury sphygmomanometer or calibrated oscillometer electronic sphygmomanometer at each participating center according to the American Heart Association standardized protocol [19]. The mean of three BP measurements from each visit was used as the BP value. The electronic device was validated by comparing the device readings alternating with five mercury sphygmomanometer values. Hypertension was defined as BP of ≥140/90 mmHg, self-reported hypertension, or current use of antihypertensive drugs such as calcium-channel blockers, angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, beta-blockers, and diuretics. DM was defined as self-reported DM, fasting plasma glucose of ≥126 mg/dL, or use of glucose-lowering drugs. Smoking status was binarized as current smoker or former/never smoker. Alcohol drinkers were defined as patients who drink alcohol more than twice a week. All data including baseline demographic information and laboratory data were gained from the electronic data management system (PhactaX). Laboratory data such as hemoglobin, blood urea nitrogen (BUN), creatinine, uric acid, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), calcium, phosphate, and parathyroid hormone (PTH) levels were measured from overnight fasting venous samples. Fibroblast growth factor 23 (FGF-23) was measured by enzyme-linked immunosorbent assay (Immutopics). The TyGI was calculated as follows: ln (fasting triglycerides × fasting glucose/2).

Outcome measures

CAC scores were measured at enrollment and at the 4-year follow-up. CAC was assessed via electrocardiography-gated coronary 64-slice multidetector chest computed tomography. The CAC density score was calculated by dividing the Agatston score by the total area score by experienced cardiac radiologists from each participating center. The primary outcome was CAC progression, which was defined as an annualized percentage change in CAC scores ≥15% [20–24]. As in previous studies [25], the percentage change of CAC score was defined as [(CAC score at follow-up) – (CAC score at enrollment)] / (CAC score at enrollment + 1). Adding 1 to the CAC score at enrollment in the denominator allows for the inclusion of patients with a baseline CAC score of 0. Furthermore, the annualized percentage change in CAC score was calculated as (percent change of CAC score + 1)^1/4 – 1. The value “1/4” in this equation was derived from (12/follow-up months), where the number of follow-up months is 48. Secondary outcomes included major adverse cardiovascular events (MACE), such as myocardial infarction, nonfatal stroke, and all-cause mortality.

Statistical analysis

Continuous variables were stated as mean ± standard deviation and categorical variables as number (percentage). Baseline characteristics and changes in CAC scores were compared between groups using analysis of variance for continuous variables and the chi-square test for categorical variables. Multivariable logistic regression analyses were performed to evaluate the association between the TyGI and CAC progression in each group. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to provide the relative risk for CAC progression. Statistical analyses in this study were performed using IBM SPSS version 26 (IBM Corp.), and R software version 4.3.2 (R Foundation for Statistical Computing). A p-value of <0.05 was considered significant.

Results

Baseline characteristics

The mean age of study subjects was 52.8 ± 11.9 years and 688 (59.6%) were male. The mean TyGI was 8.8 ± 0.6 in all patients, 8.2 ± 0.3 in the low TyGI group, 8.8 ± 0.2 in the intermediate group, and 9.5 ± 0.4 in the high group. Compared to the low TyGI group, patients with a high TyGI tended to be older, have higher BMI and systolic BP (SBP), and more comorbidities such as hypertension and DM. The high TyGI group also had higher levels of calcium, BUN, creatinine, fasting plasma glucose, total cholesterol,
and triglycerides but lower eGFR and HDL-C compared to the low TyGI group. There were no significant differences in bone mineral disease markers such as phosphate, alkaline phosphate, FGF-23, or total PTH other than calcium among different TyGI groups (Table 1).

**Baseline and change in coronary artery calcification**

During the 4-year follow-up, the median value of annualized percentage change of patients with CAC progression increased across TyGI groups (0%, 7.0%, and 13.6% in low, intermediate, and high TyGI groups, respectively; p < 0.001) (Table 2, Fig. 2). The percentage of patients with CAC progression increased across TyGI groups (28.6%, 37.5%, and 46.2% in low, intermediate, and high TyGI groups, respectively; p < 0.001) (Table 2).

**Association between triglyceride-glucose index and coronary artery calcification progression in patients with chronic kidney disease**

In multivariable logistic regression analysis, the high TyGI group was associated with increased risk of CAC progression (OR, 2.11; 95% CI, 1.14–3.88; p = 0.02) compared to

| Table 1. Baseline characteristics at enrollment among patients stratified by TyGI |
|---------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Characteristic                  | Total            | Low              | Intermediate     | High             | p-value          |
| No. of subjects                 | 1,154 (100)      | 382 (33.1)       | 387 (33.5)       | 385 (33.4)       | <0.001           |
| Age (yr)                        | 52.8 ± 11.9      | 50.5 ± 12.1      | 53.7 ± 11.9      | 54.1 ± 11.4      | <0.001           |
| Male sex                        | 688 (59.6)       | 193 (50.5)       | 233 (60.2)       | 262 (68.1)       | <0.001           |
| SBP (mmHg)                      | 126.0 ± 14.7     | 123.7 ± 13.9     | 126.5 ± 14.4     | 127.8 ± 15.4     | <0.001           |
| DBP (mmHg)                      | 76.8 ± 10.4      | 76.2 ± 10.3      | 76.9 ± 9.9       | 77.3 ± 10.8      | 0.36             |
| Body mass index (kg/m²)         | 24.7 ± 3.3       | 23.2 ± 3.1       | 24.6 ± 2.9       | 26.1 ± 3.4       | <0.001           |
| Comorbidities                   |                  |                  |                  |                  |                  |
| Hypertension                    | 1,101 (95.4)     | 351 (91.9)       | 371 (95.9)       | 379 (98.4)       | <0.001           |
| Diabetes mellitus               | 307 (26.6)       | 49 (12.8)        | 102 (26.4)       | 156 (40.5)       | <0.001           |
| Statin use                      | 583 (50.5)       | 152 (39.8)       | 220 (56.8)       | 211 (54.8)       | <0.001           |
| Alcohol                         | 349 (30.2)       | 89 (23.3)        | 105 (27.1)       | 155 (40.3)       | <0.001           |
| Smoking                         | 382 (33.1)       | 238 (61.5)       | 42 (10.9)        | 102 (26.5)       | <0.001           |
| Exercise                        | 359 (31.1)       | 111 (29.1)       | 124 (32.0)       | 124 (32.2)       | 0.80             |
| Laboratory value                |                  |                  |                  |                  |                  |
| Calcium (mg/dL)                 | 9.24 ± 0.43      | 9.16 ± 0.39      | 9.25 ± 0.44      | 9.29 ± 0.43      | <0.001           |
| Phosphate (mg/dL)               | 3.54 ± 0.56      | 3.48 ± 0.53      | 3.56 ± 0.56      | 3.58 ± 0.58      | 0.053            |
| Alkaline phosphate (U/L)        | 85.4 ± 62.9      | 82.0 ± 59.0      | 87.0 ± 65.3      | 87.3 ± 64.1      | 0.42             |
| FGF-23 (RU/mL)                  | 20.1 ± 29.4      | 17.9 ± 23.6      | 21.6 ± 38.4      | 20.7 ± 23.6      | 0.21             |
| Total PTH (pg/mL)               | 53.6 ± 39.0      | 53.3 ± 37.0      | 52.7 ± 40.5      | 55.0 ± 39.8      | 0.80             |
| Blood urea nitrogen (mg/dL)     | 23.1 ± 10.9      | 21.7 ± 10.4      | 24.2 ± 11.5      | 23.4 ± 10.6      | 0.005            |
| Creatinine (mg/dL)              | 1.38 ± 0.81      | 1.28 ± 0.60      | 1.41 ± 0.75      | 1.45 ± 1.03      | 0.004            |
| eGFR (mL/min/1.73 m²)           | 62.3 ± 28.8      | 67.0 ± 30.2      | 60.3 ± 28.6      | 59.7 ± 27.1      | <0.001           |
| Fasting plasma glucose (mg/dL)  | 107.1 ± 31.1     | 92.7 ± 11.5      | 104.7 ± 20.4     | 123.9 ± 43.1     | <0.001           |
| Total cholesterol (mg/dL)       | 174.9 ± 36.0     | 170.0 ± 33.2     | 173.5 ± 35.5     | 181.7 ± 38.0     | <0.001           |
| Triglyceride (mg/dL)            | 152.2 ± 94.2     | 79.0 ± 20.7      | 129.8 ± 26.6     | 247.4 ± 102.9    | <0.001           |
| LDL-C (mg/dL)                   | 97.4 ± 30.1      | 94.9 ± 27.4      | 99.8 ± 30.3      | 97.3 ± 32.2      | 0.08             |
| HDL-C (mg/dL)                   | 50.8 ± 15.0      | 58.0 ± 16.3      | 50.7 ± 13.5      | 43.7 ± 11.2      | <0.001           |
| TyGI                            | 8.8 ± 0.6        | 8.2 ± 0.3        | 8.8 ± 0.2        | 9.5 ± 0.4        | <0.001           |

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

DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor 23; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PTH, parathyroid hormone; SBP, systolic blood pressure; TyGI, triglyceride-glucose index.
Table 2. Change in CAC scores stratified by TyGI

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>CAC score</th>
<th>Percent change in CAC scores</th>
<th>CAC progression</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>4-yr follow-up</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>382</td>
<td>0.0 (0.0–8.0)</td>
<td>0.0 (0.0–42.4)</td>
<td>0.0 (0.0–19.7)</td>
<td>109 (28.5)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>387</td>
<td>0.5 (0.0–62.2)</td>
<td>8.3 (0.0–155.3)</td>
<td>7.0 (0.0–26.9)</td>
<td>145 (37.5)</td>
</tr>
<tr>
<td>High</td>
<td>385</td>
<td>5.3 (0.0–76.6)</td>
<td>28.5 (0.0–187.2)</td>
<td>13.6 (0.0–33.4)</td>
<td>178 (46.2)</td>
</tr>
</tbody>
</table>

Data are expressed as median (interquartile range) or number (%). CAC, coronary artery calcification; TyGI, triglyceride-glucose index.

Table 3. ORs for CAC progression according to the TyGI

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>OR (95% CI)</th>
<th>p-value</th>
<th>Model 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>OR (95% CI)</th>
<th>p-value</th>
<th>Model 3&lt;sup&gt;c&lt;/sup&gt;</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TyGI</td>
<td></td>
<td></td>
<td></td>
<td>TyGI (per 1-point increase)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>Reference</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>1.50 (1.11–2.03)</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>2.15 (1.59–2.91)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TyGI (per 1-point increase)</td>
<td>1.39 (1.08–1.79)</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CAC, coronary artery calcification; CI, confidence interval; OR, odds ratio; TyGI, triglyceride-glucose index.
<sup>a</sup>Unadjusted. <sup>b</sup>Adjusted for age, sex, body mass index, waist, smoking, alcohol, and exercise. <sup>c</sup>Model 2 + hypertension, diabetes mellitus, systolic blood pressure, estimated glomerular filtration rate, urine protein to creatinine ratio, and low- and high-density lipoprotein cholesterol.

Figure 2. Percentage change in CAC among different TyGI groups. Percentage change of patients with CAC progression increased across TyGI groups (with the mean values of 16.7%, 20.8%, and 24.9% in low, intermediate, and high TyGI groups, respectively; p < 0.001). CAC, coronary artery calcification; TyGI, triglyceride-glucose index.

TyGI was related to increased risk of CAC progression (OR, 1.55; 95% CI, 1.06–1.76; p = 0.003) after adjusting for confounding factors (Table 3).

Association between the triglyceride-glucose index and major adverse cardiovascular events in patients with chronic kidney disease

To determine the clinical significance of the relationship between the TyGI and CAC progression, MACE was evaluated within each TyGI group using multivariable Cox analysis. Compared to the low TyGI group, the intermediate and high TyGI groups exhibited an increased risk of MACE (hazard ratio [HR], 1.50; 95% CI, 1.11–2.03; p = 0.009 and HR, 2.15; 95% CI, 1.59–2.91; p < 0.001) after adjusting for age, sex, BMI, smoking, alcohol consumption, hypertension, DM, SBP, eGFR, LDL-C, and HDL-C.

Subgroup analyses

To assess how different subgroups influence the relationship between the TyGI and CAC progression, patients were stratified by age, sex, BMI, eGFR, SPB, and DM (Fig. 3). These analyses revealed an interaction between age and...
TyGI (p for interaction = 0.02), between DM and TyGI (p for interaction = 0.003), and between eGFR and TyGI (p for interaction = 0.001). These findings suggest that the impact of the TyGI is influenced by age, kidney function, and DM.

Sensitivity analyses

To validate the findings in this study, sensitivity analyses were conducted using an alternative definition of CAC progression. This alternative definition involves the square-root transformation of the difference between baseline and follow-up CAC scores [√CAC score (follow-up) − √CAC score (baseline)], with a threshold set at greater than 2.5. This threshold was chosen to minimize the effect of interscan variability. Multivariable Cox analyses were carried out, adjusting for the same variables as previously described, and revealed that the highest tertile of the TyGI and a one-unit increase in TyGI were both associated with a significantly elevated risk of CAC progression (Table 4).

Discussion

As mentioned in other traditional studies, the baseline characteristic analysis showed a significant relationship between a high TyGI and well-known CVD risk factors including comorbidities [11–13,26–29]. In addition, in the current study, the baseline and follow-up CAC score and their changes increased in a stepwise manner according to the TyGI. Furthermore, the proportion of patients with CAC progression during follow-up was higher in the high TyGI group. This study also demonstrated an independent association between the TyGI and CAC progression in patients with CKD. This relationship was consistent even after adjusting for widely known CVD risk factors including eGFR. In the continued exploration of the clinical significance of the relationship between the TyGI and CAC progression, our investigation revealed that elevated TyGI levels were associated with increased risks of MACE when compared to CKD patients with lower TyGI levels.

There are several cross-sectional and population-based studies supporting the positive correlation between the
TyGI and CAC progression [11–13]. The mechanism of association between a high TyGI and CAC progression is uncertain. While the TyGI is an important surrogate marker for insulin resistance, previous studies demonstrated that insulin resistance contributes to atherogenesis and progression of atherosclerosis by triggering macrophage apoptosis and endothelial and vascular smooth muscle cell damage [30–32]. Hyperinsulinemia can induce oxidative stress and interfere with the proper functioning of endothelial cells. Also, consequent reduction in nitric oxide availability may contribute to both functional and structural injury to blood vessels [33]. Moreover, hyperinsulinemia can trigger osteogenic differentiation and the formation of calcifications in vascular cells [34]. Furthermore, insulin resistance can accelerate the accumulation of advanced glycosylation end-products, thereby promoting the progression of CAC [35]. Atherosclerosis and valvular calcification commonly manifest in individuals with CKD [36]. Although it is challenging to determine the causal relationship from this observational cohort study, there have been studies suggesting that CAC progression may be related to the deterioration of kidney function [17,37]. A plausible explanation may be that the process, as described above, wherein a high TyGI may lead to CAC progression, could further contribute to the development of CKD. This could represent an ongoing process in patients with CKD. Further prospective studies are needed to elucidate the mechanism underlying this association in patients with CKD.

There is a lack of a clinical causal relationship between the TyGI and CAC since there are no data or clinical trials on improved obesity and coronary calcium score, proven TyGI-lowering medications, or improvement in CAC. Therefore, further studies are needed to determine the precise causal relationship between the TyGI and CAC progression.

There were several limitations in this study. First, this was an observational study; therefore, it was difficult to control for all possible confounding factors. Second, the study was limited to one ethnicity, and so the results cannot be generalized to other populations with different ethnicities. Despite these limitations, this study included a large population of patients with CKD and CAC measurements; and to our knowledge, it is the first to determine a correlation between CAC progression and a high TyGI in patients with CKD using longitudinal data, unlike other cross-sectional studies. Another notable aspect of this study is the simplicity of obtaining the TyGI through routine blood tests. This index could be valuable as an alternative indicator, not only for predicting the presence of CAC but also for doing so without radiation exposure.

In conclusion, this study showed an independent association of a high TyGI with CAC progression regardless of other CVD risk factors in patients with CKD. A high TyGI may be a useful predictor of CAC progression implying CVD risk and kidney outcomes in patients with CKD.

### Additional information

The approval numbers of each Institutional Review Board are as follows: Seoul National University Hospital (No. 1810-132-982), Seoul National University Bundang Hospital (No. B-1901-514-409), Severance Hospital (No. 4-2019-0152), Kangbuk Samsung Hospital (No. 2019-04-011), The Catholic University of Korea, Seoul St. Mary’s Hospital (No. KC19OEDI0263), Gachon University Gil Medical Center (No. GAIRB2019-154), Nowon Eulji Medical Center, Eulji

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**Table 4. ORs for CAC progression with different definitions according to the TyGI**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>p-value</th>
<th>Model 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>p-value</th>
<th>Model 3&lt;sup&gt;c&lt;/sup&gt;</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
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<td>OR (95% CI)</td>
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<td>OR (95% CI)</td>
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<tr>
<td>TyGI</td>
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<tr>
<td>Low</td>
<td>Reference</td>
<td>-</td>
<td>Reference</td>
<td>-</td>
<td>Reference</td>
<td>-</td>
</tr>
<tr>
<td>Intermediate</td>
<td>1.82 (1.34–2.48)</td>
<td>&lt;0.001</td>
<td>1.48 (1.00–2.21)</td>
<td>0.05</td>
<td>1.57 (0.86–2.90)</td>
<td>0.15</td>
</tr>
<tr>
<td>High</td>
<td>2.29 (1.69–3.11)</td>
<td>&lt;0.001</td>
<td>1.67 (1.11–2.51)</td>
<td>0.01</td>
<td>1.85 (0.94–3.68)</td>
<td>0.08</td>
</tr>
<tr>
<td>TyGI (per 1-point increase)</td>
<td>1.82 (1.50–2.22)</td>
<td>&lt;0.001</td>
<td>1.53 (1.17–2.00)</td>
<td>0.002</td>
<td>1.61 (1.01–2.60)</td>
<td>0.048</td>
</tr>
</tbody>
</table>

CAC, coronary artery calcification; CI, confidence interval; OR, odds ratio; TyGI, triglyceride-glucose index.

<sup>a</sup> Unadjusted.  <sup>b</sup> Adjusted for age, sex, body mass index, waist, smoking, alcohol, and exercise.  <sup>c</sup> Model 2 + hypertension, diabetes mellitus, systolic blood pressure, estimated glomerular filtration rate, urine protein to creatinine ratio, and low- and high-density lipoprotein cholesterol.
University (No. 201904-01), Chonnam National University Hospital (No. 2021-07-111), Inje University Pusan Paik Hospital (No. 2018-01-203), Hallym University Dongtan Sacred Heart Hospital (No. 2019-11-006), National Health Insurance Service Ilsan Hospital (No. 2019-12-008), Seoul National University Boramae Medical Center (No. 2019-76), Pusan National University Hospital (No. 1912-019-086), and Chungnam National University Hospital (No. 2021-07-111).

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 potential conflicts of interest relevant to this article.

Funding

This work was supported by the Research Program funded by the Korea Disease Control and Prevention Agency (2011E3300350, 2012E3301100, 2013E3301600, 2013E3301601, 2013E3301602, 2016E3300200, 2016E3300201, 2016E3300202, 2019E3201000, 2019E320101, 2019E320102, and 2022-11-007). The funding sources had no role in the design and conduct of the study, collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

Acknowledgments

The authors thank the clinical research coordinators of each participating institution and the Medical Research Collaborating Center, Seoul National University Hospital for the data management and data quality control. This is a group study by multi-center collaboration by the KNOW-CKD Investigator Group.

Data sharing statement

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

Authors’ contributions

Conceptualization, Investigation: YEK, THY
Methodology: YEK, HWK, JTP, SHH, SWK, THY
Data curation: HWK
Formal analysis: YEK, HWK, JTP, SHH, SWK, THY
Supervision: THY, HWK, JTP, SHH, SWK, SS, KBL, JL, KHO
Writing—original draft: YEK, THY
Writing—review & editing: YEK, THY
All authors critically reviewed and approved the final manuscript.

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1. Manuscript Submission

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(2) A conflict of interest disclosure statement (see relevant section 4.2 below).
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(6) The terms sex (when reporting biological factors) and gender (identity, psychosocial or cultural factors) should be correctly used. The sex and/or gender of study participants, the sex of animals or cells should be reported, and the methods used to determine sex and gender should be described. If the study was done involving an exclusive population, for example in only one sex, authors should justify why, except in obvious cases (e.g., ovarian cancer).
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(9) Articles should be written in English (using American English spelling) and meet the following basic criteria: the material is original; the information is important; the writing is clear, concise and grammatically correct; the study methods are appropriate; the data are valid; and the conclusions are reasonable and supported by the data. The articles should be readable to native English users, and we recommend using professional language editing service (e.g., American Journal Experts) prior to submission to avoid delays with the review processes.
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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.

2.3. Special Articles
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 and 8 references. A maximum of 2 figures or tables may be included.

2.5. Editorials
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.

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

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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”).
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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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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://wwwdeclarationofistanbul.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.

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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).

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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.
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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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Select one of the visual abstract templates provided (https://www.krcp-ksn.org/file/KRCP_Visual_Abstracts_v1.0.pptx). There are multiple layouts to accommodate author preferences as well as graphical constraints. The visual abstract should include a title, methods, outcome and a concluding sentence. Please fill in the template as it’s laid out and do not alter the basic components of the template.

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12. After acceptance

12.1. Article-in-press publication

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

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CKD, chronic kidney disease; Hb, hemoglobin

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2. Based on IQVIA MAT 3Q 2023, V03G
* Kalimate® Powder is the 1st released calcium polystyrene sulfonate agent in 1984 in Korea, through the licensing with the originator, Nikken(now Kowa) from Japan.
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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\(^2,3\)

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