KIDNEY RESEARCH AND CLINICAL PRACTICE

HIGHLIGHTS

Fructose in the kidney: from physiology and pathology

Executive Summary of the Korean Society of Nephrology 2021 Clinical Practice Guideline for Optimal Hemodialysis Treatment

Probiotics partially attenuate the severity of acute kidney injury through an immunomodulatory effect

Metabolic risks in living kidney donors in South Korea

Modeling of endothelial cell dysfunction using human induced pluripotent stem cells derived from patients with end-stage renal disease
Aims and Scope

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

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

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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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The image on the front cover: Kim et al showed the human induced pluripotent stem cells from the patients with ESRD and healthy controls. Please see the text for more details (pp. 698–711).
The intestinal microbiota, a community of 100 trillion microorganisms (more than 1,000 species that consist of mostly bacteria but also viruses, fungi, and protozoa), plays an important role in maintaining homeostasis (especially in regard to mucosal immunity and nutrient metabolism) in the human gastrointestinal tract [1]. The intestinal epithelial barrier can be divided into three components; a biological barrier, a physical barrier, and an immune barrier [2]. The biological barrier is composed of bacterial and fungal symbionts that are closely attached to the intestinal mucosal surface and compete with pathogenic bacteria. The physical barrier refers to intestinal epithelial cells with apical tight junctions (TJs). Changes in TJs can lead to increased permeability, allowing bacteria, endotoxins, and macromolecules to enter the circulatory system. The immune barrier is the third system for maintaining microbial homeostasis. Dendritic cells in the lamina propria activate T cells to evoke an adaptive immune response. Innate lymphoid cells located in the gut epithelium have key defensive functions to produce or activate the release of immune-activating cytokines.

Recent studies suggest that altered structure and composition of gut microbiota known as dysbiosis are linked to disrupted homeostasis and pathogenesis of various diseases, including inflammatory bowel disease, obesity, diabetes, cancer, Alzheimer disease, nonalcoholic fatty liver disease, and chronic obstructive pulmonary disease [1–3]. Therefore, several hypotheses of the “Gut-Brain Axis,” “Gut-Liver Axis,” “Gut-Lung Axis,” and others have been proposed to explain the bidirectional complex communication between the gut and other organs. Although the exact mechanism underlying crosstalk between gut microbiota and distant organs remains uncertain, it has been proposed that the pathogenesis might be mediated by altering the function of the intestinal barrier, modifying local and systemic inflammation, controlling the production of metabolites, and affecting immune responses [1,3,4].

From the point of view of kidney disease, several recent experimental and clinical data have revealed the existence of a gut-kidney axis and the pivotal role of gut microbiota in kidney injury [3,5]. Increasing urea concentration in chronic kidney disease (CKD) leads to alterations in the intestinal flora. The potential mechanisms for change in...
microbiome composition include decreased fiber intake, phosphate binder use, decreased colonic transit time, and comorbidities such as diabetes. Alterations in the intestinal flora can increase the production of gut-derived toxins and alter the intestinal epithelial barrier. These changes can accelerate the process of kidney injury. Probiotics are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” and have been used as a potential therapeutic option in several chronic inflammatory disease models, including CKD [6].

To date, only a limited number of studies have investigated the impact of probiotics in the gut-kidney axis during acute kidney injury (AKI). Yang et al. [7] investigated the beneficial effects and distant organ influences of prior probiotic supplementation on AKI in a renal ischemia/reperfusion injury (IRI) animal model. Pretreatment of mice with *Bifidobacterium bifidum* BGN4 (BGN4) attenuated AKI-induced dysbiosis and gut barrier disruption. In addition, BGN4 administration significantly reduced the severity of renal IRI and distant organ (liver) injury. These results were associated with expansion of regulatory T cells and reduced interleukin-17A expression in the colon, mesenteric lymph nodes, and kidney, suggesting that BGN4-induced immunomodulation contributes to its renoprotective effect. Previously, the authors demonstrated a bidirectional relationship between the kidney and intestine in AKI that renal IRI provoked intestinal dysbiosis and the dysbiotic microbiota was an important modifier of postsischemic kidney outcome due to immune modulatory effects [8]. Moreover, they also reported that *Lactobacillus salivarius* BP121 attenuated cisplatin-induced kidney injury by decreasing inflammation, oxidative stress, and serum levels of uremic toxins and by modulating the gut environment [9]. Recently, some studies have presented the renoprotective effects of probiotics and gut microbiota-derived metabolites (e.g., short-chain fatty acids [SCFAs] and D-serine). *Lactobacillus casei* Zhang delayed kidney disease progression in a renal IRI mouse model and also in patients with CKD by increasing the levels of SCFAs and via nicotinamide metabolism, which together modulate the inflammatory response of local macrophages and tubular epithelial cells [6]. Furthermore, administration of SCFAs (acetate, propionate, and butyrate) attenuated inflammation in kidney epithelial and immune cells and ameliorated renal IRI through modula-

![Figure 1](506 www.krcp-ksn.org)

*Figure 1.* The bidirectional relationship between the gut microbiota and kidney and the proposed protective role of probiotics and their metabolites in acute kidney injury.
tion of the inflammatory process, most likely via epigenetic modification. Gut microbiota-derived D-serine was shown to reduce tubular damage in AKI. In addition, a microbial cocktail of *Escherichia*, *Bacillus*, and *Enterobacter* exhibited a protective effect in nephrotoxin-induced AKI [10]. Although these studies indicate the bidirectional relationship between the intestinal microbiota and kidney through the gut-kidney axis in AKI, the precise mechanisms by which these processes act remain unclear.

Based on the studies mentioned above, crosstalk in the gut-kidney axis during AKI can be explained as follows. (1) AKI induces intestinal dysbiosis and barrier disruption (often referred to as “leaky gut”); (2) dysbiosis and leaky gut alter mucosal immune responses, which leads to accumulation of neutrophils and proinflammatory macrophages and activation of the Th17 pathway; (3) a shift in mucosal immunity toward proinflammatory status can aggravate kidney and distant organ injury via systemic inflammation; and (4) probiotics or their metabolites might have a potential renoprotective effect by restoring the intestinal microbiota and gut environment through immune modulation (Fig. 1).

In summary, the interactions between the gut microbiota and the kidney are important in AKI, and probiotics are a potential therapeutic approach for AKI (Fig. 1). However, the research results were insufficient because they have not been applied to humans, and it remains unclear whether administration of probiotics after AKI development has a protective effect on renal function. Future studies are needed to clarify the role of the intestinal microbiota in AKI and to explore whether modification of the gut microbiota using probiotics or supplementation with their metabolites is a potential therapeutic option.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

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**References**

Fabry disease is a rare lysosomal storage disorder caused by absent or reduced activity of α-galactosidase A, and the subsequent systemic accumulation of predominantly globotriaosylceramide (GL3, also called GB3) is caused by mutations in the α-gal A-encoding gene on the X chromosome (Xq22.1). This deposition within the lysosomes triggers pathogenic pathways in the vascular endothelium and activities of cells of different tissues in vital organs (renal, cardiac, and nervous systems) that lead to cell death and irreversible organ damage.

The severity of clinical expression in hemizygous males correlates with α-gal residual activity, and the classical phenotype develops in individuals with <1% of residual enzyme activity. At a young age, classic male patients show severe general symptoms (neuropathic pain, angiookeratoma, hypohidrosis, and corneal opacity). Kidney involvement is a significant feature of Fabry disease, and untreated classical male patients develop end-stage renal disease in the third to fifth decade of life. On the other hand, manifestations of heterozygous females can range from asymptomatic, mild, or severe due to skewed X inactivation [1].

Fabry disease can be divided into a classic phenotype and a late-onset variant (nonclassical or atypical). This clinical phenotype is usually considered to be defined (at least partially) by the genotype. Classic Fabry disease manifests with typical symptoms of absent or low enzyme activity levels that begin in childhood. Late-onset Fabry disease is characterized by a more variable disease course (adulthood onset) with residual enzyme activity. In this issue of Kidney Research and Clinical Practice, Kim et al. [2] report the clinical and pathologic findings of patients with Fabry disease who underwent kidney biopsy before or after enzyme replacement therapy [ERT]. In particular, the before-treatment group showed pathologic GL3 accumulation in kidney tissues, even in those without microalbuminuria. Apparent nephropathy, including GL3 accumulation, can occur in patients with normal glomerular filtration rate and no or minimal microalbuminuria.

In a recent study in 14 patients with Fabry disease between 4 and 19 years of age with normal glomerular filtration rates, the amount of GL3 accumulation in the podocytes correlated with age [3]. In a study by Tøndel et al. [4], loss of segmental foot processes was observed in patients
### Table 1. Renal pathologic changes after enzyme replacement therapy (ERT)

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>No. of patients (male)</th>
<th>Age (yr)</th>
<th>ERT duration (y)</th>
<th>Podocytes</th>
<th>Mesangium</th>
<th>Endothelium</th>
<th>Interstitium</th>
<th>Major finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim et al. [2]</td>
<td>2021</td>
<td>9</td>
<td>19–58</td>
<td>1.2–8</td>
<td>22</td>
<td>67</td>
<td>78</td>
<td>33</td>
<td>Segmental FPE and GL3 deposits can be persistent in Fabry nephropathy despite ERT</td>
</tr>
<tr>
<td>Skrune et al. [6]</td>
<td>2017</td>
<td>20 (12)</td>
<td>21 (7–62)</td>
<td>9.4 (4.4–11.7)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Adult male patient who started ERT at 18 years of age showed clearance of GL3 deposits from podocytes, yet a pediatric male patient who started ERT at 7 years of age showed better clearance</td>
</tr>
<tr>
<td>Najafian et al. [4]</td>
<td>2016</td>
<td>5 (5)</td>
<td>31 (18–46)</td>
<td>1 (11–12 mo)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>73% decline in podocyte GL3 content and 63% reduction in podocyte volume after 11–12 months of agalsidase beta</td>
</tr>
<tr>
<td>Tøndel et al. [4]</td>
<td>2013</td>
<td>12 (11)</td>
<td>16.5 (7–33)</td>
<td>5 (4.2–5.8)</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>Significant correlation between reduction in podocyte GL3 inclusions and cumulative dose of agalsidase alfa or beta</td>
</tr>
<tr>
<td>Lubanda et al. [8]</td>
<td>2009</td>
<td>21</td>
<td>35.7 (19–55)</td>
<td>8</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>90</td>
<td>Lower dosage than 0.3 mg/kg can maintain GL3 clearance in some patients with Fabry disease, but other patients seem to require a dosage higher to prevent recurrence of GL3 accumulation in cells</td>
</tr>
<tr>
<td>Thurberg et al. [5]</td>
<td>2002</td>
<td>58 (56)</td>
<td>28.4 ± 11.4</td>
<td>1 (11 mo)</td>
<td>0*</td>
<td>100</td>
<td>96</td>
<td>100</td>
<td>Significantly more patients treated with agalsidase beta achieved GL3 clearance from glomerular capillary endothelial cells, arterial/arteriolar endothelial cells, mesangial cells, and interstitial cells compared to placebo</td>
</tr>
</tbody>
</table>

FPE, foot process effacement. GL3, globotriaosylceramide.

*Some patients (18%) showed reduction of GL3.

### Table 2. Proposed assessments in Fabry disease

<table>
<thead>
<tr>
<th>Organ system</th>
<th>Diagnostic tool</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>eGFR (MDRD, CKD-EPI), ACR/PCR, kidney ultrasound, kidney biopsy</td>
</tr>
<tr>
<td>Cardiac</td>
<td>EKG, echocardiography, 24 hr Holter, cardiac MRI, troponin, BNP</td>
</tr>
<tr>
<td>Neurologic</td>
<td>Neurologic status, carotid/vertebral Doppler, nerve conduction studies, brain MRI</td>
</tr>
<tr>
<td>Others</td>
<td>Genetic counseling, Lyso GL3, Ophthalmologic diagnosis, ENT, Pulmonology (spirometry including response to bronchodilators and chest X-ray), Gastrointestinal (endoscopy or radiographic findings), Skeletal (bone mineral density)</td>
</tr>
</tbody>
</table>

ACR, albumin-creatinine-ratio; BNP, brain natriuretic peptide; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; ENT, ear, nose, and throat; GL3, globotriaosylceramide; MDRD, modification of diet in renal disease; MRI, magnetic resonance imaging; PCR, protein-creatinine-ratio.
with Fabry disease with albuminuria in the normal range of less than 30 mg/day. Similarly, the present study showed that podocyte foot process effacement (FPE) is one of the earliest signs of renal damage in Fabry disease.

Thurberg et al. [5] reported that patients receiving 11 months of ERT demonstrated a 100% reduction (complete clearance) in GL3 in peritubular capillary endothelial, mesangial, and interstitial cells. In contrast, only 18% of patients showed a reduction of GL3 in podocytes that responded after 11 months of ERT. This result might reflect the rate at which these cells turn over. In contrast to endothelial and mesangial cells, podocytes are differentiated terminally and proliferate poorly in response to injury or loss. We summarized several studies of renal pathologic changes after ERT for patients with Fabry disease (Table 1) [2,4–8]. Higher dose and early initiation of ERT might be positively associated with clearance of GL3 deposits from podocytes [4,6,8].

For assessment of disease severity and renal effect of ERT, we recommend histopathologic examination of the glomerular, tubulointerstitial, and vascular compartments. Therefore, kidney biopsy is pivotal. Because it can serve as a standard for treatment, kidney biopsy is a crucial screening test for women who suffer from classical complications. Therefore, kidney biopsy can be performed in a Fabry disease patient group to assess the degree of damage to the underlying tissues before enzymatic treatment and for clinical judgment of Fabry disease with atypical expression. ERT leads to a rapid and marked decrease in mesangial and endothelial cell GL3 inclusions, whereas podocyte inclusions and proteinuria persist despite treatment. Early intervention is crucial because ERT is less effective in more advanced diseases and results in irreversible damage.

Since Fabry disease is a progressive multisystem disease, various organs should be tested upon diagnosis (Table 2) [9,10]. A renal examination can be performed by a nephrologist (including estimated glomerular filtration rate and albuminuria), but involvement of other vital organs (especially the cardiac and neurologic) must be determined by an appropriate specialist.

In summary, Kim et al. [2] suggest the pivotal role of kidney biopsy in patients with Fabry disease as a screening tool for kidney damage and as an ERT response evaluation tool. In addition, they demonstrate segmental FPE and GL3 accumulation in renal pathologic findings even in patients with normoalbuminuria. Kidney biopsy is a vital tool in assessing renal involvement and can lead to early initiation of ERT, which can change the course of Fabry disease.

Conflicts of interest

All authors have no conflicts of interest to declare.

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References


In the kidney, a set of proteins expressed in the epithelial cells of the thick ascending loop of Henle and the distal convoluted tubule directly or indirectly play important roles in the regulation of serum magnesium levels. Magnesium reabsorption in the thick ascending loop of Henle occurs through a passive paracellular pathway, while in the distal convoluted tubule, the final magnesium concentration is established through an active transcellular pathway. The players involved in magnesium reabsorption include proteins with diverse functions including tight junction proteins, cation and anion channels, sodium chloride cotransporter, calcium-sensing receptor, epidermal growth factor, cyclin M2, sodium potassium adenosine triphosphatase subunits, transcription factors, a serine protease, and proteins involved in mitochondrial function. Mutations in the genes that encode these proteins impair their function and cause different rare diseases associated with hypomagnesemia, which may lead to muscle cramps, fatigue, epileptic seizures, intellectual disability, cardiac arrhythmias, and chronic kidney disease. The purpose of this review is to describe the clinical and genetic characteristics of these hereditary kidney diseases and the current research findings on the pathophysiological basis of these diseases.

Keywords: Hypomagnesemia, Magnesium handling, Mutation, Rare diseases, Renal tubulopathies

Introduction

Magnesium (Mg$^{2+}$), the second-most abundant intracellular cation, is an indispensable ion for many cellular functions including energy metabolism and nucleic acid and protein synthesis [1]. Mg$^{2+}$ is also a regulator of sodium, potassium, and calcium channels. Therefore, serum Mg$^{2+}$ levels, which are usually 0.70 to 1.1 mmol/L, need to be precisely controlled. Hypomagnesemia is defined as serum Mg$^{2+}$ level below 0.7 mmol/L. The predominant laboratory tests used for the diagnosis of hypomagnesemia are the serum Mg$^{2+}$ concentration and the 24-hour urinary Mg$^{2+}$ tests. In cases in which the serum Mg$^{2+}$ level is low, a 24-hour urine Mg$^{2+}$ higher than 24 mg/day suggests renal Mg$^{2+}$ wasting as the cause of the hypomagnesemia, while values lower than 24 mg/day indicate deficient Mg$^{2+}$ intake and/or gastrointestinal losses. Magnesium homeostasis is determined by intestinal absorption, renal re-absorption, and storage in bone. In the kidney and intestine, these processes involve a combination of paracellular and transcellular epithelial transport routes.
Hypomagnesemia may cause muscle cramps, fatigue, appetite loss, and disruptions in calcium and potassium homeostasis [1]. Acute hypomagnesemia may lead to more serious consequences like epileptic seizures, intellectual disability, and cardiac arrhythmias. Causes of hypomagnesemia include type 2 diabetes, gastrointestinal diseases, alcoholism, use of diuretics or other medications, dietary deficiency, and genetic defects. Over the last two decades, clinical and genetic studies of patients with rare hereditary disorders of Mg\(^{2+}\) handling have enabled the identification of important components of epithelial Mg\(^{2+}\) transport in the kidney [2].

After passing the glomerular filter, 90% to 95% of the filtered Mg\(^{2+}\) is subsequently reabsorbed along the nephron [1]. Approximately 10% to 25% of the Mg\(^{2+}\) is reabsorbed by the proximal tubule (PT), 50% to 70% is reabsorbed by the thick ascending limb of the loop of Henle (TAL), and 5% to 10% is reabsorbed by the distal convoluted tubule (DCT) (Fig. 1). In the PT, Mg\(^{2+}\) reabsorption occurs in a passive paracellular mode. The mechanisms that control this process are unknown. In the TAL, Mg\(^{2+}\) reabsorption also takes place through a passive paracellular pathway, which is facilitated by tight junction proteins claudin-16 and claudin-19 (Fig. 2) [3,4]. The basolateral calcium-sensing receptor (CaSR) controls the paracellular transport of calcium (Ca\(^{2+}\)) and Mg\(^{2+}\) by regulating the claudin-16/claudin-19 channel function through a signaling pathway [5]. In contrast, the main K\(^{+}\) secretory channel in the kidney, the renal outer medullary K\(^{+}\) channel (ROMK), which is located in the apical membrane of the TAL, plays a key role in the generation of the lumen-positive potential in TAL [1]. The renal chloride (Cl\(^{-}\)) channel CIC-Kb, which is located in the basolateral membrane of the TAL and also the DCT, facilitates the efflux of Cl\(^{-}\) to the interstitium [6]. The DCT establishes the final Mg\(^{2+}\) concentration through active transcellular transport.
reabsorption, which is highly regulated, via transient receptor potential melastatin type 6 (TRPM6) Mg\(^{2+}\) channels located in the apical membrane [7] (Fig. 3). TRPM6 needs to form heterotetramers with its close homolog TRPM7 to function [8]. The protein(s) that facilitates Mg\(^{2+}\) efflux from the basolateral side to the blood compartment has not been identified [9]. Cyclin M2 (CNNM2) and solute carrier family member A1 (SLC41A1) have been proposed as likely candidates for this function, but this subject is still under debate [10–12]. Other proteins involved, albeit indirectly, in Mg\(^{2+}\) transport in the DCT include epidermal growth factor (EGF), a hormone that regulates the expression of TRPM6 on the apical membrane, basolateral Na\(^{+}\), K\(^{+}\)-adenosine triphosphatase (ATPase), and K\(^{+}\) channel Kir4.1, and the apical K\(^{+}\) channel Kv1.1 and Na\(^{+}\)Cl\(^{-}\) cotransporter (NCC) [13–16]. The last four proteins participate in the generation of the driving force needed for Mg\(^{2+}\) transport [9]. Mutations in any of the above proteins, except SLC41A1, which are involved directly or indirectly in Mg\(^{2+}\) transport, have been shown to cause hypomagnesemia (Table 1).

In this review, we present the current knowledge of hereditary kidney diseases associated with hypomagnesemia. We discuss the clinical characteristics and genetic information for each disease and describe the pathophysiological basis that has been proposed for some of the diseases, although in general these remain incompletely understood. We classified hypomagnesemias in three groups according to the implicated genes (Table 1). Group 1 includes hypomagnesemias associated with genes that encode proteins directly involved in Mg\(^{2+}\) transport and the regulatory proteins. Group 2 contains hypomagnesemias associated with genes encoding proteins involved in transport of other ions or their regulators, which indirectly affect Mg\(^{2+}\) handling. Group 3 includes hypomagnesemias associated with genes required for mitochondrial function, which also indirectly result in Mg\(^{2+}\) loss.

**Group 1 hypomagnesemias**

**Familial hypomagnesemia with hypercalciuria and nephrocalcinosis types 1 and 2**

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is a rare autosomal recessive tubular disorder characterized by excessive urinary loss of Mg\(^{2+}\) and Ca\(^{2+}\), bilateral nephrocalcinosis, and progressive chronic kidney disease (CKD) [17]. FHHNC patients typically present during early childhood or before adolescence with recurrent urinary tract infections, polyuria, polydipsia, nephrolithiasis, and failure to thrive [18–21]. FHHNC patients may show a pronounced decline in glomerular filtration rate at the time of diagnosis, and approximately one-third of cases progress to chronic renal failure during childhood or adolescence [21,22]. In contrast to patients with other hypomagnesemias, FHHNC patients have high serum levels of...
parathyroid hormone (PTH) before the onset of chronic renal failure [1,19,23]. In some cases, patients present amelogenesis imperfecta [24,25]. Clinical signs of severe hypomagnesemia such as seizures and muscular tetany are rare.

FHHNC is caused by recessive mutations in CLDN16 (FHHNC type 1, OMIM #248250) or CLDN19 (FHHNC type 2, OMIM #248190) [3,4]. Patients with mutations in CLDN19 also present ocular abnormalities such as severe myopia, nystagmus, and macular colobomata [4,21,26]. CLDN16 and CLDN19 encode the tight junction proteins claudin-16 and claudin-19, respectively, which are strongly expressed in the kidney [3,4]. Claudin-19 is also expressed in peripheral neurons and retina [4,27]. Claudin-16 and claudin-19 form heteromeric paracellular cation channels in the TAL that regulate Ca\textsuperscript{2+} and Mg\textsuperscript{2+} transport [28,29]. The reabsorption of Mg\textsuperscript{2+} is greatly dependent on the transepithelial potential as a driving force, which is created by a transepithelial NaCl concentration gradient. CLDN16 and CLDN19 mutations have been shown to impair the permeating function of these channels, resulting in a reduced lumen-positive potential and the simultaneous loss of the driving force for Mg\textsuperscript{2+} reabsorption [22,28].

### Hypomagnesemia with secondary hypocalcemia

Hypomagnesemia with secondary hypocalcemia (HSH, OMIM #602014) is a rare autosomal recessive disorder characterized by severe hypomagnesemia associated with hypocalcemia. The levels of serum Mg\textsuperscript{2+} in patients with HSH are usually much lower than in patients with other types of hereditary hypomagnesemias [30]. The disease usually presents in early infancy, with neurological symptoms in-

<p>| Table 1. Inherited diseases associated with hypomagnesemia |
|---------------------------------|-----------------|---------------|----------|----------|</p>
<table>
<thead>
<tr>
<th>Disorder</th>
<th>Associated gene</th>
<th>Protein\textsuperscript{a}</th>
<th>Inheritance</th>
<th>OMIM #</th>
</tr>
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<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
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<tr>
<td>FHHNC type 1</td>
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<td>Claudin-16</td>
<td>AR</td>
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<td>AR</td>
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<tr>
<td>Hypomagnesemia with secondary</td>
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<td>Mg\textsuperscript{2+} channel TRPM6</td>
<td>AR</td>
<td>602014</td>
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<td>hypocalcemia</td>
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<td>Cyclin M2</td>
<td>AD/AR</td>
<td>616418/613882</td>
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<td>Cl- channel CIC-Kb</td>
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<td>SLC12A3</td>
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<td>Calcium-sensing receptor CaSR</td>
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<td>K\textsuperscript{+} channel Kir4.1</td>
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<td>Transcription factor HNF1β</td>
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<td>Nuclear serine protease FAM111A</td>
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<td>Large deletions</td>
<td>-</td>
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<td>HUPRA syndrome</td>
<td>SARS2</td>
<td>seryl-tRNA synthetase</td>
<td>AR</td>
<td>613845</td>
</tr>
</tbody>
</table>

\textsuperscript{a}All the genes associated with hereditary hypomagnesemias encode proteins, except MT-TI, which encodes a tRNA.

AD, autosomal dominant; ADTKD-HNF1B, autosomal dominant tubulointerstitial kidney disease subtype HNF1B; AR, autosomal recessive; EAST/SeSAME, epilepsy, ataxia, sensorineural deafness and tubulopathy syndrome/seizures, sensorineural deafness, ataxia, mental retardation and electrolyte imbalance; FHHNC, familial hypomagnesemia with hypercalciuria and nephrocalcinosis; HHH, hypertension, hypercholesterolemia and hypomagnesemia; HSMR, hypomagnesemia, seizures, and mental retardation disorder; HUPRA, hyperuricemia, pulmonary hypertension, renal failure and alkalosis; NISBD2, neonatal inflammatory skin and bowel disease type 2; TNHP, transient neonatal hyperphenylalaninemia and primapterinuria; tRNA, transfer RNA.

Claverie-Martin, et al. Inherited hypomagnesemias
cluding tetany and severe seizures that are refractory to anticonvulsant therapy [30,31]. HSH patients have very low serum levels of PTH. Persistent low levels of serum Mg\(^{2+}\) likely cause secondary hypocalcemia by inhibiting PTH secretion and inducing resistance at the receptor sites [30]. The main defect in HSH is a reduction in intestinal Mg\(^{2+}\) absorption, which is in contrast to all other identified hereditary hypomagnesemias, along with reduced Mg\(^{2+}\) reabsorption in the DCT with renal Mg\(^{2+}\) wasting [31,32].

HSH is caused by recessive loss-of-function mutations in the TRPM6 gene, which encodes the TRPM6 cation channel [31,32]. TRPM6 is predominantly expressed in the apical membrane of the intestinal and renal DCT epithelial cells, where it is involved in Mg\(^{2+}\) reabsorption [7]. Its channel activity and expression are regulated by several factors including EGF and adenosine triphosphate (ATP) [14]. TRPM6 mutations identified in HSH patients disrupt Mg\(^{2+}\) conductance through the channel, both in the colon and DCT, causing hypomagnesemia [7]. However, the mechanisms leading to this disease are not entirely known. TRPM6 interacts with its close homolog TRPM7 to form functional heteromeric TRPM6/TRPM7 Mg\(^{2+}\) channels [8]. TRPM6 and TRPM7 contain a transmembrane ion channel segment covalently joined to a cytosolic serine/threonine protein kinase domain at the carboxy terminus. The kinase domain is cleaved from the channel segment and, after its translocation to the nucleus, it regulates the transcription of many genes involved in development [33]. Therefore, it has been suggested that TRPM6 may play a much wider role in the cell than only in Mg\(^{2+}\) homeostasis [33].

Isolated recessive hypomagnesemia

Isolated recessive renal hypomagnesemia is a rare disorder characterized by hypomagnesemia and normocalciuria [34]. Hypomagnesemia is due to renal Mg\(^{2+}\) wasting. Patients show seizures and neurodevelopmental delay during childhood. Only two affected girls from a consanguineous family have been reported, and no other biochemical abnormalities were identified in these patients. A homozygous missense mutation in the EGF gene coding for pro-EGF was identified as the underlying genetic defect [34]. Pro-EGF is a type I membrane-bound precursor protein that is proteolytically cleaved to generate the soluble EGF peptide hormone. EGF binds with high affinity to the renal epidermal growth factor receptor (EGFR) at the basolateral membrane of the DCT. EGFR activation triggers a signaling cascade leading to an increase of TRPM6 channels on the apical membrane and increased Mg\(^{2+}\) reabsorption [14]. The disease-causing EGF mutation results in diminished sorting of pro-EGF, preventing adequate secretion of the EGF hormone [34]. This leads to inadequate stimulation of the EGFR, and therefore insufficient activation of the TRPM6 channel, which results in reduced reabsorption of Mg\(^{2+}\).

Neonatal inflammatory skin and bowel disease type 2

Using whole-exome sequencing, a rare homozygous missense mutation (p.Gly428Asp) in EGF was identified in a child with an inflammatory syndrome affecting the skin, bowel, and lungs (OMIM #616069) [35]. The pregnancy was complicated by polyhydramnios and the child was born prematurely. Laboratory tests revealed low serum levels of magnesium. The child showed failure to thrive and died at 2.5 years of age from widespread cutaneous and pulmonary infections in addition to electrolyte imbalance. Results of a skin biopsy and immunofluorescence microscopy studies revealed that the mutation p.Gly428Asp reduces EGFR plasma membrane localization [35].

Hypomagnesemia, seizures, and mental retardation disorder type 1

Hypomagnesemia, seizures, and mental retardation disorder (HSMR) type 1 is a complex rare condition characterized by renal Mg\(^{2+}\) loss that results in hypomagnesemia, infantile or juvenile epileptic seizures, and intellectual disability [36,37]. Patients also show autistic features, aggressive behavior, variable degrees of delayed psychomotor development, speech limitations, impaired motor skills, and in some cases obesity [37,38]. HSMR type 1 is caused by loss-of-function mutations in the CNNM2 gene, which encodes the transmembrane protein CNNM2 [36–38]. Most HSMR type 1 patients carry heterozygous mutations that are generated de novo or inherited in an autosomal dominant pattern (OMIM #616418) [36–38]. However, a recessive mode of inheritance has been reported for several families [37,39]. Patients with recessive CNNM2 mutations show a severe phenotype, including brain malformations, refractory epilepsy, and acute intellectual disability (OMIM #613882).
CNNM2 is expressed in many organs and tissues including brain and kidney. In the kidney, CNNM2 is predominantly expressed at the basolateral membrane of the DCT cells, where it is associated with Mg\(^{2+}\) reabsorption [36,40]. Whether CNNM2 is itself an Mg\(^{2+}\) transporter or a regulator of Mg\(^{2+}\) transport is unclear [11]. However, CNNM2 pathogenic mutations reduce its expression in the plasma membrane, resulting in defective Mg\(^{2+}\) reabsorption and Mg\(^{2+}\) wasting [11,38].

Furthermore, recent studies revealed that Mg\(^{2+}\)–ATP binding to the intracellular C-terminus of CNNM2 is required for protein dimerization and Mg\(^{2+}\) efflux [41]. Disease-causing mutations that are located in the Mg\(^{2+}\)–ATP-binding site abolish ATP binding and Mg\(^{2+}\) efflux activity. The basis of the neurological defects remains unknown.

**Group 2 hypomagnesemias**

**Bartter syndrome type 3**

Bartter syndrome (BS) includes a group of several tubulopathies characterized by renal salt wasting, hypokalemia, hypochloremic metabolic alkalosis, hyperreninemia, hyperaldosteronism, and low to normal blood pressure [36,38]. Patients usually present during the first years of life with failure to thrive, polyuria, and polydipsia. The main pathogenic mechanism in these tubulopathies is defective salt reabsorption predominantly in the TAL. Five different types of BS have been identified based on the gene involved [43]. Patients with BS type 3 or classic BS (OMIM #607364) develop hypomagnesemia during childhood or later in life [44]. This disorder is characterized by a great clinical variability, and there is a correlation between the severity of mutations and younger age at diagnosis [44,45].

BS type 3 is caused by recessive loss-of-function mutations of the CLCNKB gene, which encodes the kidney-specific Cl\(^{-}\) channel ClC-Kb that is involved in NaCl reabsorption in the renal tubule [6]. The ClC-Kb protein is expressed in the basolateral membrane of epithelial cells in the TAL and the DCT. In these tubular segments, Cl\(^{-}\) exits the cell through ClC-Ka and ClC-Kb channels. Mutations in CLCNKB alter the intracellular Cl\(^{-}\) regulation, which subsequently interferes with the generation of the lumen-positive potential and results in salt wasting and possibly hypomagnesemia.

**Gitelman syndrome**

Gitelman syndrome (GS, OMIM #263800) is an autosomal recessive salt-losing tubulopathy characterized by hypokalemic metabolic alkalosis, low or normal blood pressure, hypocalciuria, and hypomagnesemia with renal Mg\(^{2+}\) wasting [46]. GS is the most common cause of hereditary hypomagnesemia and is usually detected during adolescence or adulthood. GS may be asymptomatic or associated with mild symptoms including chronic fatigue, muscle weakness, thirst, salt craving, nocturia, and cramps, which can considerably reduce the quality of life [47]. Severe complications such as cardiac arrhythmias have been reported in some cases [46].

GS is caused by recessive inactivating mutations of the SLC12A3 gene encoding the thiazide-sensitive NCC, which is localized in the apical membrane of DCT cells, where it plays a fundamental role in the reabsorption of Na\(^{+}\) and Cl\(^{-}\) [48,49]. NCC mutations lead to reduced reabsorption, which results in Na\(^{+}\) and Cl\(^{-}\) wasting, hypovolemia, and subsequent hyperaldosteronism with metabolic alkalosis [50]. The pathogenesis of hypocalciuria and hypomagnesemia may be explained by the compensatory paracellular reabsorption of Na\(^{+}\) and Ca\(^{2+}\) in the PT due to volume reduction and by decreased apical expression of TRPM6 [51]. There is phenotypic variability in GS, including in patients with the same SLC12A3 mutation.

**Autosomal dominant hypocalcemia with hypercalciuria**

Autosomal dominant hypocalcemia with hypercalciuria (ADHH, OMIM #601198) is a rare disorder of calcium homeostasis characterized by variable levels of hypocalcemia and low or normal serum levels of PTH [52]. Patients also present with hypomagnesemia, hypermagnesuria, hyperphosphatemia, and hypercalciuria [52,53]. Hypocalcemia is a derived effect of hypomagnesemia as a result of parathyroid failure or PTH resistance [30]. ADHH patients may develop hypocalcemic symptoms (paresthesias, carpopedal spasm, and seizures), and some have renal and basal ganglia calcifications, but others are asymptomatic [52].

ADHH is caused by heterozygous gain-of-function mutations in the CASR gene [52,54]. This gene encodes the extracellular CaSR, a G protein-coupled receptor that is highly expressed in parathyroid glands and kidneys [53].
In the kidney, CaSR is highly expressed in the basolateral membrane of the TAL, where it regulates Ca\(^{2+}\) reabsorption independently of PTH [53,55]. A signaling pathway including two microRNAs and the tight junction proteins claudin-14 and claudin-16 mediates the effect of CaSR on renal Ca\(^{2+}\) and Mg\(^{2+}\) excretion [5]. Activating mutations of CaSR increase the expression of claudin-14, which binds to claudin-16 and blocks the cation permeability of the claudin-16/claudin-19 channel.

**Episodic ataxia type 1**

Loss-of-function mutations in the KCNA1 gene are typically associated with an autosomal dominant neurological disorder called episodic ataxia type 1 (EA1, OMIM #160120), which is characterized by recurring episodes of ataxia and myokymia from early childhood [13]. The clinical phenotype in EA1 patients can include seizures, epilepsy, and, in some cases, paroxysmal kinesigenic dyskinesia, cataplexy, myokymia, and hypomagnesemia [56]. These symptoms can appear alone or in combination with EA1. A genotype-phenotype correlation analysis in a large cohort of EA1 patients revealed high inter- and intrafamilial variability of symptoms, but the penetrance of hypomagnesemia has not been evaluated [57].

The KCNA1 gene encodes the α subunit of the voltage-gated potassium channel Kv1.1, which is abundantly expressed in specific neurons and plays an important role in regulating neuronal excitability in the central and peripheral nervous system [57]. In the kidney, KCNA1 is exclusively expressed at the apical membrane of the DCT cells alongside the Mg\(^{2+}\) transporter TRPM6 [58]. Previous studies suggested that potassium (K\(^{+}\)) secretion via Kv1.1 provides the electrochemical gradient needed for Mg\(^{2+}\) reabsorption by the TRPM6 channel [58,59]. Interestingly, two specific KCNA1 heterozygous mutations, p.Asn255Asp and p.Leu328Val, have been associated with hypomagnesemia, leading to muscle cramps and tetanic episodes [58,59]. Electrophysiological analyses showed that both amino acid substitutions result in nonfunctional Kv1.1 channels [58-60]. The reduction in the K\(^{+}\) conductance polarizes the apical membrane of DCT cells, reducing the electrical driving force and leading to renal Mg\(^{2+}\) loss. The frequency of hypomagnesemia in patients with KCNA1 mutations may be greater than reported, since serum Mg\(^{2+}\) levels have not been examined in all EA1 patients. Additional research is needed to understand the association of KCNA1 mutations with hypomagnesemia.

**Isolated dominant hypomagnesemia**

Isolated dominant hypomagnesemia is a rare autosomal dominant disorder characterized by hypomagnesemia, hypocalciuria, and occasionally chondrocalcinosis (OMIM #154020) [61]. Some patients suffer from muscle cramps, episodes of convulsions, or chondrocalcinosis [61,62]. This disease has been identified in only three families who carry the same missense mutation, p.Gly41Arg, in the FXYD2 gene and appear to be descendants of a common ancestor [62,63]. FXYD2 encodes the kidney-specific regulatory γ-subunit of basolateral Na\(^{+}\), K\(^{-}\)-ATPase, which is composed of a catalytic α subunit and an auxiliary β subunit. The Na\(^{+}\), K\(^{-}\)-ATPase complex maintains the electrochemical gradients of Na\(^{+}\) and K\(^{+}\) across the basolateral plasma membrane that provide the driving force for transepithelial Mg\(^{2+}\) transport particularly in the DCT [15]. There are two splice variants of the γ-subunit: FXYD2a, which is expressed mainly in the TAL and PT, and FXYD2b, which is expressed exclusively in the basolateral membrane of the DCT and collecting duct [64]. Expression studies showed that the p.Gly41Arg mutation causes incorrect trafficking of the mutant γ FXYD2b subunit, preventing its interaction with the α and β Na\(^{+}\), K\(^{-}\)-ATPase subunits [62]. This leads to destabilization and reduction of Na\(^{+}\), K\(^{-}\)-ATPase activity in the DCT, which eventually results in decreased Mg\(^{2+}\) uptake and renal Mg\(^{2+}\) loss.

**Hypomagnesemia, seizures, and mental retardation disorder type 2**

Recently, three children from non-consanguineous families were reported who presented with generalized seizures in infancy associated with severe intellectual disability, massive renal Mg\(^{2+}\) wasting, and hypomagnesemia (HSMR type 2; OMIM #618314) [65]. Other findings included significant developmental delay and limited motor skills. Mutational analysis identified heterozygous de novo mutations in the ATP1A1 gene encoding the catalytic α1 subunit of the Na\(^{+}\), K\(^{-}\)-ATPase [65]. This ubiquitously expressed subunit is the major α isoform in the kidney and is present in practically all cell types of the central nervous system [15]. Dominant and de novo mutations in ATP1A1 are also the cause of
autosomal dominant Charcot-Marie-Tooth disease type 2 (CMT2) [66]. To the best of our knowledge, serum Mg$^{2+}$ levels have not been studied in CMT2 patients. Functional studies of the mutant Na$, K^+$-ATPase a1 subunits revealed loss of pump activity and anomalous cation permeability, leading to membrane depolarization [65]. These discoveries emphasize the essential task of the catalytic a1 subunit of the Na$, K^+$-ATPase in renal tubular Mg$^{2+}$ transport and neuronal activity. Since ATP1A1 is expressed in the TAL and DCT, it could affect transport in both tubular segments.

**Epilepsy, ataxia, sensorineural deafness, and tubulopathy/seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance syndrome**

Epilepsy, ataxia, sensorineural deafness, and tubulopathy (EAST) syndrome or seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (SeSAME) syndrome (OMIM #612780) is an autosomal recessive disease characterized by early-onset epilepsy, delayed psychomotor development, ataxia, sensorineural deafness, and a salt-wasting tubulopathy with or without mental retardation [67,68]. The renal phenotype develops during the course of the disease and comprises polyuria, hypokalemia, metabolic alkalosis, hypocalciuria, and hypomagnesemia [69,70]. Plasma renin and aldosterone levels are increased and blood pressure is at the low end of the normal range. Urinary findings include K$, Mg^{2+}$, and Na$^+$ wasting.

This complex disorder is caused by loss-of-function mutations in the KCNJ10 gene encoding Kir4.1, one of the components of the inwardly rectifying K$^+$ channel Kir4.1/ Kir5.1 [16,67,68]. KCNJ10 is mainly expressed in glial cells of the brain, the stria vascularis of the inner ear, and the kidney [69]. The renal phenotype of patients with EAST/SeSAME syndrome closely resembles that of patients with GS, suggesting that KCNJ10 mutations mainly impair transport in the DCT. In the kidney, Kir4.1 is expressed in the basolateral membrane of the DCT and is involved in K$^+$ recycling, which is needed for the activity of Na$, K^+$-ATPase, and generation of a negative transmembrane potential [16]. Mutations that inactivate Kir4.1 function lead to a depolarization of the basolateral membrane and to a reduction of the driving force for anion channels and Na$^+$-coupled exchangers. This alteration in membrane voltage could also affect other transport processes, such as those for Cl$^-$ and Mg$^{2+}$, which could explain the Mg$^{2+}$ loss detected in patients with EAST/SeSAME syndrome.

**Autosomal dominant tubulointerstitial kidney disease caused by HNF1B mutations**

Autosomal dominant tubulointerstitial kidney disease (ADTKD) comprises a group of rare kidney disorders characterized by tubular damage and interstitial fibrosis without glomerular lesions [71]. Affected individuals usually develop CKD and end-stage renal disease in adulthood. Heterozygous mutations in several genes cause ADTKD, and this disease is subdivided into several subtypes based on the mutated gene [71].

ADTKD subtype HNF1B (ADTKD-HNF1B, OMIM #137920) is associated with hypomagnesemia [72,73]. The phenotypes detected in ADTKD-HNF1B patients are very heterogeneous and may appear during pregnancy, in childhood, or in adulthood [73]. Symptoms include renal cysts, kidney malformations, abnormalities of the genital tract and liver, and maturity-onset diabetes of the young (MODY) type 5 [72,74]. Hypomagnesemia and hypermagnesuria are observed in approximately 50% of patients [72,73].

ADTKD-HNF1B is caused by heterozygous mutations of the HNF1B gene, which encodes the developmentally regulated transcription factor hepatocyte nuclear factor-1β (HNF1β) [72,74,75]. These mutations are inherited in a dominant inheritance pattern or appear de novo. HNF1β regulates tissue-specific gene expression in epithelial cells of several organs, including the kidneys, pancreas, liver, and urogenital tract [76]. In the adult kidney, HNF1β is expressed in epithelial cells of all tubular segments. However, its role in renal Mg$^{2+}$ reabsorption seems to take place in the DCT, since hypomagnesemia in ADTKD-HNF1B patients is frequently accompanied by hypocaliuria [72,73]. Several transcriptional targets of HNF1β have been identified, including the FXYD2 gene [72], which is involved in reabsorption of Mg$^{2+}$ in the DCT, as described above. Therefore, inactivating mutations of HNF1β would lead to reduced expression of FXYD2 and, consequently, to renal Mg$^{2+}$ wasting and hypomagnesemia [72]. HNF1β also regulates the transcription of KCNJ10, a gene that can affect Mg$^{2+}$ transport in the DCT, as described above [77].
Transient neonatal hyperphenylalaninemia and primapterinuria

Transient neonatal hyperphenylalaninemia and primapterinuria (TNHP, OMIM #264070) is an autosomal recessive disorder characterized by mild transient hyperphenylalaninemia and elevated urinary levels of 7-biopterin [78]. Affected individuals may also develop hypomagnesemia with renal Mg²⁺ wasting and MODY type diabetes [79]. TNHP is caused by mutations in the PCBD1 gene, which encodes the bifunctional protein pterin-4 α-carbinolamine dehydratase (PCBD1). PCBD1 functions as a cytosolic enzyme that is implicated in the regeneration of the essential cofactor tetrahydrobiopterin as well as a coactivator of HNFIβ-mediated transcription within the nucleus [80]. Gene expression studies combined with immunohistochemical analysis showed that in the kidney, PCBD1 is expressed predominantly in the DCT [79]. PCBD1 interacts with the dimerization domain of HNFIβ and regulates the formation of a transcriptionally active tetrameric complex [81]. Binding of PCBD1 to HNFIβ stimulates the FXYD2 promoter in the DCT, and PCBD1 mutations identified in TNHP patients cause defective dimerization and degradation of the PCBD1 protein, leading to decreased FXYD2 promoter activity [79]. The reduced expression of FXYD2 would cause hypomagnesemia in these patients.

Kenny-Caffey syndrome type 2

Kenny-Caffey syndrome type 2 (KCS type 2, #OMIM 127000) is characterized by severe short stature, impaired skeletal development, eye abnormalities, hypomagnesemia, and hypoparathyroidism [82,83]. KCS type 2 patients may also have frequent episodes of low Ca²⁺ levels in serum triggered by hypoparathyroidism. This multisystem disease is caused by heterozygous missense mutations in the FAM111A gene, which encodes the nuclear trypsin-like serine protease FAM111A [82,83]. FAM111A is involved in the regulation of PTH production, calcium homeostasis, bone development and growth, but the specific mechanisms have not been determined [82,83]. FAM111A mutations identified in patients usually appear de novo, but some cases with autosomal dominant inheritance have been described [84]. These mutations affect the peptidase domain of FAM111A and may impair its catalytic activity [83,85].

FAM111A, first identified as an antiviral restriction factor, is ubiquitously expressed, and its nuclear localization suggests that it might be involved in transcriptional regulation [82,83,86]. FAM111A mutations result in hyperactivation of the FAM111A intrinsic protease activity, which could cause abnormal degradation of DNA-binding proteins or FAM111A depletion through hyper-autoproteolytic cleavage [87–89]. Hyperactive FAM111A is cytotoxic, disrupting nuclear structure and pore distribution in a protease-dependent manner [89]. Targets of FAM111A protease activity have been recently identified and include nucleoporins and the associated germinal center-associated nuclear protein transcription/replication factor [89]. Hypomagnesemia in KCS type 2 patients could be attributed to degradation of transcription factors involved in magnesium homeostasis. Tan et al. [90] suggested a potential role of FAM111A in regulation of CaSR, since KCS type 2 shares phenotypic characteristics with ADHH-like hypocalcemia and hypoparathyroidism.

Group 3 hypomagnesemias

Mitochondria are abundant in kidneys, as the kidney requires high amounts of energy to enable the reabsorption of ions in different tubular segments [91]. Mitochondrial dysfunction leads to reduced ATP synthesis and loss of renal function. Because the function and biogenesis of mitochondria are under the genetic control of both mitochondrial DNA (mtDNA) and nuclear DNA, mutations in either genome can be the cause of disease [92]. mtDNA mutations are usually inherited from the patient’s mother but they can also appear de novo. The clinical features of these patients can be very variable since they depend not only on the type of mutation but also on the number of mitochondria affected [92,93]. Mitochondrial diseases manifest in infancy and are multisystemic, and there are no effective treatment options for the patients. Some mitochondrial diseases are associated with hypomagnesemia but the mechanism underlying how they cause hypomagnesemia remains unsolved. These diseases may affect the TAL, the DCT, or both.
Hypertension, hypercholesterolemia, and hypomagnesemia

This disorder was identified in a large pedigree and includes hypertension, hypercholesterolemia, and hypomagnesemia (OMIM #500005) [94]. Affected individuals also have hypocalciuria and hypokalemia. Each of these traits was transmitted via the maternal lineage with a pattern indicating mitochondrial inheritance. Other clinical characteristics include migraine headache, sensorineural hearing loss, and hypertrophic cardiomyopathy, all usually associated with mitochondrial dysfunction. Sequence analysis of the mitochondrial genome in the maternal lineage of the pedigree identified a C to T transition in the MT-TI gene, which encodes isoleucine transfer RNA (tRNA\(^{Ile}\)) [94]. Further analysis of affected members revealed that all copies of the mtDNA contain this mutation. The affected C is located immediately 5' to the tRNA\(^{Ile}\) anticodon, and biochemical studies showed that the substitution of T significantly weakens ribosome binding [95]. These studies suggest that this mutation results in the loss of mitochondrial function. Hypomagnesemia associated with hypocalciuria points to a primary defect in the DCT [96].

Kearns-Sayre syndrome

Kearns-Sayre syndrome (KSS, OMIM #530000) is a rare progressive multisystem disease characterized by ophthalmoplegia, ptosis, and pigmentary retinopathy. The onset is typically before 20 years of age, and patients typically present one of the following: cerebellar ataxia, cardiac conduction defects, deafness, short stature, cognitive involvement, tremor, and cardiomyopathy [97]. Some cases develop hypoparathyroidism and renal tubular dysfunction resulting in severe hypomagnesemia, hypocalcemia with hypermagnesuria, and hypokalemia [98,99]. KSS is caused by large deletions in mtDNA, which can reduce considerably ATP production [93]. In most cases, the deletions arise de novo but some are transmitted through maternal inheritance. Patients’ cells usually contain a mixture of wild-type and mutant mtDNA molecules in variable quantities, which is critical in determining the level of cellular dysfunction [92]. A defect in Mg\(^{2+}\) reabsorption in the TAL or DCT, due to ATP depletion, could be the cause of hypomagnesemia in these patients.

Hyperuricemia, pulmonary hypertension, renal failure, and alkalosis syndrome

Hyperuricemia, pulmonary hypertension, renal failure and alkalosis (HUPRA) syndrome (OMIM #613845) is an autosomal recessive disease characterized by early-onset progressive renal failure, hyperuricemia, metabolic alkalosis, pulmonary hypertension, developmental delay, and, in some cases, hypomagnesemia [100]. This rare disorder has been diagnosed in only six children from three families and it is caused by homozygous missense mutation in SARS2 on chromosome 19, which encodes the mitochondrial serine tRNA synthetase (SARS2) [100,101]. SARS2 catalyzes the serine aminoacylation of two mitochondrial tRNAs. Only two mutations have been identified in patients with HUPRA syndrome, p.Asp390Gly and p.Arg402His. The p.Asp390Gly mutation significantly reduces the aminoacylation of one mitochondrial tRNA leading to its degradation, which would cause alterations in the synthesis of mitochondrial proteins and consequently in energy supply [100]. Decreased energy production may account for diminished Na\(^{+}\), K\(^{+}\)-ATPase activity in the TAL and DCT, which could explain the salt wasting and hypomagnesemia observed in HUPRA patients.

Concluding remarks

Mg\(^{2+}\) is an essential ion that plays a key role in the regulation of many cellular functions. In the kidney, proteins expressed in the apical and basolateral membranes of the TAL and DCT maintain serum Mg\(^{2+}\) levels within a narrow physiological range. Mutations in the genes that encode these proteins cause different types of hypomagnesemia. Consequently, serum Mg\(^{2+}\) levels should be determined in patients presenting with seizures, muscle cramps, and arrhythmias.

In this review, we have described the current research findings on hereditary hypomagnesemias. Some of these diseases share common characteristics and thus precise diagnosis requires the identification of the causative mutation. Therefore, we suggest a diagnostic approach for clinicians based on previously reported data [2,102] (Fig. 4). Many questions are still unanswered. For instance, the underlying mechanisms for hypomagnesemia in most hereditary diseases are still unclear. In addition, the physiological functions of CNNM2...
Hypomagnesemia and metabolic alkalosis

Yes

Hypokalemia and metabolic alkalosis

No

Cardiac arrhythmias

GS (SLC12A3)

Developmental delay

BS3 (CLCNKB)

Epilepsy, ataxia, intellectual disability, sensorineural deafness

EAST/SeSAME (KCNJ10)

Renal cysts/malformations, genital tract and liver abnormalities, MODY

ADTKD-HNF1B (HNF1B)

Transient hyperphenylalaninemia, primapterinuria, MODY

TNHP (PCBD1)

Refractory seizures, intellectual disability

HSMR2 (ATP1A1)

No ocular defects

FHHNC1 (CLDN16)

Serum Mg²⁺ < 0.2 mmol/L

HSH (TRPM6)

Seizures, intellectual disability

IRH (EGF)

Neonatal inflammation of skin and bowel

NISBD2 (EGFR)

Refractory epilepsy, intellectual disability, brain malformations

HSMR1 (CNNM2)

Isolated dominant hypomagnesemia

IDH (FXYD2)

Tetanic episodes

EA1 (KCNA1)

Impaired skeletal development

KCS2 (FAM111)

Severe ocular defects

FHHNC2 (CLDN19)


drug therapies usually involve supportive and involve oral or intravenous magnesium supplementation. However, this approach has been found to be unsuccessful in patients with other hypomagnesemias like FHHNC, HSMR, and ADTKD-HNF1B.

**Conflicts of interest**

All authors have no conflicts of interest to declare.
Funding

This work was financially supported by the “Instituto de Salud Carlos III-Subdirección General de Evaluación y Fomento de la Investigación” and the European Regional Development Fund “Another way to build Europe” (grant number PI20/00652, Project RenalTube).

Disclosure

Figures were created using the web application BioRender.com.

Authors’ contributions

Conceptualization: All authors
Writing original draft: FCM, APR
Review and editing: All authors
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Fructose in the kidney: from physiology to pathology

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The Warburg effect is a unique property of cancer cells, in which glycolysis is activated instead of mitochondrial respiration despite oxygen availability. However, recent studies found that the Warburg effect also mediates non-cancer disorders, including kidney disease. Currently, diabetes or glucose has been postulated to mediate the Warburg effect in the kidney, but it is of importance that the Warburg effect can be induced under nondiabetic conditions. Fructose is endogenously produced in several organs, including the kidney, under both physiological and pathological conditions. In the kidney, fructose is predominantly metabolized in the proximal tubules; under normal physiological conditions, fructose is utilized as a substrate for gluconeogenesis and contributes to maintain systemic glucose concentration under starvation conditions. However, when present in excess, fructose likely becomes deleterious, possibly due to excessive uric acid, which is a by-product of fructose metabolism. A potential mechanism is that uric acid suppresses aconitase in the Krebs cycle and therefore reduces mitochondrial oxidation. Consequently, fructose favors glycolysis over mitochondrial respiration, a process that is similar to the Warburg effect in cancer cells. Activation of glycolysis also links to several side pathways, including the pentose phosphate pathway, hexosamine pathway, and lipid synthesis, to provide biosynthetic precursors as fuel for renal inflammation and fibrosis. We now hypothesize that fructose could be the mediator for the Warburg effect in the kidney and a potential mechanism for chronic kidney disease.

Keywords: Fructose, Glycolysis, Inflammation, Mitochondria, Proximal tubules, Uric acid, Warburg effect

Introduction

Fructose is a natural sugar present in fruits and honey and is a fundamental nutrient for wild animals. Bears, squirrels, birds migrating over long distances, and freshwater Pacu fish actively eat fruits to accumulate fat, presumably as a protection against periods of food shortage [1]. Fructose contributes to lipid and glycogen syntheses for energy storage, and to the development of insulin resistance to prevent glucose utilization in the peripheral tissue, and glucose delivery to the central nervous system. In addition, fructose also stimulates salt reabsorption to raise blood pressure (as discussed in the following section) [2]. Fructose is also metabolized under hypoxic conditions and often exhibits the protective effect. A recent study examined how the naked mole rat could survive long periods of hypoxic conditions...
and found that it was attributed to their ability to produce fructose endogenously in several organs, which is subsequently metabolized to provide several biosynthetic precursors required for cell survival, including nucleic acids, amino acids, lipids and energy [3]. Likewise, the reason why the fetus exposed to hypoxia can survive during early pregnancy is that the developing placenta also produces endogenous fructose, likely aiding fetal organ growth in wild animals as well as humans [4–6].

In modern society, fructose, as a component of high-fructose corn syrup or table sugar, is preferentially added to sugar-sweetened soft drinks and sodas. A dramatic increase in fructose consumption is, however, associated with a high prevalence of the metabolic syndrome, stimulating a heated debate over the potential danger of sugar-sweetened beverages (SSB) [7,8]. Likewise, several clinical studies have sought the role of fructose in the kidney, but the issue remains controversial. Interestingly, more than two SSB per day is associated with an incidence and a prevalence of chronic kidney disease (CKD) [9,10], but less than one SSB per day was not [11,12]. Fructose may therefore impair renal function in a dose-dependent manner. An intervention study also showed that a low-fructose diet lowered blood pressure and reduced systemic inflammation in subjects with CKD [13].

A recent scientific discovery is that fructose is produced endogenously, and is involved in the pathogenesis of several types of disorders. Acute kidney injury, diabetic nephropathy, cardiac hypertrophy, aging, and salt-sensitive hypertension are now recognized to be mediated by endogenous fructose.

This article summarizes the basis of fructose physiology, discusses a potential mechanism by which fructose causes kidney disease, and finally proposes our hypothesis that fructose mediates the Warburg effect in CKD.

Current concepts and update regarding dietary fructose metabolism

It has long been assumed that the liver is the primary site for dietary fructose metabolism, but recent studies have demonstrated that the small intestine plays a substantial role in dietary fructose metabolism [14]. After sucrose is digested by sucrase into fructose and glucose, fructose is absorbed by enterocytes via the glucose transporter (GLUT) 5 at the apical membrane of enterocytes. Since the intestine contributes ~25% of systemic gluconeogenesis both after prolonged fasting and in diabetes [15], intestinal epithelium likely utilizes dietary fructose as a substrate for gluconeogenesis (Fig. 1). However, when present in excess, fructose saturates the intestinal metabolic capacity. Excessive fructose either spills over to the colon or is transported through the GLUT2 from the basal membrane into the portal vein and then to the liver [14]. Interestingly, intestinal fructose metabolism determines an individual’s preference for sweet tastes and sugar intake but does not contribute to the development of metabolic syndrome [16]. In the colon, fructose is likely digested by microbiota that use fructose carbons to generate tricarboxylic acid (TCA) cycle intermediates, essential amino acids, and short-chain fatty acids [14]. In turn, fructose spilling over from intestinal shield acts on the hepatocyte via GLUT2 and drives the metabolic syndrome [16]. Hepatic fructose metabolism is associated with increased hepatic fatty acid and malonyl-CoA synthesis, reduced fatty acid oxidation, and modification of the mitochondrial proteome [17]. Similar to the enterocyte, excessive fructose in the kidney likely escapes into the systemic circulation. In the kidney, fructose in systemic circulation is filtered through the glomerulus into the urinary space, and urinary fructose is reabsorbed by the proximal tubular cells (Fig. 1).

Fructose transporters are predominantly expressed in the proximal tubules

After fructose is metabolized in the liver, only small amount of fructose escapes from the liver to reach the systemic circulation, and therefore serum fructose concentrations range from 0.1 to 0.8 mM [18]. After filtration through the glomerulus, urinary fructose is either reabsorbed in the proximal tubular epithelial cells (Fig. 2), or excreted in the urine.

GLUT5, a high-affinity facilitative transporter, is considered to play a major role in fructose transport and is expressed at the apical membrane of the epithelial cells in the straight portion of the proximal tubule [19,20]. An alternate fructose transporter is the sodium glucose cotransporter 5 (SGLT5), which is a high-affinity transporter for fructose and mannose in humans and mice [21,22]. This transporter is exclusively expressed in the kidney, and likely located in the S2 segment of the proximal tubular cells [23]. The
rat sodium-dependent glucose transporter 1 (rNaGLT1) is also expressed at the apical membrane of epithelial cells in both the convoluted and straight proximal tubules in the rat, and also mediates fructose transport [24]. While GLUT9 (SLC2A9) is a member of the facilitative GLUT gene family, it is now primarily described as a urate transporter (URAT) that can exchange both fructose and glucose for urate. The two splice variants of GLUT9, GLUT9a (full length) and GLUT9b (ΔN) are both present in the human kidney [25]. The GLUT9a (540 amino acids) splice variant is expressed in the basolateral membrane of the proximal tubular epithelial cells and favors urate transport back into the circulation from the tubular cells [26]. In turn, the GLUT9b (512 amino acids) splice variant is expressed at the apical site, and likely transfers urate from tubular fluid into cells [26,27] and the collecting ducts [25,28] in humans.

Alternatively, GLUT2 may transport fructose from the basolateral membrane of the proximal tubular cells into the systemic circulation [23,29], perhaps when fructose is abundant in the cytosol (Fig. 2). Given GLUT2 is a facilitative transporter operated by a passive diffusion process, it may act to excrete fructose when the intracellular fructose concentration is greater than that of the blood.

**Physiology of fructose metabolism in the proximal tubules**

The straight segment of the proximal tubules exclusively expresses GLUT5 so that it may be the primary site for fructose metabolism. However, both fructokinase and aldolase B, another key enzyme for fructose metabolism, are also present in the convoluted proximal tubules [30,31], suggesting that fructose metabolism is not restricted in the straight segment, but is also likely operated in the convoluted segment of renal tubules.

Fructose are likely utilized as substrates for gluconeogenesis in the proximal tubules, where gluconeogenesis is dominant over glycolysis. In fact, several gluconeogenesis enzymes, including phosphoenolpyruvate carboxykinase, fructose bisphosphatase, and enzymes of the glucose 6-phosphatase system are dominantly activated [32,33], while glycolysis enzymes are less activated [34–36] in the proximal tubules compared to other parts of nephron.

In 1961, by utilizing in situ perfusion in the rat, Salomon et al. [37] directly measured the difference between arteriovenous fructose and glucose concentrations after bolus infusion of 25 mg of fructose into the peripheral vessels. The reduction of fructose concentrations after passage of blood through the kidney was associated with equivalent increases in the renal venous glucose; the extent of fructose
disappearance and the appearance of glucose averaged approximately 19%. In 1982, Björkman and Felig [38] found that intravenous infusion of fructose in humans at 2 mmol/min for 135 minutes resulted in a rise in glucose concentration in the renal vein (0.17 ± 0.05 mmol/L). The results indicated that 20% of intravenously infused fructose was taken up by the kidney, and the net glucose release from the kidney could be derived from 55% of the net renal uptake of fructose.

**Figure 2. Fructose transporters and metabolism in the proximal tubules.** In the proximal tubules, urinary fructose is reabsorbed at the apical membrane of the epithelial cells via several types of fructose transporters. The glucose transporter (GLUT) 5 is considered to be a major transporter for fructose. The sodium glucose cotransporter 5 (SGLT5) is a high-affinity kidney-specific transporter for fructose and mannose in humans and mice, while the rat sodium-dependent glucose transporter-1 (rNaGLT1) is also expressed in both convoluted and straight proximal tubules in the rat and also mediates fructose transport. Under physiological conditions or during starvation, fructose is utilized as a substrate for gluconeogenesis. In turn, during satiation or when fructose is in excess, fructose is metabolized in the cytosol to produce several fructose metabolites, including uric acid. The GLUT2 facilitative transporter is expressed in the basal membrane. When fructose concentrations are higher in the cytosol than in the blood of peritubular capillary, GLUT2 transports intracellular fructose into the blood in the peritubular capillary. Likewise, GLUT9a, another facilitative transporter, is expressed in the basolateral membrane and favors urate transport back into the circulation from the tubular cells.

**Proximal tubular cells go wrong with excessive fructose**

When the proximal tubular cells are overloaded with excessive fructose after satiation, fructose metabolism likely becomes dysregulated and causes pathological reactions (Figs. 2, 3). In experimental studies, normal rats developed mild tubulointerstitial injury with inflammation and fibrosis when fed a high-fructose diet [19,39]. In the case of preex-
also involved in this process, as uric acid directly impairs endothelial function \[46,47\].

Likewise, macrophages are also a target for fructose given they express GLUT5 (Fig. 3). Several experimental studies have shown that a high-fructose diet causes renal inflammation with macrophage infiltration in rodents \[19,40,43\]. *In vitro* studies confirmed the ability of fructose to act directly on macrophages via GLUT5 to release inflammatory cytokines \[48,49\]. Macrophages exhibit two phenotypes, a proinflammatory M1 and an anti-inflammatory M2 phenotype; fructose is likely an ideal fuel for M1 macrophage, which relies on glycolysis. However, the mechanism by which fructose activates macrophages may be somewhat complex. A recent study of fructose-induced inflammation demonstrated that oxidative metabolism, but not glycolysis, plays a dominant role in macrophage activation \[48\].

While a basic concept is that uric acid suppresses aconitase in the TCA cycle and hence reduces mitochondrial respiration, the investigators identified an alternative pathway for fructose to stimulate the TCA cycles. They found that glutamine was incorporated into the TCA cycles in response to fructose, and supplies \(\alpha\)-ketoglutarate that can bypass this step allowing oxidative phosphorylation to occur in human monocytes and mouse macrophages. Therefore, macrophages likely utilize either glycolysis or oxidative phosphorylation, perhaps depending on cellular conditions. While it remains unclear how macrophages switch these pathways, a key trigger may be oxygen availability. Under severe hypoxia, cytochrome c oxidase activity decreases \[50\], and glycolysis is dominant \[51\]. In contrast, under aerobic conditions, cytochrome c activity is activated, and glycolysis is completely replaced by oxidative phosphorylation \[51\].

**Fructose causes salt-sensitive hypertension**

Fructose intake is likely associated with hypertension in humans \[52\]. Experimental studies have shown that rats fed a high-fructose diet had elevated blood pressure in response to additional salt intake (Fig. 2). The proximal tubules play a key role in salt handling, as the majority of Na\(^+\) filtered through glomerulus is reabsorbed into the tubular epithelial cells via Na\(^+\)/H\(^+\) exchangers (NHEs) located in the apical membrane \[53,54\]. Fructose-induced salt sensitivity can be accounted for by the ability of fructose to stimulate both

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**Figure 3. Postulate mechanism of fructose-induced kidney disease.** Fructose, arising either from the diet or from endogenous production under pathological conditions, acts on the tubular epithelial cells, endothelial cells, and macrophages through fructose transporters, such as glucose transporter 5 (GLUT5), to cause inflammation and fibrosis in the kidney. AR, aldose reductase; eNOS, endothelial NO synthase; ICAM-1, intercellular adhesion molecule-1; MO, macrophage.
the expression and the activity of NHEs, and increase Na+ reabsorption in the proximal tubules [53]. While NHEs are regulated by angiotensin II, fructose sensitizes the proximal tubules to angiotensin II by upregulating NHE expression [53,54]. In addition, urate also plays a key role in the development of salt-sensitive hypertension in response to fructose as it causes arteriopathy, tubulointerstitial injury, and a reduction in NO in endothelial cells [43,46,47,55].

Renal proximal tubular cells turn on glycolysis when injured

The proximal tubular cells normally prefer lipids over glucose for energy generation, so glycolysis is not operative in this cell type. It is because enzymes for gluconeogenesis are dominantly activated over glycolytic enzymes; and therefore, fructose metabolism is physiologically linked with gluconeogenesis, but not with glycolysis [56]. However, this is unlikely the case when the tubular cells are damaged. In fact, damaged proximal tubular cells are often associated with mitochondrial alteration, resulting in a metabolic switch from mitochondrial oxidative phosphorylation to glycolysis with amplified expression of glycolytic enzymes [57]. Thus, both fructose and glucose are metabolized in damaged proximal tubular cells.

Does the combination of fructose with glucose accelerate glycolysis in renal proximal tubular epithelial cells?

The combination of fructose with glucose modifies the activation of glucokinase, the enzyme that catalyzes the first step of glycolysis. In hepatocytes, glucokinase is positively regulated by fructose 1-phosphate (Fru1P) whereas it is inhibited by fructose 6-phosphate (Fru6P) [58,59]. The mechanism for Fru1P-mediated glucokinase activation is the release of glucokinase from glucokinase regulatory protein (GKRP), which sequesters glucokinase in the nucleus [60,61]. Even at low concentrations, intracellular fructose is rapidly metabolized to Fru1P. Therefore, Fru1P-induced glucokinase activation may explain how fructose facilitates glucose utilization. Consistent with these findings, Shiota et al. [62] showed that small amounts of fructose enhanced hepatic glucose uptake in the dog. Furthermore, fructose metabolism also increases fructokinase activity, which completes intracellular adenosine triphosphate (ATP). Since ATP negatively regulates the glycolytic pathway by inhibiting phosphofructokinase and pyruvate kinase, the ATP depletion due to fructokinase activation enhances glycolysis. However, these processes may not occur in the kidney, given glucokinase (hexokinase IV) is expressed only in hepatocytes and pancreatic β cells, [63] while renal proximal tubular cells express hexokinase I and II [57,64].

Endogenous fructose may be a unifying pathway in the development of chronic kidney diseases

Interestingly, fructose is produced endogenously in the kidney, particularly under condition of ischemia/hypoxia, high osmotic stress, aging, pressure overload, and diabetes. A potential mechanism for fructose synthesis is the activation of the polyol pathway, in which glucose is reduced by aldose reductase to sorbitol, which is then oxidized by sorbitol dehydrogenase to fructose. Therefore, fructose can be readily produced when glucose is constantly supplied. A key step in this process is the activation of aldose reductase, which can be stimulated by several factors, including hypoxia, osmotic stress, and diabetes, and may also account for the findings that several factors induce endogenous fructose production in several pathological conditions.

Cardiac surgery often results in postoperative acute kidney injury due to ischemia. Our research group studied pediatric patients who underwent cardiac bypass surgery and found that urinary fructose concentrations were elevated in patients with ischemic acute kidney injury (iAKI) compared with patients without iAKI [65]. Mice with iAKI also exhibited increased renal fructose concentrations [65], suggesting that ischemia could stimulate endogenous production of fructose in the kidney. Similarly, compared with nondiabetic mice, diabetic mice also had higher fructose levels in the kidney due to the activation of the polyol pathway [66]. Importantly, both studies demonstrated that blocking fructose metabolism ameliorated tubular injury induced by either ischemia or diabetes in mice lacking the fructokinase gene [65,66].

In the senescent kidney, endogenous fructose production likely contributes to the development of glomerular injury. A mouse study showed that glomerular injury accompanied by glomerular hypertrophy, collagen IV deposition, and mesangiolysis was observed in aging wild-type
mice while aging fructokinase-knockout mice developed significantly less glomerular injury [67]. In turn, high salt intake also stimulates endogenous fructose production in the liver, while blocking fructokinase slows fat accumulation in the epididymis [68]. In addition, in the mouse heart, pressure-overload-induced cardiac hypertrophy was ameliorated by blocking fructose metabolism [69].

**Pathways downstream from fructose metabolism may contribute to kidney disease**

In the kidney, inflammation and fibrosis are accompanied with several pathological steps, including cell proliferation, enzymatic activation, and protein synthesis, which require several biological factors, including energy sources, nucleotides, lipids, and redox balance, which can be efficiently provided by aberrant glycolysis. Fructose is metabolized through several pathways and contributes to the progression of CKD (Fig. 4).

**Glycolysis**

The first enzyme involved in fructose metabolism is fructokinase (known as ketohexokinase [KHK]), which phosphorylates fructose to produce Fru1P (Fig. 4). There are two spliced isoforms of KHK, and each is produced by mutual exclusion of the adjacent exons 3C and 3A within the KHK gene [70]. The “A” isoform is ubiquitously expressed but has low activity due to relatively low affinity for its substrate (Km 8 mM) [71]. Expression of the “C” isoform is primarily restricted to metabolic tissues, including the liver, kidney, and intestine, and this form has much higher affinity for fructose (Km 0.8 mM) [71,72]. Fru1P is subsequently metabolized by aldolase B and triokinase to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate that enter the glycolytic pathway downstream of phosphofructokinase. Subsequently, glyceraldehyde-3-phosphate is metabolized to pyruvate in the glycolytic pathway to produce ATP and nicotinamide adenine dinucleotide. Pyruvate is further converted into lactate by lactate dehydrogenase. Importantly, this reaction is usually stimulated by low oxygen, but is accelerated by fructose even under aerobic condition [73]. Lactate seems to be an energy for cancer growth [74].

**Pentose phosphate pathway**

The pentose phosphate pathway (PPP) is activated by fructose and comprises two distinct phases, the oxidative pathway and the non-oxidative pathway (Fig. 4). Glucose-6-phosphate, a fructose metabolite, is metabolized by three sequential reactions in the oxidative pathway to NADPH, which supplies reducing equivalents, and reduces glutathione through the action of glutathione reductase. In turn, two forms of fructose carbon backbones, Fru6P and glyceraldehyde-3-phosphate, are catalyzed by transketolase to enter the non-oxidative pathway for nucleotide formation through ribose 5-phosphate, while erythrose 4-phosphate is metabolized into amino acids. Alternatively, activated hexokinase can convert fructose into Fru6P, which may be a link between glycolysis and the nonoxidative PPP in cancer cells [75].

**Lipogenesis**

Lipids are required as an energy source, for membrane formation, and as signaling molecules (Fig. 4). Fructose is metabolized in the glycolytic pathway to provide acetyl-CoA as the building block of carbon chains for de novo lipogenesis, and also promotes fatty acid synthesis to form palmitate. In turn, glyceraldehyde-3-phosphate, carrying a fructose-based carbon backbone, is also utilized to form triglycerides. Fructose also stimulates intracellular signaling pathways, including those mediated by carbohydrate-responsive element-binding protein [76] and GKRP [60]. A recent study using a mouse model demonstrated that fructose-mediated fatty liver disease was likely mediated by impairment of fatty acid oxidation due to an increased acetylation of long-chain specific acyl-CoA dehydrogenase and carnitine palmitoyl-transferase 1α [17].

**Uric acid production and the Warburg effect**

Fructokinase activation rapidly sequesters phosphate, consequently activating adenosine monophosphate (AMP) deaminase to cleave AMP to inosine monophosphate (IMP) (Fig. 4). However, phosphate levels subsequently increase due to the slower reaction of aldolase with Fru1P. This reaction is further accentuated by the increased IMP, which is an aldolase B inhibitor [77]. Sequential enzymatic acti-
We found that uric acid could prevent the entry of fructose metabolites into mitochondrial oxidation in the human hepatocellular carcinoma cell line HepG2. A potential mechanism for this effect is the suppression of mitochondrial aconitase activity by uric acid, and disconnection of fructose metabolites from mitochondrial oxidation. Since aconitase lies at the junction of acetyl-CoA oxidation, blocking aconitase leads to acetyl-CoA shuttling out of the mitochondria, resulting in the accumulation of citrate in the cytosol. Citrate is then utilized for lipid synthesis by sequential ATP-citrate lyase and fatty-acid synthase. As a result, fructose leads to a state of metabolic imbalance that favors glycolysis over mitochondrial respiration, resembling the Warburg effect in cancer.

The Warburg effect is shared by non-cancer disorders

In 1924, Otto Warburg initially described that cancer cells,
as opposed to normal cells, exhibit a unique ability to ferment glucose to lactate even in the presence of sufficient oxygen [80]. This process is now recognized as a key mechanism of cancer growth and is called the “Warburg effect.” However, we need to be cautious for when interpreting this effect, as general scientists tend to share a misconception regarding oxidative metabolism in the mitochondria [81]. Warburg’s own experiments revealed persistent oxygen consumption in tumor tissues. The rate of mitochondrial respiration was low relative to what might have been predicted given the high rate of glucose uptake, but respiration itself did not appear to be impaired [81]. The Warburg effect was long considered a unique characteristic of cancer; however, recent studies indicate that aberrant glycolysis is not specific to cancer, but rather is shared by other non-cancer disorders [82].

The Warburg effect is involved in multiple processes in several disorders, and the cardiovascular, immune, and neuronal systems are now found to be all modulated by aerobic glycolysis [82]. Although glycolysis produces less ATP than mitochondrial oxidative phosphorylation, the process of aerobic glycolysis is much faster than that of mitochondrial respiration [83]. As a result, aerobic glycolysis can produce more ATP than mitochondrial oxidative phosphorylation in the same amount of time [84]. More importantly, the Warburg effect may impact more than energy production, and may regulate several cellular functions, including cell proliferation, extracellular matrix production, autophagy, and apoptosis [85], and may consequently participate in multiple biological processes.

**The Warburg effect is involved in kidney diseases**

Recent studies have documented that autosomal-dominant polycystic kidney disease (ADPKD) is mediated by aberrant glycolysis. Rowe et al. [86] demonstrated that cultured mouse embryonic fibroblasts derived from *Pkd<sup>−/−</sup>* mice exhibited activated glycolysis, given the cells preferentially utilized greater amounts of glucose and excreted more lactate into the culture medium than cells from wild-type mice. Mice lacking *Pkd* in the renal tubules, as a mouse model of ADPKD, exhibited glycolysis activation while blocking glycolysis with 2-deoxy-D-glucose (2DG), a glucose analog, attenuated tubular cell proliferation, leading to the reduction in kidney size and cyst formation [87].

In diabetic nephropathy, mitochondrial overproduction of superoxide due to the activation of the electron transport chain is considered a unifying mechanism, but this hypothesis remains controversial [88]. Recent studies have demonstrated that mitochondrial function is suppressed in diabetic nephropathy, and the restoration of normal mitochondrial health improves renal, cardiovascular, and neuronal outcomes. In addition, mitochondrial TCA cycle metabolites are also significantly reduced in patients with diabetic nephropathy compared to healthy controls [89]. In turn, glycolytic activation is upregulated in the renal cortex in type 2 diabetes [90], suggesting that activation of glycolysis is dominant over mitochondrial oxidation and plays a pathological role in diabetic nephropathy.

A shift to glycolysis has also been observed in two animal models; one is a model of unilateral ureteral obstruction and the other is a transforming growth factor (TGF)-β1-induced renal fibrosis model. Specifically, Ding et al. [91] found that myofibroblast activation in the kidneys was associated with enhanced renal glucose uptake and lactate production that could be attenuated by blocking glycolysis by 2DG treatment. In these models, a key factor is likely TGF-β1 as this growth factor was capable of switching metabolic profile favoring glycolysis over mitochondrial respiration in fibroblasts. In addition to TGF-β1, PDGF also causes the Warburg effect [92], consistent with the notion that growth factors disproportionately activate glycolysis relative to mitochondrial oxidation [93].

**Could natural fruit exacerbate kidney disease?**

One might ask whether fruit can be also deleterious to the kidney, given fruit contains substantial amount of fructose. This question arises from the assumption that fructose in natural fruit is theoretically metabolized, resulting in uric acid production, and therefore a large amount of fruit may be deleterious. In this regard, several clinical studies have examined the effect of fruit on renal function, and in many cases, the effect of consuming fruits together with vegetables, low salt, and other dietary modifications were assessed [94,95]. These studies generally found that our assumption was flawed and fruit was protective due to the improvement of metabolic acidosis, reduction of blood pressure, and prevention of cardiovascular diseases. However, the effect of a defined amount of fruits was examined in those studies,
and it remains uncertain if a large amount of fruits could cause renal disease.

The mechanism by which fruits are protective of the kidney may be that metabolism of fructose in fruit is inhibited by vitamin C and other nutrients. For example, fructose-associated uric acid production is linked with xanthine oxidase activation and oxidant stress, which can be blocked by flavonoids/catechins and vitamin C in fruits [96]. Vitamin C also enhances urinary urate excretion through URAT-1 [97,98] and lessens the effects of uric acid. In addition, the potassium present in many fruits can ameliorate urate-induced endothelial dysfunction [99].

In this regard, we previously discussed this issue and reviewed the effect of variety of natural fruits on hyperuricemia or gout [100]. One thing to bear in mind is that the effects of fruit are often inconsistent in clinical studies, in part due to difference in study designs, although other factors may impact the results. Fruit intake is often estimated from the results of face-to-face interviews or questionnaires, which usually rely on memory, and may not always be accurate [101]. The composition of fruits may vary significantly depending on growing, harvesting, and storage conditions, and how they are prepared for consumption. For example, black currants become sweeter at higher growing temperatures and their taste varies with season, with the concentrations of fructose, glucose, and vitamin C found

![Fructose Diagram]

**Figure 5. Current hypothesis of the pathophysiology of fructose in the kidney.** In addition to dietary fructose, fructose can be produced endogenously as a result of several pathological conditions in the kidney. During starvation, fructose is utilized for gluconeogenesis in the proximal tubular cells and excreted into the systemic circulation to maintain serum glucose concentrations. In turn, when in excess or during satiation, fructose is associated with aberrant energy production, biomass synthesis, and redox balance with uric acid production, resulting in the Warburg effect in the kidney. Together with such reactions, fructose also causes endothelial NO synthase (eNOS) uncoupling and release inflammatory cytokines. Fructose also stimulates the Na/H exchanger to accelerate sodium absorption, leading to salt-sensitive hypertension (HT).

ICAM-1, intercellular adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1, mOXP0HS, mitochondrial oxidative phosphorylation; Osm, osmolarity.
to also differ depending upon the season [102]. In addition, humidity and latitude influence the maturation process of many fruits, and often determine the sugar content; together, these various influences may result in sweeter fruit in the fall than in the spring, and greater fructose content in mature fruit than less mature fruit [103]. These factors may add a layer of complexity to the effects of fruit intake on human renal disease.

Conclusions

Several risk factors, including hypoxia, high blood glucose concentrations, senescence, and cardiac pressure overload, are found to share endogenous fructose production in the kidney as a common underlying factor (Fig. 5). A unique characteristic of fructose metabolism, as opposed to glucose metabolism, is the production of uric acid. Excessive uric acid production links to inflammation, endothelial dysfunction, vascular injury, and insulin resistance, while also favoring glycolysis over mitochondrial respiration, similar to the Warburg effect in cancer. The Warburg effect creates a pool of biosynthetic precursors that contribute to several pathological processes, including nucleotide synthesis, amino acid production, lipids and lactate, and excessive endogenous fructose is likewise a mechanism of CKD (Fig. 5). Further studies exploring the role of endogenous fructose in CKD are warranted.

Conflicts of interest

Takahiko Nakagawa has equity with XORTX therapeutics, which is developing novel xanthine oxidase inhibitors. The authors have no other conflicts of interest to declare.

Funding

This work was supported by a grant from the Korean Society of Nephrology (BAXTER, 2017) and a National Research Foundation of Korea (NRF) grant funded by the Korean government MIST, (2020R1A2C3007759).

Authors’ contributions

Conceptualization: TN
Writing–original draft: TN
Writing–review & editing: DHK
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Chronic kidney disease (CKD) is associated with increased risk of cardiovascular (CV) events, and the disease burden is rising rapidly. An important contributor to CV events and CKD progression is high blood pressure (BP). The main mechanisms of hypertension in early and advanced CKD are renin-angiotensin system activation and volume overload, respectively. Sodium retention is well known as a factor for high BP in CKD. However, a BP increase in response to total body sodium or volume overload can be limited by neurohormonal modulation. Recent clinical trial data favoring intensive BP lowering in CKD imply that the balance between volume and neurohormonal control could be revisited with respect to the safety and efficacy of strict volume control when using antihypertensive medications. In hemodialysis patients, the role of more liberal use of antihypertensive medications with the concept of functional dry weight for intensive BP control must be studied.

Keywords: Antihypertensive medication, Chronic kidney disease, Diuretics, Hemodialysis, Hypertension, Renin-angiotensin system, Sodium

Introduction

In chronic kidney disease (CKD), cardiovascular (CV) risk is increased linearly by 7% per 10 mL/min/1.73 m$^2$ decrease in glomerular filtration rate (GFR) [1]. Death from CV disease (CVD) is much more common than that from progression to end-stage renal disease (ESRD) [2]. In addition, CKD is a poor prognostic factor in CVD patients [3].

CKD is defined as persistently elevated urine albumin excretion (≥30 mg/g [3 mg/mmol] creatinine [Cr]), persistently reduced estimated GFR (eGFR < 60 mL/min per 1.73 m$^2$), or both for greater than 3 months, in accordance with current Kidney Disease: Improving Global Outcomes (KDIGO) guidelines [4]. Kidney damage in many diseases usually can be ascertained by the presence of albuminuria, defined as albumin-to-Cr ratio of >30 mg/g in two of three
spot urine specimens based on KDIGO guidelines [5]. In addition to chronicity, demonstration of damaged renal parenchyma is mandatory for diagnosis of CKD stages 1 and 2. For CKD stage 3 or higher, chronic decreased kidney function as determined by GFR of <60 mL/min/1.73 m² is sufficient for diagnosis regardless of the presence of renal damage.

Approximately one-third of CKD stage 3 and less than one-half of CKD stage 4 patients have albuminuria [6–8]. Increased blood pressure (BP) aggravates renal damage and proteinuria, which results in more rapid loss of kidney function. This decreased renal function contributes to higher BP, completing a vicious cycle [9]. Moreover, increased BP not only exacerbates renal function deterioration but also damages CV systems.

The differences in the rationale or approach between prevention of renal progression and CVD are frequently discussed among cardiologists, hypertension specialists, and nephrologists. This article will review recent updates in BP control in CKD with a major focus on CVD prevention in addition to renal outcomes with viewpoints of salt retention or volume status control and the use of diuretics and non-diuretics as antihypertensive medications (AHMs) for intensive BP control. This review will not cover specific target BPs according to specific disease populations of CKD.

### Updates on tissue or skin sodium

In addition to classic pressure natriuresis relationships, recent views highlighted that sodium balance is largely dependent on neurohormonal modulation [10]. Accumulation of sodium in soft tissue measured by skin and/or muscle sodium has been demonstrated to be a buffer between sodium intake and changes in volume status, which was regarded as an explanation of the time lag or loose connection between sodium intake and BP response as shown in Fig. 1 [11]. Titze et al. [12] reported that glycosaminoglycan (GAG) in tissue in the epidermal skin layer plays a role as a sodium buffer, assuming that GAG binding to sodium ions makes it osmotically inactive. The skin buffer concept by hypertonicity is useful to explain the time lag or discrepancy between salt overload and BP increase.

![Figure 1. Hypothetical distribution of skin sodium according to tonicity of skin sodium.](image)

(A) A three-compartment model with hypertonic sodium in the skin, muscle, or artery that could buffer the exchangeable sodium overload. Exact mechanisms on how sodium could be concentrated in the skin remain unknown. (B) A two-compartment model with isotonic skin sodium exhibiting distribution in the other interstitial tissues or edema. The dotted line indicates a sodium gradient across the cell membrane. The broken line means sodium distribution by Starling force. The separated bone compartment suggests different exchangeability kinetics from the other.

ECF, extracellular fluid; ICF, intracellular fluid.
However, a recent study by Rossitto G et al. [13] showed that sodium concentration in the skin is importantly isotonie but could be mistakenly identified as hypertonic due to technical reasons during skin magnetic resonance imaging. In this respect, the amount of skin sodium could be regarded as an extension of the expansion of extracellular fluid (ECF) volume or interstitial edema. Inflammation was explained not only through hypertonic stress, but also by the biomechanical stress of edema. A high salt-related increase in peripheral resistance was found to be due to compression from perivascular swelling or edema both in terms of vasoconstriction and vasodilation [13].

Hypertonic or nonosmolar sodium accumulation in tissue compartments can be denied when sodium in tissues is isotonic and a reflection of ECF volume [14]. However, isotonic edema is also associated with increased GAG and biomechanical stress as well as crosstalk between GAG and macrophages. This results in prohypertensive effects in the kidney, vasculature, and brain [15]. Angiotensin II (Ag II) also has proinflammatory effects through macrophage infiltration in the renal interstitium, leading to sodium retention and BP elevation, as well as renal damage mediated by T lymphocytes [16]. The direct connection between high sodium intake and Ag II induction, both of which are crucial components of the salt-induced hypertension model, seems to be weak [16].

In CKD, a diverse level of skin or tissue sodium was reported, which now could be interpreted as silent edema or volume expansion by high salt intake. In a prospective observational study performed in a CKD population, high salt intake measured by 24-hour urine sodium excretion was significantly correlated with CKD progression and CVD events [17]. The harmful effect of sodium intake seems to be more obvious in CKD compared to the general population [18]. Systematic reviews have exhibited that dietary sodium reduction demonstrate short-term reductions in BP in CKD populations [19]. However, it is necessary to have more clinical data evaluating the effects of dietary sodium reduction on CV events in CKD populations [4]. It is essential to be reminded of inconsistencies among studies examining the relationship of dietary sodium intake with health outcomes in persons with diabetes, suggesting that the effects of dietary sodium intake changes on health benefits and harms depend on different causes and severities of CKD [4,19–21].

The clinical evidence on whether so-called salt toxicity is independent of BP changes is debated [22]. There is also ample, recent evidence to indicate that physical factors are clearly the subordinates of the neurohumoral mechanisms. Essentially, the question is then whether the normal physiological situation is best described as (i) neurohumoral modulation of the pressure natriuresis mechanism, or (ii) neurohumoral control occasionally modulated by pressure natriuresis. The latter possibility appears attractive.

Renal salt excretion in sodium balance under normal conditions

Slight changes in osmolarity by salt intake, inducing immediate movement of water from the intracellular to the extracellular compartments, thirst, and secretion of antidiuretic hormones resulted in increasing and maintaining ECF volumes almost without apparent changes in sodium concentration [23]. Even small volume increases resulted in relatively large pressure elevation through the secondary increase in total peripheral resistance [24].

When a bout of dietary salt is loaded in a normal subject, approximately half is excreted on day 1. With thirst and renal water resorption, body weight and ECF volume increases are associated with a variable degree of BP changes according to individual salt sensitivity [25]. After 3 to 4 days when the sodium balance becomes zero, the original steady state is recovered as long as no more bout of salt was maintained [25,26]. Although the exact details are being debated, renal excretion of sodium has been found to be related to the function of ECF volume and BP. According to Walser’s analysis, the time constant of the relationship between ECF volume and renal salt excretion determines the speed at which an individual can adapt to a change in dietary intake [27]. Daily urinary sodium excretion is proportional to the time constant and the amount of sodium excess. While the time constant is approximately 0.79 per day−1 in normal subjects, it appears to be reduced by aging and CKD, indicating that it takes longer than 3 to 4 days for the kidney to recover its sodium balance to zero [27]. Sodium retention might be similar to the setting when a drug in maintenance dose is repeated within the elimination half-life so that increased sodium amount or volume will be maintained.
Sodium retention, volume overload, and hypertension in chronic kidney disease

The sodium excretion rate appears to be reduced by aging and CKD, and the mechanisms of decreased sodium excretion are a mixture of reduced glomerular filtration of sodium and increased tubular reabsorption of sodium independently, or both situations in combination. CKD is an important contributor to salt sensitivity. In a small study for male CKD patients on sequential low salt and high salt diets, the salt sensitivity index was calculated by the increase in mean arterial pressure in mmHg divided by the increase in 24-hour sodium excretion in mEq/day, which is the inversely slope of the classic pressure natriuresis relationship [28]. In this study, the log of the salt sensitivity index was linearly associated with Cr clearance (r = −0.89, intercept = −0.74). For example, the salt sensitivity index can be calculated as 1.4 mmHg / (100 mEq of sodium per day) at a Cr clearance of 75 mL/min and was increased to 2.0 mmHg and 3.6 mmHg at a Cr clearance of 50 mL/min and 25 mL/min, respectively. In an animal study, high dietary sodium intake in CKD contributes to sodium retention through aldosterone-independent activation of the mineralocorticoid receptor-mediated through small GTPase Rac1 [29,30]. In addition to Rac1, high dietary salt in salt-sensitive rodents appears to increase serum and glucocorticoid-induced kinase 1 independent of aldosterone, which could be another pathway through which dietary salt directly increases distal nephron sodium reabsorption [30,31]. ECF volume expansion through a repeated sufficient amount of salt intake within the half time that the sodium balance reaches zero can lead to a compensatory decrease in tubular reabsorption of sodium, reestablishment of the steady state of sodium balance, a variable degree of hypertension, and accompanied with or without other manifestations of ECF volume expansion. Once the new steady state is achieved, tubular reabsorption of sodium reaches almost the original sodium resorption status before increased salt intake, producing infinitely small error and infinite gain of control (correction/error) where the correction is the change of tubular resorption of sodium during the process [32].

In an insightful and comprehensive study by Essig et al. [33], CKD stage 3 exhibited higher BP with ECF volume expansion, even with greater use of AHM, compared to CKD stage 1. Compared to CKD stage 2, CKD stage 3 exhibited comparable BP and ECF volume expansion. This was the case even with more AHM including diuretics and less sodium excretion compared to CKD stage 2. The steady status of CKD stages 2 and 3 in Fig. 2 did not directly exhibit the dynamics such as salt sensitivity index for each stage due to the limitation of the cross-sectional study, but it suggested that the required amount of salt restriction and required AHM should be increased to allow for comparable levels of BP and ECF volume excesses (Fig. 2).

In general, the prevalence of masked hypertension (MH) is higher in CKD compared to the general population and is related to low eGFR and proteinuria [34]. The prevalence of normal, MH, and sustained hypertension in CKD stages 1 and 2 was reported as 58%, 27%, and 15%, respectively [35]. MH with elevated nighttime BP is reported to be associated with target organ damage [35]. Even in CKD stages 1 and 2, it is reasonable to have a high level of suspicion.
of MH in patients with prehypertension. To detect MH through increased nighttime BP in CKD stages 1 and 2, ambulatory BP monitoring (ABPM) seems to be the best method. Moreover, the proportion of CKD patients with masked uncontrolled hypertension and sustained uncontrolled hypertension is expected to increase when using a lower systolic BP (SBP) threshold as per the recommendation of recent guidelines [4].

The association of MH and lower eGFR was observed only in patients with increased nighttime BP even though the mechanisms of increased MH in CKD are multifactorial [35]. Meanwhile, the mechanisms of increased nighttime BP in CKD are salt sensitivity, increased sympathetic activity, and proteinuria [36].

Management of hypertension in chronic kidney disease

Role of diuretics and volume control

ECF volume overload was reported as a group in early CKD, but routine assessments on an individual patient level are not practical [33]. The existence of an ECF volume increase can be detected by noticing impressive BP reduction when diuretics are added and/or salt was restricted, specifically in a situation of uncontrolled high BP through the use of AHMs other than diuretics.

There is insufficient data on the role of diuretics as the first-line therapy for the management of hypertension in CKD populations, and several guidelines on hypertension have shown different opinions and views on the use of diuretics [4]. Previously, National Institute for Health and Care Excellence (NICE) guidelines recommended that diuretics be used in non-proteinuric CKD [37] as a first-line therapy. Since albuminuria is a common indicator to diagnose CKD stages 1 and 2, and some patients of CKD stages 3 and 4 do not possess albuminuria [7], the first-line use of diuretics seems to be more useful in CKD stages 3 and 4 with salt retention and reduced GFR. The role of diuretics in CKD stages 3 and 4 is increasing, and more potent loop diuretics and some thiazide-like diuretics such as chlorothalidone, metolazone, and indapamide appear to be effective for optimal BP control [4]. However, because the glomerular stretch in the nephron level triggers a vicious cycle of CKD progression even in CKD stages 3 and 4, renin-angiotensin system blockade (RASB) could theoretically have a major role as a first-line therapy, even in non-proteinuric CKD stage 3 and 4 patients [37,38].

Despite an increase in ECF volume in CKD, most guidelines prefer an RASB as the first-line therapy as long as BP is well controlled. The ACC/AHA (American College of the Cardiology/American Heart Association) 2017 guidelines recommend an angiotensin-converting enzyme (ACE) inhibitor for CKD and emphasize initial combination therapy and a target BP of <130/80 mmHg [39,40] so that second-line drugs can be used as initial combination therapy. As the second-line drug, the National Kidney Foundation’s Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines recommend diuretics [32], but ESH-ESC (European Society of Hypertension/European Society of Cardiology) 2018 guidelines advocated for calcium channel blockers (CCB) [41]. As for CCB, it was reported to be associated with mortality in CKD patients with glomerulonephritis [42]. But in a meta-analysis, CCB has similar effects to RASB in terms of long-term BP, mortality, heart failure, stroke or cerebrovascular events, and renal function [43]. For comparable BP levels and salt intake, there can be a different volume status according to the proportion of diuretics and other AHMs (Fig. 3A vs. 3B). These discrepancies call to attention the need for more individualized approaches balanced by volume status, side effects, CVD risk, and comorbid CVD profiles to select second-line drugs as well as further studies demonstrating clinical evidence. For example, increased BP and/or ECF volume is the main factor for left ventricular hypertrophy (LVH) in CKD [44]. In CKD stages 3 and 4, additional furosemide on top of an RASB exhibited a greater regression of LVH [45]. Similarly, among patients treated with an ACE inhibitor or angiotensin II receptor blocker, the combination of salt restriction and a diuretic can provide a greater antiproteinuric effect as well as improved BP reduction than either intervention alone [46]. Therefore, RASB will be the basis for antihypertensive drug therapy in CKD, and the choice between diuretics and CCB should be individualized.

For additional use of diuretics in CKD, there was a recent observational study showing the benefit of spironolactone in CVD and renal outcomes in CKD stages 3 and 4 [47]. However, the risk of hyperkalemia should be more preemptively managed through the use of dietary and/or pharmacologic interventions using potassium-wasting diuretics.
and oral potassium binders [48]. More options for diuretics such as a higher dose of loop diuretics in twice a day use or in combination with chlorthalidone and spironolactone could be considered for optimal BP control [48,49].

**Newer drugs associated with volume control**

As the elderly population rapidly increases, there are more and more cardiorenal syndrome patients with CKD. From the randomized controlled trial (RCT) for heart failure in which volume control by diuretics is an essential component in standard drug therapy, two interesting drugs potentially related to volume control or sodium excretion are more and more frequently indicated in heart failure with CKD. First, the sodium glucose co-transporter 2 inhibitor (SGLT2I) has several mechanisms to protect the heart and kidneys. Among them, sodium excretion in the proximal tubule followed by increased sodium delivery to the macula densa and tubuloglomerular feedback results in a short-term increase in the excretion of renal sodium [50]. BP reduction as the one of the important mechanisms of cardiorenal protection has been proven in CKD patients [51,52]. Although precise interactions or differential effects between loop diuretics and SGLT2I require further studies or analyses, it was reported in terms of heart failure that SGLT2I prevents the increase of the dose of loop diuretics, and that it can overcome the resistance of loop diuretics [53]. Second, theoretically, the natriuretic components of angiotensin receptor neprilysin inhibitor (ARNI) could facilitate renal excretion of sodium. In CKD patients, neprilysin inhibitor components have stable pharmacokinetic properties, and the reduction in loop diuretic dose was reported in RCT for heart failure [54]. Since diabetes and cardiorenal syndrome are frequent comorbidities in CKD, the roles of SGLT2I and ARNI in volume control require further analysis.

**Intensive blood pressure control in chronic kidney disease**

KDOQI guidelines underscore the purpose of antihyper-
tensive therapy for prevention of both CKD progression and CVD [32]. In a meta-analysis, lower achieved BP (SBP < 120 mmHg) in those with CKD reported a smaller risk reduction for CV events compared to patients without CKD [55]. The impact of SPRINT is important in terms of clinical evidence in CKD, and the results from SPRINT were supported by subsequent meta-analysis examining death exclusively in the CKD subgroups of RCTs, which found a benefit of lower achieved or target BP [4,56]. In the KDIGO 2021 guidelines, it was suggested that adults with high BP and CKD be treated with a target SBP of <120 mmHg. Despite this intensive target BP, some risk-benefit ratios need to be individualized, and intensive BP control could be harmful, for example, when BP was measured in a non-standardized manner, when it is uncertain if the patient has a silent coronary obstruction, when the patient cannot tolerate intensive target BP or is extremely old (>90 years old), and when patient is bed-ridden or when life expectancy is limited.

Practically, in general, CKD stage 3 and 4 patients are very concerned about GFR decline in terms of fear of dialysis. First of all, it is very difficult for a patient to understand that higher GFR associated with higher BP could indicate a poor prognosis and that hyperfiltration or glomerular stretch at the level of the nephron is harmful. The acute decline of GFR especially by RASB, a functional side effect, could be uncomfortable for patients. Clearly informing patients of this potential issue and warning that 10% to 20% of the initial Cr increase is normal and reversible seems to be essential for patient adherence [57]. In some CKD patients who have comorbidities such as heart failure or coronary artery disease that requires the use of RASB to reduce CV mortality, an initial Cr increase more than >30% could be acceptable [58].

The initial decline in GFR has become a greater challenge for physicians because intensive BP lowering is increasingly considered for better CVD outcomes. After all, recommendations not to retry RASB in cases of an initial Cr increase >30% and in cases of failure to return to baseline after dosage reduction or cessation do not seem to have a solid scientific basis. Avoidance of an initial Cr increase more than >30% could be regarded as a consistent application of the “primum non nocere” principle and a compromise to alleviate patient apprehension [32,59]. For this issue, volume status and a preexisting or combination regimen of RASB and diuretics vs. CCB seems to be differently related to the degree of initial decline [60]. In SPRINT, the difference in the rate of such (eGFR) decline was very small with comparable biomarker changes, even though initial decline during the first 6 months was significantly faster in the intensive group compared with the standard group [5,61,62]. Moreover, RASB in patients with advanced CKD was reported to be rather safe [63], even though there are studies from selected small populations reporting that habitual use does not appear to be beneficial and might even be harmful [63,64]. Despite the risk reduction of dialysis therapy (27.9% vs. 36.1%), stopping RASB was associated with a higher absolute 5-year risk of all-cause mortality (54.5% vs. 40.9%) and major adverse CV events (59.5% vs. 47.6%) [65].

Strict volume control for intensive BP control could have increased risk of hypovolemia. Hypovolemia and/or hypotension are the most common factors for acute kidney injury (AKI). The nonrecovery of kidney function following an episode of AKI is a major contributing factor for the prevalence of CKD and the progression of CKD to an advanced stage [66,67]. Hypovolemic patients in CKD stages 3 and 4 reported to have lower BP and lower AHM use including diuretics. This means that, in some CKD patients, patient-related factors such as fewer comorbidities, decreased lean body mass, anemia, and other unknown causes might be more important in determining volume status than is use of diuretics [68]. Diuretic use was associated with poor renal outcomes independently of BP, volume status, and other covariates [68]. Since patient factor-driven hypovolemia is associated with lower BP, whether inducing minimal hypovolemia by diuretics therapy to comparable level to the patient factor-driven hypovolemia could be beneficial or not for intensive BP control needs future study (Fig. 3F). The role of sodium restriction or weight reduction in the context of intensive BP control also requires further studies.

Blood pressure variability in hypertension management in chronic kidney disease

There are several types of BP variabilities (BPV) such as visit-to-visit BPV in clinics, day-to-day BPV with home BP monitoring (HBPM), and short-term BPV with ABPM. The mechanisms for increased BPV in CKD are largely unknown, but impaired baroreceptor sensitivity, altered sym-
pathetic nervous system activity, oxidative stress, inflammation, and increased arterial stiffness were suggested [69]. Few studies have been performed to correlate high sodium intake and BPV [70].

The Spanish ABPM registry showed that BPV increases as CKD progresses from stage 1 to 5 [71], and short-term systolic BPV by ABPM was associated with renal outcome independently of 24-hour BP in a CKD population with a mean eGFR of 50 mL/min/1.73 m² [72].

For clinic BP, among 114,900 patients with CKD, BPV was associated with all-cause mortality, hemorrhagic stroke, ESKD, and heart failure [73]. There were also studies showing that systolic BPV predicts the risk of death, but not CKD progression to dialysis in CKD [74].

However, few studies have reported on the therapeutic implications of BPV in CKD. CCB was reported to be associated with lower BPV in CKD compared to beta-blockers or RASB [75,76]. In SPRINT, similar patterns were observed, with lower BPV in participants receiving chlorthalidone and CCB and higher BPV variability among those on RASB [73].

There could also be a marked reduction in GFR when starting RASB in CKD despite the slight BP reduction. Diuretics or RASB and diuretics in combination are more commonly associated with side effects and a worse BPV profile than CCB in CKD [77,78].

BPV can affect variability in GFR because volume changes and impaired GFR autoregulation can be common in CKD, and variability in GFR can predict CV outcomes [79] as well as renal outcomes [80]. However, since studies of GFR variability have not reported the relationship between BPV and GFR variability, BPV could be a significant confounding factor.

Blood pressure control in hemodialysis patients

This review confined to BP control under a standard reimbursement protocol with a thrice a week hemodialysis (HD) protocol spanning about 4 hours seems to be limited for adopting various dialysis protocols for better BP control. For the volume status description, dry weight is defined operationally as the lowest postdialysis weight with minimal signs or symptoms of hypovolemia even though the exact definition of dry weight remains uncertain or multiple definitions have been suggested [81]. Theoretically, reducing volume overload by ultrafiltration (UF) is such an efficient measure to manage BP as to stop the use of AHMs in up to 90% of patients undergoing HD even though the long-term consequences have not been studied, as shown in Fig. 3C [82,83]. The finding that 36% of normovolemia and 54% of hypervolemia patients in whom AHM were prescribed in 45% and 54%, respectively, as schematized in Fig. 3D and 3E, were hypertensive suggests a huge gap in real practice, with more frequent AHM use and low probability of hypovolemia [84]. This finding suggests that the dry weight concept when used in an effort to simply avoid or stop AHM for BP control must be reconsidered [85]. There might be a concept of “functional dry weight” permitting greater use of AHM instead of “absolute dry weight” as long as BP is controlled [86]. In fact, AHM can be considered the first-line to lowering BP in patients receiving HD [87]. It is reasonable to choose AHMs based on patient characteristics, CV indications, and availability as well as intradialytic BP patterns with regard to drug dialyzability and elimination routes [88,89]. In theory, drugs with hepatic clearance could provide stable antihypertensive effects, and dialyzable drugs would be better in lowering BPs increased by volume uptick that will be removed during dialysis simultaneously with volume. However, an optimal combination strategy of drugs with hepatic and renal clearances in which dose reduction is required remains unknown (Fig. 4). It is very important to limit interdialysis weight gain (IDWG), which results in higher BP levels and BPV requiring additional AHMs.

Optimal predialysis BP for mortality risk was 130 to 160 mmHg when adjusted for confounding factors including AHM [90]. BP measurement methods seem to be limited to demonstrate the relationship between volume status and BP because HBPM or ABPM could predict CVD outcomes better than casual BP in HD patients and because BPV during inter and intradialytic periods was much higher in HD patients compared to non-dialysis patients [91]. Therefore, for more reliable BP control, reference methods to monitor BP should be HBPM or ABPM, and further studies for optimal BP thresholds to prevent hard outcomes are necessary. Therapeutic implications of BPV in HD patients are not established but, in a large-scale observational study, lower visit-to-visit BPV was associated with greater UF volume, dry weight attainment, and AHM other than β-blockers or RASB, suggesting the roles of volume control and CCB for more stable BP control [92].
Volume overload and high BP are the most important contributors to LVH. In HD patients, there are other remarkable factors related to LVH such as anemia, arterial-venous dialysis accesses with high cardiac output, arterial stiffness, and bone mineral hormones [93]. LVH and accompanied cardiac fibrosis are strongly associated with cardiac diastolic dysfunction in which high left ventricular (LV) filling pressure is required to maintain LV end-diastolic volume that will be transferred to stroke volume by LV contraction. LVH are strongly associated with CV prognosis in HD patients, and a 10% decrease in LV mass predicts a 28% decrease in CV outcomes [94,95]. It should be essential to achieve individualized target BPs for prevention of severe LVH or diastolic dysfunction to prevent intradialytic hypotension (IDH) and CVD until the proven BP target beneficial for LVH is available.

Establishing a balance between AHMs and volume control is most challenging for treatment of severe IDH. If an effective AHM prescription during the phase of advanced CKD stage is available in a patient, the same AHM regimen with a UF amount equivalent to the role of diuretics during the CKD phase seems reasonable to be maintained during HD (Fig. 3D) [88]. The so-called “wet strategy” with some degree of permissive hypervolemia with profuse use of AHM as long as the target BP is achieved caring of residual renal function might be an option for IDH [96]. It is mandatory to decrease IDWG for which sodium restriction to the level to avoid IDH concomitantly with ultrafiltration rate adjustment is needed [97]. Routine increase in IDWG to avoid IDH is not recommended.

**Conclusion**

In conclusion, sodium retention can be demonstrated in both early and late CKD. The BP response to sodium retention could be modulated by neurohormones to maintain stable BP to a certain limit. RAS seems to be involved in a common pathophysiology for nephron injury in CKD with reduced renal mass. Recent clinical trial data favoring intensive BP lowering in CKD imply that the balance be-
between volume and neurohormonal control could be shifted by using more AHMs other than diuretics than previously believed. More liberal use of AHMs could be allowed for effective BP control and CV protection even after HD is initiated with the concept of functional dry weight.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Authors’ contributions**

Conceptualization: JS, CHL
Visualization: JS, CHL
Writing–original draft: JS
Writing–review & editing: CHL, JS
All authors read and approved the final manuscript.

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Angiotensin receptor-neprilysin inhibitor in patients with heart failure and chronic kidney disease

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Despite significant advances in the management of heart failure with reduced ejection fraction (HFrEF), there remains an enormous health problem with high morbidity and mortality over the last few decades. The neprilysin inhibitor enhances the activity of natriuretic peptides, producing vasodilation, natriuresis, and diuresis. Angiotensin receptor blockers inhibit the renin-angiotensin-aldosterone system. Sacubitril/valsartan, a first-in-class angiotensin receptor-neprilysin inhibitor (ARNI), has been shown to improve cardiovascular outcomes in HFrEF and delay the progression of chronic kidney disease (CKD) in patients with HFrEF. The PARADIGM-HF study showed a reduction in diuretic need in the ARNI group. While the use of diuretics is effective in volume control in patients with HFrEF, their use has the potential to adversely affect renal function. Therefore, ARNI therapy could benefit patients with heart failure and CKD by reducing cardiovascular morbidity and mortality and possibly retarding the progression of CKD, although more clinical evidence is required in patients with severe CKD and end-stage renal disease.

Keywords: Chronic kidney disease, Heart failure, Neprilysin, Renin-angiotensin-aldosterone system

Introduction

Heart failure (HF) and chronic kidney disease (CKD) are expected to continue to increase worldwide as the number of elderly people increases [1,2]. The heart and kidneys are closely related and interdependent, which is expressed by the term cardiorenal syndrome [3]. The presence of concomitant HF and CKD accelerates the presentation and progression of the disease. Patients with both HF and CKD are at an increased risk of hospitalization, need for intensive care or renal replacement, and death [4]. A large meta-analysis of patients with HF found that up to 55% of HF patients had a reduced estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m², and there was a stepwise increase in mortality risk with an increase in CKD stages [5]. There are two major risks for patients with CKD: cardiovascular morbidity or mortality and an increased risk of progression to end-stage renal disease (ESRD) requiring...
dialysis or kidney transplantation [6,7]. Therefore, the comprehensive goal for the management of CKD patients is to prevent cardiovascular disease and attenuate progression to ESRD. As CKD progresses, the clinical manifestation of cardiovascular disease changes from atherosclerotic disease to nonatherosclerotic disease [8,9] and the incidence of HF and sudden cardiac death increases. Unfortunately, the treatment of patients with concomitant HF and CKD is challenging as CKD progresses. Patients with HF and CKD may frequently fail to respond to conventional HF therapies and experience an increased risk of toxicity to guideline-directed medical therapy (GDMT) of HF [10].

Previous studies have shown that inhibition of the renin-angiotensin-aldosterone system (RAAS) decreases the risk of cardiovascular events and slows the progression of CKD with proteinuria [11], suggesting both the cardiovascular and renal benefits of RAAS inhibition in CKD patients. Recently, dual inhibition of neprilysin and RAAS has shown superior cardiovascular and renal benefits compared to conventional RAAS inhibitors, including angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) in patients with HF [12,13]. The first-in-class angiotensin receptor-neprilysin inhibitor (ARNI), sacubitril/valsartan, is rapidly replacing RAAS inhibitors as a frontline medical therapy in patients with heart failure with reduced ejection fraction (HFrEF) [14]. This review explores the background of ARNI in HF and offers guidance on how to use ARNI in clinical practice, especially in patients with concomitant HFrEF and CKD.

**Classification of heart failure and guideline-directed medical therapy**

HF was categorized according to left ventricular ejection fraction (LVEF) in the 2016 European Society of Cardiology Guidelines for HF as follows: HF with preserved ejection fraction (HFrEF), LVEF ≥ 50%; HFrEF, LVEF < 40%; and HF with mid-range ejection fraction, LVEF 40% to 49% [15]. More recently, a new revised 2021 universal classification of HF has been proposed, including HFrEF, LVEF ≤ 40%; HF with mildly reduced ejection fraction, LVEF 41% to 49%; HFrEF, LVEF ≥ 50%; and HF with improved ejection fraction: a baseline LVEF ≤ 40%, a ≥10% increase from baseline LVEF, and a second measurement of LVEF > 40% [16]. There is no robust evidence that any treatment can modify the natural history of patients with HFrEF, probably due to the heterogeneity of its etiologies [17]. In contrast, there is plenty of evidence for medical therapy for HFrEF, which has shown survival improvement in large randomized controlled clinical trials, including ACEIs, ARBs, beta-blockers, mineralocorticoid antagonists (MRAs), and an ARNI [18].

RAAS inhibition has been the mainstay of treatment strategies for patients with HFrEF [19,20]. Randomized controlled trials have proven that the RAAS plays an important role in the pathophysiology of HFrEF. The blocking points in RAAS for each ARNI, ACEI, ARB, and MRA are systematically demonstrated in Fig. 1. The updated guidelines for HF treatment recommend the use of an ARNI, ACEI, or ARB to reduce morbidity and mortality in patients with chronic HFrEF and advise that patients who can tolerate an ACEI or ARB should change to an ARNI to further reduce adverse cardiovascular outcomes [21,22]. Furthermore, the 2021 American College of Cardiology Expert Consensus has suggested that an ARNI is the preferred method for RAAS inhibition over ACEIs or ARBs if there are no compelling contraindications, suggesting a superior role of ARNI in the management of HFrEF in other RAAS inhibitors [14].

**Dual angiotensin receptor-neprilysin inhibition**

In patients with HFrEF, RAAS is upregulated, which leads to excessive production of natriuretic peptides. Consequently, natriuretic peptides modulate the response to RAAS by aiding natriuresis and vasodilation [23]. Neprilysin is responsible for the breakdown of vasoactive peptides. Neprilysin inhibition increases endogenous levels of vasoactive peptides, resulting in increased vasodilation, natriuresis, and diuresis, as well as a reduction in cardiac fibrosis and hypertrophy. However, neprilysin inhibition also impairs the degradation of angiotensin II, which induces compensatory upregulation of RAAS and sympathetic nervous activity [24]. Therefore, the best strategy to suppress RAAS would be to inhibit the breakdown of natriuretic peptides and block the RAAS simultaneously [25], which led to the development of ARNI. Neprilysin inhibitors are not combined with ACEI, since a previous study has shown a higher risk of angioedema with the combination of neprilysin inhibitor and ACEI [26]. The first-in-class ARNI, sacubitril/valsartan, are the only ARNIs approved for clinical use and have shown many benefits in patients with HFrEF. The
indications, contraindications, and cautions for sacubitril/valsartan use are summarized in Table 1.

**Cardiovascular effects of angiotensin receptor-neprilysin inhibitor**

The long-term benefits of sacubitril/valsartan on cardiovascular morbidity and mortality over other RAAS inhibitors in patients with chronic HFrEF was first described in the Prospective Comparison of ARNI with ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF) trial, which showed that sacubitril/valsartan was superior to enalapril in reducing the risk of HF hospitalization and cardiovascular death by 20% [12]. According to the result of PARADIGM-HF, guidelines have recommended sacubitril/valsartan as a replacement for ACEIs or ARBs [15,18]. Claggett et al. [27] suggested that the life expectancy of patients receiving ARNI might increase by 1 to 2 years compared with patients receiving ACEI, supporting a strong recommendation to use sacubitril/valsartan for patients with HFrEF. Furthermore, the Prospective Study of Biomarkers, Symptom Improvement, and Ventricular Remodeling During Entresto Therapy for Heart Failure (PROVE-HF) trial [28], Comparison of Sacubitril-Valsartan versus Enalapril on Effect on NT-proBNP in Patients Stabilized from an Acute Heart Failure Episode (PIONEER-HF) trial [29], and the Comparison of Pre- and Post-discharge Initiation of LCZ696 Therapy in HFrEF Patients After an Acute Decompensation Event (TRANSITION) study [30] have shown that sacubitril/valsartan was effective and safe in a wide range of HFrEF, including those with acute decompensated HF, newly diagnosed HF, and HF without prior ACEI or ARB use, all of which supports the expansion of ARNI application in a broad range of patients with HFrEF.

In contrast to the promising results from patients with HFrEF, the Prospective Comparison of ARNI with ARB Global Outcomes in HF with Preserved Ejection Fraction (PARAGON-HF) trial in patients with HFpEF showed that sacubitril/valsartan did not result in a significantly lower rate of total HF hospitalizations and cardiovascular deaths among patients with HFpEF (LVEF > 45%), even though there was a suggestion of possible benefit with sacubitril/valsartan and in women and in patients with lower LVEF (ejection fraction < 57%) [31]. The Angiotensin Receptor Neprilysin Inhibition Versus Individualized RAAS blockade (PARALLAX) trial which randomized 2,572 patients with an HFpEF (LVEF > 40%) showed mixed results, in which only one of two co-primary endpoints showed significant improvement in the sacubitril/valsartan group compared to the comparator (enalapril, valsartan, or placebo), and the
reduction in N-terminal prohormone of brain natriuretic peptide (NT-proBNP) was 16% greater in the sacubitril/valsartan group (adjusted geometric mean ratio, 0.84; 95% confidence interval [CI], 0.80–0.88), while there was no significant difference between groups in the 6-minute walk distance [32]. However, severe adverse events were lower in the sacubitril/valsartan group than in the individualized medical therapy group; first hospitalization due to HF (hazard ratio [HR], 0.49; 95% CI, 0.30–0.81; p = 0.005) and composite of death due to HF or HF hospitalization (HR, 0.64; 95% CI, 0.42–0.97; p = 0.034) were lower, although they were not the primary endpoints of the PARALLAX trial [32]. The U.S. Food and Drug Administration has recently approved the indication of sacubitril/valsartan in patients with HFpEF with LVEF below normal to reduce worsening HF (total HF hospitalizations and urgent HF visits), although further clarification is still needed for HFpEF subgroups who can benefit mostly. Randomized clinical trials assessing the clinical outcomes of sacubitril/valsartan are summarized in Table 2.

Renal effects of angiotensin receptor-neprilysin inhibitor

Inhibition of RAAS reduces urinary albumin excretion and delays the progression of CKD to ESRD. However, treatment with RAAS inhibitors is limited in patients with CKD, as the risk of serum creatinine increase or hyperkalemia is greater in CKD patients than in those without this medical condition [11]. RAAS inhibition by ACEIs or ARBs decreases intra-glomerular pressure by preventing angiotensin II-induced predominant vasoconstriction of the efferent arteriole, contributing to a decrease in albuminuria and eGFR [33].

Three natriuretic peptides are present in humans; atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide [23]. ANP and BNP are synthesized in cardiac myocytes, whereas C-type natriuretic peptide is mainly expressed in endothelial cells [23]. ANP increases renal perfusion through systemic vasodilation, and there is evidence that sacubitril mainly acts by enhancing ANP instead of BNP [34]. Concomitant inhibition of angiotensin II and neprilysin induces selective vasorelaxation of preglomerular afferent arterioles and relative vasoconstriction of the postglomerular efferent arteriole, contributing to increased intracapillary hydraulic pressure and eGFR [35]. Sacubitril/valsartan may also affect renal tubular reabsorption. By increasing ANP, it inhibits sodium reabsorption in the renal proximal tubule, which may account for the benefits of ARNI therapy in patients with HF [35]. Sacubitril/valsartan has been shown to prevent fibrosis, mitochondrial damage, oxidative stress, and apoptosis in kidney and heart tissues of cardiorenal syndrome rat models [36]. The urine albumin creatinine ratio (ACR) modestly increases after ARNI initiation [37–39], increasing concerns regarding deterioration of kidney function after ARNI use. However, in contrast to worse renal outcome related to the increase in albuminuria with enalapril therapy, an increase in the ACR was not related to worse renal

<table>
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<tr>
<th>Table 1. Indications, contraindications, and cautions for the administration of sacubitril/valsartan</th>
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<tr>
<td><strong>Indications</strong></td>
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<td>· HFrEF (EF ≤ 40%)</td>
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<td>· NYHA class II–IV</td>
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<tr>
<td>· Administered in conjunction with other heart failure therapies, in place of an ACEI or other ARB</td>
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<td><strong>Contraindications</strong></td>
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<tr>
<td>· Within 36 hours of an ACEI use</td>
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<td>· A history of angioedema related to previous ACEI or ARB therapy</td>
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<tr>
<td>· Concomitant use of ACEI</td>
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<td>· Concomitant use of aliskiren in patients with diabetes</td>
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<tr>
<td>· Hypersensitivity to any component</td>
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<td>· Severe hepatic impairment (Child-Pugh C)</td>
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<td>· Pregnancy</td>
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<td>· Lactation</td>
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<tr>
<td><strong>Cautions</strong></td>
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<tr>
<td>· Renal impairment</td>
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<tr>
<td>· Moderate (eGFR, 30–59 mL/min/1.73 m²): no starting dose adjustment</td>
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<tr>
<td>· Severe (eGFR, &lt;30 mL/min/1.73 m²): half the usually recommended starting dose</td>
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<tr>
<td>· Hepatic impairment</td>
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<tr>
<td>· Mild (Child-Pugh A): no starting dose adjustment</td>
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<tr>
<td>· Moderate (Child-Pugh B): half the usually recommended starting dose</td>
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<tr>
<td>· Renal artery stenosis</td>
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<td>· Systolic blood pressure &lt; 100 mmHg</td>
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<td>· Volume depletion</td>
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The indications, contraindications, and cautions for sacubitril/valsartan follow the U.S. Food and Drug Administration-approved labeling indications. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; HFrEF, heart failure with reduced ejection fraction; NYHA, New York Heart Association.
outcome with ARNI therapy, suggesting the increase in the ACR is mediated by a mechanism that does not result in low renal filtration [37]. Despite a similar increase in ACR, the Prospective Comparison of ARNI with ARB on Management of Heart Failure with Preserved Ejection Fraction (PARAMOUNT) trial reported a slower deterioration of eGFR in patients with HFrEF after sacubitril/valsartan use.

A plausible explanation for this specific dissociation phenomenon between albuminuria and renal function deterioration is the selective vasorelaxation of preglomerular afferent arterioles with ARNI use, leading to an increase in intracapillary hydraulic pressure, which may contribute to increased albumin ultrafiltration and a modest increase in albuminuria without renal function deterioration [35].

The renal safety of sacubitril/valsartan has been reported consistently in patients with HFrEF [12,37] and HFrEF, which included a significant number of patients with stage 2 and 3 CKD (eGFR, 30–59 mL/min/1.73 m²). PARADIGM-HF post-hoc analysis [37] and PARAGON-HF [38] showed that sacubitril/valsartan led to a slower rate of decrease in eGFR and improved renal outcomes in patients with HFrEF and HFrEF. In a study of patients with acute decompensated HF, sacubitril/valsartan showed similar renal event rates to those of enalapril [29]. In a meta-analysis, Kang et al. [40] reported that compared to other RAAS inhibitors, sacubitril/valsartan significantly increased the eGFR and decreased blood pressure, suggesting that it may have renal and cardiovascular benefits in patients with HF and CKD. The efficacy and safety of sacubitril/valsartan have also been studied in patients with other cardiovascular or renal diseases, although many recent studies have investigated patients with HF. Sacubitril/valsartan demonstrated a low prevalence of renal side effects including hyperkalemia, hypokalemia, and creatinine elevation in patients with hypertension despite its superior blood pressure-lowering effect compared to olmesartan [41,42]. The United Kingdom Heart and Renal Protection-III (UK HARP-III) trial investigating 414 patients with CKD (eGFR, 20–60 mL/min/1.73 m²) without HF showed that sacubitril/valsartan had similar effects on kidney function and albuminuria to irbesartan, but it has the additional effect of lowering blood pressure and cardiac biomarkers [43]. Randomized clinical trials assessing the renal outcomes of sacubitril/valsartan are summarized in Table 3.

Hyperkalemia is a potentially serious complication in

| Table 2: Effect of sacubitril/valsartan on clinical outcomes in patients with heart failure (HF) |
|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Reference | Comparator | Definition of clinical events | Population | Subgroup |
| PARADIGM-HF [12] | Enalapril | CV death, HF hospitalization | Chronic HFrEF | All |
| PARAGON-HF [31] | Valsartan | CV death, HF hospitalization | Chronic HFrEF | LVEF ≥ 40% |
| PARALLAX [32] | Enalapril, valsartan, or placebo | CV death, HF hospitalization | Chronic HFrEF | LVEF > 40% |
| PIONEER-HF [28] | Enalapril | HF death, HF hospitalization | ADHF | LVEF ≤ 40% |

ADHF, acute decompensated heart failure; CI, confidence interval; CV, cardiovascular; eGFR, estimated glomerular filtration rate (in mL/min/1.73 m²); HFrEF, heart failure with reduced ejection fraction; HFrEF, heart failure with preserved ejection fraction; HR, hazard ratio; LVEF, left ventricular ejection fraction.
CKD patients receiving RAAS inhibitors, which can impact clinical outcomes directly and can limit the use of GDMT [44]. The benefits of MRA in patients with HFrEF are well established [45,46]. However, physicians are reluctant to initiate MRA in patients with CKD due to concerns of hyperkalemia, even though it is recommended to initiate MRA in conjunction with ACEIs, ARBs, or an ARNI to reduce morbidity and mortality in patients with New York Heart Association classes II–IV symptoms [18]. In the PARADIGM-HF trial, potassium levels of >6.0 mmol/L occurred in 4% of the patients treated with sacubitril/valsartan and in 6% of the patients with enalapril, and the difference was statistically significant [12]. Moreover, sacubitril/valsartan has been reported to attenuate the risk of hyperkalemia when MRAs are combined with other inhibitors of the RAAS system, suggesting the safer use of MRAs when combined with ARNI [47].

Efficacy and safety of angiotensin receptor-neprilysin inhibitor in advanced chronic kidney disease

After oral administration, sacubitril/valsartan was divided into valsartan and prodrug sacubitril. Valsartan is primarily excreted via the biliary route, and renal impairment does not affect its pharmacokinetics [48]. Sacubitril is rapidly converted to the active neprilysin inhibitor sacubitrilat [49]. Kidney function has an insignificant impact on the disposition of sacubitril, which is excreted through the urine and feces in less than 2% of the total administered dose [49], whereas sacubitrilat is eliminated primarily via the kidney, suggesting that its exposure is increased with renal function decline [50].

The optimal treatment of HF in patients with stage 4 or 5 CKD (eGFR, <30 mL/min/1.73 m²) is unclear as there is little evidence regarding this. The area under the concentration-time curve increased by 2.7-fold in patients with eGFR of <30 mL/min/1.73 m², which raises concerns about the safety and toxicity of sacubitril/valsartan in patients with stage 4 or 5 CKD [50]. Unfortunately, most of the previous randomized clinical trials that guided the management of HFrEF with an ARNI defined CKD as baseline eGFR of <60 mL/min/1.73 m² and excluded patients with severe CKD (eGFR, <30 mL/min/1.73 m²) [12,30,39,51–53].

There have been a few studies published on the use of
sacubitril/valsartan in patients with stage 4 or 5 CKD, or ESRD. In a real-world study, Chang et al. [54] showed that patients with stage 4 or 5 CKD treated with sacubitril/valsartan had 28% fewer cardiovascular deaths or HF hospitalizations than those treated with standard HF treatment, including 102 patients with eGFR of <30 mL/min/1.73 m² among the whole study population of 932 patients with HFrEF [54]. Quiroga et al. [55] investigated 66 patients with stage 1 to 4 CKD and HFrEF (17% of stage 4 CKD) and found that sacubitril/valsartan was safe in patients with CKD, suggesting stability in CKD progression after 6 months.

There is a paucity of data on the evidence of ARNI in patients with ESRD on maintenance dialysis. Heyse et al. [56] presented a case report of a 67-year-old man with HFrEF due to ischemic cardiomyopathy and renal insufficiency undergoing hemodialysis, who tolerated a moderate dose of 49/51 mg twice daily, and finally showed symptomatic improvement with a reduction in HF biomarkers and left ventricular filling pressure. Only one study evaluated the use of sacubitril/valsartan in patients with HFrEF and ESRD, which showed that sacubitril/valsartan reduced cardiac biomarkers and improved LVEF; the most common adverse event was hypotension, which was corrected with down-titration of the drug dosage [57].

Clinical application of angiotensin receptor-neprilysin inhibitor

Patients with CKD tend to receive GDMT inappropriately, probably due to concerns about hypotension, renal function deterioration, and hyperkalemia [58]. Patients with CKD were at a higher risk for noncompliance during the run-in period of the PARADIGM-HF trial, supporting the need for closer monitoring during the up-titration of sacubitril/valsartan or conversion to sacubitril/valsartan in CKD patients [59]. In patients with moderate CKD (eGFR, 30–59 mL/min/1.73 m²), no dose adjustment is required at the start of sacubitril/valsartan. However, the starting dose of sacubitril/valsartan should be reduced in patients with severe CKD (eGFR, <30 mL/min/1.73 m²). The PARADIGM-HF study showed a reduction in diuretic need in the ARNI group, suggesting that treatment with ARNI may reduce the requirement for loop diuretics doses compared to other RAAS inhibitors [60]. Failure to down-titrate the diuretic doses in patients taking sacubitril/valsartan in response to reduced clinical need, which may result in over-diuresis that can contribute to hypotension or renal function deterioration [60]. This possibility highlights the significance of assessment and adjustment of diuretic doses prior to and following the initiation of an ARNI. Renal function and potassium levels are recommended to be evaluated within 1 to 2 weeks after ARNI initiation or dose escalation, and the schedule for subsequent monitoring should be determined by the patient’s kidney function and volume status [14]. The recommended following intervals for renal function monitoring are monthly for the first 3 months and every 3 months thereafter [14].

Gaps in the evidence and future directions

The burden of HF in patients with CKD is considerable. However, many clinical trials in HF patients have excluded patients with severe CKD or ESRD, which results in uncertain efficacy and safety of the treatments in the advanced CKD population. ARNI seems to be a promising treatment option that could reduce the risk of cardiovascular morbidity and mortality in patients with CKD, but randomized clinical trials with ARNI have also excluded patients with advanced CKD. Future trials of HF interventions should focus on pre-specified subgroups with eGFR of <30 mL/min/1.73 m².

Newer treatments for HF, such as sodium-glucose cotransporter 2 (SGLT2) inhibitors, are being tested in large clinical trials in both HF and CKD populations [61–64]. Among patients with CKD, the risk of a composite endpoint of a decline in the eGFR of more than 50%, ESRD, or renal or cardiovascular deaths were reduced by 39% with dapagliflozin than with placebo [61]. However, the benefits of SGLT2 inhibitors for HFrEF management in patients with severe CKD remain unclear. Currently, the use of dapagliflozin and empagliflozin is recommended in patients with eGFR of ≥30 mL/min/1.73 m² and ≥20 mL/min/1.73 m², respectively, since the glucosuric effects of SGLT2 inhibitors may be reduced in those with a lower eGFR. There are little data assessing the combination of an ARNI and an SGLT2 inhibitor, even though the benefit of SGLT2 inhibition was consistent in patients already treated with an ARNI in both Dapagliflozin And Prevention Of Adverse Outcomes In Heart Failure (DAPA-HF) and Empagliflozin.
Outcome Trial in Patients With Chronic Heart Failure with Reduced Ejection Fraction (EMPEROR-Reduced) \[65,66\]. Further evidence to guide the concomitant use of ARNI and SGLT2 inhibitors is needed. Sotagliflozin, a dual sodium-glucose cotransporter 1 (SGLT1) and SGLT2 inhibitor, resulted in significantly lower cardiovascular death and hospitalizations and urgent visits for HF than placebo in patients with diabetes and recent worsening HF \[67\]. However, further studies are needed on the concomitant use of SGLT1/SGLT2 inhibitors and ARNIs.

Conclusions

The heart and kidneys were highly interdependent. CKD is associated with two major risks: fatal or nonfatal cardiovascular diseases and an increased risk of progression to ESRD requiring treatment with renal replacement therapy. ARNI therapy could benefit patients with HF and CKD by reducing cardiovascular morbidity and mortality and possibly retarding the progression of CKD, although more clinical evidence is required in patients with severe CKD and ESRD.

Conflicts of interest

All authors have no conflicts of interest to declare.

Authors’ contributions

Conceptualization, Investigation, Project administration: IJC, SMK
Formal analysis: IJC
Writing–original draft: IJC
Writing–review & editing: SMK
All authors read and approved the final manuscript.

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Acute kidney injury (AKI) is a common condition in critically ill patients, and may contribute to significant medical, social, and economic consequences, including death. Although there have been advances in medical technology, including continuous renal replacement therapy (CRRT), the mortality rate of AKI is high, and there is no fundamental treatment that can reverse disease progression. The decision to implement CRRT is often subjective and based primarily on the clinician’s judgment without consistent and concrete guidelines or protocols regarding when to initiate and discontinue CRRT and how to manage complications. Recently, several randomized controlled trials addressing the initiation of renal replacement therapy in critically ill patients with AKI have been completed, but clinical application of the findings is limited by the heterogeneity of the objectives and research designs. In this review, the advantages and disadvantages of CRRT initiation, clinical guideline recommendations, and the results of currently published clinical trials and meta-analyses are summarized to guide patient care and identify future research priorities.

Keywords: Acute kidney injury, Continuous renal replacement therapy, Guideline, Meta-analysis, Randomized controlled trial

Introduction

Acute kidney injury (AKI) is a common complication in critically ill patients [1,2], and it increases the risk of morbidity and mortality, including progression to chronic kidney disease [3,4], major adverse cardiac events [5–7], infection [8,9], and gastrointestinal bleeding [10]. The number of AKI cases requiring renal replacement therapy (RRT) in intensive care units (ICUs) continues to increase worldwide [11–14]. Despite remarkable medical advances, including the development of continuous RRT (CRRT), the mortality rate of patients with severe AKI requiring RRT is as high as 60% to 70% [15–18]. No treatment can completely resolve the course of AKI, and conservative and supportive treatments remain the mainstay of clinical management. Supportive CRRT is an important tool to improve the prognosis of patients with severe AKI [19–21]; however, there are no clear guidelines for its use or concrete evidence supporting the various clinical protocols. Despite the absence of clear criteria or guidelines for selection of CRRT rather than in-
termittent hemodialysis as the RRT modality, use of CRRT is recommended in cases of hemodynamic instability even when the criteria are appropriate for intermittent hemodialysis [2]. The question of when to initiate CRRT has been discussed extensively in leading journals in the field of intensive care medicine and nephrology in recent years, but it remains controversial.

When should CRRT be initiated? Specifically, initiation of CRRT as an emergency intervention is indicated in cases of life-threatening, medically refractory complications of AKI (Table 1) [22]. This is a situation where the patient will die if CRRT is not started immediately. However, in clinical practice, this situation does not occur often, and there are not many cases where CRRT is implemented based only on this indication. Unless there is an obvious emergent indication, it is important for the clinician to use their judgment and consider the severity of the accompanying clinical situation, dysfunction of other organs (brain, heart, lung, liver, and gastrointestinal tract), and possibility of renal function recovery. In addition, the preferred treatment option selected by the patient, caregiver, and physician must be considered, as well as the cost of treatment and circumstances of each institution, including its internal protocols/guidelines and available infrastructure (resources). The basic premise is that the benefits of implementing CRRT should outweigh the associated risks [23].

This concise review summarizes the results of research addressing implementation of CRRT and compiles current guidelines regarding indications for and initiation of CRRT in critically ill patients with AKI. The review focuses on the findings of the latest randomized controlled trials (RCTs). The aim is to provide relevant insights to supplement the clinician’s judgment.

**Advantages of early continuous renal replacement therapy**

The advantages of early CRRT implementation in the absence of traditional indications include avoidance and/or early control of fluid accumulation and overload, acid-base and electrolyte/metabolic derangement, complications of uremia, and unnecessary or excessive diuretic exposure. Early CRRT also can support beneficial immunomodulation and increase clearance of inflammatory mediators (Table 2) [22]. Thus, early CRRT is necessary to maintain fluid, electrolyte, and acid-base homeostasis, as well as to treat and prevent life-threatening AKI-related complications and deterioration of organs other than the kidneys. Notably, early CRRT becomes necessary when there is hemodynamic instability, fluid overload, catabolism, and/or sepsis with severe AKI to the extent that it is difficult to apply intermittent hemodialysis [2]. These criteria are different from those

| Table 1. Indications and contraindications for CRRT initiation in critically ill patients with AKI |
|---------------------------------|-------------------------------------------------|
| **Absolute indications (in the absence of contraindications for CRRT)** |
| Refractory hyperkalemia | Refractory metabolic acidosis |
| Refractory metabolic acidosis | Refractory pulmonary edema due to volume overload not responding to diuretics |
| Symptomatic uremia or its complications (bleeding, pericarditis, encephalopathy, etc.) |
| Overdose or toxicity of dialyzable drugs (salicylates, ethylene glycol, methanol, etc.) |
| **Relative indications (in the absence of life-threatening complications of AKI)** |
| Hemodynamic instability |
| Advanced dysfunction of organs other than the kidneys (brain, heart, lung, liver, and gastrointestinal tract) |
| Need for administration of a large volume of fluid (massive volume challenge, massive transfusion, medications, nutritional support, etc.) |
| Severity of the underlying disease |
| **Contraindications** |
| Patient or legal representative does not want CRRT |
| **Relative contraindications** |
| Futility prognosis |
| Patient receiving palliative care |

AKI, acute kidney injury; CRRT, continuous renal replacement therapy.
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applied when initiating intermittent dialysis in end-stage renal disease, and the indications can be diverse and broad depending on the clinician’s judgment and criteria.

Disadvantages of early continuous renal replacement therapy

One significant limitation of early CRRT is the high risk of a variety of complications (Table 2) [22]. Complications such as bleeding, infection, and pneumothorax can be caused by placement of the central venous catheter, and anticoagulant treatment is required to maintain the circuit and filter. In addition, CRRT typically is initiated for patients who are hemodynamically unstable and unable to withstand intermittent dialysis. However, complications such as blood pressure decrease, tachycardia, and other arrhythmia can occur during CRRT and worsen the clinical situation or delay patient recovery. In addition, micronutrients, trace elements, and/or therapeutic drugs can be cleared more rapidly through the CRRT circuit, resulting in low or inconsistent concentrations in blood and tissues and potentially leading to adverse events and/or loss of therapeutic effect. The use of antibiotics or anticonvulsants can be problematic if blood concentrations cannot be monitored. In severe AKI requiring CRRT due to sepsis, the most important treatment objective is appropriate use of antibiotics. If the blood concentration of antibiotics is not maintained, it can affect patient prognosis.

Another potential issue with the use of CRRT is that therapy (and the accompanying risk of complications) can be initiated in patients whose renal function would have improved more rapidly had they received conservative management alone.

Finally, the use of CRRT creates hardship for the patient and requires significant use of hospital personnel and equipment. The patient must enter the ICU for CRRT and cannot move throughout the application of CRRT, one CRRT machine is required per patient, and real-time monitoring is needed. Additionally, it is necessary to have ICU staff available to change the CRRT fluid or respond to alarms, and resources such as filters, CRRT fluids, and catheters are consumed continuously. The biggest drawback is that the cost of providing CRRT significantly increases the cost of patient care. As such, it is necessary to make decisions that consider the potential benefits (whether survival or secondary outcomes) of early initiation of CRRT and the resulting economic or medical burden.

Timing of continuous renal replacement therapy

In cases where the advantages and disadvantages of CRRT are understood, the question is the need for CRRT without absolute indication. If the response is yes, when should CRRT be initiated?

As noted above, the potential benefits of early initiation of CRRT must be balanced with the risks and burdens associated with CRRT [24]. In clinical practice, the final outcome has been favorable in only a few clinical scenarios (Fig. 1).
Given RRT as an invasive treatment with significant physical, economic, medical, and social impacts, early initiation of RRT should be considered only after assessing the likelihood of patient survival and potential to achieve secondary outcomes, in addition to the survival of multiple organs, including the kidney. Considering the high cost and burden of treatment, the overall effect of RRT is favorable only in cases where RRT must be started early to ensure that the patient survives. In such cases, both survival and secondary outcomes are improved, resulting in an overall favorable effect. However, it is difficult to predict the optimal time of RRT initiation to achieve the greatest beneficial effect. Notably, early RRT should not be initiated for cases that are predicted to have unfavorable outcomes. For example, early RRT initiation can ‘do harm’ in situations where the patient is expected to die regardless of RRT or to survive without RRT. Therefore, predicting the final outcome of RRT initiation is a complex process that requires the consideration of different factors. It is necessary to understand and identify situations in which the patient will die if RRT is not started immediately and to make a quick decision.

Several observational studies and meta-analyses have reported that the early application of CRRT reduces mortality in critically ill patients with AKI [17,26–35]. However, the primary studies were observational, and the meta-analyses were based on observational studies; thus, their quality of evidence is low, and they have several limitations. First, only patients who started RRT were enrolled, and the prognosis was compared by classifying RRT as “early” or “late.” Comparative analyses including patients who did not undergo RRT (i.e., patients whose renal function recovered or those who died without RRT) were not completed [36]. Second, the criteria or definitions for classifying “early” and “late” were arbitrary and differed by study. Third, the patients and their underlying diseases were heterogeneous. Fourth, various residual confounders could not be adjusted for, and some biases could not be controlled; hence, care should be taken when interpreting the results. Furthermore, more recent research results have been published indicating that early application of CRRT did not affect patient prognosis or recovery of renal function [37–41].

Figure 1. In the absence of absolute indications, predicting the prognosis of critically ill AKI patients undergoing early initiation of RRT is complex. Reproduced from the article of Prowle and Davenport (Kidney Int 2015;88:670-673) [25] with the permission from Elsevier.

AKI, acute kidney injury; RRT, renal replacement therapy.
Current recommendations for initiation of continuous renal replacement therapy

The current clinical practice guidelines are summarized in Table 3. In 2012, the Kidney Disease: Improving Global Outcomes (KDIGO) study group [42] recommended emergency RRT in cases of potentially fatal changes in fluid, electrolytes, and/or acid-base balance. The trend of broader clinical situations and laboratory test results in addition to the values of serum creatinine or blood urea nitrogen should be examined, and it is recommended to assess and judge whether there are conditions to be modified through RRT. These recommendations were based on expert opinion, not evidence-based grading. In 2015, the French Intensive Care Society presented a similar expert opinion in which the results were deemed insufficient to define the appropriate timing for initiation of RRT beyond life-threatening indications [43]. Although the definitions of “early” and “late” initiation of CRRT were described, the quality of evidence was low. In the following year, the Japanese Clinical Practice Guideline was released, which also highlighted the lack of evidence to support decision making [44]. In 2019, the National Institute for Health and Care Excellence guidelines for AKI were revised, but the content was not significantly different from that released in 2013 [45]. To date, the published guidelines have emphasized the need for high-quality evidence from high-quality clinical trials to improve decision making.

Recent studies addressing when to start continuous renal replacement therapy

Randomized controlled trials

Well-designed RCTs published within the last 5 years have addressed the effect of the timing of RRT initiation on patient outcomes (Table 4). The Early Versus Late Initiation of RRT in Critically Ill Patients with Acute Kidney Injury (ELAIN) trial [46] was conducted in 231 patients. The early group had no conventional indications for CRRT, but the therapy was started within 8 hours of a diagnosis of KDIGO stage 2 AKI. The delayed group started CRRT within 12 hours of a diagnosis of KDIGO stage 3 AKI or when a conventional indication was present. Significant improvements in outcomes among the early CRRT group compared with the delayed group were reported with respect to 90-day mortality (39.3%
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<tr>
<td><strong>Country</strong></td>
<td>Germany</td>
<td>France</td>
<td>France</td>
<td>Multinational (15)</td>
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<td><strong>Centers</strong></td>
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<td>Multicenter (31)</td>
<td>Multicenter (29)</td>
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<td><strong>Patients randomized</strong></td>
<td>231</td>
<td>620</td>
<td>488</td>
<td>3,019</td>
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<td><strong>Patient population</strong></td>
<td>Mixed medical &amp; surgical ICU (94.8% surgical)</td>
<td>Mixed medical &amp; surgical ICU (79.7% medical)</td>
<td>Mixed medical &amp; surgical ICU</td>
<td>Mixed medical &amp; surgical ICU</td>
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<tr>
<td><strong>Inclusion criteria</strong></td>
<td>KDIGO stage 2 AKI and plasma NGAL &gt; 150 ng/mL and at least one of the following: severe sepsis; use of vasopressors; refractory fluid overload; and/or nonrenal organ dysfunction</td>
<td>KDIGO stage 3 AKI and receiving mechanical ventilation and/or vasoactive support</td>
<td>Adults with severe AKI and septic shock</td>
<td>Critically ill patients and kidney dysfunction and those with severe AKI (KDIGO stage 2 or 3)</td>
</tr>
<tr>
<td><strong>Intervention</strong></td>
<td>Early RRT KDIGO stage 2 (within 8 hr)</td>
<td>KDIGO stage 3 (within 6 hr)</td>
<td>Failure stage of RIFLE (within 12 hr)</td>
<td>Fulfills eligibility criteria (within 12 hr)</td>
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<td></td>
<td>Delayed RRT KDIGO stage 3 (within 12 hr) or conventional indications for RRT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Conventional indications for RRT&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Conventional indications for RRT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Until the occurrence of one or more of the applicable criteria&lt;sup&gt;a&lt;/sup&gt;</td>
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<td><strong>Median time from randomization to RRT (hr)</strong></td>
<td>6/25.5</td>
<td>2/57.0</td>
<td>7.6/51.5</td>
<td>6.1/31.1</td>
</tr>
<tr>
<td><strong>Percentage receiving RRT</strong></td>
<td>100.0/90.8</td>
<td>98.0/51.0</td>
<td>97.0/62.0</td>
<td>96.8/61.8</td>
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<td><strong>RRT modality</strong></td>
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<td>iHD, CRRT</td>
<td>iHD, CRRT</td>
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<td><strong>Primary outcome</strong></td>
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<td>60-day mortality</td>
<td>90-day mortality</td>
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<tr>
<td></td>
<td>Early RRT (%)</td>
<td>39.3</td>
<td>48.5</td>
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<td></td>
<td>Delayed RRT (%)</td>
<td>54.7</td>
<td>49.7</td>
<td>54</td>
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<tr>
<td><strong>RRT dependence among survivors at day 90 (%)</strong></td>
<td>13.4/15.1</td>
<td>2/5 (at day 60)</td>
<td>2/3</td>
<td>10.4/6.0</td>
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<td><strong>Hospital stay (day)</strong></td>
<td>51/82&lt;sup&gt;g&lt;/sup&gt; (p &lt; 0.001) (p = 0.03)</td>
<td>29/32&lt;sup&gt;g&lt;/sup&gt; (p = 0.03)</td>
<td>22/21&lt;sup&gt;g&lt;/sup&gt; (p = 0.03)</td>
<td>28/29&lt;sup&gt;g&lt;/sup&gt; (p &lt; 0.001)</td>
</tr>
<tr>
<td><strong>Adverse event (%)</strong></td>
<td>Not significant</td>
<td>Hypophosphatemia (22/15)</td>
<td>Hyperkalemia (0/4) (p = 0.03)</td>
<td>23/17 (p &lt; 0.001)</td>
</tr>
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AKI, acute kidney injury; AKIKI, Artificial Kidney Initiation in Kidney Injury trial; CRRT, continuous renal replacement therapy; ELAIN, Early Versus Late Initiation of RRT in Critically Ill Patients with Acute Kidney Injury trial; ICU, intensive care unit; IDEAL-ICU, Initiation of Dialysis Early Versus Delayed in the Intensive Care Unit trial; iHD, intermittent hemodialysis; KDIGO, Kidney Disease: Improving Global Outcomes; NGAL, neutrophil gelatinase-associated lipocalin; RRT, renal replacement therapy; STARRT-AKI, Standard versus Accelerated Initiation of Renal-Replacement Therapy in Acute Kidney Injury trial.

<sup>a</sup>Serum urea > 36 mmol/L; K > 6.0 mmol/L; Mg > 4 mmol/L; urine output < 200 mL/12 hours or anuria; organ edema resistant to diuretics. <sup>b</sup>Severe hyperkalemia (>6.0 mmol/L); severe pulmonary edema refractory to diuretics; severe acidosis (pH < 7.15); urea > 40 mmol/L; oligo-anuria > 72 hours. <sup>c</sup>Severe hyperkalemia (>6.5 mmol/L); severe pulmonary edema refractory to diuretics; severe acidosis (pH < 7.15); no renal function recovery after 48 hours. <sup>d</sup>At least two of the following: 2-fold increase in serum creatinine from baseline; urine output < 6 mL/kg in the preceding 12 hours; whole-blood NGAL > 400 ng/mL. <sup>e</sup>Serum potassium > 6.0 mmol/L; pH < 7.20 or serum bicarbonate < 12 mmol/L; severe respiratory failure based on the PaO<sub>2</sub>/FiO<sub>2</sub> ratio < 200 and clinical perception of volume overload; persistent AKI for at least 72 hours after randomization. <sup>f</sup>Hospital stay was censored at day 90 or at patients’ deaths where applicable. <sup>g</sup>Hospital stay of survivors.
vs. 54.7%; hazard ratio [HR], 0.66; 95% confidence interval [CI], 0.45–0.97; p = 0.03), hospital stay (51 days vs. 82 days; HR, 0.34; 95% CI, 0.22–0.52; p < 0.001), and renal function recovery (53.6% vs. 38.7%; odds ratio, 0.55; 95% CI, 0.32–0.93; p = 0.02). However, excluding the patients who died within 3 months, the proportion of patients who recovered renal function at 90 days did not differ between the two groups. In this small RCT performed in a single-center, most patients underwent surgery, 91% received RRT even though they were assigned to the delayed group, and the difference in time to initiate RRT between the groups was only 20 hours. Although the dialysis modality was unified as CRRT, it was difficult to view the study as well-controlled and designed. In addition, patients assigned to the early group might have recovered spontaneously without CRRT, leaving the possibility of skewed study results.

The Artificial Kidney Initiation in Kidney Injury (AKIKI) trial [47] of 620 patients with severe AKI admitted to 31 ICUs in France compared an early group that underwent CRRT within 6 hours of reaching KDIGO stage 3 AKI without conventional indication and a delayed group that underwent CRRT when a conventional indication was present. There were no differences in 60-day mortality between the early and delayed groups (48.5% and 49.7%, respectively; p = 0.79) or in the secondary outcomes, such as ventilator- and vasoactive-free days, ICU and hospital stays for 28 days, and dialysis dependence on day 60. In the delayed group, 51% of patients received RRT compared with 98% in the early group (p < 0.001). The number of RRT-free days was greater, and diuresis, an indicator of renal function improvement, appeared earlier in the delayed group than in the early group (p < 0.001). However, with respect to the RRT modality used in the study, intermittent hemodialysis and CRRT were mixed, and there are limitations in generalization of the study findings.

Two years later, the Initiation of Dialysis Early Versus Delayed in the Intensive Care Unit (IDEAL-ICU) trial [48] reported the results of a study of 488 patients admitted into 29 ICUs in France for septic shock and associated severe AKI. The early group underwent RRT within 12 hours after meeting the Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease (RIFLE) criterion F without a conventional indication, and the delayed group underwent RRT if their condition did not improve within 48 hours after meeting the RIFLE criterion F or showed a conventional indication. The mortality rate at 90 days was not reduced in the early group compared to the delayed group (58% and 54%, respectively; p = 0.38). Ventilator- and vasoactive-free days and duration of stay in the ICU and hospital were also not different between the two groups, but the proportion of RRT recipients in the delayed group was smaller than that in the early group (97% vs. 62%, respectively; p < 0.001). Given that the RIFLE criteria were not applied in earlier studies and the application of mixed intermittent dialysis and CRRT, direct comparison of the available study results is of limited utility.

The most recently completed Standard versus Accelerated Initiation of Renal-Replacement Therapy in Acute Kidney Injury (STARRT-AKI) trial [49] was a multinational, multicenter, open-label RCT that targeted 3,019 patients with severe AKI at 168 hospitals in 15 countries over a 4-year period. There was no significant difference in mortality at 90 days between the group who started RRT within 12 hours after reaching KDIGO stage 2 or 3 AKI and the group that received RRT within 72 hours after randomization due to continued AKI or the presence of a conventional indication (early group vs. delayed group: 43.9% vs. 43.7%; p = 0.92). However, among survivors at 90 days, the early group showed about 1.7-fold greater RRT dependence than the delayed group (risk ratio [RR], 1.7; 95% CI, 1.2–2.4), and the percentage of patients who experienced adverse events during the RRT maintenance period was significantly greater in the early group than in the delayed group (23% vs. 17%, p < 0.001). Among the RCTs on related topics, the largest and relatively pragmatic design has been advocated, but the limitation is that individual clinicians’ bias was involved in determining the eligibility of the study subjects. Furthermore, the median time to initiate RRT was 6.1 hours (interquartile range [IQR], 3.9–8.8 hours) from full eligibility judgment in the early group, whereas that in the delayed group, for which there was no obligation to select RRT and the judgment of individual clinicians was followed, was 31.1 hours (IQR, 19.0–71.8 hours).

It is difficult to make general recommendations to guide clinical practice due to the heterogeneity of the designs of the published RCTs. The four aforementioned RCTs (1) enrolled diverse study subjects (a mix of medical and surgical ICU patients); (2) applied different criteria for “early” and “delayed” CRRT initiation or were not consistent in their application (for example, the key entry criterion for both the
AKIKI and IDEAL-ICU trial—stage 3 AKI—was the criterion for late initiation of RRT in the ELAIN trial; (3) the RRT modality was mixed; (4) patients with emergent indications, such as refractory hyperkalemia, metabolic acidosis, and pulmonary edema, were excluded from the AKIKI and IDEAL-ICU trials, whereas the majority of the patients in the ELAIN trial had fluid overload or pulmonary edema prior to enrollment, and the results cannot be said to reflect typical clinical practice; (5) most importantly, the criteria for initiating RRT used in the studies were contingent and specified for the study, and there was inconsistency with the situation of actual patients requiring RRT; and (6) the protocols for initiation or discontinuation of RRT differed by trial. Therefore, it is difficult to apply the research results to typical patient care and clinical situations.

In consideration of these points, RCTs should continue to be conducted and well-controlled with practical and applicable research designs. Furthermore, if a proven clinical or laboratory marker or tool that reliably can distinguish patients who are likely to require RRT from patients who can recover without RRT or predictive models using scoring or artificial intelligence are available, effective, and efficient treatment using limited resources will be possible, and unnecessary exposure to RRT will be minimized. Further research is expected in the future.

The results of the Artificial Kidney Initiation in Kidney Injury 2 (AKIKI2) trial that is currently in progress (ClinicalTrials.gov identifiers: AKIKI-2 [NCT03396757]) are expected to provide significant contributions to the body of knowledge regarding CRRT in AKI [50]. The AKIKI2 trial is a prospective, multicenter, open-label, two-arm randomized trial that comprises observational and randomization stages. Patients with KDIGO stage 3 AKI who need a vasopressor will be included in the observational stage (expected to be about 810 patients), and patients with serum urea concentration of 40 mmol/L or greater or oliguria/anuria for more than 72 hours will be randomized to receive RRT within 12 hours (the standard group). In contrast, in the delayed group, when the serum urea concentration reaches 50 mmol/L or more or a severe condition (e.g., severe hyperkalemia, severe metabolic or mixed acidosis, or acute pulmonary edema due to fluid overload resulting in severe hypoxemia) occurs, RRT will be initiated, and randomization of about 270 patients is expected. The primary outcome will be defined as the number of RRT-free days by day 28.

Meta-analyses

The results of the available meta-analysis comparing the clinical effects of early or delayed initiation of RRT in critically ill patients with AKI are as follows.

In a meta-analysis of 1,636 patients from nine RCTs published from 1985 to 2016, early RRT initiation did not reduce mortality (RR, 0.98; 95% CI, 0.78–1.23), and secondary outcomes (ICU or hospital length of stay, renal function recovery, and RRT dependence) were not affected [51,52]. Even when five studies specific to CRRT were analyzed separately, early CRRT initiation did not significantly affect the outcome. In the analysis of other outcomes, except for overall mortality, the recommendation grade was weak due to poor quality and importance.

Similarly, another meta-analysis of nine RCTs comprising a total of 1,627 patients reported that earlier initiation of RRT had no benefit regarding mortality [53]. In subgroup analyses, in-hospital mortality decreased following early RRT initiation in surgical patients (RR, 0.78; 95% CI, 0.64–0.95) and CRRT patients (RR, 0.80; 95% CI, 0.66–0.95). Nevertheless, early intervention might not significantly improve the outcome but can increase the risk of side effects.

A study that analyzed a total of 1,479 patients from five RCTs, one prospective cohort study, and nine retrospective cohort studies published from 1971 to 2016, found that early RRT reduced 28-day mortality, ICU, and hospital lengths of stay, and RRT duration [54]. This was especially true if RRT was initiated within 12 hours or 24 hours in patients who developed AKI after cardiac surgery. Even after analyzing eight studies targeting only CRRT, the prognostic improvement effect of early RRT was confirmed (RR, 0.36; 95% CI, 0.19–0.67). However, there are limitations to generalization because many retrospective studies were included, the number of patients was small, and different criteria were applied to each study.

According to the results of a review study comprising five RCTs and 1,084 participants published in the Cochrane Database of Systematic Reviews [55], early RRT seems to reduce the risk of death and improve renal function recovery. However, considering the 95% CI, early RRT can worsen the outcome, and an increase in adverse events due to early RRT was noted. In addition, the RR for death was 0.65 (95% CI, 0.31–1.36) in the three RCTs for CRRT only, which was not statistically significant. The same was true for the recov-
ery of renal function (RR, 1.36; 95% CI, 0.79–2.34). Overall, most studies presented low-quality evidence, underscoring the need for adequately powered RCTs.

In a meta-analysis of nine studies published between April 2008 and December 2019, comprising 1,879 subjects [56], the timing of RRT initiation in the absence of an urgent indication did not affect the survival of critically ill patients with severe AKI (28-day mortality; HR, 1.01; 95% CI, 0.87–1.17). The results were consistent regardless of sex, age, Sequential Organ Failure Assessment score, sepsis, or chronic kidney disease. Other outcomes and adverse events, such as death at 60 and 90 days, in-hospital death, duration of hospitalization, RRT-free days, RRT dependence upon discharge, and ventilator- and vasopressor-free days, were not affected by the timing of CRRT initiation.

A meta-analysis of 10 RCTs published between 2002 and 2020, including the most recently reported STARRT-AKI trial and comprising 4,753 patients, showed no correlation between the timing of RRT initiation and all-cause mortality or freedom from dialysis [57]. In the subgroup analyses, if the patient was in the surgical ICU or underwent CRRT, early initiation showed a benefit for the aforementioned outcomes. However, the studies used in this meta-analysis were heterogeneous, and there might have been biased; thus, the results should be interpreted with caution.

**Recommendations for future research**

To overcome the limitations of the previous studies and enable generalization of research results to clinical practice, we propose the following recommendations. First, the study population must be homogeneous; for instance, patients can be enrolled exclusively from medical or surgical ICUs or be limited to those receiving CRRT. The studies discussed in this paper included a mixture of patients receiving intermittent hemodialysis or CRRT, with few targeting CRRT only, hindering separate assessment of the findings for the two types of patients. Therefore, future RCTs should enroll more unified and homogenized study groups. Second, it is necessary to apply the inclusion and intervention (early or delayed) criteria realistically and conduct the research in a typical clinical situation where applicable. In other words, all patients with emergent indications should be included in the study. Third, multicenter and multinational studies should be conducted using well-designed protocols with minimal dependence upon subjective criteria and the judgments of clinicians and physicians when assigning treatments. Fourth, an RCT targeting older adults requiring CRRT is needed, considering the rapid aging of populations in many countries.

**Conclusions**

CRRT is important in the treatment and management of AKI in critically ill patients. Except for implementation in patients with life-threatening emergent indications, the proper timing of CRRT initiation remains controversial. In the current situation where it is difficult to generalize the conflicting results of recent research to patients seen in typical clinical practice and in the absence of reliable tools to

![Figure 2. Clinical decision tree for initiation of RRT in critically ill patients with AKI.](https://www.krcp-kdn.org) Reproduced from the article of Ostermann et al. ([Contrib Nephrol](https://www.krcp-kdn.org) 2016;187:106-120) [22] with the permission from S. Karger AG.

AKI, acute kidney injury; RRT, renal replacement therapy.
predict the prognosis associated with RRT, a personalized patient-oriented approach should be used. In other words, it is important to determine the optimal timing of CRRT initiation by assessing the clinical situation and disease progression and not relying solely upon simple indicators such as serum creatinine concentration or urine volume.

The 17th Acute Disease Quality Initiative Consensus Group stated that acute RRT must be considered when metabolic and fluid demands exceed total kidney capacity and presented a conceptual model of demand-capacity balance [58]. The demand for kidney function is determined by nonrenal comorbidities, the severity of the acute disease, and the solute and fluid burden; the combination of these factors in given patients creates distinct scenarios requiring variable management. Demand-capacity imbalance is dynamic, varies from patient to patient, and should be evaluated regularly. In addition, selection of the preferred RRT modality is determined by the technological capability/availability at the healthcare facility, the inherent risk of the procedure, and current needs of the patient. In the case of CRRT, improved hemodynamic and intracranial pressure stability are expected benefits, but there are risks of infection, and patient immobility is required. Notably, if the demand-capacity balance or treatment priorities change and an alternative technique are judged to be a better fit, transition to a different modality should be considered. All these processes underscore the need for a “precision & personalized” approach, and this concept should be applied in real-world clinical practice in the future.

Recent research suggests that a careful waiting strategy should be properly applied according to patient (Fig. 2) [22], but CRRT should not be delayed if there is a life-threatening emergent condition. However, a wait-and-see approach with supportive care can be appropriate as there is no clear evidence that early CRRT initiation improves outcome, and CRRT is not a harmless treatment [59]. Therefore, future research related to the timing of CRRT initiation should focus on the development of algorithms to help clinicians make appropriate decisions that go beyond early or late CRRT initiation. It is expected that many ongoing studies will provide real-world data to support such algorithms and improve outcomes of critically ill patients with AKI in clinical practice.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Authors’ contributions**

Conceptualization: JNA, YRS
Data curation, Formal analysis: All authors
Writing–original draft: JNA
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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Executive Summary of the Korean Society of Nephrology 2021 Clinical Practice Guideline for Optimal Hemodialysis Treatment

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The Korean Society of Nephrology (KSN) has published a clinical practice guideline (CPG) document for maintenance hemodialysis (HD). The document, 2021 Clinical Practice Guideline on Optimal HD Treatment, is based on an extensive evidence-oriented review of the benefits of preparation, initiation, and maintenance therapy for HD, with the participation of representative experts from the KSN under the methodologists' support for guideline development. It was intended to help clinicians participating in HD treatment make safer and more effective clinical decisions by providing user-friendly guidelines. We hope that this CPG will be meaningful as a recommendation in practice, but not on a regulatory rule basis, as different approaches and treatments may be used by health care providers depending on the individual patient's condition. This CPG consists of eight sections and 15 key questions. Each begins with statements that are graded by the strength of recommendations and quality of the evidence. Each statement is followed by a summary of the evidence supporting the recommendations. There is also a link to full-text documents and lists of the most important reports so that the readers can read further (most of this is available online).

Keywords: Evidence-based practice, GRADE approach, Hemodialysis units, Hospital, Practice guideline

Introduction

Over the past 60 years, due to the advancement of hemodialysis (HD) technology and the introduction of medical insurance, dialysis treatment has become widespread, enabling many patients with end-stage kidney disease (ESKD) to maintain their lives. The treatment of dialysis patients has also evolved considerably. Depending on the circumstances, various clinical practice guidelines (CPGs) for initiating and maintaining HD have been published internationally. However, the clinical field, the technology of HD, and the target patients covered in previously published CPGs are subject to change. In addition, because the clinical evidence for HD has been reinforced in follow-up studies after
the publication of previous CPGs, there is now a demand for reevaluation of these CPGs in accordance with current conditions. In response, the Korean Society of Nephrology (KSN) established the Work Group and tasked it with planning, developing, reviewing, and disseminating appropriate HD treatment guidelines in accordance with international standards. The level of evidence was evaluated using the Grading of Recommendations Assessment Development and Evaluation (GRADE) methodology. The importance of each result is evaluated first, and then the level of evidence for each result is determined as high, moderate, low, or very low. The meaning of each evidence level is shown in Table 1. The recommendation grade was divided into four levels: strong, conditional, against, and inconclusive (Table 2). Key questions that cannot be adapted and developed directly due to poor existing research are expressed as “expert consensus.”

When to begin dialysis is influenced by a variety of factors, including signs and symptoms of uremia, biochemical tests, and the patient’s GFR. As the precise timing will likely affect the cost of dialysis services and clinical outcomes, certain factors related to mortality, degree of improvement in symptoms and functions, quality of life, and other medical expenses should be considered.

No published studies have investigated the timing of the initiation of dialysis based on patient symptoms. One randomized study (IDEAL; Initiating Dialysis Early and Late) compared the clinical outcomes of relatively early- and late-starting groups based on GFRs [1], and three subanalyses of this randomized study have been reported [2–4]. Only a comparison between the early-start group and the late-start group based on GFR was available; early (10–14 mL/min/1.73 m²) and late (5–7 mL/min/1.73 m²)

### Chapter 1. Start of hemodialysis

**Recommendation 1.1**

We recommend that whether and when to start HD be decided through a careful discussion between the patient and the healthcare provider about the benefits/harms of the treatment and the patient’s values and preferences about HD initiation because an early start of HD, as determined by the glomerular filtration rate (GFR), in patients with chronic kidney disease (CKD) stage G5 does not produce any differences in clinical outcomes from a late start.

_Strong recommendation, moderate quality of evidence_

<table>
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<th>Table 1. GRADE quality levels of evidence and meaning</th>
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<td>Low</td>
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GRADE, Grading of Recommendations Assessment Development and Evaluation.

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GRADE, Grading of Recommendations Assessment Development and Evaluation.
Each statement is shown as a combination of the strength of the recommendation and level of evidence.
In the case of a consensus statement based on expert opinion, the recommendation grade and level of evidence are not indicated.
Time definitions were defined. The analysis found no significant difference between the two groups in major clinical outcomes, such as mortality, quality of life, hospitalization, and infection [1].

In addition, based on various retrospective studies, including domestic research, we synthesized evidence in a meta-analysis [5-14]. After classifying retrospective studies according to design and similarity of selected groups, no benefit or harm was apparent for the relatively early-start patients compared to the late-start group. However, because the heterogeneity between the retrospective studies used in the synthesis of evidence was high, and there was no consistency in the quality evaluation, all were evaluated at a moderate level of evidence.

**Recommendation 1.2**

1. We recommend the preparation of an arteriovenous access prior to HD initiation to avoid central venous catheter insertion. (Strong recommendation, low quality of evidence)

2. We consider it reasonable that the timing of an arteriovenous access preparation be individualized according to patient comorbidities and GFR decline. (Expert consensus)

The purpose of preparing arteriovenous access for HD using an arteriovenous fistula (AVF) or arteriovenous graft (AVG) is to avoid unnecessary central venous catheter insertion at the timing of dialysis initiation. Central venous catheter insertion may be associated with catheter-related infection, central vein stenosis, pneumothorax, and additional medical expenses, which are typically unnecessary.

Most studies of the preparation of arteriovenous access investigated clinical outcomes by types of arteriovenous access and timing of preparation. No randomized controlled trials have been reported, and most research was observational in nature and based on cohort data. Low mortality and low hospitalization rates were reported in native AVF groups [15-18], but selection bias cannot be excluded due to the nature of the observational studies.

Although survival benefits of patients with a native AVF had been reported in some studies, it has also been associated with maturation failure [17]. Preparation of AVF and AVG has been described as a trade-off for elderly patients. The use of AVGs was superior with respect to maturation, leading to reduced duration of central venous catheter placement and less intervention for delayed vascular access maturation. However, AVG was accompanied by more vascular access abandonment and secondary operation after maturation. Compared to AVG, AVF involved longer vascular access survival and less secondary intervention after maturation.

Although there is no direct evidence regarding the optimal timing of referral of arteriovenous access preparation, the recent Kidney Disease Outcomes Quality Initiative (KDOQI) stated that referral for dialysis access assessment and subsequent creation should occur when the GFR is 15-20 mL/min/1.73 m², based on expert opinion. They also stated that earlier referral should occur in patients with unstable and/or rapid rates of GFR decline (>10 mL/min/year) [19], based on a well-designed Monte Carlo simulation model [20].

**Chapter 2. Frequency and dose of hemodialysis**

*Recommendation 2.1*

We recommend maintaining a dialysis at a frequency of at least three sessions per week and for 4 hours or more for patients with minimal residual renal function. (Strong recommendation, moderate quality of evidence)

Since Scribner introduced intermittent maintenance HD in patients with ESKD in 1960, a typical HD schedule has been three sessions for 10-12 hours per week. In Korea, the frequency of dialysis is three sessions a week, for 12 hours. Various frequencies of HD treatments, such as daily home HD sessions, are not mentioned in this guideline due to medical insurance issues in Korea. It is difficult to define the appropriate number and duration of dialysis sessions separately. We therefore examined and summarized related studies about relevant sessions and the time of dialysis.

In two randomized controlled studies of HD patients who received dialysis three times a week, no significant differences in mortality (odds ratio [OR], 1.02; 95% confidence interval [CI], 0.88-1.18; \( p = 0.79 \)) and hospitalization rates (OR, 1.38; 95% CI, 0.67-2.87; \( p = 0.38 \)) were reported between the two groups (patients receiving more than 4 hours and less than 4 hours of HD per session) [21,22]. However, in the four cohort studies in which a meta-analysis was possible, the mortality rate (OR, 1.34; 95% CI, 1.15-
1.55; p < 0.01) was higher in the group receiving less than 4 hours of dialysis compared to the group receiving more than 4 hours [23–26]. Based on these findings, the dialysis frequency in patients with minimal residual renal function should be at least three times a week, with sessions lasting at least 4 hours [27]. Charra et al. [28] reported improved blood pressure control through long HD (3 × 8 hours/week). Marshall et al. [29] found that the mortality rate was lower among patients receiving more than 4.5 hours of HD per session.

In addition, studies show that it is possible to try initiating twice-weekly HD in patients who retain significant residual kidney function. A meta-analysis of three studies found that the mortality rate tended to increase in HD patients without residual renal function, suggesting it should only be attempted while monitoring carefully for changes in residual renal function [30–32].

**Recommendation 2.2**

We recommend a target dose of 1.4 single-pool Kt/V (spKt/V) for patients receiving thrice-weekly HD.

(Strong recommendation, moderate quality of evidence)

The adequacy of HD has been traditionally measured by evaluating the clearance of small molecules such as urea. Since the advent of the Kt/V measure, which consists of dialyzer clearance (K), dialysis time (t), and volume of distribution (V), many observational studies have consistently reported that dialysis with an increased Kt/V was significantly associated with survival benefits in patients on HD [25,33–42].

The representative study for this issue is the HEMO (Hemodialysis) Study published in 2002 [43]. In this randomized clinical trial involving 1,846 patients undergoing thrice-weekly HD, the high-dose group maintaining a mean spKt/V of 1.71 enjoyed no significant benefit of morbidity and mortality compared to the standard group maintaining a mean spKt/V of 1.32.

Because the aforementioned observational studies reported that increased mortality was associated with an inadequate dialysis dose, maintaining appropriate dialysis time under a qualified dialysis system is recommended to obtain a spKt/V of 1.4. However, as the HEMO Study showed no improvement in morbidity and mortality with high-dose dialysis, increasing the dialysis dose beyond the recommended level is unnecessary.

The urea reduction ratio (URR) and equilibrated Kt/V (eKt/V) offer alternatives for assessing dialysis adequacy. The URR is simple and easy to calculate, but does not assess dialysis adequacy accurately because it does not take into account the volume of urea distribution. The eKt/V value is lower than that of spKt/V, because it is calculated by considering the redistribution of urea after dialysis. In the HEMO Study, the mean eKt/V in a standard group maintaining a mean spKt/V of 1.32 was 1.16. In general, the corresponding eKt/V is 1.2 when the targeting dialysis dose of spKt/V is 1.4.

**Chapter 3. Dialysis membrane and modality for hemodialysis**

**Recommendation 3.1**

We recommend the use of high-flux dialysis membranes in adult HD patients. However, the cost and availability of high-flux membrane need to be considered.

(Strong recommendation, high quality of evidence)

To date, three large-scale randomized clinical trials, the HEMO [43], MPO (Membrane Permeability Outcome) [44], and EGE [45] trials, have compared high- vs. low-flux HD membranes. These trials have not revealed a statistically significant benefit in reducing all-cause death.

However, the HEMO Study [43] reported a significant reduction of cardiovascular (CV) death as a secondary endpoint (0.072 vs. 0.059 patient-year), and a significant benefit in the composite outcome defined as CV death and hospitalization due to CV disease. Furthermore, a subgroup analysis showed a significant reduction of mortality risk by 37% in subgroup of patients treated with dialysis for more than 3.7 years prior to randomization. In the MPO Study [44], a statistically significant reduction in all-cause mortality was evident in the high-flux group compared to the low-flux group among participants with serum albumin equal to or lower than 4 g/dL (relative risk [RR], 0.49; 95% CI, 0.28–0.87). This study also showed that improved survival was associated with high-flux dialyzers among those with diabetes. Although the EGE Study [45] did not show a reduction of composite CV events, post hoc analysis suggested a benefit associated with high- vs. low-flux dialysis membrane on improving CV event-free survival among...
those with AVTs and those with diabetes.

Meta-analysis of 12 prospective clinical trials [43–54] comparing high- vs. low-flux HD membranes, excluding observational studies, showed a 13% reduction (RR, 0.87; 95% CI, 0.76–0.99) in all-cause deaths and a 19% reduction (RR, 0.81; 95% CI, 0.70–0.95) in CV deaths. Furthermore, β2-microglobulin concentrations were reduced by 9.90 mg/L. However, no differences in hospitalization and Kt/V were shown.

In randomized clinical trials comparing online HDF with high-flux HD, including the Turkish OL-HDF (Online Hemodiafiltration) [55] and the FRENCHIE (French Convective vs. Hemodialysis in the Elderly) [56], no significant effect on overall mortality and CV mortality was demonstrated. However, in the Turkish OL-HDF Study, which was divided into two groups with a 17.4 L (the median amount of supplementation) group and a high-efficiency group with 17.4 L or more, the latter experienced significantly reduced overall mortality rate (p = 0.03).

The ESHOL (Estudio de Supervivencia de Hemodiafiltración On-Line) Study [57], a randomized clinical trial comparing high-efficiency online HDF with HD, reported a 30% reduction in overall mortality (hazard ratio [HR], 0.70; 95% CI, 0.53–0.92; p = 0.01) and a 33% reduction in CV mortality (HR, 0.67; 95% CI, 0.44–1.01; p = 0.06) in the high-flow online HDF. Of the patients in the HD group, 8.1% used low-flux HD membranes.

In both the Turkish OL-HDF and FRENCHIE studies, no differences in overall hospitalization rates were observed between the two groups, but in the ESHOL Study, the hospitalization rate was lower in the high-flow online HDF group (RR, 0.78; 95% CI, 0.67–0.90; p < 0.01). In terms of quality of life, a meta-analysis performed on six prospective clinical trials, excluding observational studies [58–63], found no significant difference between the online HDF and HD groups.

Chapter 4. Anticoagulation for the Hemodialysis

Recommendation 4.1
We recommend using unfractionated heparin (UFH) as the standard for systemic anticoagulation in HD patients without an increased bleeding risk because no differences could be found in the bleeding outcomes or circuit thrombosis between UFH and low-molecular-weight heparin (LMWH).

(Strong recommendation, low quality of evidence)

UFH is a conventional anticoagulant for HD in patients without active bleeding, a recent history of bleeding events, moderate to severe thrombocytopenia or heparin allergy. Typically, a loading dose of 1,000 to 2,000 units is administered at the start of HD, followed by a continuous infusion of 500 to 1,500 units per hour that is stopped approximately 30 minutes before the end of the HD session. The heparin dose can be adjusted empirically according to the clinical situation. Compared to UFH, LMWH, which can be administered as a bolus, has been shown to produce superior lipid profiles and less osteoporosis, and its use in HD patients in Europe is increasing [64]. We intended to verify whether LMWH could reduce bleeding events or HD circuit thrombosis compared to the conventional UFH in HD patients without higher bleeding risks.

Three meta-analyses that addressed the efficacy and safety of LMWH and UFH were identified at the time of literature search [65–67]. We selected clinical studies with parallel or cross-over designs that randomly allocated patients on HD or HDF into LMWH and UFH groups over a period of at least one month. Several studies were excluded from the analysis because of the following reasons: a less-than-1-week study period (Borm et al. [68], Koutsikos et al. [69] in the meta-analysis by Lim et al. [66] and Palamaner Subash Shantha et al. [67]); a dose-finding study design (Ryan et al. [70]); and no random allocation (Al-Saran et al. [71], Bramham et al. [72], Yang et al. [73] in a meta-analysis by Lazrak et al. [65], and Sabry et al. [74]). A meta-analysis was performed using six studies [75–80], although the poor blinding in these studies produced only moderate levels of evidence. The RR for any bleeding events was 0.74 (95% CI, 0.24–2.31), indicating no difference between the LMWH and UFH groups. The reported cases of major bleeding
were too low to perform subgroup analyses. Circuit thrombosis was defined as the number of cases of clotting in the dialyzer and circuit lines. Meta-analysis using three studies [76,77,79] resulted in an RR of 0.99 (95% CI, 0.56–1.77) for the LMWH group compared with UFH group, indicating no difference between the two anticoagulants. However, the level of evidence was assessed to be low due to heterogeneity among the studies and possible risks of bias.

**Recommendation 4.2**
1. We recommend not to use heparin for anticoagulation in HD patients with a high risk of bleeding.
   (Against recommendation, low quality of evidence)
2. We suggest using nafamostat mesylate, instead of heparin, for anticoagulation in HD patients with a high risk of bleeding.
   (Conditional recommendation, low quality of evidence)

Only a few studies conducted in Korea present low-level evidence for anticoagulation strategies for the HD patients with a risk of bleeding.

In a multi-center phase III trial assessing the influence of the anticoagulation efficacy and safety of nafamostat [81], 58 HD patients were considered to be at high risk of bleeding due to hemorrhagic complications, including postoperative status and gastrointestinal bleeding. Among 49 patients assessed during their clinical course, none experienced progression of preexisting hemorrhagic lesions while using nafamostat, and an improvement in preexisting hemorrhagic lesions was evident in 37 patients (71%). In a cross-over arm involving the use of heparin in the same patients at preoperative stages or at recovery from hemorrhagic complication, aggravation of a preexisting lesion was observed in a single patient (4%); Only six patients (28%) experienced improvement in preexisting lesions, while 15 patients (68%) remained stationary. Nafamostat also proved to be superior to heparin in the degrees of residual blood in the dialyzer and blood clotting in the venous drip chamber. The incidence of adverse reactions was comparable in both groups.

In a randomized trial conducted in a single center in Korea [82], 17 HD patients with intracerebral hemorrhages were divided into two groups; one treated with heparin (n = 9), and the other with nafamostat (n = 8). Follow-up imaging of hemorrhagic lesions with computed tomography revealed that, compared with heparin, nafamostat significantly prevented the aggravation of preexisting hemorrhagic lesions (p = 0.02), while no specific descriptions of blood clots or the adverse events were presented.

Despite the lack of large-scale trials, we recommend not using heparin as an anticoagulant in HD patients with a high risk of bleeding, based on limited data that use of heparin may aggravate preexisting hemorrhagic lesions. Provided that regional anticoagulation with nafamostat efficiently prevents both aggravations of preexisting lesion and thrombosis in the extracorporeal blood circuits, we suggest the use of nafamostat, instead of heparin, for anticoagulation in HD patients at high risk of bleeding.

**Chapter 5. Volume and fluid status in hemodialysis patients**

**Recommendation 5.1**
1. We suggest that the weight-gain ratio between dialysis sessions not exceed 4% compared with the dry weight before dialysis.
   (Strong recommendation, moderate quality of evidence)
2. We consider it reasonable that patients whose body weight before dialysis exceeds 4% compared with the dry weight require an assessment of excess body fluids, dietary compliance, and nutritional status along with the provision of dietary education.
   (Expert consensus)

Excessive weight gain between dialysis sessions can lead to excess fluid volume and increase CV events and mortality by inducing excessive ultrafiltration [83,84]. However, because weight gain between dialysis sessions is indicative of adequate nutritional intake, nephrologists should use a multifactorial approach to the evaluation of patients with weight gain between dialysis sessions. Both the United States Renal Data System (USRDS) Study [85] and the DOPPS (Dialysis Outcomes and Practice Patterns Study) [86], large-scale observational studies in the early 2000s, reported that if the rate of weight gain between dialysis is excessively high compared to the dry weight, the risk of death is significantly higher than that of the control group. The USRDS Study reported that the risk of death was higher in patients with an interdialytic weight gain [IDWG] of >4.8% compared with a control group (IDWG ≤ 2.3%). For patients in the DOPPS, an IDWG of >5.7% was considered a high risk compared with an IDWG of ≤5.7%.
in the control group. Based on these results, the 2015 dialysis treatment guidelines in Japan recommended a weight-gain ratio of less than 6% between dialysis sessions. However, the ultrafiltration rate per time of dialysis was not adjusted in these studies, the effect size of the mortality risk was small, and the definition of IDWG between dialysis sessions was not unified [83,84].

Weight gain between dialysis sessions is closely correlated with chronic volume overload, but the two concepts are not identical. Recent research suggests that, in patients with large weight gain during dialysis, there is a need to assess body-fluid levels simultaneously using different methods such as bioimpedance spectroscopy [87], correcting for anemia and nutritional status [88,89], and suggesting individualized approaches. The study included 38,614 HD patients who underwent total-body-fluid assessments. Even if the IDWG between dialysis was low (2.4% or less), the patients with chronic volume overload experienced significantly higher mortality [87]. As a result of the 2017 Japan DOPPS, in the group with a serum albumin level of 3.8 g/dL or less, the association with death was significant only in the group with a weight gain between dialysis sessions of less than 2.4% [88]. In a study by Lee et al. [90], the weight-gain ratio between dialysis sessions was 4.0% in a dialysis group compared with 2.6% in a control group, with the dialysis group showing a significant CV event risk with an HR of 1.93 compared with that of a control group after adjustment for residual renal function. In addition, the frequency of intradialytic hypotension during dialysis increased significantly from 3% or more of IDWG, and this phenomenon during dialysis was associated with death [91]. After an observational study of DOPPS on the effect of weight gain between dialysis sessions on prognosis was published in 2003, recent trends and prognosis of weight gain between dialysis sessions were published in 2017 [92]. The 2017 DOPPS study, which included approximately 22,000 patients, showed that, compared with results from 2003, the number of patients with a high rate of weight gain between dialysis sessions was decreasing. Nephrologists and dialysis staff should examine whether patients with excessive weight gain between dialysis sessions have poor compliance with a low-salt diet and water restrictions, and whether these cause excessive volume overload [86,93,94]. Conversely, patients with a low IDWG should be assessed for their nutritional status and need for greater intake.

Sodium and water accumulation can lead to volume overload and hypertension, both of which are major risk factors for left ventricular hypertrophy [95-98]. In dialysis patients, antihypertensive drugs and ultrafiltration are the treatment of choice to remove volume overload, which is often not treated in clinical situations [99,100]. Katzarski et al. [101] reported that 90% of patients could control blood pressure without antihypertensive drugs if patients receive long HD (3 × 8 hours/week) and maintain an ideal healthy weight. In addition, some studies, which increased the frequency of dialysis to above usual levels, effectively controlled blood pressure, and edema and left ventricular hypertrophy were also improved [99,102,103].

However, increasing the frequency and duration of dialysis is subject to medical insurance restrictions. Lowering sodium dialysate levels below conventional levels is one method of removing sodium and water. Even at conventional sodium concentrations in dialysate, sodium moves back into the body, increasing blood pressure and water retention and leading to weight gain between dialysis [104]. According to a report studied in Korea, the sodium concentration of the dialysate was 140 mEq/L, 23%; 138 mEq/L, 64%; and 136, 137, and 139 mEq/L [105].

Recently, Dunlop et al. [106] published a meta-analysis comparing low sodium dialysate levels (Na of <138 mEq/L) to neutral conditions (Na of 138–140 mEq/L) and high sodium dialysate (Na of >140 mEq/L) in HD patients. This study shows that a low sodium dialysate level was associated with decreased weight gain, but increased risks of hypotension [106].

We conducted a literature search to compare the effects of conventional and low sodium dialysate on IDWG. Three randomized control studies and five before-and-after studies were reviewed [107-114]. We found that low dialysate sodium-reduced IDWG (mean difference [MD], −0.27kg; 95% CI, −0.57 to 0.17; p = 0.01), predialysis blood pressure (MD, −3.52; 95% CI, −5.46 to −1.57; p < 0.01) and use of antihypertensive medications (standardized MD, −0.60; 95%
Low dialysate sodium was associated with low serum sodium concentration (MD, –1.59; 95% CI, –2.40 to –0.78; p < 0.01). The use of low sodium dialysate comes with increased side effects, such as hypotension, muscle cramps, and headaches during dialysis. The meta-analysis revealed that the frequency of hypotension during dialysis was significantly increased (RR, 1.49; 95% CI, 1.09–2.03; p = 0.01). This meta-analysis confirmed that low sodium dialysis solutions significantly reduced IDWG and blood pressure before dialysis compared with a group using conventional sodium dialysate.

Chapter 6. Blood pressure control in hemodialysis patients

Recommendation 6.1
1. There is insufficient evidence to assign optimal blood pressure target for HD patients. (Inconclusive, very low quality of evidence)
2. We consider it reasonable that antihypertensive medications should be prescribed for hypertensive HD patients considering multi-factors. (Expert consensus)

Lowering blood pressure significantly reduces CV morbidity and mortality rate in HD patients, which is a phenomenon similar to one associated with antihypertensive medications in the general population. However, no optimal blood pressure has been suggested [115]. Some traits require careful interpretation of the effects of lowering blood pressure. Most randomized controlled trials are based on a specific drug, not a target blood pressure. In a systematic review, it was difficult to pool blood pressure targets, because reductions in blood pressure achieved by patients varied widely among the trials, and also because baseline blood pressures were heterogenous among the studies. It is therefore insufficient to decide whether the effect of antihypertensive medication is from drug-specific effects or from reduced blood pressure under certain standards.

In one prospective observational cohort study performed in South Korea, a U-shaped HR pattern for patient mortality was observed among 2,299 HD patients over 4.5 median years of follow-up. The lowest risk was shown at 130–150 mmHg of systolic blood pressure. When continuous blood pressure was categorized, groups of patients with systolic blood pressure under 110 mmHg and over 170 mmHg were associated with an increased HR for mortality [116].

In a Western study based on 9,333 HD patients in an observational cohort with a median follow-up of 1.5 years, a similar U-shaped HR pattern of patient mortality was observed. However, the lowest risk was observed at close to 165 mmHg, which was different from the results of the Korean study [117]. Observational investigations of blood pressure and patient mortality among HD patients reported a U-shaped HR pattern, which represents an increased mortality risk at the tails of the blood pressure distribution. Nevertheless, this evidence is insufficient to suggest a consistent threshold of blood pressure at which an elevated mortality risk is likely. A multi-faceted approach is needed, because several factors can affect blood pressure treatment as confounders; these include interdialytic blood pressure variability [118], intradialytic antihypertensive drug removal through dialysis membranes [119], body-fluid changes [120], reduced vascular elasticity, and postdialysis blood pressure increment, which can also manifest as intradialytic hypertension [121].

Recommendation 6.2
We suggest lowering the dialysate temperature to reduce intradialytic hypotension. (Conditional recommendation, moderate quality of evidence)

Intradialytic hypotension is a common complication and requires appropriate management because it affects the morbidity and mortality of HD patients. Several methods have been applied to the prevention of intradialytic hypotension. One is the lowering the dialysate temperature. Standard temperature dialysis typically involves maintaining the dialysate at 36.5°C–37.0°C, which is similar to body temperature. Methods that lower the dialysate temperature are dialysis with a fixed reduction of dialysate temperature (usually 35.0°C–35.5°C) and isothermic dialysis through body temperature monitoring using a biofeedback system [122]. According to seven randomized controlled trials [123–129] and three prospective studies [130–132], intradialytic hypotension incidence decreased when dialysis was performed by lowering the dialysate temperature [123–128,130,131]. Moreover, little change in blood pressure reduction was evident during or after dialysis, and the lowest blood pressure during dialysis was also higher than that of standard dialysis.
In patients undergoing HD, the purpose of dialysis is to remove uremic substances and water caused by CKD, and to control uremic symptoms, maintain stable electrolyte balance, and prevent deterioration of nutritional status, thereby improving health and quality of life. Maintaining an adequate dialysis dose means maintaining the patient’s well-being, adequate volume status, and balanced biochemical levels. Multiple studies have reported that dialysis adequacy improves patient survival and quality of life [43,133–136]. However, no randomized controlled trials or prospective observational studies report outcomes for test items and intervals in patients on maintenance HD. Moreover, we found no studies of Korean patients on maintenance HD. However, in a recently published retrospective study in Canada, monthly routine blood testing in HD patients was not associated with a lower risk of death, CV events, or hospitalizations compared with testing every 6 weeks [137]. This guideline recommends performing a test as described above in accordance with expert opinion, considering that most dialysis centers conduct blood tests monthly.

Previously published foreign practice guidelines recommend that dialysis doses be measured monthly, as most dialysis centers perform blood tests, including those for electrolytes, monthly and as tests performed in patients undergoing maintenance HD are uncomplicated and inexpensive [138,139]. The KDOQI guideline published in 2006 recommended that the dialysis dose be measured at regular intervals of no less than monthly (A). Less-frequent measurements may compromise the timeliness with which deficiencies in the delivered dose of HD are detected and therefore may delay implementation of corrective action [138]. European best practice guidelines published in 2007 also recommend that delivered dialysis doses should be measured at least monthly (opinion) [139]. The UK Renal Association CPG published in 2019, recommends measuring and monitoring dialysis doses on a monthly base for the majority of center-based dialysis patients (1B) [64]. However, in this guideline, we recommended that dialysis doses be measured at least every 3 months according to expert agreement, taking into account the medical reality and cost of testing in Korea.

In this guideline, monthly complete blood counts, liver function tests (including total protein and albumin levels), and routine blood chemistry (blood urea nitrogen, creat-
inine, sodium, potassium, calcium, phosphate, uric acid, and glucose) are recommended. Most HD centers in Korea perform blood tests monthly. Moreover, the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) CPG for anemia in CKD recommends measuring hemoglobin concentrations at least monthly in patients with grade 5 CKD [140]. This guideline recommends applying those guidelines to Korean patients on maintenance HD, according to expert agreement.

The KDIGO 2017 CPG update for CKD-mineral bone disorder states that it is reasonable to monitor PTH levels every 3–6 months in patients with grade 5 CKD [141,142]. We recommend monitoring PTH levels at least every 3 months, according to expert agreement in this guideline. As other guidelines, including the KDIGO, suggest screening for hepatitis virus infection in patients on dialysis every 6 months [143–145], this guideline recommends screening for hepatitis viral markers at least every 6 months, according to expert agreement in this guideline.

Chapter 8. Nonstandard setting of hemodialysis (elderly, children)

**Recommendation 8.1**

1. We suggest that preparation for appropriate renal replacement therapy be considered for elderly patients who progress to ESKD.

   (Conditional recommendation, moderate quality of evidence)

2. We consider it reasonable that in elderly patients with ESKD, the optimal treatment should find an individualized balance between appropriate renal replacement therapy and conservative treatment.

   (Expert consensus)

With the advancement of renal replacement therapy, the overall survival rate of patients with ESKD is improving, but it is not yet clear whether renal replacement therapy offers any survival benefit in elderly patients compared with conservative treatment. Although randomized control studies are not available due to the nature of the study, comparing the dialysis group with the conservative treatment group makes it difficult to compare the selection bias of elderly patients with a relatively healthy group, the lead-time bias of the patients with dialysis, and the lack of studies of patients who perform conservative treatment. A meta-analysis of 89 observational studies from 1976 to 2014 on elderly patients with ESKD, including a total of 294,921 patients, reported a 1-year survival rate of 77.9% in the HD group and 70.6% in the conservative treatment group. Although the report that HD may have some benefits [146], it was unable to judge the role of conservative treatment because only 724 patients (0.2%) were included in the conservative treatment group. In a 2017 meta-analysis, the dialysis group showed a superior survival rate compared with the conservative treatment group (HR, 0.53; 95% CI 0.30–0.91; p = 0.02), but there was significant heterogeneity among studies [147]. Most of the studies before 2010 included in the meta-analysis were small and retrospective studies [148–150], and one small prospective study did not distinguish HD from peritoneal dialysis [151].

In prospective observational studies of elderly patients after 2015, dialysis treatment was associated with benefits compared with conservative treatment in entire patient groups [152–154], although comorbidities increased [152] and the benefit was not significant in patients older than 85 years [153] or 80 years [154]. In retrospective studies, the benefit of dialysis was greater than that of conservative treatment, but setting an appropriate control group would be important [155–157]. A Canadian study of reimbursement data using propensity-score matching showed a benefit of dialysis in the first 3 years (HR, 0.59; 95% CI, 0.46–0.77; p < 0.01), but no difference between dialysis and conservative treatment was found after 3 years [157]. In this practice guideline, we conducted a meta-analysis of 11 studies in which the mean age of elderly patients in the dialysis group was 76.0 ± 5.3 years. The meta-analysis showed that dialysis was more beneficial regarding survival than conservative treatment in elderly patients (HR, 0.42; 95% CI, 0.37–0.47; p < 0.01). As the evidence for survival gain by dialysis treatment grows [158,159], preparations for appropriate renal replacement therapy are needed when elderly patients progress to ESKD.

**Recommendation 8.2**

1. For HD of children younger than 5 years old, we consider it reasonable that the minimal nurse-to-patient ratio be 1:1.

   (Expert consensus)

2. For HD of older children, we consider it reasonable that the minimal nurse-to-patient ratio be 1:2.

   (Expert consensus)
Dialysis in infants and children requires exceptional skill and expertise. Pediatric HD requires devices appropriate for the patient’s body size, neonatal or pediatric dosages of medications, proper management of vascular access problems, and meticulous monitoring of volume status and vital signs. Infants and young children undergoing HD are sensitive to small changes in body water volume or blood pressure because their effective blood volume is smaller than that of adults [160]. As children may not recognize or verbally express the symptoms of side effects of HD, vital signs should be measured more frequently, and patients need to be monitored more carefully than adults during pediatric HD. For safe HD in children, more frequent clinical assessment is necessary [161] and often requires a nurse-to-patient ratio of 1:1 [162]. While there has been no CPG [163], a survey of clinical practices in the UK reported that a typical nurse-to-patient ratio was 1:1 for HD in children younger than 5 years old and 1:2–1:3 in the case of older children at most centers [164]. For HD in young children or patients with significant neurocognitive disability, a nurse-to-patient ratio of 1:1 is required. An infant may need the care of two nurses. HD requiring isolation also needs one nurse for each patient. For children who can communicate or adolescents whose development is normal, a nurse-to-patient ratio of 1:2 may be safe. For pediatric dialysis, there should be at least two registered nurses per duty, and a nurse-to-patient ratio should be 1:2 or higher [165].

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Access to the full-text version

The full-text version of this CPG is available on the KSN website (http://www.ksn.or.kr, http://krcp-ksn.org).

Conflicts of interest

All authors have no conflicts of interest to declare. The Work Group has been making the best efforts to avoid any actual and potential conflicts of interest to ensure a neutral and fair process in guideline development. All members of the Work Group obtained a conflict-of-interest disclosure form before participating in the development of the CPG and when completing the CPG to determine whether there was a financial or non-financial conflict of interest. In the case of reports of corporate research sponsorship or consulting, detailed report contents were confirmed after review by the Work Group. To determine whether the amount of money and the content of the recommendation can be affected, and if an amount exceeding the standard may affect the content of the recommendation, we recommend that the opinion of the relevant member be excluded when determining the direction and strength of the recommendation. This principle was applied from the beginning to the end of the development.

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Hepatocyte growth factor and soluble cMet levels in plasma are prognostic biomarkers of mortality in patients with severe acute kidney injury

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Background: Hepatocyte growth factor (HGF)/cMet pathway is necessary for repair and regeneration following acute kidney injury (AKI). We evaluated the clinical potential of plasma HGF and soluble cMet as prognostic biomarkers for severe AKI requiring continuous renal replacement therapy (CRRT).

Methods: One hundred thirty-six patients with severe AKI who participated in the VENUS (volume management under body composition monitoring in critically ill patients on CRRT) trial between 2017 and 2019 were enrolled in this study. We investigated associations between plasma HGF and cMet concentrations and all-cause mortality.

Results: Plasma HGF and soluble cMet levels were positively correlated. Patients were divided into three groups based on their HGF and soluble cMet concentrations. The day D 0, D2, and D7 highest concentration HGF groups had significantly higher in-hospital mortality after adjusting for sex, body mass index, Acute Physiology and Chronic Health Evaluation II, and age-adjusted Charlson comorbidity index score, especially on D7 (hazard ratio, 4.26; 95% confidence interval, 1.71–10.62; p = 0.002). D7 soluble cMet level was also associated with mortality. Receiver operating characteristic curve analysis indicated that D7 HGF and soluble cMet levels were best at predicting mortality. Addition of plasma HGF and soluble cMet to conventional prognostic indices significantly improved the predictive value for mortality on D7. However, plasma HGF and soluble cMet were not associated with fluid status.

Conclusion: Plasma HGF and soluble cMet levels were significant predictors of the outcomes of severe AKI patients undergoing CRRT. There was no correlation between plasma HGF and soluble cMet levels and fluid balance.

Keywords: Acute kidney injury, Biomarkers, Continuous renal replacement therapy, Hepatocyte growth factor, Soluble c-Met
**Introduction**

Acute kidney injury (AKI) occurs in 10% of hospitalized patients and 30% of critically ill patients each year, and its incidence is increasing [1]. Severe AKI is one of the leading causes of death in critically ill patients. Despite recent technical advances in AKI management, mortality rates associated with severe AKI are approximately 40% to 50% [2,3].

Reliable biomarkers are important for treating AKI patients and predicting outcomes. Several studies have reported various novel biomarkers for AKI that can detect kidney injury before serum creatinine (sCr), such as tissue inhibitor of metalloproteinase (TIMP)-2, insulin-like growth factor-binding protein 7 (IGFBP7) [4], and urinary matrix metalloproteinase 7 [5]. However, few studies have investigated prognostic predictors of the outcomes of patients with severe AKI undergoing continuous renal replacement therapy (CRRT).

Hepatocyte growth factor (HGF) and its tyrosine kinase receptor, cMet, have important roles in regulating wound healing, cell proliferation, and tissue fibrosis [6-8]. Several experimental studies have indicated that HGF has multiple protective effects on physiological and pathophysiological processes in the kidney, including accelerated DNA synthesis and improved kidney cell regeneration, apoptosis, and necrosis (including that of proximal tubular cells, mesangial cells, podocytes, and endothelial cells) [7,8]. Activation of the HGF/cMet axis can improve acute and chronic kidney disease (CKD) by inhibiting oxidative stress, apoptosis, fibrosis, and inflammation [9,10]. We have also confirmed that cMet activation by agonistic antibodies can improve renal fibrosis [11].

A recent study showed that plasma HGF level predicted all-cause mortality and cardiovascular mortality in the general population [12]. Additionally, HGF and cMet can predict clinical outcomes of patients with various cancers [13,14]. Recent studies have reported that HGF and cMet are biomarkers for CKD [15,16]. However, the clinical predictive abilities of HGF and soluble cMet in AKI patients have not been well studied.

In this study, we investigated whether plasma HGF and soluble cMet levels were associated with fluid balance.

**Methods**

**Study design and population**

A total of 136 severe AKI patients admitted to seven hospitals in Korea who participated in the VENUS trial between 2017 and 2019 were enrolled in this study [3]. The VENUS trial was designed to determine whether bioelectrical impedance analysis-guided fluid management could help achieve euvoletic status in patients treated with CRRT more efficiently than fluid management guided by a generally used quantification method. All participants were selected from patients who were scheduled to undergo CRRT for at least 72 hours. There was no mandatory standardized CRRT protocol. CRRT initiation was decided by each institution’s physicians. CRRT settings, effluent dose, and target I/O balance were freely applied to maintain stable blood pressure to meet metabolic demands. In general, blood flow was set to 100–130 mL/min, and target effluent dose was set to 35 mL/kg/hr.

Exclusion criteria were the following: age younger than 18 years; imminent death (<24 hours); maintenance dialysis used before current hospitalization; any other major illness that, according to the investigators’ judgment, would substantially increase the risk associated with the subject’s participation in this study; and withdrawal of patient consent.

**Clinical data and sample collection**

Clinical baseline characteristics data at the time of study enrollment, including age, sex, mean arterial pressure (MAP), body mass index (BMI), laboratory findings, contributing factors for AKI, all-cause mortality, outcome events include sepsis, cardiogenic diseases, pulmonary diseases, and cancer, among others, and Acute Physiology and Chronic Health Evaluation II (APACHE II) scores, and age-adjusted Charlson comorbidity index (Age-CCI) scores, were collected. Age-CCI is the combination of the age equivalence index and Charlson comorbidity index (CCI) [17]. For patients over 40 years old, the cumulative score was 1 point for each additional 10 years of age, and the score for age was added to the CCI. A completely resolved condition or current inactive surgery history was not considered a comorbid disease [18].
Laboratory evaluations included measurements of complete blood cell counts, electrolytes, sCr, total protein, albumin, calcium, and high-sensitivity C-reactive protein. Blood samples were centrifuged to extract plasma. The plasma was frozen, stored at –70°C, and thawed before analysis.

All procedures were performed in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All patients provided written informed consent at the time of their enrollment. The APACHE II score was designed to measure the disease severity of adult patients admitted to the intensive care unit (ICU) during the first 24 hours after admission. It is calculated using the patient’s age and 12 routine physiological measurements (alveolar-arterial oxygen gradient [AaDO₂] or partial pressure of oxygen [PaO₂] depending on the fraction of inspired oxygen [FiO₂], temperature, MAP, pH, heart rate, respiratory rate, sodium level, potassium level, creatinine level, hematocrit, white blood cell count, and Glasgow Coma Scale score). Approval to perform the study was obtained from the Institutional Review Boards of all participating centers (No. 20-2019-79). The trial protocol has been registered at http://www.clinicaltrials.gov (NCT03330626).

**Measurement of plasma soluble cMet and hepatocyte growth factor**

Enzyme-linked immunosorbent assay kits were used to measure the plasma concentrations of HGF (DY294; Becton Dickinson, Minneapolis, MN, USA) and soluble cMet (KHO2031; Invitrogen, Vienna, Austria) on day (D) 0, D2, and D7. Plasma HGF concentrations were measured after a 10-fold dilution and plasma soluble cMet concentration was measured after a two-fold dilution. Enzyme-linked immunosorbent assays was performed according to the manufacturers’ instructions. All laboratory investigators were blinded to the sample source, and all measurements were performed in duplicate.

**Assessment of fluid status**

Fluid status was measured by bioimpedance analysis using InBody S10 (InBody, Seoul, Korea) on D0, D2, and D7. Measurements of total body water (TBW), intracellular water (ICW), and extracellular water (ECW) were obtained with InBody S10. Fluid state was based on ECW/TBW and TBW/height squared (H²).

**Clinical outcomes**

Primary outcome was all-cause mortality after CRRT. We investigated the associations between HGF concentration and soluble cMet concentration and all-cause mortality. In this study, survival duration was calculated from the day of enrollment in the study to the day of discharge from the hospital or death. The relationship between all-cause mortality after 2 years of follow-up was also assessed, starting from the first enrolled patient. Secondary outcome was fluid status according to HGF and soluble cMet concentrations.

**Statistical analysis**

Patients were classified into three groups based on their D0 HGF and soluble cMet concentrations. Table 1 shows the HGF and soluble cMet concentrations of each group. Categorical variables, which were expressed as frequencies and proportions, were compared using the chi-square tests. After testing for normality, normally distributed continuous variables were expressed as mean ± standard deviations and compared using the Student t test or one-way analysis of variance. Nonnormally distributed variables were expressed as medians (interquartile ranges) and compared using the Mann-Whitney U or Kruskal-Wallis tests. To investigate the impact of D0, D2, and D7 plasma soluble cMet and HGF levels on mortality, Kaplan-Meier survival curves were constructed. Cox proportional hazard models with plasma soluble cMet or HGF levels were used for multivariate survival analyses. To examine the prognostic value of plasma HGF and soluble cMet levels at multiple time points, we calculated the statistical significance of differences between the areas under the curve (AUC) at three-time points (D0, D2, and D7). Survival duration was calculated from day 0 to evaluate the predictive effects of D2 and D7 soluble cMet and HGF. Receiver operating characteristic (ROC) curves and AUC were generated to evaluate the accuracy and predictive capability of each indicator of survival. To examine the incremental prognostic value before and after inclusion of plasma HGF and soluble cMet levels with traditional indices, including APACHE II and Age-CCI, we calculated the statis-
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total (n = 136)</th>
<th>Group 1 (n = 45)</th>
<th>Group 2 (n = 46)</th>
<th>Group 3 (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma HGF (pg/mL)</td>
<td>1,881.2</td>
<td>4,270.6</td>
<td>16,821.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma cMet (pg/mL)</td>
<td>627.4</td>
<td>889.5</td>
<td>720.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.50</td>
<td>0.89</td>
<td>0.02</td>
<td>0.91</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>17 (12.5)</td>
<td>20 (14.4)</td>
<td>20 (14.4)</td>
<td>0.64</td>
</tr>
<tr>
<td>Comorbidity</td>
<td>17 (12.5)</td>
<td>16 (11.3)</td>
<td>18 (13.0)</td>
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<tr>
<td>Diabetes mellitus</td>
<td>21 (15.6)</td>
<td>14 (10.0)</td>
<td>10 (22.2)</td>
<td>0.71</td>
</tr>
<tr>
<td>Hypertension</td>
<td>60 (44.1)</td>
<td>20 (44.4)</td>
<td>24 (48.9)</td>
<td>0.25</td>
</tr>
<tr>
<td>Congestive heart disease</td>
<td>12 (8.8)</td>
<td>8 (11.3)</td>
<td>5 (11.1)</td>
<td>0.57</td>
</tr>
<tr>
<td>Cerebrovascular attack</td>
<td>12 (8.8)</td>
<td>7 (15.9)</td>
<td>5 (11.1)</td>
<td>0.57</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>73 (53.7)</td>
<td>66.4 (62.0–70.6)</td>
<td>69.2 (64.9–73.4)</td>
<td>0.71</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>153 (113.0)</td>
<td>117.4 (92.3–133.8)</td>
<td>112.3 (90.2–144.6)</td>
<td>0.71</td>
</tr>
<tr>
<td>Baseline sCr (mg/dL)</td>
<td>83.2 (80.5–85.9)</td>
<td>84.8 (82.7–89.9)</td>
<td>84.5 (80.6–90.1)</td>
<td>0.71</td>
</tr>
<tr>
<td>Factor contributing to AKI</td>
<td>19.1 (15.1–23.5)</td>
<td>21.1 (14.4–28.8)</td>
<td>19.9 (14.4–28.8)</td>
<td>0.71</td>
</tr>
<tr>
<td>Hospital LOS (day)</td>
<td>7 (5.37)</td>
<td>23 (15.0)</td>
<td>29 (16.4)</td>
<td>0.71</td>
</tr>
<tr>
<td>WBC (10³/µL)</td>
<td>7.3 (4.29)</td>
<td>3 (6.7)</td>
<td>3 (6.7)</td>
<td>0.71</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>113.0</td>
<td>117.4</td>
<td>102.4</td>
<td>0.64</td>
</tr>
<tr>
<td>Platelet (10³/µL)</td>
<td>28.0 (23.3–25.6)</td>
<td>25.6 (24.0–26.3)</td>
<td>24.9 (23.7–26.1)</td>
<td>0.64</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>3 (0.6–2.6)</td>
<td>2 (0.4–2.4)</td>
<td>2 (0.4–2.2)</td>
<td>0.57</td>
</tr>
<tr>
<td>Baseline cMet (mg/dL)</td>
<td>8.3 (5.0–13.2)</td>
<td>7.2 (5.0–13.2)</td>
<td>6.9 (5.0–13.2)</td>
<td>0.64</td>
</tr>
<tr>
<td>Platelet count (×10³/µL)</td>
<td>3 (0.6–2.6)</td>
<td>2 (0.4–2.4)</td>
<td>2 (0.4–2.2)</td>
<td>0.57</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>3.9 (1.0–2.6)</td>
<td>3.8 (1.0–2.6)</td>
<td>3.8 (1.0–2.6)</td>
<td>0.57</td>
</tr>
<tr>
<td>Baseline cMet (mg/dL)</td>
<td>8.3 (5.0–13.2)</td>
<td>7.2 (5.0–13.2)</td>
<td>6.9 (5.0–13.2)</td>
<td>0.64</td>
</tr>
<tr>
<td>Platelet count (×10³/µL)</td>
<td>3 (0.6–2.6)</td>
<td>2 (0.4–2.4)</td>
<td>2 (0.4–2.2)</td>
<td>0.57</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>3.9 (1.0–2.6)</td>
<td>3.8 (1.0–2.6)</td>
<td>3.8 (1.0–2.6)</td>
<td>0.57</td>
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Clinical parameter

<table>
<thead>
<tr>
<th>D0 HGF</th>
<th>Group 1 (n = 45)</th>
<th>Group 2 (n = 45)</th>
<th>Group 3 (n = 45)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>66 (48.5)</td>
<td>20 (41.3)</td>
<td>26 (57.8)</td>
<td>0.28</td>
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<tr>
<td>Group 1</td>
<td>24 (52.2)</td>
<td>9 (20)</td>
<td>19 (41.3)</td>
<td>0.09</td>
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<td>Group 2</td>
<td>10 (31.2)</td>
<td>11 (24.4)</td>
<td>7 (15.6)</td>
<td>0.31</td>
</tr>
<tr>
<td>Group 3</td>
<td>22 (44.9)</td>
<td>21 (46.7)</td>
<td>8 (17.8)</td>
<td>0.14</td>
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</table>

Clinical parameter

<table>
<thead>
<tr>
<th>D0 cMet</th>
<th>Group 1 (n = 45)</th>
<th>Group 2 (n = 45)</th>
<th>Group 3 (n = 45)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>26 (57.8)</td>
<td>19 (41.3)</td>
<td>26 (57.8)</td>
<td>0.28</td>
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<tr>
<td>Group 1</td>
<td>10 (43.3)</td>
<td>7 (36.8)</td>
<td>6 (33.3)</td>
<td>0.74</td>
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<tr>
<td>Group 2</td>
<td>10 (43.3)</td>
<td>9 (47.4)</td>
<td>10 (55.6)</td>
<td>0.40</td>
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<tr>
<td>Group 3</td>
<td>6 (26.7)</td>
<td>3 (15.8)</td>
<td>10 (55.6)</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Results

Baseline characteristics

The study cohort consisted of 136 patients divided into three groups based on their HGF and soluble cMet concentrations on D0. Table 1 provides clinical baseline characteristics based on D0 HGF and soluble cMet concentrations. There were no significant differences in diabetes, hypertension, BMI, sCr, or blood urea nitrogen among the three groups based on D0 HGF and soluble cMet concentrations. In the group with the highest D0 HGF concentration, the soluble cMet concentration was significantly higher (p = 0.02), MAP was significantly lower (p = 0.009), and more patients were on vasopressors (p = 0.02). The laboratory findings of patients in the highest D0 HGF group indicated higher lactate levels in this group (p < 0.001) and a lower pH (p = 0.004). The group with the highest D0 soluble cMet concentration had a higher APACHE II score than the other two groups (p = 0.03).

Variations and correlations between hepatocyte growth factor and soluble cMet concentrations after continuous renal replacement therapy

Both HGF and soluble cMet concentrations tended to
decrease after CRRT. HGF concentrations on D7 were significantly reduced compared to those on D0 (Fig. 1A). Concentrations of HGF and soluble cMet on D0 were positively correlated with each other ($R^2 = 0.126; p = 0.03$) (Fig. 1B). HGF and soluble cMet concentrations were not significantly correlated on D2 ($R^2 = 0.107$ and $p = 0.09$) or D7 ($R^2 = 0.06$ and $p = 0.42$) (Fig. 1C, D).

Effects of plasma hepatocyte growth factor levels on clinical outcomes

Kaplan-Meier survival curves and log-rank tests were used to investigate the associations between plasma HGF concentrations on D0, D2, and D7 and mortality. We divided patients into three groups and analyzed survival rates. Survival rate distribution of the first and second groups based on HGF and soluble cMet levels were similar, but different from those of the third group that had the highest concentration of both HGF and soluble cMet. Therefore, we combined the first group and the second group for analysis (Supplementary Fig. 1, available online). The risk of all-cause mortality was increased in patients with the highest D0 HGF levels ($p = 0.04$) (Fig. 2A). D2 and D7 HGF levels displayed similar associations with in-hospital mortality, especially D7 HGF level ($p = 0.01$ and $p = 0.001$, respectively) (Fig. 2B, C).

In the 2-year follow-up data, plasma D0, D2, and D7 HGF levels were still closely related to all-cause mortality (Supplementary Fig. 2A–C, available online). Next, we performed multivariate Cox proportional analysis to investigate the independent effects of D0, D2, and D7 HGF levels on patient outcomes (Table 2). Elevated HGF values remained an independent variable associated with clinical outcomes after adjusting for confounding variables, including sex, BMI, APACHE II score, and Age-CCI (D0 HGF: hazard ratio [HR] 1.71, 95% confidence intervals [CI] 1.02–2.86, $p = 0.04$; D2 HGF: HR 2.57, 95% CI 1.37–4.83, $p = 0.003$; D7 HGF: HR 4.26, 95% CI 2.13–8.49, $p = 0.0002$).

**Figure 1. Variations and correlations between HGF and soluble cMet levels.** (A) Trends of HGF and soluble cMet concentrations in plasma after CRRT. (B) Plasma HGF level converted to a natural logarithm was positively correlated with plasma soluble cMet level on D0 after CRRT was initiated. Pearson correlation coefficient was $R^2 = 0.126$ with a $p$-value of 0.03. No correlation was observed between plasma HGF levels and cMet levels on D2 (C) or D7 (D) after initiating CRRT.

CRRT, continuous renal replacement therapy; D, day; HGF, hepatocyte growth factor.
Figure 2. Survival rates of AKI patients who underwent CRRT according to plasma HGF and soluble cMet concentrations. (A) Patients in group 3 had a significantly lower survival rate than patients in group 1 and group 2 according to plasma HGF concentration on D0 (log-rank p = 0.04). (B and C) Patients in HGF group 3 had a significantly lower survival rate than those in groups 1 and 2 on D2 (log-rank p = 0.01) and D7 (log-rank p = 0.001). Patients in group 3 were divided according to plasma soluble cMet concentrations on D0, D2, and D7. (D and E) No difference in mortality rates was found between cMet groups 1 and 2 and group 3 on D0 and D2. (F) The risk of all-cause mortality was significantly increased in the patient group with the highest D7 cMet levels (p = 0.005).

AKI, acute kidney injury; CRRT, continuous renal replacement therapy; D, day; HGF, hepatocyte growth factor.

Additionally, we evaluated the effects of changes in HGF concentrations between different time points during the week after CRRT initiation on clinical outcomes. Changes in HGF concentrations were calculated as differences between D2 and D0, between D7 and D0, and between D7 and D2. Changes in HGF concentrations were not related to mortality (Supplementary Fig. 3A-C, available online).

Association between plasma soluble cMet level and clinical outcomes

We used the same method to investigate the associations between plasma soluble cMet concentrations on D0, D2, and D7 and in-hospital mortality. D0 and D2 soluble cMet levels did not predict all-cause mortality (Fig. 2D–E). On D7, the risk of all-cause mortality (Fig. 2F) was significantly increased in the patient group with the highest soluble cMet level (p = 0.005). In the 2-year follow-up data, plasma D0 soluble cMet level was still closely related to all-cause mortality (Supplementary Fig. 2D–F). Multivariate Cox regression analysis showed that the group with the highest soluble cMet level had a significantly higher mortality rate than the other groups after adjusting for other risk factors such as sex, BMI, APACHE II score, and Age-CCI (HR, 4.18; 95% CI, 1.69–10.32; p = 0.002) (Table 3). Additionally, changes in soluble cMet concentrations between D2 and D0, between D7 and D0, and between D7 and D2 were not associated with all-cause mortality (Supplementary Fig. 3D–F).
Table 2. Multivariate Cox proportional analysis model for HGF (D0, D2, D7)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>D0 Group 1 + 2 (n = 91)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Group 3 (n = 45)</td>
<td>1.65 (1.00–2.71)</td>
<td>0.05</td>
<td>1.74 (1.05–2.88)</td>
<td>0.03</td>
</tr>
<tr>
<td>Continuous variable (n = 136)</td>
<td>1.30 (1.05–1.61)</td>
<td>0.02</td>
<td>1.41 (1.11–1.79)</td>
<td>0.004</td>
</tr>
<tr>
<td>D2 Group 1 + 2 (n = 75)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Group 3 (n = 37)</td>
<td>2.10 (1.16–3.81)</td>
<td>0.01</td>
<td>2.46 (1.33–4.54)</td>
<td>0.004</td>
</tr>
<tr>
<td>Continuous variable (n = 112)</td>
<td>1.56 (1.14–2.12)</td>
<td>0.006</td>
<td>1.60 (1.17–2.18)</td>
<td>0.003</td>
</tr>
<tr>
<td>D7 Group 1 + 2 (n = 57)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Group 3 (n = 28)</td>
<td>3.87 (1.60–9.35)</td>
<td>0.003</td>
<td>4.35 (1.76–10.75)</td>
<td>0.001</td>
</tr>
<tr>
<td>Continuous variable (n = 85)</td>
<td>1.42 (0.98–2.05)</td>
<td>0.06</td>
<td>1.54 (1.03–2.31)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

CI, confidence interval; D, day; HGF, hepatocyte growth factor; HR, hazard ratio.
Model 1: adjusted for sex and body mass index; model 2: adjusted for Acute Physiology and Chronic Health Evaluation II score in addition to model 1 adjusted variables; model 3: adjusted for age-adjusted Charlson comorbidity index score in addition to model 1 adjusted variables.

Table 3. Multivariate Cox proportional model for cMet (D0, D2, D7)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>D0 Group 1 + 2 (n = 91)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Group 3 (n = 45)</td>
<td>1.24 (0.75–2.04)</td>
<td>0.40</td>
<td>1.22 (0.74–2.03)</td>
<td>0.45</td>
</tr>
<tr>
<td>Continuous variable (n = 136)</td>
<td>1.16 (0.92–1.46)</td>
<td>0.21</td>
<td>1.18 (0.92–1.51)</td>
<td>0.20</td>
</tr>
<tr>
<td>D2 Group 1 + 2 (n = 75)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Group 3 (n = 37)</td>
<td>1.77 (0.97–3.21)</td>
<td>0.06</td>
<td>1.61 (0.88–2.96)</td>
<td>0.12</td>
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<tr>
<td>Continuous variable (n = 112)</td>
<td>1.30 (0.98–1.73)</td>
<td>0.07</td>
<td>1.32 (0.97–1.79)</td>
<td>0.08</td>
</tr>
<tr>
<td>D7 Group 1 + 2 (n = 57)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Group 3 (n = 28)</td>
<td>3.25 (1.36–7.76)</td>
<td>0.008</td>
<td>3.84 (1.59–9.24)</td>
<td>0.003</td>
</tr>
<tr>
<td>Continuous variable (n = 85)</td>
<td>2.07 (1.18–3.64)</td>
<td>0.01</td>
<td>2.54 (1.37–4.72)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

CI, confidence interval; D, day; HR, hazard ratio.
Model 1: adjusted for sex and body mass index; model 2: adjusted for Acute Physiology and Chronic Health Evaluation II score in addition to model 1 adjusted variables; model 3: adjusted for age-adjusted Charlson comorbidity index score in addition to model 1 adjusted variables.
Ability of plasma hepatocyte growth factor and soluble cMet to predict outcomes at different time points

Levels of HGF and soluble cMet that best predicted mortality were investigated using ROC curves. The AUCs (95% CI) of D0, D2, and D7 HGF were 0.576 (0.440–0.711), 0.540 (0.392–0.689), and 0.716 (0.598–0.838), respectively (Fig. 3A). The AUCs (95% CI) of D0, D2, and D7 soluble cMet were 0.579 (0.440–0.719), 0.570 (0.429–0.711), and 0.678 (0.553–0.825), respectively (Fig. 3B).

Plasma hepatocyte growth factor and soluble cMet as biomarkers for predicting mortality

Next, we evaluated whether the addition of plasma HGF and soluble cMet to conventional prognostic markers such as APACHE II and Age-CCI improved prediction of mortality (Table 4). In ROC analysis, the addition of log(D7 HGF) and log(D7 cMet) increased the AUC. The AUCs (95% CIs) of the APACHE II (model 1), APACHE II + log(D7 HGF) (model 2), and APACHE II + Age-CCI + log(D7 HGF) + log(D7 cMet) (model 3) were 0.523 (0.393–0.653), 0.710 (0.588–0.832), and 0.749 (0.635–0.863), respectively. Additionally, the IDI and cNRI for predicting mortality (model 1 vs. model 3) were 0.166 (0.070–0.262; p < 0.001) and 75.1% (29.9%–120.3%; p < 0.001), suggesting that addition of plasma log(D7 HGF) and log(D7 cMet) to conventional predictors of mortality significantly increased predictive value.

Prediction of clinical outcomes according to combined hepatocyte growth factor and soluble cMet concentrations

The ability to predict patient outcomes using the combination of HGF and soluble cMet concentrations was evaluated. Group 1 had a significantly higher mortality rate than the other groups (p = 0.04). Furthermore, the difference in mortality between these groups was statistically significant when using D7 HGF and cMet concentrations (p = 0.02) (Supplementary Fig. 4A–C, available online).

Fluid status according to hepatocyte growth factor and soluble cMet concentrations

We investigated fluid status according to HGF and soluble cMet concentrations using parameters of fluid status measured using InBody S10. There was no significant difference

Figure 3. Comparison of the ROC curves for plasma H GF and soluble c Met at various time points. (A) ROC curve and AUC for plasma HGF concentrations at various time points. (B) ROC curve and AUC for plasma soluble cMet concentrations at various time points.

AUC, area under the curve; D, day; HGF, hepatocyte growth factor; ROC, receiver operating characteristic.
between ECW/TBW and plasma HGF and soluble cMet levels on D0, D2, or D7 (Fig. 4A). There was no significant difference between plasma HGF levels on D0 and D7 and soluble cMet levels on D0, D2, and D7. TBW/H² increased significantly (p = 0.02) only in the group of patients with the highest D2 HGF level (Fig. 4B).

### Discussion

In this study, we found that increased plasma HGF and soluble cMet levels could predict the outcomes of patients with severe AKI undergoing CRRT. Plasma HGF and soluble cMet levels on D7 were the most valuable predictors of patient outcomes. The inclusion of plasma HGF and soluble cMet levels with conventional prognostic index markers improved the predictive power of the composite indices. Finally, there was no correlation between plasma HGF and soluble cMet levels and fluid balance. To our knowledge, this is the first study to show that increased plasma HGF and soluble cMet levels of patients with severe AKI can predict clinical outcomes.

HGF and its receptor cMet are key components of a signaling pathway with critical roles in cellular regeneration, proliferation, differentiation, invasion, angiogenesis, anti-apoptosis, tissue fibrosis, and wound healing [19,20]. The HGF/cMet signaling pathway has therefore been identified as a target to alleviate the various types of AKI and prevent progression to CKD. The expression of HGF has been shown to be increased in various acute and CKDs including experimental acute ischemic injury, toxic elements, and unilateral nephrectomy [21,22].

HGF may act as an endocrine and paracrine effector for kidney repair during kidney injury. Administration of exogenous HGF promotes tubular repair and recovery [22]. Previously, we demonstrated that administration of cMet agonistic antibodies halted the progression of CKD [11]. Our group also confirmed that cMet agonistic antibodies attenuate apoptosis in AKI [23]. Conversely, disruption of HGF signaling aggravates renal interstitial fibrosis after obstructive injury [24]. However, many studies have reported that elevated plasma HGF and soluble cMet concentrations are associated with poorer clinical prognosis. Santalahti et al. [12] reported that levels of HGF and placental growth factor in plasma could predict mortality in a general population. Elevated circulating HGF levels have been observed in many patho-

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**Table 4. Incremental values of HGF and soluble cMet compared to traditional risk factors for predicting mortality due to severe acute kidney injury (n = 84)**

<table>
<thead>
<tr>
<th>Day</th>
<th>Model</th>
<th>ROC analysis (DeLong test)</th>
<th>IDI analysis</th>
<th>Category-free NRI analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC (95% CI)</td>
<td>p-value</td>
<td>IDI (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>0</td>
<td>APACHE II</td>
<td>0.523 (0.393–0.653)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>APACHE II + Age-CCI</td>
<td>0.581 (0.426–0.736)</td>
<td>0.55</td>
<td>0.034 (–0.012 to 0.000)</td>
</tr>
<tr>
<td>0</td>
<td>APACHE II + log(HGF)</td>
<td>0.584 (0.448–0.720)</td>
<td>0.49</td>
<td>0.043 (0.009 to 0.069)</td>
</tr>
<tr>
<td></td>
<td>APACHE II + Age-CCI + log(HGF)</td>
<td>0.631 (0.422–0.878)</td>
<td>0.45</td>
<td>0.043 (0.009 to 0.069)</td>
</tr>
<tr>
<td>0</td>
<td>APACHE II + log(HGF)</td>
<td>0.584 (0.448–0.720)</td>
<td>0.49</td>
<td>0.043 (0.009 to 0.069)</td>
</tr>
<tr>
<td>2</td>
<td>APACHE II + Age-CCI + log(HGF)</td>
<td>0.631 (0.422–0.878)</td>
<td>0.45</td>
<td>0.043 (0.009 to 0.069)</td>
</tr>
<tr>
<td>7</td>
<td>APACHE II + Age-CCI + log(HGF)</td>
<td>0.631 (0.422–0.878)</td>
<td>0.45</td>
<td>0.043 (0.009 to 0.069)</td>
</tr>
</tbody>
</table>

**Legend:**
- **APACHE II:** Acute Physiology and Chronic Health Evaluation II
- **AUC:** Area under the curve
- **IDI:** Integrated discrimination improvement
- **NRI:** Net reclassification improvement
- **log:** Natural logarithm
- **Age-CCI:** Age-adjusted Charlson comorbidity index

**Note:** Incremental values refer to the improvement in model discrimination when adding HGF or soluble cMet levels to traditional risk factors.
Figure 4. Euvolemic status on D7 after CRRT according to HGF and soluble cMet concentrations. (A) Comparison of ECW/TBW and plasma HGF or soluble cMet on D0, D2, and D7. (B) Comparison of TBW/H² and plasma HGF or soluble cMet levels. CRRT, continuous renal replacement therapy; D, day; ECW, extracellular water; HGF, hepatocyte growth factor; H², height squared; TBW, total body water.
logic liver diseases, including hepatitis and hepatocellular carcinoma, and have been shown to be correlated with more severe liver cirrhosis [25]. Likewise, HGF levels are associated with sepsis and correlated with established markers of endothelial cell injury. Elevated HGF level in sepsis patients is a significant indicator of a poor prognosis [26]. Moreover, an increase in soluble cMet concentration has been shown to be associated with a poor clinical prognosis. As reported in various malignant tumors, soluble cMet concentration is associated with progression, metastasis, and a poor prognosis [27–29]. Our group previously demonstrated increased urinary cMet in diabetic nephropathy patients was strongly associated with progression to end-stage renal disease [15].

There are several explanations for why elevated HGF and soluble cMet concentrations are highly related to a poorer prognosis. First, the greater the extent of the disease, the greater the activation of the HGF/cMet signaling pathway for cell regeneration. Overexpression of the HGF/cMet pathway and increased plasma HGF and soluble cMet concentrations may be closely related to disease progression. In addition, an increase in soluble cMet inhibits the phosphorylation of cMet, thereby inhibiting the HGF/cMet signaling pathway [30].

One study reported a marked increase in urinary HGF levels in patients with AKI [23]. Another study showed that HGF concentration in serum was significantly increased in AKI patients relative to participants receiving hemodialysis (approximately 20-fold) [31]. Clinical trials have indicated that HGF and other biomarkers in urine (such as neutrophil gelatinase-associated lipocalin [NGAL]) have the potential to predict AKI [32]. These studies suggested that HGF was correlated with AKI severity. Despite these data, these studies only examined AKI patients with urine volumes that were maintained and measured at one-time point; furthermore, cMet concentration was not considered. Additionally, the mortality rate of patients was not assessed.

We previously reported that the expression of HGF and soluble cMet in plasma was significantly increased in AKI patients [23]. In this study, we evaluated if plasma HGF and soluble cMet concentrations could predict clinical longitudinal outcomes of severe AKI patients undergoing CRRT. We confirmed that mortality rates during hospitalization were significantly higher in patients with severe AKI and patients with the highest HGF levels on D0, D2, and D7. Soluble cMet level on D7 was a significant predictor of patient mortality. After adjusting for sex, BMI, APACHE II score, Age-CCI, HGF and soluble cMet levels were still significant predictors of patient mortality. A large amount of HGF may be released as an injury protection mechanism to activate downstream signaling pathways. HGF is released not only by damaged kidneys but also distant organs (lung, liver, spleen) and can participate in tubular repair both as an endocrine factor and paracrine substance [33]. This suggests that plasma HGF and soluble cMet levels can be used as predictors of the prognosis of patients with severe AKI and of clinical risk and recovery after AKI.

A previous study showed that a panel of urine biomarkers measured on D1, D7, and D14 yielded significantly different results for those recovering from AKI compared with those patients who did not recover [34]. We analyzed the correlation between plasma HGF and soluble cMet concentrations and mortality at multiple time points after the initiation of CRRT (D0, D2, and D7). We found that plasma HGF as a clinical predictor was not affected by CRRT and that plasma HGF was a better predictor of mortality than soluble cMet. Furthermore, we examined whether HGF and soluble cMet levels on D0, D2, and D7 were reliable biomarkers of mortality using ROC curves and AUCs. D7 HGF and soluble cMet concentrations were prognostic biomarkers with high sensitivity and specificity. The best time to measure plasma HGF and soluble cMet levels may be on D7 after the start of CRRT for severe AKI; repeated measurements can improve prediction accuracy.

An individual biomarker is rarely sufficient for clearly defining a particular pathologic state [35,36]. Vaidya et al. [32] measured kidney injury molecule-1, HGF, NGAL, and interleukin-18 levels simultaneously in the same aliquot of urine. The specificity and sensitivity of the combination of these urinary biomarkers for the diagnosis of AKI were significantly higher than those of single biomarkers. The urinary [TIMP-2] · [IGFBP7] test can be used to identify critically ill patients at high risk for imminent AKI [5]. Therefore, multiple biomarkers measured in the same biological sample at the same time are extremely useful for predicting outcomes. Circulating HGF and cMet are attractive potential alternative biomarkers for ligand overexpression and receptor overexpression, respectively [37]. In this study, we analyzed the ability of combined plasma HGF and soluble cMet levels to predict mortality in patients with severe AKI. We found that the mortality rates of groups with high HGF and soluble
cMet concentrations on D2 and D7 were significantly higher than those of other groups; furthermore, the inclusion of soluble cMet and HGF with conventional prognostic indices such as APACHE II and Age-CCI significantly improved predictive power.

Rhee et al. [38] demonstrated that TBW/H² (≥13 L/m²) and ICW/H² were independently associated with higher in-hospital mortality for patients with AKI undergoing CRRT. That study showed that the fluid status could be assessed using ECW/TBW in critically ill patients requiring CRRT and that ECW/TBW could predict mortality [39]. All patients who participated in this study were admitted to the ICU, and their fluid balance was maintained during CRRT. We used ECW/TBW to evaluate the correlation between fluid status and plasma HGF or soluble cMet levels; however, no significant correlation was found.

The present study had some limitations. First, although ethnicity can influence several clinical outcomes, data were acquired only from a Korean population, and this study was limited to Korean tertiary hospitals. Second, only mortality during hospitalization was assessed and long-term outcomes were not considered. Finally, plasma HGF and soluble cMet concentrations could be affected by CRRT. It is necessary to identify HGF and soluble cMet levels in CRRT waste liquid or blood before and after CRRT to confirm that the CRRT waste liquid does not contain HGF or soluble cMet in future studies.

In summary, plasma HGF measurements at multiple time points predicted the clinical outcomes of patients with severe AKI undergoing CRRT. Furthermore, plasma soluble cMet measurements at a specific time point were able to predict clinical outcomes. For accurate mortality predictions, the most valuable time to perform plasma HGF and soluble cMet measurements is D7 after CRRT, and repeated measurements can improve accuracy. We found no correlation between plasma HGF and soluble cMet levels and fluid balance in this study.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

This work was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare, Republic of Korea (grant number: HI17C1827) in addition to a grant from the Korea Healthcare Technology R&D Project, Ministry for Health and Welfare, Republic of Korea (HI17C1693).

Acknowledgments

The authors would like to thank the study participants. The biospecimens and data used for the present study were provided by the Biobank of Seoul National University Hospital, a member of the Korea Biobank Network (KBN4 A03).

Authors’ contributions

Conceptualization: LL, JPL
Funding acquisition: JPL
Investigation, Methodology: LL, JNA, JHK, SMZ, DJS, JPL
Formal analysis, Visualization: LL, JNA
Supervision, Validation: JL, DKK, DRR, SK
Writing–original draft: LL, JPL
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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References


Fabry nephropathy before and after enzyme replacement therapy: important role of renal biopsy in patients with Fabry disease

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Background: In Fabry disease, the presence of globotriaosylceramide (GL3) deposits in various kidney cells leads to progressive renal dysfunction. However, kidney biopsy studies in patients with Fabry disease are limited. In the present study, the pathologic findings of patients with Fabry nephropathy receiving enzyme replacement therapy (ERT) and untreated patients without albuminuria were investigated.

Methods: The present study included 15 patients with Fabry disease who underwent renal biopsy while receiving ERT (group 1: n = 9, age 19–58 years, two males and seven females) or before ERT initiation (group 2: n = 6, age 11–66 years, one male and five females). All patients in group 2 were normoalbuminuric.

Results: Group 1 showed improved clinical symptoms, such as acroparesthesia. The ERT duration was 1.2 to 8 years and seven of the nine patients showed GL3 deposits in various kidney cells and segmental foot process effacement (FPE) of podocytes. GL3 deposits and FPE were not observed in the two remaining patients in group 1. Group 2 showed segmental FPE and podocyte GL3 deposits. Most patients in group 2 also showed GL3 deposits in the mesangium, endothelium, or tubular epithelium.

Conclusion: The study results showed that segmental FPE and GL3 deposits can persist in Fabry nephropathy despite ERT. In addition, segmental FPE and GL3 deposits were observed in various kidney cells in normoalbuminuric patients with Fabry disease. These findings indicated that kidney biopsies at baseline and follow-up evaluation of Fabry nephropathy are essential for timely ERT initiation and ERT response assessment.

Keywords: Enzyme replacement therapy, Fabry disease, Globotriaosylceramide, Nephropathy
**Introduction**

Fabry disease is an X-linked lysosomal storage disease caused by mutations in the gene encoding the enzyme α-galactosidase A (α-GLA gene) [1]. Deficient or absent activity of α-GLA causes progressive accumulation of undegraded glycosphingolipid products, predominantly globotriaosylceramide (GL3), within lysosomes in the cells of many tissues, including the kidney [2,3]. Fabry disease causes progressive cardiovascular, neurological, and renal complications, all potentially fatal [4-6]. Renal manifestation (proteinuria and progressive renal failure) occurs early in the course of Fabry disease and tends to be more severe in males than in females [7-9].

Enzyme replacement therapy (ERT) has been reported to slow the deterioration of renal function in patients with Fabry nephropathy [10]. However, ERT was shown in previous studies to have a limited effect on progressive renal damage when initiated at late stages [11,12]. Although kidney biopsy is one of the best methods to investigate the effect of ERT on Fabry nephropathy, the procedure is not easily performed due to its invasiveness. However, previous kidney biopsy studies have shown that long-term ERT could induce and sustain clearance of GL3 deposits from various types of kidney cells [13,14]. However, de novo or persistent podocyte GL3 accumulation and progressive foot process effacement (FPE) were reported in young patients with Fabry disease despite 3 to 5 years of ERT [15]. The authors also showed that podocyte accumulation of GL3 and FPE might be present in patients with Fabry disease without clinically evident kidney involvement (normal glomerular filtration rate [GFR] and normoalbuminuria) [15].

Due to the heterogeneous kidney biopsy findings in patients with Fabry disease, the effects of ERT on kidney pathology in patients with Fabry disease who had received ERT were investigated in the present study. In addition, the pathologic kidney findings in normoalbuminuric patients with Fabry disease who were naïve to ERT were evaluated.

**Methods**

A total of 15 patients (3 males and 12 females) with Fabry disease who underwent kidney biopsy at Pusan National University Yangsan Hospital were retrospectively investigated in this study. Fabry disease was diagnosed based on clinical manifestations and α-GLA gene mutation and/or α-GLA analysis findings. Among the cases, nine were the classical type and six were the late-onset type. The patients were divided into two groups. Group 1 (n = 9, patients No. 1–9) had received ERT (agalsidase α or β) at the time of kidney biopsy, and group 2 (n = 6, patients No. 10–15) had not received ERT at the time of kidney biopsy. The drug history for angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB), which can affect the degree of proteinuria, was also investigated.

All research and data collection processes were conducted in accordance with the Declaration of Helsinki and current ethical guidelines. This study protocol was approved by the Institutional Review Board (IRB) of Pusan National University Yangsan Hospital (No. 05-2020-226). The need for informed consent was waived by the IRB due to the retrospective nature of the analysis and only anonymized information contained in medical charts and records was used.

The urinary albumin-to-creatinine ratio and protein-to-creatinine ratio were measured in morning urine samples (means of two consecutive samples). An albumin-to-creatinine ratio of <30 mg/g was considered to indicate normoalbuminuria. Albumin-to-creatinine ratios of 30–300 mg/g and >300 mg/g were considered to indicate microalbuminuria and macroalbuminuria, respectively. Renal function was measured using the estimated GFR (eGFR). The eGFR was calculated from the serum creatinine level using the Chronic Kidney Disease-Epidemiology formula for adults [16] or the Schwartz formula for children [17]. Both eGFR values have been validated in patients with Fabry disease [18].

The kidney biopsy findings were examined by an experienced nephropathologist. For standard light microscopy, the biopsy tissue was stained with hematoxylin and eosin, periodic acid-Schiff, and silver methenamine. The biopsy specimens were evaluated using light microscopy based on changes in the glomeruli (global or focal glomerulosclerosis), tubes (tubular atrophy), interstitium (interstitial fibrosis), and vessels (hyaline change in the media). GL3 deposits in podocytes, mesangial cells, glomerular endothelial cells, and tubular epithelial cells were scored as present or absent on electron microscopy (EM). The degree of segmental FPE was evaluated using EM and expressed as the percentage of the length with FPE out of the entire length of the capillary loops.
Results

Table 1 shows the clinical features of the 15 patients with Fabry disease (three males and 12 females). All patients underwent renal biopsy while receiving ERT (group 1, patients No. 1–9) or before ERT initiation (group 2, patients No. 10–15). The age range was 19 to 58 years in group 1 and 11 to 66 years in group 2. Most patients had acroparesthesia, vortex keratopathy, and angiokeratoma of various degrees. Most patients in group 1 showed improved clinical symptoms, such as acroparesthesia (pain score from 2.8 ± 1.9 to 0.7 ± 0.7) while receiving ERT.

The baseline characteristics of the 15 patients with Fabry disease are summarized in Table 2. None of the 15 patients had diabetes mellitus. Two patients (No. 1 and 5) had hypertension. The ERT duration was 1.2 to 8.0 years in group 1. ACEI or ARB (duration, 1.0–4.5 years) was used in six of the nine patients in group 1 at the time of kidney biopsy. Conversely, neither of these drugs were used in any patient in group 2 at the time of renal biopsy. The plasma globotriaosylsphingosine (lyso-Gb3) levels were 10.4 ± 14.6 ng/mL in group 1 and 29.8 ± 54.1 ng/mL in group 2. In group 1, the mean albumin-to-creatinine ratio was 125.9 ± 179.3 mg/g (range, 5.4–530.0 mg/g). Five of the nine patients (patients No. 1, 2, 3, 7, and 8) showed normoalbuminuria. Three patients (patients No. 4, 5, and 6) showed microalbuminuria. Only one patient (patient No. 9) showed macroalbuminuria. In group 2, the mean albumin-to-creatinine ratio was 13.8 ± 5.8 mg/g (range, 6.6–21.5 mg/g). All patients in group 2 showed normoalbuminuria. The mean protein-to-creatinine ratio was 242.3 ± 254.7 mg/g (range, 43.2–731.0 mg/g) in group 1 and 81.1 ± 24.4 (range, 55.0–125.0 mg/g) in group 2. The mean eGFR was 112.9 ± 20.1 mL/min/1.73 m^2 (range, 80–137 mL/min/1.73 m^2) in group 1 and 111.0 ± 22.4 mL/min/1.73 m^2 (range, 82–137 mL/min/1.73 m^2) in group 2.

Light microscopy showed glomerular, tubular, interstitial, and vascular changes in some patients, either alone or in combination (Table 3 and Fig. 1) as follows: global glomerular sclerosis, 5 of 9 patients in group 1 and 2 of 6 patients in group 2; segmental glomerular sclerosis, 3 of 9 patients in group 1 and 2 of 6 patients in group 2; tubular atrophy, 1 of 9 patients in group 1 and 1 of 6 patients in group 2; interstitial fibrosis, 4 of 9 patients in group 1 and 3 of 6 patients in group 2; and vasculopathy, 1 of 9 patients in group 1 and 2 of 6 patients in group 2. EM showed segmental FPE and GL3 deposits in the podocytes, mesangium, endothelium, and tubular epithelium in most patients in

Table 1. Genotype and clinical features of the patients with Fabry disease at the time of kidney biopsy

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex/age (yr)</th>
<th>Classification</th>
<th>Mutation</th>
<th>Acroparesthesia</th>
<th>Vortex keratopathy</th>
<th>Angiokeratoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Female/58</td>
<td>Late onset</td>
<td>c.640-11T&gt;A</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>Female/19</td>
<td>Classic</td>
<td>c.614C&gt;T</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Female/31</td>
<td>Classic</td>
<td>c.782_delG</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Male/34</td>
<td>Classic</td>
<td>c.782_delG</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Female/56</td>
<td>Late onset</td>
<td>c.782_delG</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Male/51</td>
<td>Late onset</td>
<td>c.56T&gt;C</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Female/19</td>
<td>Classic</td>
<td>c.658C&gt;T</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>Female/21</td>
<td>Classic</td>
<td>c.56T&gt;C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Female/35</td>
<td>Classic</td>
<td>c.676T&gt;G</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Female/28</td>
<td>Classic</td>
<td>c.861G&gt;A</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>Female/11</td>
<td>Classic</td>
<td>c.1024C&gt;T</td>
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<td>+</td>
<td>+</td>
</tr>
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<td>12</td>
<td>Male/19</td>
<td>Classic</td>
<td>c.680G&gt;A</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Female/66</td>
<td>Late onset</td>
<td>c.640-11T&gt;A</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>Female/55</td>
<td>Late onset</td>
<td>c.196G&gt;C</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>15</td>
<td>Female/51</td>
<td>Late onset</td>
<td>c.272T&gt;C</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

All patients underwent renal biopsy while receiving ERT (group 1, patients No. 1-9) or before ERT initiation (group 2, patients No. 10-15).

ERT, enzyme replacement therapy
### Table 2. Baseline characteristics of the patients with Fabry disease at the time of kidney biopsy

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex/age (yr)</th>
<th>DM/HTN</th>
<th>Disease duration (yr)</th>
<th>ACEI/ARB duration (yr)</th>
<th>Plasma lyso-Gb3 (ng/mL)</th>
<th>Serum creatinine (mg/dL)</th>
<th>eGFR(^a) (mL/min/1.73 m(^2))</th>
<th>Urine ACR (mg/g)</th>
<th>Urine PCR (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Female/58</td>
<td>--/+</td>
<td>15</td>
<td>8.0</td>
<td>3.6</td>
<td>0.81</td>
<td>80</td>
<td>6.6</td>
<td>60.4</td>
</tr>
<tr>
<td>2</td>
<td>Female/19</td>
<td>--/-</td>
<td>10</td>
<td>NA</td>
<td>4.0</td>
<td>1.5</td>
<td>0.66</td>
<td>129</td>
<td>8.9</td>
</tr>
<tr>
<td>3</td>
<td>Female/31</td>
<td>--/-</td>
<td>20</td>
<td>1.5</td>
<td>4.2</td>
<td>0.65</td>
<td>119</td>
<td>11.2</td>
<td>88.0</td>
</tr>
<tr>
<td>4</td>
<td>Male/34</td>
<td>--/-</td>
<td>21</td>
<td>2.0</td>
<td>3.8</td>
<td>28.8</td>
<td>0.69</td>
<td>124</td>
<td>119.1</td>
</tr>
<tr>
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<td>--/+</td>
<td>9</td>
<td>1.6</td>
<td>4.0</td>
<td>4.2</td>
<td>0.60</td>
<td>102</td>
<td>160.0</td>
</tr>
<tr>
<td>6</td>
<td>Male/51</td>
<td>--/-</td>
<td>9</td>
<td>4.5</td>
<td>4.5</td>
<td>45.2</td>
<td>1.04</td>
<td>83</td>
<td>282.0</td>
</tr>
<tr>
<td>7</td>
<td>Female/19</td>
<td>--/-</td>
<td>8</td>
<td>NA</td>
<td>5.2</td>
<td>2.0</td>
<td>0.56</td>
<td>137</td>
<td>5.4</td>
</tr>
<tr>
<td>8</td>
<td>Female/21</td>
<td>--/-</td>
<td>9</td>
<td>NA</td>
<td>2.6</td>
<td>2.6</td>
<td>0.72</td>
<td>120</td>
<td>9.7</td>
</tr>
<tr>
<td>9</td>
<td>Female/35</td>
<td>--/-</td>
<td>19</td>
<td>1</td>
<td>1.2</td>
<td>7.6</td>
<td>0.55</td>
<td>122</td>
<td>530.0</td>
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<tr>
<td><strong>Group 2</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Female/28</td>
<td>--/-</td>
<td>14</td>
<td>NA</td>
<td>4.8</td>
<td>0.60</td>
<td>124</td>
<td>6.6</td>
<td>66.5</td>
</tr>
<tr>
<td>11</td>
<td>Female/11</td>
<td>--/-</td>
<td>5</td>
<td>NA</td>
<td>0.50</td>
<td>128(^b)</td>
<td>12.5</td>
<td>73.8</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Male/19</td>
<td>--/-</td>
<td>9</td>
<td>NA</td>
<td>111.0</td>
<td>0.67</td>
<td>137</td>
<td>13.2</td>
<td>90.0</td>
</tr>
<tr>
<td>13</td>
<td>Female/66</td>
<td>--/-</td>
<td>10</td>
<td>NA</td>
<td>3.5</td>
<td>0.72</td>
<td>88</td>
<td>19.6</td>
<td>55.0</td>
</tr>
<tr>
<td>14</td>
<td>Female/55</td>
<td>--/-</td>
<td>8</td>
<td>NA</td>
<td>1.0</td>
<td>0.53</td>
<td>107</td>
<td>9.3</td>
<td>76.2</td>
</tr>
<tr>
<td>15</td>
<td>Female/51</td>
<td>--/-</td>
<td>11</td>
<td>NA</td>
<td>3.8</td>
<td>0.83</td>
<td>82</td>
<td>21.5</td>
<td>125.0</td>
</tr>
</tbody>
</table>

All patients underwent renal biopsy while receiving ERT (group 1, patients No. 1–9) or before ERT initiation (group 2, patients No. 10–15).

ACEI, angiotensin-converting enzyme inhibitor; ACR, albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; ERT, enzyme replacement therapy; lyso-Gb3, globotriaosylsphingosine; HTN, hypertension; NA, not applicable; PCR, protein-to-creatinine ratio.

\(^a\)The eGFR was estimated using the Chronic Kidney Disease-Epidemiology formula [16] except for patient No. 11. \(^b\)The eGFR was estimated using the Schwartz formula [17].

### Table 3. Electron microscopy findings of the renal biopsy specimens from the patients with Fabry disease

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex/age (yr)</th>
<th>Global sclerosis(^a)</th>
<th>Segmental sclerosis(^a)</th>
<th>Tubular atrophy (%)</th>
<th>Interstitial fibrosis (%)</th>
<th>Vasculopathy(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Female/58</td>
<td>+ (1/21)</td>
<td>+ (1/21)</td>
<td>NA</td>
<td>&lt;20</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Female/19</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>Female/31</td>
<td>+ (1/20)</td>
<td>–</td>
<td>NA</td>
<td>&lt;20</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>Male/34</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>Female/56</td>
<td>–</td>
<td>+ (2/16)</td>
<td>NA</td>
<td>&lt;20</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>Male/51</td>
<td>+ (2/9)</td>
<td>+ (1/9)</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>Female/19</td>
<td>+ (1/12)</td>
<td>–</td>
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<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>Female/21</td>
<td>+ (1/12)</td>
<td>–</td>
<td>NA</td>
<td>NA</td>
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</tr>
<tr>
<td>9</td>
<td>Female/35</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
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<td>–</td>
<td>–</td>
<td>NA</td>
<td>NA</td>
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<td>Female/11</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>Male/19</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>&lt;20</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>Female/66</td>
<td>+ (6/16)</td>
<td>–</td>
<td>&lt;20</td>
<td>NA</td>
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<tr>
<td>14</td>
<td>Female/55</td>
<td>–</td>
<td>+ (2/27)</td>
<td>NA</td>
<td>&lt;20</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>Female/51</td>
<td>+ (1/11)</td>
<td>+ (2/11)</td>
<td>NA</td>
<td>&lt;20</td>
<td>+</td>
</tr>
</tbody>
</table>

All patients underwent renal biopsy while receiving ERT (group 1, patients No. 1-9) or before ERT initiation (group 2, patients No. 10-15).

ERT, enzyme replacement therapy; NA, not applicable.

\(^a\)Number of affected glomeruli. \(^b\)Defined as a hyaline change in the media.
groups 1 and 2 (Table 4) as follows: segmental FPE, 7 of 9 patients in group 1 and 6 of 6 patients in group 2; podocyte GL3 deposits, 7 of 9 patients in group 1 and 6 of 6 patients in group 2; mesangial GL3 deposits, 3 of 9 patients in group 1 and 5 of 6 patients in group 2; endothelial GL3 deposits, 2 of 9 patients in group 1 and 4 of 6 patients in group 2; and tubular epithelial GL3 deposits, 3 of 9 patients in group 1 and 5 of 6 patients in group 2. Notably, patients No. 1 and 7 in group 1 showed no FPE or GL3 deposits (Fig. 2). The seven remaining patients (patients No. 2, 3, 4, 5, 6, 8, and 9) in group 1 showed segmental FPE and podocyte GL3 deposits of various degrees despite ERT. Conversely, all patients in group 2 (patients No. 10–15) showed segmental FPE and podocyte GL3 deposits, indicating that segmental FPE may be present in patients with Fabry disease with no clinical signs of renal involvement (Fig. 3). Most patients in group 2 also showed GL3 deposits in the mesangium, endothelium, or tubular epithelium.

Table 4. Electron microscopy findings of the renal biopsy specimens from the patients with Fabry disease

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex/age (yr)</th>
<th>Segmental FPE (%)</th>
<th>Podocyte GL3 deposit</th>
<th>Mesangial GL3 deposit</th>
<th>Endothelial GL3 deposit</th>
<th>Tubular epithelial GL3 deposit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>1</td>
<td>Female/58</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>Female/19</td>
<td>10</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>Female/31</td>
<td>30</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>9</td>
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<td>Female/51</td>
<td>30</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

All patients underwent renal biopsy while receiving ERT (group 1, patients No. 1-9) or before ERT initiation (group 2, patients No. 10-15). FPE, foot process effacement; ERT, enzyme replacement therapy; GL3, globotriaosylceramide; NA, not applicable.
Discussion

Progressive nephropathy (Fabry nephropathy) is the main feature of Fabry disease [8]. In the kidney, progressive deposits of GL3 affect all types of kidney cells, including tubular, glomerular, endothelial, and vascular smooth muscle cells [19–21]. In untreated patients with classical mutations, Fabry nephropathy leads to end-stage renal disease from the third to the fifth decade of life [8]. Approximately 30% to 35% of females with Fabry disease have proteinuria and 1% to 4% have end-stage renal disease [9]. Therefore, kidney biopsy in Fabry nephropathy could be a useful tool for investigating its mechanism, progression, and treatment. However, studies in which the pathologic lesions of Fabry nephropathy have been investigated are limited.

In the present study, typical findings of GL3 accumulation were observed in the podocytes, mesangium, endothelium, and tubular epithelium in the majority of patients with Fabry disease. In group 1, seven of the nine patients (patients No. 2, 3, 4, 5, 6, 8, and 9) showed persistent GL3 deposits in the podocytes and segmental FPE despite 1.2 to 4.0 years of ERT. Only two patients (patients No. 1 and 7) showed no GL3 deposits and segmental FPE with 8.0 and 5.2 years of ERT, respectively. At the time of kidney biopsy, the average plasma lyso-Gb3 level in these two patients was 3.6 ng/mL, which was lower than the lyso-Gb3 level of 13.4 ng/mL in the other seven patients. Although the ERT duration was relatively shorter in the seven patients (3.4 years) than in the two patients (6.6 years), these observations indicated that ERT might not prevent or clear the GL3 deposits in the kidneys of some patients with Fabry disease. Conversely, in the present study, patients in group 1 showed lower rates of segmental FPE and GL3 deposits in podocytes, mesangium, endothelium, and tubules than patients in group 2, indicating this observation may be due to the effects of ERT. Regarding the effect of ERT on GL3 deposits in the kidney, inconclusive results have been shown in previous studies. Skrunes et al. [22] showed in 12 patients with Fabry disease that long-term ERT for up to 14 years can result in the reduction of podocyte GL3 deposits,
which correlated with the cumulative ERT dose. However, in the present study, limited clearing of arterial GL3 deposits raised concerns regarding the long-term vascular effects of ERT [22]. Lubanda et al. [23] showed that a lower dose of ERT might be sufficient in some but not all patients with Fabry disease to maintain GL3 clearance in various kidney cell types. Tøndel et al. [15] reported the de novo appearance of GL3 deposits and segmental FPE in patients with Fabry disease who showed no FPE at baseline kidney biopsy after 3 years of ERT. The authors also reported persistent GL3 deposits and progression of segmental FPE in two patients with Fabry disease treated with ERT for 5 years [15]. These findings, including the results in the present study, indicate that multifactorial mechanisms might be involved in progressive Fabry nephropathy in addition to GL3 accumulation in the kidney cells.

The most important finding observed in the present study was the segmental FPE in the patients with Fabry disease who had normalalbuminuria and were naïve to ERT and ACEI/ARB (patients No. 10–15). To date, early segmental FPE in normoalbuminuric patients with Fabry disease has been reported in only a few studies [15,24,25]. Podocytes are highly specialized epithelial cells that cover the glomerular basement membrane (GBM) with numerous interdigitating foot processes [26]. The GBM and podocytes are key components of the glomerular filtration barrier. Proteinuria is associated with significant changes in podocyte architecture, which include loss of the podocyte FPE [26]. FPE is regarded as a stereotypical reaction of podocytes to injury or damage. Although whether FPE in Fabry nephropathy is secondary to GL3 deposits or associated with mechanisms other than GL3 deposition is unknown, a strong association between podocyte GL3 accumulation and FPE was demonstrated in a previous study. Najafian et al. [27] recently showed that podocyte GL3 volume was associated with podocyte injury and loss, evidenced by increased foot process width and decreased podocyte number density. The authors also showed that increased podocyte GL3 volume and foot process width were associated with increased urinary protein excretion as well as decreased GFR. Finally, they suggested that podocyte injury plays an important role in the progression of Fabry nephropathy and a need for ERT before critical podocyte loss occurs. The results of the present study also indicated that FPE might be an early sign of Fabry nephropathy, preceding the onset of overt albuminuria. Because overt albuminuria has been reported a strong predictor and to indicate the irreversible stage of progressive renal dysfunction in proteinuric renal disease as well as Fabry nephropathy, these early morphological changes could be clinically significant as a prealbuminuric marker of Fabry nephropathy. However, the results need to be interpreted cautiously because biopsy sampling errors may be present; possibly segmental FPE and GL3 deposition could be missed during biopsy sampling. Thus, further kidney biopsy studies that include a large number of patients with Fabry disease are needed to confirm the present and previous study results.

This study had several limitations. First, due to the retrospective design, comparing the kidney biopsy findings before and after ERT in the same patient was not possible. Thus, longitudinal studies in which the kidney biopsy findings are compared before and after ERT in each patient are necessary to determine if the ERT could lead to amelioration of Fabry nephropathy. Second, in the female patients with Fabry disease, the clinical characteristics are heterogeneous due to skewed X-chromosome inactivation. In the present study, most patients were females. The situation for females would be more complex because their podocyte involvement and injury are affected by mosaicism resulting from skewed X-chromosome inactivation. Therefore, in the future, performing X-chromosome inactivation analysis will help to understand the association between the X-chromosome inactivation and degree of kidney injury in female patients with Fabry disease.

Despite the small number of patients with Fabry disease included in the study, important clinical implications were determined. The study results showed that GL3 deposits in the kidney and segmental FPE may be present in patients with Fabry disease experiencing improved clinical symptoms while receiving ERT. This observation indicates the suboptimal effect of the ERT regimen in these patients. Although the only clearance of GL3 deposits confers unequivocal long-term prevention or stabilization of Fabry nephropathy [15], the study results showed that ERT response may be asynchronous between clinical symptoms, such as acroparesthesia and Fabry nephropathy. Thus, the important role of kidney biopsy in the assessment of the response to ERT was proven in this study. In addition, GL3 deposits in the kidney and segmental FPE in normoalbuminuric patients with Fabry disease were observed. To
date, albuminuria and GFR reduction are the only clinical markers of renal dysfunction in Fabry disease [8]. Thus, the initiation of ERT is often delayed until proteinuria or GFR reduction occurs, during which the reversibility of renal damage is already difficult to achieve [8]. Generally, kidney biopsy is not recommended for kidney disease that does not show proteinuria [28]. However, the results of the present study showed that kidney biopsy is essential even in patients with Fabry disease who present with normal-albuminuria to confirm kidney involvement of Fabry disease and initiate timely ERT intervention.

In conclusion, segmental FPE and GL3 deposits can be persistent in Fabry nephropathy despite ERT, indicating the response to ERT could be asynchronous between clinical symptoms and Fabry nephropathy. In addition, segmental FPE and GL3 deposits were observed in various kidney cell types in normoalbuminuric patients with Fabry disease, indicating that albuminuria is not sufficiently sensitive to detect early kidney injury in Fabry nephropathy. These results indicate that kidney biopsies at baseline and follow-up evaluation of Fabry nephropathy are essential for timely ERT initiation and ERT response assessment.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

This research was supported by grants from the National Research Foundation of Korea (2019R1F1A1058972).

**Acknowledgments**

We thank the patients and their families for participating in the study.

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Background: A healthy microbiome helps maintain the gut barrier and mucosal immune tolerance. Previously, we demonstrated that acute kidney injury (AKI) provoked dysbiosis, gut inflammation, and increased permeability. Here, we investigated the renoprotective effects of the probiotic *Bifidobacterium bifidum* BGN4 and the underlying mechanisms thereof.

Methods: C57BL/6 mice were subjected to bilateral renal ischemia-reperfusion injury (IRI) or sham operation. In the probiotic-treated group, BGN4 was administered by gavage once daily, starting 2 weeks before injury.

Results: Administration of BGN4 significantly increased gut microbiome diversity and prevented expansion of the *Enterobacteriaceae* and *Bacteroidetes* that were the hallmarks of AKI-induced dysbiosis. Further, BGN4 administration also significantly reduced other IRI-induced changes in the colon microenvironment, including effects on permeability, apoptosis of colon epithelial cells, and neutrophil and proinflammatory macrophage infiltration. Mononuclear cells co-cultured with BGN4 expressed significantly increased proportions of CD103⁺/CD11c⁺ and CD4⁺CD25⁺ Treg cells, suggesting a direct immunomodulatory effect. BGN4 induced Treg expansion in colon, mesenteric lymph nodes (MNL), and kidney. BGN4 also reduced CX₃CR₁⁺Ly6C⁺high monocyte infiltration and interleukin (IL)-17A suppression in the small intestine, which may have attenuated AKI severity, kidney IL-6 messenger RNA expression, and AKI-induced liver injury.

Conclusion: Prior supplementation with BGN4 significantly attenuated the severity of IRI and secondary liver injury. This renoprotective effect was associated with increased Foxp3 and reduced IL-17A expression in the colon, MNL, and kidney, suggesting that BGN4-induced immunomodulation might contribute to its renoprotective effects. Probiotics may therefore be a promising strategy to reduce AKI severity and/or remote organ injury.

Keywords: Acute kidney injury, BGN4, Immunology, Microbiota, Probiotics
Introduction

Acute kidney injury (AKI) is a frequent complication in intensive care settings, especially among high-risk patients, including those with underlying comorbidities [1–3]. The occurrence of AKI is associated with an increased length of hospital stay, and greater mortality and economic burden. A recent meta-analysis found AKI to be a coronavirus disease 2019-associated complication, with an estimated incidence of 8.4%; 3.6% of patients needed renal replacement therapy, and these patients had a 13-fold increased risk of mortality [4].

The human gut harbors more than 100 trillion microbial cells that maintain complete symbiosis with the host and play an important role in the development and shaping of the immune system [5,6]. The symbiotic relationship is altered in diverse pathological conditions and dysbiosis may be a key event in disease pathogenesis, given AKI provokes intense systemic inflammation with frequent distant organ injury [7,8]. Crosstalk between the kidney and gut in AKI has been postulated; however, unlike research into the pathogenesis of diabetes, obesity, or inflammatory bowel disease, there have been only a few studies that demonstrated links between the kidney and gut in AKI. Recently, we demonstrated a bidirectional relationship between the kidney and gut during AKI [9], such that AKI-induced dysbiosis was associated with increased gut permeability and bacterial translocation, intestinal inflammation, and reduced concentrations of short-chain fatty acids (SCFAs). Prior depletion of the gut microbiota by oral antibiotics resulted in less severe kidney injury and this renoprotective effect was associated with reduced intestinal Th17 cells and Th1 responses, along with expansion of Treg cells and M2 macrophages. We also demonstrated that intestinal microbiota act as an important modifier of post-AKI severity by showing that colonizing germ-free mice with microbiota harvested from post-AKI mice worsened kidney IRI, suggesting that the intestinal microbiota might be a novel therapeutic target [9,10].

Probiotics are “live microorganisms” that have beneficial effects and have shown promising results in several chronic inflammatory conditions [8]. However, despite recent reports indicating possible renoprotective effects of probiotics or bacterial products, there have been few studies of the use of pre- or probiotics in AKI [11–15].

In this study, we investigated whether administration of probiotics had renoprotective effects in IRI-induced AKI. We chose *Bifidobacterium bifidum* BGN4 (BGN4) as the test probiotic. The genus *Bifidobacterium* is the predominant component of the intestinal microbiota and supports various functions including carbohydrate fermentation, vitamin synthesis, or immune modulation [16–18]. The effects of BGN4 on the severity of AKI and distant organ injury, as well as on the intestinal microenvironment, were investigated.

Methods

Experimental animal model

Six-week-old male C57BL/6 mice were obtained from Orient Bio Laboratory Animal Incorporation (Seoul, Korea). The mice were housed in a level 1 specific-pathogen-free facility, given *ad libitum* access to sterile food and water. The mice were randomized to the probiotic supplement + IRI or IRI-only groups. Probiotics were provided by BIFIDO Co. (Seoul, Korea), and BGN4 at a concentration of $2 \times 10^9$ was administered by gavage to each mouse in the probiotic supplement + IRI group once a day for 5 days a week (Fig. 1A). Two weeks later, the mice were subjected to IRI, for which the mice were administered intraperitoneal anesthesia using 50 mg/kg ketamine and 0.04 mL/g xylazine and then subjected to bilateral renal pedicle clamping for 25.5 minutes through a flank incision. A sham operation was also performed in a group of mice that underwent anesthesia and flank incision. All mice were placed on a heating pad and monitored until they woke up.

This study was approved by the Institutional Review Board of the Korea University College of Medicine Laboratory Animal Research Center. The study protocol was approved by the Animal Care Committee of Korea University (No. KOREA-2016-0260), and all animal experiments were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and the NIH publication “Principles of Laboratory Animal Care.”

Serum chemistry and histological analyses

Plasma creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) concentrations were measured using a Beckman...
Figure 1. Microbiome and colon environmental alteration in acute kidney injury with BGN4 supplementation. (A) Treatment and experiment component of analysis using number of operational taxonomic units in each group. (C) Microbiome diversity index analysis in terms of richness and evenness using Simpson’s methods. (D) Quantitative analysis of the microbial communities at the genus level was performed using 16S RNA isolated from stool samples. Only the genera with frequencies of >1% and with significant differences between the groups were included. (E) Western blot analyses of claudin-1, occludin, and β-actin expression in the colon. (F) Western blot band intensities of claudin-1 and occludin in the colon normalized to those of β-actin. (G) Representative images of colon apoptosis using deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining of the colon 1 day after IRI with semiquantitative comparison of the colon apoptosis-positive cells in each group. The number of positive cells per high-power field (HPF) was compared (×200). (H) Representative images of Ly6G and F4/80 inflammatory cell infiltration into the colon (×100). Semiquantitative comparison of each group according to the number of positive cells. (I) Intestinal permeability was measured by detecting the activity of fluorescein-isothiocyanate (FITC) 4 hours after the oral administration of FITC-dextran 1 day after IRI. n = 3–5 per group. *p < 0.05 compared with the sham vs. IRI. #p < 0.05 compared with the IRI vs. BGN4 + IRI. (Continued to the next page)
ent grades assigned as follows: no visible necrosis (grade 0), 0%–25% (grade 1), 25%–50% (grade 2), 50%–75% (grade 3), or 75%–100% (grade 4) necrosis. To detect macrophage/neutrophil infiltration, the kidney and colon tissues were stained with monoclonal antibodies against F4/80 (1:100; mch-497-GA; Bio-Rad Laboratories, Hercules, CA, USA) and Ly6G (1:200; 14-59-85; eBioscience, San Diego, CA, USA). To detect regulatory T cells (Tregs), immunohistochemical staining of the kidney and colon was performed using Foxp3 (1:1,000, ab215206; Abcam, Cambridge, UK). The mean numbers of positive cells per 10 high-power fields were compared. Colon epithelial cell apoptosis was assessed after terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) of epithelial cells by counting TUNEL-positive cells in 8–10 high-power fields (×200). Liver tissues obtained from each group were stained using hematoxylin and eosin (H&E). Images were collected at 20× magnification using a Slide Scanner automated image capture system (Axio Scan Z1; Zeiss Korea, Seoul, Korea).

**Western blot analyses**

Proteins were extracted from whole-colon tissue samples using the bicinchoninic acid method, and the expression
of tight-junction proteins was examined using anti-mouse antibodies against claudin-1 (1:200; ab15098; Abcam) and occludin (1:200; ab168986; Abcam). The band intensities were measured using Image Studio Lite software (LI-COR; Biosciences, Lincoln, NE, USA). The target protein levels were normalized to those of β-actin.

**In vitro analyses**

Splenocytes $1.5 \times 10^7$ per well were incubated with BGN4 in 2% fetal bovine serum for 72 hours in a 5% CO$_2$ incubator at 37°C, and CD103$^+$CD11c$^+$ or CD4$^+$CD25$^+$ Treg cells (BioLegend, San Diego, CA, USA) were evaluated.
Intestinal permeability

Fluorescein-isothiocyanate (FITC)-conjugated dextran (FITC-dextran; catalog number FD4; Sigma-Aldrich, St. Louis, MO, USA) dissolved in phosphate-buffered saline (100 mg/mL) was administered by gavage (44 mg/100 g) after overnight water starvation, and the fluorescence activity of FITC in the blood was measured 4 hours later after first anesthetizing the mice. This method was performed as described previously [7].

Flow cytometry

Flow cytometric analyses of leukocytes in the mesenteric lymph nodes (MNL), spleen, kidney, and intestine were performed. The cells were stained with fluorochrome-labeled monoclonal antibodies (anti-CD4, anti-CD25, Fixable Viability Dye, anti-Foxp3, anti-CD45, anti-CD11c, anti-CD103, anti-CX3CR1, and anti-Ly6C; all eBioscience, BioLegend, or BD Biosciences [Franklin Lakes, NJ, US]) and analyzed using a four-color flow cytometer (FACSCanto II; BD Biosciences) and FlowJo software (Tree Star Inc., Ashland, CA, USA).

Real-time reverse transcription–polymerase chain reaction

For detection of Foxp3 messenger RNA (mRNA) expression, total RNA was purified using TRIzol extraction reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s protocol, and complementary DNA was synthesized using standard procedures. Real-time polymerase chain reaction was performed in an iCycler IQ Real-Time PCR Detection System Bio-Rad Laboratories (Hercules, California, USA) using the iQ SYBR Green Supermix (Bio-Rad Laboratories) for Foxp3. We used 18S ribosomal RNA (rRNA) as the reference gene (RT2 PCR Primer Set; Applied Biosystems, Foster City, CA, USA), and the fold difference was compared to that of the mice subjected to sham operation.

Stool microbiome analyses

More than two stool pellets per mouse were obtained and stored at −70°C. Microbiome analyses of pyrosequenced 16S rRNA were completed using the Quantitative Insights Into Microbial Ecology 2 (QIIME 2) database, which is a next-generation microbiome bioinformatics platform [19].

Statistical analyses

The data are presented as the mean ± standard error of the mean. An unpaired t test and one-way analysis of variance (with Bonferroni post hoc test) were used for comparisons between two and three or more groups, respectively. A p-value of <0.05 was considered statistically significant. The data were analyzed using GraphPad Prism version 8.5 (GraphPad Software Inc., La Jolla, CA, USA).

Results

BGN4 modifies acute kidney injury-induced dysbiosis and enhances mucosal barrier function

The BGN4-supplemented group showed distinctive microbiome features in the principal coordinate analyses (PCoA) (Fig. 1B). One day after kidney IRI, the gut microbiome was characterized by relative expansion of Enterobacteriaceae and Bacteroidaceae families despite comparable diversity index (Fig. 1C). In contrast, BGN4 administration for 2 weeks prior to IRI increased microbiome evenness as measured using Simpson’s diversity index, but did not significantly increase diversity (Fig. 1D), and also prevented the relative expansion of both marker species. These changes in the microbiome of the BGN4-supplemented group were associated with preservation of gut barrier integrity following IRI. Expression of the barrier-enhancing junctional protein claudin-1 was reduced after IRI and was partially restored in BGN4-supplemented mice, although the expression of junctional protein occluding-1 did not change (Fig. 1E, F).

Apoptosis of colon epithelial cells was assessed semi-quantitatively using TUNEL staining. The number of apoptotic cells increased significantly in IRI mice, but was reduced in BGN4-supplemented mice (Fig. 1G). In addition, the increase in the numbers of infiltrating Ly6G+ neutrophils and F4/80+ macrophages occurring after kidney IRI was significantly reduced in BGN4-pretreated mice (Fig. 1H). And finally, the increase in gut permeability after IRI was partially reversed in BGN4-pretreated mice (Fig. 1I).
BGN4 attenuates severity of ischemia-reperfusion injury and distant organ injury

Administration of the probiotic BGN4 for 2 weeks prior to IRI significantly attenuated kidney injury. Post-IRI tubular injury scores, as well as the number of neutrophils and macrophages and kidney interleukin (IL)-6 mRNA expression were significantly reduced in BGN4-pretreated mice (Fig. 2A, B). Plasma creatinine concentrations showed that BGN4 supplement mitigated IRI severity. We also found that BGN4 attenuated kidney-IRI-induced liver injury and reduced plasma LDH concentrations compared with those mice from the IRI-only group (Fig. 2C, D). The AKI-related distant organ injury was evaluated using liver histology H&E staining and plasma AST and ALT concentrations (Fig 2E). In BGN4-supplemented mice, no significant morphological alterations were noted in the liver, and the plasma AST and ALT concentrations indicated AKI-related liver damage was attenuated.

BGN-induced immunomodulatory effects might contribute to renoprotective effects

To investigate the mechanisms underlying the renoprotective effects of BGN4 pretreatment in experimental kidney IRI, we assessed the direct impact of BGN4 on immune cell function in splenocytes co-cultured with BGN4. This in vitro experiment showed that BGN4 facilitated expansion of CD103+ CD11c+ regulatory dendritic cells and of CD4+ CD25+ regulatory T cells (Fig. 3A). Immunohistochemical examinations indicated increased kidney and colon Foxp3 staining in BGN4-pretreated mice compared to INI-only mice, and Foxp3 mRNA expression increased in both the colon and kidneys, suggesting that BGN4-induced Treg expansion may provide renoprotective effects in IRI (Fig. 3B, C). An increase in Foxp3+ Treg cells as measured using flow cytometry was evident in the kidney, MNL, and colon of BGN4-pretreated mice, consistent with the ameliorated CXCR4+Ly6CintLy6C+CD11b+ monocyte infiltration (Fig. 3D, E). Flowcytometry analysis showed CD4+IL-17A+ T17 cell fraction was increased in IRI compared with sham, whereas the same fraction was decreased in the BGN4 + IRI. This result demonstrated BGN4 has a potential effect on the regulation of the Th 17 pathway (Fig. 3F).

Discussion

Accumulating evidence suggests that the gut microbiota and their metabolites regulate inflammation, oxidative stress, and fibrosis, and play pivotal roles in host physiology and pathology in kidney disease through activation of the gut-kidney axis. Here, we investigated the benefits of prior probiotic supplementation on AKI and the distant organ effects of AKI, and on the gut environment, in particular. Pretreatment of mice with BGN4 protected against AKI by reinforcing gut permeability and modulated the immune response to attenuate the severity of the AKI and distant liver injury.

Bifidobacterium is the dominant gut microorganism, accounting for more than 80% of the microbiota in the intestinal tract of healthy breast-fed infants; B. bifidum is the second most abundant species among Bifidobacterium. In contrast to breast-fed infants, formula-fed infants have a significantly lower prevalence of Bifidobacterium species. In addition, the abundance of B. bifidum decreases as an individual grows older. Various experiments have demonstrated that B. bifidum has a convincing beneficial effect. Ku et al. [17] demonstrated the immunomodulatory effect of BGN4 and its pharmaceutical application in a previous study. The BGN4 bacterium was first isolated from the feces of a breast-fed infant by Professor Geun Eog Ji and has since been used as a probiotic since 2000 [17,18]. In this study, BGN4 supplementation enriched the gut microbiome as shown by diversity evenness. Simpson’s microbiome diversity index is an indicator that considers both the numbers of microbiome components and the abundance of the components. Along with the change in the microbiome, BGN4 supplementation induced changes in the intestinal environment toward a more renoprotective condition. Notably, BGN4 supplementation appeared to maintain colon barrier function by reinforcing tight-junction proteins, as well as reducing colon epithelial cell apoptosis and attenuating mucosal barrier disruption.

This study confirmed that the beneficial effects of probiotics are a function not only of prevention of pathogenic bacterial spread but also of direct strengthening of the intestinal barrier capacity. Preconditioning with BGN4 supplements can make the intense colon environment, protect the distant organ injury from AKI. In addition to directly strengthening mucosal barrier function, BGN4 produced
Figure 2. BGN4 attenuated ischemia-reperfusion injury (IRI) and distant organ injury. (A) Representative images of PAS-stained kidney tissue sections (×100), indicating the Ly6G- and F4/80-positive cells per high-power field (HPF). Tubular injury score, numbers of Ly6G and F4/80 positive cells of each group were compared. (B) The relative fold differences of kidney interleukin (IL)-6 messenger RNA expression were compared. (C) Serum creatinine concentrations in each group were compared. (D) Serum lactate dehydrogenase concentrations of each group were compared. (E) Live histology for each group as assessed using H&E staining (×200). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations of each group were compared. n = 3–5 per group. *p < 0.05 compared with the sham vs. IRI. **p < 0.05 compared with the IRI vs. BGN4 + IRI. (Continued to the next page)
immunomodulatory effects in the colon. The immunomodulatory effect of probiotics has been well studied in gastrointestinal disease like irritable bowel syndrome and allergic dermatitis. Previously, BGN4 was shown to induce macrophage differentiation [20], and to have anti-allergic effects by decreasing antigen-specific serum immunoglobulin E and IL-4 and increasing IL-12 concentrations [21]. We discovered another immune modulatory effect of BGN4, which was expansion of the colon regulatory T cell population. The protective role of regulatory T cell expansion has been examined in previous studies [9,22,23]. Administration of probiotics prior to IRI in the experimental setting may not be directly applicable to management of acute renal disease in a clinical setting. We did not conduct experiments assessing probiotic supplementation after IRI, and additional studies to assess the therapeutic potential of post-IRI BGN4 supplementation on IRI are needed.

Bifidobacteria are commensal bacteria that produce SCFAs. Supplementation with SCFAs attenuates kidney damage after IRI, colitis, and lung injury [12]. Supplementation with acetate-producing bacterial probiotics such as *Bifidobacterium adolescentis* and *Bifidobacterium longum* in AKI

Figure 2. (Continued from the previous page)
**Figure 3. BGN4 modulates immune response in ischemia-reperfusion injury (IRI).** (A) Flow cytometry of splenocyte cultured with BGN4 for 72 hours. Comparison of percent CD11c+CD103+ regulatory dendritic cells and CD4+CD25+ regulatory T cells. (B) Representative images of kidney and colon Foxp3 staining (×100), positive cells per high-power fields (HPF). Positive cells of each group were compared and shown a semiquantitative graph. (C) Relative fold difference of kidney and colon Foxp3 messenger RNA expression. (D) Flow cytometry of colon, mesenteric lymph node (MNL), and kidney of Foxp3+CD4+ regulatory T cells in each group were compared. (E) Flow cytometry of colon CX3CR1+Ly6Clo intermediate Ly6C+high monocyte. (F) Flow cytometry of small intestine interleukin (IL)-17A+ cells in each group were compared. n = 3–7 per group. *p < 0.05 compared with the sham vs. IRI. †p < 0.05 compared with the IRI vs. BGN4 + IRI. FITC, fluorescein-isothiocyanate; IHC, immunohistochemistry. (Continued to the next page)
Figure 3. (Continued from the previous page)
has also been shown to attenuate AKI-induced increases in serum creatinine \[12\]. Although we could not measure serum SCFAs (due to lack of facilities) in our study, we found that BGN4 pretreatment exerted a renoprotective effect by inhibiting inflammation and colon cell apoptosis. Intestinal permeability was reduced in mice supplemented with BGN4 prior to renal IRI, indicating that probiotics strengthen the colon mucosal barrier function. Post-IRI systemic inflammation and harmful effect were blocked as the colon barrier function was enhanced by probiotic supplementation. The serum LDH concentration is a marker of tissue damage and has been reported to be directly correlated with cardiovascular mortality and all-cause mortality in metabolic syndrome patients \[24\]. The increase in serum LDH concentrations occurring after AKI was also attenuated with BGN4 preconditioning. These findings demonstrated the beneficial effects of probiotics may in part be a function of reduced systemic inflammation. Nishida et al. \[25\] demonstrated that the renoprotective effect of recombinant thioredoxin-1 and albumin fusion protein in IRI also influenced AKI-associated distant organ injury, including injury to the lung and liver. Similarly, the renoprotective effect of probiotic supplementation reduced AKI-related liver injury in our study. Although the liver histology as assessed using H&E staining did not detect distinct histological differences between the IRI-only and BGN4-supplemented groups, the increase in plasma AST and ALT concentrations after IRI was attenuated in the BGN4 + IRI group. As probiotics function to strengthen the colon barrier and modulate the immune system, probiotics such as BGN4 may play a protective role in AKI-induced distant organ damage and in other diseases.

In conclusion, AKI-induced gut barrier disruption and colon inflammation may be among the mechanisms involved in systemic inflammation, kidney injury, and other remote organ injuries. Probiotic BGN4-mediated renoprotective effects may have occurred due to strengthening of the gut barrier and modulation of mucosal immune tolerance mechanisms. Probiotics may be a promising strategy to reduce the severity of AKI and/or remote organ injury.

**Conflicts of interest**

All authors have no conflicts of interest to declare.
Funding
This study was supported by a Young Investigator Research Grant from the Korean Nephrology Research Foundation (KSN 2018), and a grant from the Korea University Anam Hospital, Seoul, Republic of Korea (K2014071).

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References


Renal outcomes of laparoscopic versus open surgery in patients with rectal cancer: a propensity score analysis

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Background: A laparoscopic approach is widely used in abdominal surgery. Although several studies have compared surgical and oncological outcomes between laparoscopic surgery (LS) and open surgery (OS) in rectal cancer patients, there have been few studies on postoperative renal outcomes.

Methods: We conducted a retrospective cohort study involving 1,633 patients who underwent rectal cancer surgery between 2003 and 2017. Postoperative acute kidney injury (AKI) was diagnosed according to the serum creatinine criteria of the Kidney Disease: Improving Global Outcomes classification.

Results: Among the 1,633 patients, 1,072 (65.6%) underwent LS. After matching propensity scores, 395 patients were included in each group. The incidence of postoperative AKI in the LS group was significantly lower than in the OS group (9.9% vs. 15.9%; p = 0.01). Operation time, estimated blood loss, and incidence of transfusion in the LS group were significantly lower than those in the OS group. Cox proportional hazard models revealed that LS was associated with decreased risk of postoperative AKI (hazard ratio [HR], 0.599; 95% confidence interval [CI], 0.402–0.893; p = 0.01) and postoperative transfusion was associated with increased risk of AKI (HR, 2.495; 95% CI, 1.529–4.072; p < 0.001). In the subgroup analysis, the incidence of postoperative AKI in patients with middle or high rectal cancer who underwent LS was much lower than in those who underwent OS (HR, 0.373; 95% CI, 0.197–0.705; p = 0.002).

Conclusion: This study showed that LS may have a favorable effect on the development of postoperative AKI in patients with rectal cancer.

Keywords: Acute kidney injury, Colorectal surgery, Laparoscopy, Rectal neoplasms
Acute kidney injury (AKI) is a clinical syndrome that affects kidney structure and function. It is characterized by abrupt loss of kidney function [1]. AKI is a serious complication that increases economic costs as well as the risk of mortality and morbidity [2,3]. The incidence of AKI among hospitalized patients and intensive care unit patients has been reported to be 3%–7% and 22%, respectively [3–5]. AKI is a common postoperative complication and postoperative AKI accounts for approximately 30% to 40% of in-hospital AKI [6]. Postoperative AKI is associated with prolonged hospital stay, increased risk of mortality, and progression to chronic kidney disease [7,8].

Laparoscopic surgery (LS) is increasingly being performed for the treatment of colorectal cancer in several centers. Its advantages include less pain, reduced intraoperative blood loss, and shorter recovery time [9]. However, there are concerns about increased intraabdominal pressure resulting from pneumoperitoneum. In patients with liver resection surgery, postoperative AKI was more common in the open surgery (OS) group than in the LS group [10]. Another study involving patients who underwent pylorus-preserving pancreaticoduodenectomy revealed that there were no significant differences in the incidence of AKI between LS and OS groups [11]. Although several studies have compared surgical and oncological outcomes between LS and OS in rectal cancer patients, there are few studies focusing on postoperative renal outcomes [12,13].

Therefore, we conducted a large retrospective cohort study to compare renal outcomes following LS and OS in patients with rectal cancer. We hypothesized that LS might have a positive effect on postoperative renal outcomes in rectal cancer patients. We performed propensity score matching analysis to minimize confounding biases.

Methods

Study design and population

This retrospective study included all patients who underwent rectal cancer surgery at the Seoul National University Bundang Hospital (Seongnam, Korea) from May 2003 to May 2017. Among the 1,678 patients identified, 45 were excluded for the following reasons: underwent local excision (n = 31) or emergency surgery (n = 4); had end-stage renal disease (n = 4); and had insufficient data (n = 6). Finally, 1,633 patients were enrolled in this study (Fig. 1).

The Institutional Review Board (IRB) of Seoul National University Bundang Hospital approved this study (No. B-1707/411-105). The requirement of written informed consent was waived by the IRB because of the retrospective nature of this study.

Data collection and definitions

Electronic medical records of the study population were reviewed to retrieve patients’ baseline characteristics, laboratory findings, and intraoperative data. Age, sex, body mass index [14], comorbidities (hypertension and diabetes mellitus), stage of malignancy, neoadjuvant chemotherapy, previous operation history, American Society of Anesthesiologists (ASA) physical status (PS) classification [15], and years of surgery were included in the baseline characteristics. Laboratory findings included hemoglobin, creatinine (Cr), sodium, potassium, and total CO₂. Intraoperative data included operation time, estimated blood loss (EBL) [16], and intraoperative hypotension. We used the code of the In-
International Classification of Disease, 10th Revision to identify underlying comorbidities. Intraoperative hypotension was defined as systolic blood pressure of ≤90 mmHg or use of inotropic agents such as dopamine, norepinephrine, or phenylephrine during the surgery. Operation time of >240 minutes was considered to be a long operation time [17]. Patients were considered to have low rectal cancer when the lower tumor margin was within 6 cm from the anal verge.

Outcomes

The primary objective of this study was to compare the incidence of postoperative AKI following LS and OS in patients with rectal cancer. AKI was defined as an absolute increase in serum Cr of ≥0.3 mg/dL over the baseline value or ≥1.5 times higher than the baseline value, according to the serum Cr criteria in the Kidney Disease: Improving Global Outcome (KDIGO) guidelines [1]. We defined the baseline serum Cr value as the lowest serum Cr level measured <90 days before the surgery. We used the peak serum Cr level, which is the highest Cr value measured <14 days after surgery, to identify the stage of AKI according to the KDIGO criteria [18,19]. Severe AKI was defined as stage 2 or stage 3 AKI according to the KDIGO criteria. We also evaluated AKI recovery three months after postoperative AKI. We defined AKI recovery as a return of the serum Cr to a value less than 1.20 times the baseline serum Cr level [20]. The lowest serum Cr value measured <90 days after the AKI event was used. We also evaluated the 5-year overall survival rate. As many mortality events occurred outside the study hospital, we reviewed the national death database of the Ministry of Interior and Security of Korea to identify the outcomes. Other outcomes included hospital stay, renal replacement therapy in-hospital days, and postoperative intensive care unit admission.

Statistical analysis

We applied propensity score matching analysis to minimize the influence of potential confounding biases and to increase comparability between the LS and OS groups. Among factors that can affect postoperative AKI, we included potentially explanatory variables that can be found through electronic medical records. The following variables were included to calculate the propensity scores using a multivariate logistic regression model: age, sex, body mass index, ASA PS classification, neoadjuvant chemotherapy, previous operation history, tumor distance from anal verge, stage of cancer, hemoglobin, Cr, sodium, potassium, total CO₂, and operation year. A 1:1 propensity score matching method was applied based on the greedy 8-1-digit matching algorithm. Propensity score matching analysis was conducted using SAS version 9.1.3 (SAS Institute, Cary, NC, USA).

Mean ± standard deviation was calculated for continuous variables. Categorical variables were reported as numbers and percentages of participants. The intergroup comparison of numerical data was performed using the Student t test. The Pearson chi-square test was used to compare categorical data. The Cox proportional hazard regression analysis was performed to identify independent associations between the type of surgery and postoperative AKI in patients with rectal cancer. Survival curves were assessed using the Kaplan-Meier method and the statistical significance was estimated using the log-rank method. The additive interaction was analyzed using the relative excess risk due to interaction, attributable proportion due to interaction, and synergistic index [21] using R version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set at a p-value of <0.05. With the exception of the propensity score matching analysis and additive interaction analysis, we performed all statistical analyses using IBM SPSS version 20 (IBM Corp., Armonk, NY, USA).

Results

A total of 1,633 patients were enrolled in the present study. The mean age of the entire cohort was 62.1 ± 12.0 years, and 1,039 patients (63.6%) were males. Among these patients, 1,072 (65.6%) and 561 (34.4%) were included in the LS and OS groups, respectively. The baseline characteristics of the two groups are summarized in Table 1. In the entire cohort, the LS group had lower rates of hypertension, diabetes mellitus, previous operation history, and stage 3 or 4 cancer than the OS group. The distance of the tumor from the anal verge and levels of hemoglobin, sodium, potassium, and total CO₂ were significantly higher in the LS group than in the OS group. The LS group had more recent cases than the OS group. After the propensity score matching, 395 patients remained in each group. The mean age of the matched patients was 61.9 ± 12.2, and 495 patients (62.7%) were males.
In the propensity-matched cohort, all the patient characteristics, including hypertension, diabetes mellitus, previous operation history, cancer staging, distance of tumor from anal verge, levels of hemoglobin, sodium, potassium, and total CO$_2$, and year of surgery, were similar in the LS and OS groups.

Table 2 shows the intraoperative and postoperative variables of the two groups. The overall incidence of AKI after rectal cancer surgery was 12.1%. Before propensity score matching, the LS group had lower rates of AKI, intensive care unit admission, postoperative transfusion, and overall mortality at 5 years than the OS group. Hospital stay, operation time, and EBL were lower in the LS group than in the OS group. Intraoperative hypotension was lower in the OS group than in the LS group. After matching, lower rates of AKI, intensive care unit admission, postoperative transfusion, and overall mortality at 5 years were observed in the LS group compared to the OS group. The LS group had shorter hospital stay and operation time and less EBL than the OS group. However, there were no significant differences in intraoperative hypotension between the two groups.

Cox proportional hazards analyses were performed to identify risk factors of postoperative AKI in patients with rectal cancer (Table 3). In crude analysis, operation time of more than 240 minutes, large volume of EBL, and postoperative transfusion were associated with an increased risk of AKI after rectal cancer, and LS was associated with a decreased risk of postoperative AKI. In the multivariable analysis, postoperative transfusion (hazard ratio [HR], 2.495; 95% confidence interval [CI], 1.529–4.072; p < 0.001) was associated with an increased risk of AKI, and LS (HR, 0.615; 95% CI, 0.403–0.936; p = 0.02) was associated with reduced risk of postoperative AKI in patients with rectal cancer.

Since postoperative transfusion was lower in the LS group, we assessed the effects of the interaction between LS and postoperative transfusion on postoperative AKI (Table 4). Patients who underwent OS and received postoperative transfusion were at 4.548-fold increased risk for postoperative AKI compared to patients who underwent LS and did not receive postoperative transfusion (p < 0.001). However, there was no statistical significance in additive scale and multiplicative scale. We also performed a two-way analysis of variance (ANOVA) to analyze the interaction between LS and postoperative transfusion (Supplementary Table 1, Table 1. Baseline characteristics of the patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Entire cohort (n = 1,633)</th>
<th>Propensity-matched cohort (n = 790)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open (n = 561)</td>
<td>Laparoscopy (n = 1,072)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>62.2 ± 12.2</td>
<td>62.0 ± 11.9</td>
</tr>
<tr>
<td>Male sex</td>
<td>349 (62.2)</td>
<td>690 (64.4)</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>23.1 ± 3.4</td>
<td>23.4 ± 3.1</td>
</tr>
<tr>
<td>Hypertension</td>
<td>83 (14.8)</td>
<td>278 (25.9)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>47 (8.4)</td>
<td>141 (13.2)</td>
</tr>
<tr>
<td>Preoperative chemotherapy</td>
<td>191 (34.0)</td>
<td>324 (30.2)</td>
</tr>
<tr>
<td>Previous operation history</td>
<td>203 (36.2)</td>
<td>462 (43.1)</td>
</tr>
<tr>
<td>Cancer staging</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, 2</td>
<td>245 (43.7)</td>
<td>657 (61.3)</td>
</tr>
<tr>
<td>3, 4</td>
<td>316 (56.3)</td>
<td>415 (38.7)</td>
</tr>
<tr>
<td>Distance of tumor from AV (cm)</td>
<td>6.0 ± 3.2</td>
<td>7.0 ± 3.1</td>
</tr>
<tr>
<td>ASA PS classification</td>
<td>1.7 ± 0.6</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.4 ± 2.0</td>
<td>13.1 ± 1.8</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.1 ± 5.5</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>139.8 ± 3.0</td>
<td>140.5 ± 2.7</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.1 ± 0.4</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>Total CO$_2$ (mmol/L)</td>
<td>24.7 ± 2.8</td>
<td>25.1 ± 2.8</td>
</tr>
<tr>
<td>Years of surgery (years from 2003)</td>
<td>6.5 ± 4.0</td>
<td>9.1 ± 3.4</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation or number (%).
AV, anal verge; ASA, American society of anesthesiologists; PS, physical status.

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Table 2. Comparison of intraoperative and postoperative parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Entire cohort (n = 1,633)</th>
<th>Propensity-matched cohort (n = 790)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open (n = 561)</td>
<td>Laparoscopy (n = 1,072)</td>
</tr>
<tr>
<td>AKI stage</td>
<td>97/561 (17.3)</td>
<td>100/1,072 (9.3)</td>
</tr>
<tr>
<td>1</td>
<td>87/97 (89.7)</td>
<td>87/100 (87.0)</td>
</tr>
<tr>
<td>2</td>
<td>10/97 (10.3)</td>
<td>12/100 (12.0)</td>
</tr>
<tr>
<td>3</td>
<td>0/97 (0)</td>
<td>1/100 (1.0)</td>
</tr>
<tr>
<td>Severe AKI</td>
<td>10/97 (10.3)</td>
<td>13/100 (13.0)</td>
</tr>
<tr>
<td>AKI recovery</td>
<td>46/97 (47.4)</td>
<td>51/100 (51.0)</td>
</tr>
<tr>
<td>RRT in hospital days</td>
<td>4/561 (0.7)</td>
<td>0/1,072 (0)</td>
</tr>
<tr>
<td>Hospital stay (day)</td>
<td>19.3 ± 15.3</td>
<td>13.4 ± 7.0</td>
</tr>
<tr>
<td>ICU admission</td>
<td>99/561 (17.6)</td>
<td>79/1,072 (7.4)</td>
</tr>
<tr>
<td>Operation time (min)</td>
<td>263.0 ± 129.4</td>
<td>225.5 ± 77.2</td>
</tr>
<tr>
<td>Estimated blood loss (mL)</td>
<td>392.8 ± 532.2</td>
<td>146.9 ± 158.9</td>
</tr>
<tr>
<td>Intraoperative hypotension</td>
<td>215/561 (38.3)</td>
<td>519/1,072 (48.4)</td>
</tr>
<tr>
<td>Postoperative transfusion</td>
<td>80/561 (14.3)</td>
<td>50/1,072 (4.7)</td>
</tr>
<tr>
<td>Overall mortality at 5 year</td>
<td>185/561 (33.0)</td>
<td>113/1,072 (10.5)</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>139.8 ± 3.0</td>
<td>140.5 ± 2.7</td>
</tr>
</tbody>
</table>

Values are presented as number (%) or mean ± standard deviation.
AKI, acute kidney injury; ICU, intensive care unit; RRT, renal replacement therapy.

Table 3. Cox proportional hazard model to identify risk factors of acute kidney injury after rectal cancer surgery in propensity-matched cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariable HR (95% CI)</th>
<th>p-value</th>
<th>Multivariable HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operation time &gt; 240 min</td>
<td>1.49 (1.01–2.20)</td>
<td>0.045</td>
<td>1.37 (0.90–2.10)</td>
<td>0.14</td>
</tr>
<tr>
<td>1-mL incremental blood loss</td>
<td>1.00 (1.00–1.00)</td>
<td>0.01</td>
<td>1.00 (1.00–1.00)</td>
<td>0.67</td>
</tr>
<tr>
<td>Postoperative transfusion</td>
<td>2.66 (1.66–4.27)</td>
<td>&lt;0.001</td>
<td>2.50 (1.53–4.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intraoperative hypotension</td>
<td>1.42 (0.96–2.09)</td>
<td>0.08</td>
<td>1.47 (0.92–2.35)</td>
<td>0.01</td>
</tr>
<tr>
<td>Laparoscopic surgery</td>
<td>0.60 (0.40–0.89)</td>
<td>0.01</td>
<td>0.62 (0.40–0.94)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval.

Table 4. Interaction analysis between laparoscopic surgery and postoperative transfusion for postoperative acute kidney injury

<table>
<thead>
<tr>
<th>Laparoscopic surgery</th>
<th>Postoperative transfusion</th>
<th>OR (95% CI) for postoperative transfusion (yes vs. no) within strata of laparoscopic surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Number*</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35/336</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>45/294</td>
<td>1.47 (0.92–2.35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR (95% CI) for laparoscopic surgery (yes vs. no) within strata of postoperative transfusion</td>
<td>1.47 (0.92–2.35)</td>
<td>2.37 (0.71–7.95)</td>
</tr>
</tbody>
</table>

Measurement of interaction on additive scale (95% CI): relative excess risk due to interaction, 2.159 (–1.177 to 5.495); attributable proportion due to interaction, 0.475 (–0.081 to 1.030); and synergistic index, 2.554 (0.453–14.393). Measurement of interaction on the multiplicative scale: OR, 1.612; 95% CI, 0.440–5.907.
CI, confidence interval; OR, odds ratio.
*With/without postoperative acute kidney injury.
available online). Two-way ANOVA showed a significant effect of LS and postoperative transfusion on postoperative AKI. However, there was no significant interaction effect between LS and postoperative transfusion (p = 0.13).

Fig. 2 shows Kaplan-Meier curves for postoperative AKI and overall survival according to the type of surgery. The cumulative incidence of postoperative AKI was lower in the LS group than in the OS group before (9.3% vs. 17.3%, p < 0.001) and after (9.9% vs. 15.9%, p = 0.01) propensity score matching. As compared with the OS group, the LS group had a higher 5-year survival rate in the entire cohort (89.5% vs. 67.0%, p < 0.001) and the propensity-matched cohort (83.5% vs. 71.4%, p < 0.001).

Fig. 3 depicts subgroup analyses for postoperative AKI among the propensity-matched patients in the LS and OS groups. Patients with middle or high rectal cancer had lower postoperative AKI risk than patients with low rectal cancer (p for interaction = 0.05). Patients with older age (>65 years),

**Figure 2.** Kaplan-Meier curves for postoperative AKI and overall survival. (A) Postoperative AKI in the entire cohort. (B) Postoperative AKI in the propensity-matched cohort. (C) Overall survival in the entire cohort. (D) Overall survival in the propensity-matched cohort. AKI, acute kidney injury; LS, laparoscopic surgery; OS, open surgery.
lower ASA PS classification (≤II), no preoperative chemotherapy, and no previous operation history demonstrated a lower postoperative AKI risk compared to their counterparts. However, there were no statistical significances on interactions.

**Discussion**

In the present study, we compared the incidence of postoperative AKI in rectal cancer patients after either LS or OS. The incidence of AKI after rectal cancer surgery was 12.1%. We found that the rate of AKI after LS was significantly lower than after OS. This finding was consistent even after propensity score matching analysis. Operation time, EBL, and incidence of postoperative blood transfusion in the LS group were significantly lower than in the OS group. LS decreased the risk of postoperative AKI in the absence of blood transfusion. Among patients with middle or high rectal cancer, the LS group had a significantly lower incidence of postoperative AKI than the OS group.

In this study, the incidence of postoperative AKI in patients with rectal cancer was 12.1% in the entire cohort and 12.9% in the propensity-matched cohort. The incidence of AKI after rectal cancer surgery varies from 3.8% to 20.3% [22,23]. A cohort study including 288 rectal cancer patients revealed that the rate of postoperative AKI is 3.8% [22], and a population-based cohort study involving 1,337 patients reported an AKI incidence of 20.3% in patients with colorectal cancer [23]. These variations may be due to differences in type of cancer, definition of AKI, inclusion criteria, and exclusion criteria.

There are concerns about renal dysfunction resulting from increased intraabdominal pressure during LS. Hypercarbia due to CO₂ insufflation has a chemical effect on cardiovascular changes. However, the PaCO₂ levels usually observed during laparoscopy do not cause these complications [24]. Thus, increased intraabdominal pressure and related hormonal modifications contribute to most of the hemody-
namic effects during laparoscopic procedures. Abdominal gas insufflation, which increases pressure on the kidney parenchyma and renal vessels, can lead to reduced cardiac output, blood flow, and urine output [25–27]. A previous animal study showed that when intraabdominal pressure was higher than 20 mmHg, venous pressure was increased and cardiac output was decreased. These changes led to a reduction in renal blood flow, and diminished urine output is observed [28]. A previous study involving 104 patients who underwent laparoscopic or open gastric bypass surgery found that urinary output during LS was significantly lower than during OS. However, postoperative blood urea nitrogen and Cr levels were not significantly different between the LS and OS groups. Increased renin-angiotensin-aldosterone system activity also contributes to renal dysfunction. In patients who underwent laparoscopic gastric bypass surgery, renal vasoconstriction and increased renin, aldosterone, and vasopressin levels were reported [29]. However, recent studies revealed that these changes do not increase the risk of AKI and that the incidence of postoperative AKI was even lower in LS patients compared to OS patients [10,30]. Our findings were consistent with those of recent trials.

Prolonged operation time may indicate complex surgical procedures that may directly or indirectly impair the kidney. Anemia and transfusion are established risk factors for AKI after cardiac surgery [31]. Anemia may aggravate kidney dysfunction by reducing kidney oxygen delivery, enhancing oxidative stress, and damaging hemostasis. Transfusion may worsen tissue oxygen delivery, encourage proinflammation, and promote tissue oxidative stress [32]. A previous study involving 1,340 patients who underwent robot-assisted laparoscopic radical prostatectomy or retropubic radical prostatectomy revealed that the EBL and red blood cell transfusion rate were significantly lower in the LS group compared to the OS group [30]. Another study involving 1,173 patients who underwent either laparoscopic liver resection or open liver resection showed that transfusion was an independent risk factor for postoperative AKI [10]. Our results support the findings of the aforementioned studies.

In multivariable analysis, LS and the lack of the need for postoperative blood transfusion were significantly associated with a decreased risk of postoperative AKI. Consistent with the findings of previous studies [10,30], blood transfusion was lower in the LS group in our study. We assumed that there was an effect of the interaction between LS and blood transfusion on postoperative AKI. However, there was no statistically significant interaction. During subgroup analyses, we demonstrated that patients with middle or high rectal cancer were at a lower risk for postoperative AKI compared to patients with low rectal cancer (p for interaction = 0.05). Compared to middle or high rectal cancer surgery, low rectal cancer surgery is performed within a more confined space and requires more complex surgical procedures, which could be a plausible explanation for our findings following subgroup analyses. We assessed renal recovery in patients who developed AKI after rectal cancer surgery. However, there was no significant difference in renal recovery between the LS and OS groups. This study was retrospectively designed and there were no protociled therapeutic interventions for postoperative AKI.

Since the laparoscopic resection of colon cancer was introduced in 1991, LS has become widely used and has progressively replaced OS for the treatment of colon cancer [33]. Previous studies provided sufficient evidence for the favorable outcome of LS in patients with colon cancer [34,35]. However, there is a lack of evidence for the benefits of LS in patients with rectal cancer. Although several studies have compared surgical and oncological outcomes between LS and OS in rectal cancer patients, the efficacy of the laparoscopic procedure is still controversial [36–38]. The ACOSOG Z6051 randomized clinical trial (RCT), a multicenter noninferiority randomized trial involving 486 stage II or III rectal cancer patients, demonstrated that LS failed to prove the noninferiority of pathologic outcomes compared to OS [37]. On the other hand, an RCT with 1,044 patients from 30 hospitals reported contradictory results [36]. There were no significant differences in locoregional recurrence and disease-free and overall survival between the LS and OS groups in patients with rectal cancer. A recent meta-analysis including five RCTs and seven non-RCTs provided evidence for the noninferiority of surgical outcomes following LS, compared to OS, for the treatment of rectal cancer [38]. However, the beneficial effects of the laparoscopic approach in the treatment of rectal cancer remain controversial. Moreover, the National Comprehensive Cancer Network guidelines do not recommend LS as the treatment of choice for rectal cancer [39]. In our study, the overall 5-year survival rate was higher in the LS group than in the OS group. A recent report revealed that the LS group showed a better 5-year survival rate than the OS group (82.6% vs. 76.6%, p < 0.001) [40]. In our
study, the differences in the 5-year survival rate according to type of surgery were larger. The LS group had lower rates of stage 3 or 4 cancer than the OS group (38.7% vs. 56.3%, p < 0.001). These differences may affect the result.

There have been few studies investigating the postoperative renal outcomes after rectal cancer surgery. A previous study involving 725 patients demonstrated that AKI was more common in the OS group than in the LS group. Another study including 5,420 patients also showed that postoperative AKI was significantly lower in the LS group compared to the OS group. However, both studies did not mainly focus on renal outcome. The first study did not define AKI and the second study did not compare with baseline Cr value. They did not identify risk factors for AKI and investigate renal recovery in patients with postoperative AKI. Our study has strength in analyzing the incidence of postoperative AKI and relevant factors.

The present study has some limitations. First, this cohort study is a single-center, retrospective study, and LS was not randomly performed on patients. Although we performed a propensity score matching analysis to minimize confounding biases, we could not collect all confounding factors that influenced our results. Therefore, the outcomes may be subject to unmeasured confounders. Second, even though we defined AKI using the KDIGO criteria, we could not evaluate the urine output. This study is a retrospective study. Therefore, it was impossible to measure the urine output of all study patients in the general ward. Thus, the absence of data on urine output may lead to the misclassification of AKI. Third, increased intraabdominal pressure is one of the main mechanisms that explain postoperative AKI after LS. However, we did not measure the intraabdominal pressure during surgery.

In conclusion, this large retrospective cohort study showed that the incidence of postoperative AKI was significantly lower in LS than in OS for rectal cancer surgery. During subgroup analyses, the LS group had a lower incidence of postoperative AKI than the OS group, especially among patients with middle or high rectal cancer. LS may have a positive effect on postoperative AKI in rectal cancer patients.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

This research was supported by a grant No. 2019R1A2 C1085411 from the National Research Foundation, and by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI17C1827).

Authors’ contributions

Conceptualization: SIK, JR, SK, JCJ, HJC, KYN, DWC, SBK
Investigation, Data curation: SIK, SYL, JYR, HES
Formal analysis: JHP
Funding acquisition: SK
Writing—original draft: JHP
Writing—review & editing: SIK, JR, SK, JCJ, HJC, KYN, DWC, SBK
All authors read and approved the final manuscript.

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References


Metabolic risks in living kidney donors in South Korea

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Background: Considering the growing prevalence of Western lifestyles and related chronic diseases occurring in South Korea, this study aimed to explore the progression of metabolic risk factors in living kidney donors.

Methods: This study enrolled living kidney donors from seven hospitals from 1982 to 2016. The controls were individuals that voluntarily received health check-ups from 1995 to 2016 that were matched with donors according to age, sex, diabetes status, baseline estimated glomerular filtration rate, and date of the medical record. Data on hyperuricemia, hypertension, hypercholesterolemia, and overweight/obesity were collected to determine metabolic risks. Logistic regressions with interaction terms between the medical record date and donor status were used to compare the trends in metabolic risks over time in the two groups.

Results: A total of 2,018 living kidney donors and matched non-donors were included. The median age was 44.0 years and 54.0% were women. The living kidney donors showed a lower absolute prevalence for all metabolic risk factors, except for those that were overweight/obese, than the non-donors. The proportion of subjects that were overweight/obese was consistently higher over time in the donor group. The changes over time in the prevalence of each metabolic risk were not significantly different between groups, except for a lower prevalence of metabolic risk factors ≥ 3 in donors.
Introduction

Kidney transplantation is the preferred treatment option for suitable candidates with end-stage kidney disease (ESKD) and the number of procedures has increased rapidly [1,2]. However, the number of kidneys available from deceased donors cannot meet the increasing need. The median waiting time for deceased donor kidneys has increased continuously and is now more than 4 years in both the United States and South Korea [1,3]. Updated immunologic treatments have contributed to overcoming the donor shortage by expanding the possible living donor pool, including blood group ABO- and human leukocyte antigen (HLA)-incompatible kidneys and kidneys from older-aged donors [4]. This has resulted in a subsequent increase in kidney transplantation, especially from spousal donors [5,6]. Overall, 41% of kidney transplantations are performed with living kidney donations. In South Korea, the relative proportion of living kidney donor transplantations is the 5th highest among 70 countries, with 46.4 living donors per million people in 2018 [7].

Metabolic syndrome is a collection of risk factors that elevate the chance of developing heart disease, stroke, and diabetes, including a combination of central obesity, hypertension, impaired glucose, and hypercholesterolemia [8]. According to data from the Korea National Health and Nutritional Examination Survey, the prevalence of metabolic syndrome increased from 24.9% to 31.3% over the last 10 years, especially in younger participants [9]. In addition, hyperuricemia is closely associated with metabolic syndrome [10] and closely related to associated factors including obesity, central body fat distribution, hypertriglyceridemia, and serum leptin concentration [11,12]. Recently, the prevalence of hyperuricemia increased to more than 11% in the Korean population (17.0% in males and 5.9% in females) [13]. Thus, considering metabolic complications is an important emerging issue. Finally, these metabolic abnormalities are known to increase not only cardiovascular and all-cause mortality, but also ESKD and chronic kidney disease (CKD) progression.

The number of living kidney donors with medically complex conditions or those that are at higher risk for complications is expected to increase. However, there is a lack of epidemiologic data on the metabolic risk of living donors and the impact on long-term outcomes. More information regarding metabolic risk factors will help clarify and address the risk of donation for living donors. Therefore, the goal of this study was to explore the epidemiology of living kidney donors focused on their metabolic risk using the data collected from seven national university hospitals in South Korea.

Methods

Ethical approval

This study was approved in 2019 by the Institutional Review Board at each participating clinical center (Seoul National University Hospital, H-1903-116-1019; Seoul National University Bundang Hospital, B-1905/540-402; SMG-SNU Boramae Medical Center, 20190422/30-2019-28/053; Chonbuk National University Hospital, CUH 2019-05-068; Chonnam National University Hospital, CNUH-2019-163; Kyungpook National University Hospital, 2019-04-014-001; Pusan National University Hospital, H-1905-018-079; and the National Evidence-based Healthcare Collaborating Agency [NECA], NECA-A-20-005). Informed consent was waived because of the retrospective nature of the study and because the analysis used anonymous clinical data. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Study population

A total of 2,898 living kidney donors that were documented from 1982 to 2016 in seven national university hospitals in South Korea were included in this study. All donor candidates received complete health status evaluations.

Conclusion: Over time, metabolic risks in living kidney donors are generally the same as in non-donors, except for a lower prevalence of metabolic risk factors ≥3 in donors.

Keywords: Hypercholesterolemia, Hyperuricemia, Kidney transplantation, Living donors, Risk factors
before kidney donation. Donors were selected according to standard transplantation guidelines, although some living donors were allowed to donate despite contraindications related to their medical conditions. Data on the overall epidemiology of living kidney donors in Korea were extracted from these populations.

We constructed a study cohort comprised of individuals that voluntarily received health check-ups in Seoul National University Hospital and Seoul National University Bundang Hospital from 1995 to 2016 to determine metabolic risks in living kidney donors compared to individuals from the general population. For individuals with data from multiple visits, only the data acquired in the first visit was included. Routine health examination included demographic information and a self-administered interview about underlying diseases [14,15].

After we established both living donor and matched non-donor control cohorts, we excluded donors based on the following criteria: (1) did not undergo the donor operation between 1995 and 2016, (2) missing data for matched variables or metabolic risks including uric acid, total cholesterol, body mass index (BMI), systolic blood pressure (SBP), or history of hypertension and diabetes mellitus (DM), (3) history of cancer, and (4) age of <18 years. From the non-donor control group, we excluded individuals based on: (1) history of kidney donation, (2) measured estimated glomerular filtration rate (eGFR) <50 mL/min/1.73 m² or history of ESKD/transplantation, (3) history of cancer, and (4) missing data for matched variables or metabolic risks.

Data collection and definition of metabolic risk factors

Demographic data and laboratory findings were reviewed via electrical medical records (EMRs). Clinical variables such as age, sex, body weight and height, SBP and diastolic blood pressure, and comorbidities including DM and hypertension were obtained. Laboratory findings including plasma hemoglobin, serum calcium, serum phosphorus, serum glucose, hemoglobin A1c, serum uric acid, serum albumin, blood urea nitrogen, serum creatinine, and dipstick urine albumin and urine red blood cell (RBC) count were collected. Renal function was evaluated by the eGFR that was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation using creatinine [16].

After a complete EMR review, the multicenter retrospective living donor and healthy control cohorts were linked to the nationwide claim database. South Korea provides national health insurance from the National Health Insurance Service and the National Health Insurance Database (NHID) includes complete information on claims since 2002. We also linked the NHID data to the collected EMR database with the approval of the NECA of Korea to obtain information on the history of cancer and the prescribed diabetic and hypertensive medications that were used to define DM and hypertension.

The metabolic risk factors in the main analysis were hypertension, hyperuricemia, hypercholesterolemia, and an overweight/obese status. Hypertension was defined as a previous diagnosis of hypertension, a medication history of antihypertensive drugs, or an SBP of ≥140 mmHg. Hyperuricemia was defined as uric acid of >7 mg/dL in males and 6 mg/dL in females. Hypercholesterolemia was defined as total cholesterol of ≥200 mg/dL, which is the criterion for borderline high or high cholesterol, according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP III) guidelines. Overweight/obese status was defined as a BMI of ≥25 kg/m² [17]. DM status was defined by: (1) a previous diagnosis of DM, (2) a history of insulin or oral hypoglycemic agent use, (3) random glucose > 200 mg/dL, or (4) HbA1c > 6.5%. We defined donors with three or more risks as those with “metabolic risk factors ≥ 3”, based on hypertension, hyperuricemia, hypercholesterolemia, and overweight/obese status.

Statistical analysis

The donor and matched non-donor control baseline characteristics are described using means ± standard deviation and medians and interquartile ranges (IQRs) for continuous variables. Frequency is described using percentages for categorical variables. A t test and one-way analysis of variance were used for comparisons of continuous variables and the chi-square test for categorical variables, as appropriate.

Non-donors were individually matched without replacement to living kidney donors using iterative expanding radius matching to address the sensitivity of comparing living kidney donors and non-donor controls. We matched individuals based on their age, sex, DM status, baseline eGFR, and EMR entry date, which was defined by the year nephrectomy was performed for donors and the first health
check-up for healthy non-donors (Fig. 1). Continuous variables were matched with specific ranges, including age ± 5 years, and eGFR ± 10 mL/min/1.73 m². Sex was matched directly, and the entry date was matched based on categorical values (1995–2000, 2001–2006, 2007–2011, and 2012–2016). After 1:1 direct matching was completed, a total of 2,018 living donors and the same number of healthy non-donor controls were selected. The results indicated that DM is one of the most powerful metabolic risk factors. However, potential kidney donors that have DM are often considered as ineligible donors. Recent Kidney Disease Improving Global Outcomes (KDIGO) guideline recommended that donor candidates with prediabetes or type 2 diabetes should be counseled that their condition may progress over time and may lead to end-organ complications. Therefore, although the absolute number of DM patients was small, to evaluate whether or not the risk of DM increased, we excluded DM from matching variables and performed a sensitivity analysis with the same analysis method.

To compare the progression of metabolic risks between living donors and matched non-donors, logistic regression analyses were performed using interaction terms between the entry date and kidney donor status. To overcome the limitations of 1:1 matching and matching variability, we performed sensitivity analyses with 1,000 additional matches using the bootstrap method. All statistical analyses were performed using the R program version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria), and two-sided p-values of <0.05 were considered to indicate statistical significance.

**Results**

**Baseline characteristics**

A total of 2,898 individuals underwent nephrectomy for living donor kidney transplants from 1982 to 2016 at the study sites (Table 1). The total number of living donor kidney

![Study flow diagram](#)

**Figure 1. Study flow diagram.**

SNUH, Seoul National University Hospital; SNUBH, Seoul National University Bundang Hospital; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; BMI, body mass index.

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<tr>
<td>Age at operation (yr)</td>
<td>42.0 (32.0–51.0)</td>
<td>40.5 (29.0–53.0)</td>
<td>38.0 (30.0–49.0)</td>
<td>38.0 (30.0–47.0)</td>
<td>42.0 (34.0–50.0)</td>
<td>46.0 (38.0–54.0)</td>
<td>&lt;0.001</td>
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<td>&gt;60</td>
<td>186 (6.4)</td>
<td>47 (10.6)</td>
<td>17 (4.2)</td>
<td>16 (2.9)</td>
<td>24 (4.3)</td>
<td>82 (8.8)</td>
<td>&lt;0.001</td>
<td>0.82</td>
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<td>Sex</td>
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<tr>
<td>Female</td>
<td>1,507 (52.1)</td>
<td>229 (52.5)</td>
<td>196 (47.8)</td>
<td>276 (49.9)</td>
<td>297 (52.8)</td>
<td>509 (54.7)</td>
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<tr>
<td>Male</td>
<td>1,384 (47.9)</td>
<td>207 (47.5)</td>
<td>214 (52.2)</td>
<td>277 (50.1)</td>
<td>265 (47.2)</td>
<td>421 (45.3)</td>
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<td>Medical history</td>
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<tr>
<td>Diabetes mellitus</td>
<td>55 (2.1)</td>
<td>1 (0.3)</td>
<td>0 (0.0)</td>
<td>7 (1.3)</td>
<td>16 (2.8)</td>
<td>31 (3.3)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td>Hypertension</td>
<td>381 (14.3)</td>
<td>17 (4.8)</td>
<td>22 (8.1)</td>
<td>78 (14.1)</td>
<td>91 (16.2)</td>
<td>173 (18.6)</td>
<td>&lt;0.001</td>
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<td>Blood pressure (mmHg)</td>
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<tr>
<td>SBP</td>
<td>120.0 (110.0–130.0)</td>
<td>120.0 (110.0–130.0)</td>
<td>120.0 (110.0–130.0)</td>
<td>120.0 (110.0–130.0)</td>
<td>120.0 (110.0–130.0)</td>
<td>119.0 (110.0–130.0)</td>
<td>0.25</td>
<td>0.04</td>
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<tr>
<td>≥130</td>
<td>682 (27.3)</td>
<td>69 (28.4)</td>
<td>69 (30.0)</td>
<td>156 (28.9)</td>
<td>149 (26.7)</td>
<td>239 (25.8)</td>
<td>0.58</td>
<td>0.14</td>
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<tr>
<td>≥140</td>
<td>221 (8.9)</td>
<td>17 (7.0)</td>
<td>22 (9.6)</td>
<td>51 (9.5)</td>
<td>50 (9.0)</td>
<td>81 (8.7)</td>
<td>0.84</td>
<td>0.75</td>
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<td>DBP</td>
<td>74.0 (69.0–80.0)</td>
<td>80.0 (70.0–80.0)</td>
<td>80.0 (70.0–80.0)</td>
<td>74.0 (67.0–81.0)</td>
<td>72.0 (65.0–80.0)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td>Body mass index (kg/m²)</td>
<td>23.3 (21.5–25.4)</td>
<td>23.1 (21.1–25.0)</td>
<td>23.2 (21.5–25.5)</td>
<td>23.0 (21.3–25.0)</td>
<td>23.1 (21.2–25.1)</td>
<td>23.7 (21.9–25.8)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>&lt;18.5</td>
<td>66 (2.6)</td>
<td>12 (4.6)</td>
<td>7 (2.4)</td>
<td>11 (2.0)</td>
<td>17 (3.0)</td>
<td>19 (2.1)</td>
<td>0.07</td>
<td>0.11</td>
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<td>18.5–24.9</td>
<td>1,744 (68.4)</td>
<td>183 (70.1)</td>
<td>198 (86.5)</td>
<td>378 (68.4)</td>
<td>395 (70.8)</td>
<td>590 (64.1)</td>
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<tr>
<td>25–29.9</td>
<td>660 (25.9)</td>
<td>59 (22.6)</td>
<td>78 (27.0)</td>
<td>119 (21.5)</td>
<td>132 (23.7)</td>
<td>272 (29.5)</td>
<td>0.02</td>
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<tr>
<td>≥30</td>
<td>78 (3.1)</td>
<td>7 (2.7)</td>
<td>6 (2.1)</td>
<td>11 (2.0)</td>
<td>14 (2.5)</td>
<td>40 (4.3)</td>
<td>0.03</td>
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<td>Relationship</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td>Spouse</td>
<td>502 (17.4)</td>
<td>11 (2.5)</td>
<td>32 (7.9)</td>
<td>72 (13.0)</td>
<td>119 (21.2)</td>
<td>268 (28.9)</td>
<td>&lt;0.001</td>
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<tr>
<td>Parent-child</td>
<td>1,134 (39.3)</td>
<td>213 (48.4)</td>
<td>144 (35.4)</td>
<td>190 (34.4)</td>
<td>200 (35.7)</td>
<td>387 (41.7)</td>
<td>0.30</td>
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<tr>
<td>Brother or sister</td>
<td>908 (31.5)</td>
<td>158 (35.9)</td>
<td>143 (35.1)</td>
<td>216 (39.1)</td>
<td>175 (31.2)</td>
<td>216 (23.3)</td>
<td>&lt;0.001</td>
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<tr>
<td>Relatives</td>
<td>122 (4.2)</td>
<td>33 (7.5)</td>
<td>31 (7.6)</td>
<td>20 (3.6)</td>
<td>21 (3.8)</td>
<td>17 (1.8)</td>
<td>&lt;0.001</td>
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<tr>
<td>Not-related</td>
<td>221 (7.7)</td>
<td>25 (5.7)</td>
<td>57 (14.0)</td>
<td>54 (9.8)</td>
<td>45 (8.0)</td>
<td>40 (4.3)</td>
<td>0.01</td>
<td>0.04</td>
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<td>Baseline serum laboratory findings</td>
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<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.7 (12.7–15.0)</td>
<td>13.5 (12.4–14.6)</td>
<td>13.5 (12.5–14.8)</td>
<td>13.8 (12.6–15.1)</td>
<td>13.9 (12.8–15.3)</td>
<td>13.6 (12.7–14.9)</td>
<td>0.001</td>
<td>0.04</td>
</tr>
<tr>
<td>Anemia</td>
<td>317 (12.7)</td>
<td>40 (16.7)</td>
<td>31 (14.2)</td>
<td>82 (14.9)</td>
<td>54 (9.7)</td>
<td>110 (11.8)</td>
<td>0.02</td>
<td>0.01</td>
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<tr>
<td>Glucose (mg/dL)</td>
<td>95.0 (88.0–103.0)</td>
<td>94.0 (85.0–102.0)</td>
<td>92.0 (84.0–101.0)</td>
<td>94.0 (88.0–101.0)</td>
<td>94.0 (88.0–101.0)</td>
<td>98.0 (91.0–106.0)</td>
<td>&lt;0.001</td>
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<td>Uric acid (mg/dL)</td>
<td>4.8 (3.9–5.9)</td>
<td>4.6 (3.8–5.6)</td>
<td>4.5 (3.7–5.6)</td>
<td>4.7 (3.9–5.7)</td>
<td>4.9 (4.0–6.0)</td>
<td>4.9 (4.1–6.0)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>Hyperuricemia</td>
<td>217 (9.0)</td>
<td>19 (8.7)</td>
<td>9 (4.4)</td>
<td>32 (6.2)</td>
<td>46 (8.3)</td>
<td>111 (12.0)</td>
<td>&lt;0.001</td>
<td>0.001</td>
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<tr>
<td>Cholesterol (mg/dL)</td>
<td>184.0</td>
<td>173.0</td>
<td>184.0</td>
<td>177.0</td>
<td>182.0</td>
<td>191.0</td>
<td>&lt;0.001</td>
<td>0.001</td>
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<tr>
<td>(163.0–207.0)</td>
<td>(152.0–200.0)</td>
<td>(160.0–206.0)</td>
<td>(153.0–202.0)</td>
<td>(164.0–205.0)</td>
<td>(171.5–213.0)</td>
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<tr>
<td>Hypercholesterolemia</td>
<td>772 (32.7)</td>
<td>56 (25.6)</td>
<td>52 (29.9)</td>
<td>134 (26.9)</td>
<td>172 (31.2)</td>
<td>358 (39.0)</td>
<td>&lt;0.001</td>
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<tr>
<td>Protein (g/dL)</td>
<td>7.4 (7.1–7.6)</td>
<td>7.2 (6.9–7.5)</td>
<td>7.4 (7.1–7.7)</td>
<td>7.4 (7.1–7.7)</td>
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<td>7.4 (7.1–7.6)</td>
<td>&lt;0.001</td>
<td>0.05</td>
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<td>Albumin (g/dL)</td>
<td>4.4 (4.2–4.6)</td>
<td>4.1 (3.8–4.3)</td>
<td>4.4 (4.1–4.6)</td>
<td>4.4 (4.2–4.6)</td>
<td>4.5 (4.3–4.7)</td>
<td>4.5 (4.3–4.7)</td>
<td>&lt;0.001</td>
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<td>BUN (mg/dL)</td>
<td>12.8 (10.0–15.0)</td>
<td>12.0 (10.0–16.0)</td>
<td>12.0 (10.0–15.0)</td>
<td>13.0 (11.0–16.0)</td>
<td>12.9 (10.0–15.0)</td>
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<td>0.007</td>
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Baseline renal function

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<tr>
<td>Creatinine (mg/dL)</td>
<td>0.8 (0.7–1.0)</td>
<td>0.9 (0.8–1.0)</td>
<td>0.9 (0.7–1.0)</td>
<td>0.8 (0.7–1.0)</td>
<td>0.8 (0.6–0.9)</td>
<td></td>
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</tr>
<tr>
<td>CKD-EPI eGFR (mL/min/1.73 m²)</td>
<td>99.5 (87.4–109.1)</td>
<td>89.9</td>
<td>97.1</td>
<td>96.5</td>
<td>99.2</td>
<td>102.9</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Baseline urine laboratory finding

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipstick urine albumin*</td>
<td>1,865 (87.2)</td>
<td>51 (100)</td>
<td>99 (98.0)</td>
<td>478 (94.5)</td>
<td>48 (86.2)</td>
<td>756 (82.0)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trace</td>
<td>239 (11.2)</td>
<td>0 (0)</td>
<td>1 (1.0)</td>
<td>20 (4.0)</td>
<td>71 (12.7)</td>
<td>147 (15.9)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1+</td>
<td>29 (1.4)</td>
<td>0 (0)</td>
<td>1 (1.0)</td>
<td>6 (1.2)</td>
<td>5 (0.9)</td>
<td>17 (1.8)</td>
<td>0.14</td>
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<tr>
<td>≥2+</td>
<td>5 (0.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (0.4)</td>
<td>1 (0.2)</td>
<td>2 (0.2)</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Dipstick urine RBC (/HPF)*</td>
<td>1,002 (47.7)</td>
<td>2 (7.7)</td>
<td>56 (56.0)</td>
<td>292 (57.8)</td>
<td>261 (46.9)</td>
<td>391 (42.8)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;1</td>
<td>980 (46.7)</td>
<td>21 (80.8)</td>
<td>33 (33.0)</td>
<td>194 (38.4)</td>
<td>261 (46.9)</td>
<td>471 (51.6)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1–4</td>
<td>118 (5.6)</td>
<td>3 (11.5)</td>
<td>11 (11.0)</td>
<td>19 (3.8)</td>
<td>34 (6.1)</td>
<td>51 (5.6)</td>
<td>0.55</td>
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<tr>
<td>≥5</td>
<td>2,373 (92.3)</td>
<td>256 (97.0)</td>
<td>244 (90.7)</td>
<td>479 (87.1)</td>
<td>521 (93.0)</td>
<td>873 (94.1)</td>
<td>&lt;0.001</td>
<td>0.36</td>
</tr>
<tr>
<td>Left</td>
<td>198 (7.7)</td>
<td>8 (3.0)</td>
<td>25 (9.3)</td>
<td>71 (12.9)</td>
<td>39 (7.0)</td>
<td>55 (5.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>1,356 (72.4)</td>
<td>0 (0)</td>
<td>1 (0.9)</td>
<td>106 (35.9)</td>
<td>432 (82.3)</td>
<td>817 (89.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operation method*</td>
<td>516 (27.6)</td>
<td>33 (100)</td>
<td>109 (99.1)</td>
<td>189 (64.1)</td>
<td>93 (17.7)</td>
<td>92 (10.1)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as median (interquartile range) or number (%). BUN, blood urea nitrogen; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HPF, high power field; RBC, red blood cell; SBP, systolic blood pressure.

*These variables have missing values.
transplants increased from one case in 1982 to 242 cases in 2016 with a drastic increase starting in 2010 (Fig. 2A). The mean age at donation tended to decrease until 2003 but then increased at a sharper rate after 2003. The proportion of donors aged >60 years showed a similar trend over time (Fig. 2B). There was no definite temporal change in the donor’s sex, although for most years the proportion of females remained slightly higher (Fig. 2C). Assessment of the relationship between donor and recipients throughout the study period indicated that most were parents to children (39.3%), followed by siblings (31.5%), spouses (17.4%), relatives (4.2%), and non-related individuals (7.7%). The proportion of parent-child transplants remained stable, whereas sibling donor kidney transplantation tended to decrease over time and spousal kidney transplantation increased up to 10-fold (Fig. 2D).

Baseline characteristics of living kidney donors vs. matched healthy non-donors

The baseline characteristics of all donors and non-donors are provided in Supplementary Table 1 (available online). Additionally, the baseline characteristics of matched living kidney donors and healthy non-donors, based on the entry date are described in Supplementary Tables 2 and 3 (available online). The comparison of baseline characteristics for living kidney donors and 1:1 matched healthy non-donors is shown in Table 2. The median age was 44.0 years (IQR, 34.0–51.0 years) and 54.0% were women. The incidence of hypertension was lower in living donors than in the matched healthy non-donors. There was no significant difference in SBP between the two groups. The median BMI was higher in the donor group than in the matched non-donors (23.4 kg/m² vs. 22.8 kg/m², p < 0.001) and the proportion of overweight and obese statuses was higher in the donors (29.7% vs. 27.3%). Serum uric acid, total cholesterol levels, and the proportion of hyperuricemia and hypercholesterolemia were higher in the non-donor group. Median serum creatinine was 0.8 mg/dL (IQR, 0.7–0.9 mg/dL) in both groups. For the urine albumin test, the donor group had a higher proportion of individuals with negative results (86.5%) than the non-donor group (59.4%). There was no significant difference in the urine RBC test results between groups.

Metabolic risk trends in living kidney donors vs. matched non-donors

Fig. 3 shows the trends of several metabolic risk factors in donors and non-donors. The living kidney donors showed a lower prevalence for all metabolic risk components, except overweight/obese statuses, compared to matched healthy non-donors. The proportion of overweight/obese patients was slightly higher in donors.

The prevalence of each metabolic risk factor differed over time between the living donors and matched non-donors but the differences were not significant between groups (Table 3). However, the prevalence of ≥3 metabolic risk factors was significantly different between 2001 and 2006. During this period, the proportion of metabolic risk factors ≥ 3 decreased in living kidney donors but increased in the matched non-donor controls.

The sensitivity analysis included 1,000 additional matchings using a bootstrap method. For hypertension and hyperuricemia, more than 95% of the results were consistent and interpreted to not have a significant interaction effect with the time trend. Although hypercholesterolemia, overweight status, and ≥3 composite metabolic risk factors were not significant factors in the interaction effect results that were obtained from the 1:1 matched analysis, there are limitations for the reproducibility of the results from the bootstrap samples (Supplementary Table 4, available online).

Sensitivity analysis with diabetes mellitus removed from the matched variables

Because DM is also a major metabolic risk, a sensitivity analysis was performed after excluding DM from the matched variables. The comparison of baseline characteristics for this assessment is described in Supplementary Table 5 (available online). In this analysis, the proportion of DM in matched donors and non-donors was 2.5% and 8.5%, respectively. The proportion of individuals diagnosed with DM increased more rapidly in the non-donors than in the donors (Supplementary Fig. 1, available online). However, there was no statistically significant difference in the time-trend between the donor and non-donor groups (p > 0.05 for all interactions between groups and year) (Supplementary Table 6, available online), except for the prevalence of ≥3 metabolic risk factors.

The metabolic risk trend, including hypertension, hyperuricemia, hypercholesterolemia, and overweight/obese
status was not significantly different over time compared with the primary analysis (Supplementary Tables 6, 7 and Supplementary Fig. 1; available online).

**Discussion**

The increase in metabolic syndrome in the general population is an increasing problem worldwide and metabolic risks, including hypertension, diabetes, hypercholesterolemia, and obesity, are gradually increasing in Korea [18]. This indicates that potential kidney donors also have increased metabolic risks. Therefore, this study assessed whether the metabolic risks increased in living kidney donors that were selected as donors after testing, compared with the increased metabolic risks in the general population. We investigated the epidemiologic data of living kidney donors in South Korea and the metabolic risk factors in kidney donors compared to matched healthy non-donors. Our results show that transplantation with living kidneys increased rapidly over the study period and metabolic risk factors between living donors and matched healthy non-donors were not significantly different over time, except for the proportion of individuals with ≥3 metabolic risk factors.

In the United States, the total number of living kidney donors has remained constant since 2011 and represents a declining proportion of all kidney transplants. Specifically, among a total of 16,313 kidney transplantations, 60.5% of kidney transplants came from deceased donors and 39.5% of cases came from living donors in 2018, which is quite different from Korea [1]. Data from the Korea Organ Transplan-
Table 2. Baseline characteristic comparisons between living kidney donors and matched healthy non-donors

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 4,036)</th>
<th>Donor (n = 2,018)</th>
<th>Matched non-donors (n = 2,018)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transplantation era</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1995–2000</td>
<td>306 (7.6)</td>
<td>153 (7.6)</td>
<td>153 (7.6)</td>
<td>0.68</td>
</tr>
<tr>
<td>2001–2006</td>
<td>944 (23.4)</td>
<td>472 (23.4)</td>
<td>472 (23.4)</td>
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</tr>
<tr>
<td>2007–2011</td>
<td>1,078 (26.7)</td>
<td>539 (26.7)</td>
<td>539 (26.7)</td>
<td></td>
</tr>
<tr>
<td>2012–2016</td>
<td>1,708 (42.3)</td>
<td>854 (42.3)</td>
<td>854 (42.3)</td>
<td></td>
</tr>
<tr>
<td>Age at operation (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>228 (5.6)</td>
<td>115 (5.7)</td>
<td>113 (5.6)</td>
<td>0.95</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.99</td>
</tr>
<tr>
<td>Female</td>
<td>2,178 (54.0)</td>
<td>1,089 (54.0)</td>
<td>1,089 (54.0)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1,858 (46.0)</td>
<td>929 (46.0)</td>
<td>929 (46.0)</td>
<td></td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>98 (2.4)</td>
<td>49 (2.4)</td>
<td>49 (2.4)</td>
<td>&lt;0.99</td>
</tr>
<tr>
<td>Hyper tension</td>
<td>725 (18.0)</td>
<td>319 (15.8)</td>
<td>406 (20.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>119.0 (110.0–130.0)</td>
<td>120.0 (110.0–130.0)</td>
<td>119.0 (108.0–131.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>≥130</td>
<td>1,079 (26.7)</td>
<td>526 (26.1)</td>
<td>553 (27.4)</td>
<td>0.41</td>
</tr>
<tr>
<td>≥140</td>
<td>413 (10.2)</td>
<td>169 (8.4)</td>
<td>244 (12.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP</td>
<td>73.0 (66.0–80.0)</td>
<td>73.0 (68.0–80.0)</td>
<td>72.0 (65.0–80.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.1 (21.1–25.4)</td>
<td>23.4 (21.5–25.4)</td>
<td>22.8 (20.5–25.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>199 (4.9)</td>
<td>48 (2.4)</td>
<td>151 (7.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18.5–24.9</td>
<td>2,687 (66.6)</td>
<td>1,370 (67.9)</td>
<td>1,317 (65.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>25–29.9</td>
<td>1,013 (25.1)</td>
<td>539 (26.7)</td>
<td>474 (23.5)</td>
<td></td>
</tr>
<tr>
<td>≥30</td>
<td>137 (3.4)</td>
<td>61 (3.0)</td>
<td>76 (3.8)</td>
<td></td>
</tr>
<tr>
<td>Baseline serum laboratory finding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.9 (12.9–15.2)</td>
<td>13.7 (12.7–15.0)</td>
<td>14.1 (13.1–15.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anemia</td>
<td>388 (9.6)</td>
<td>239 (11.8)</td>
<td>149 (7.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.3 (9.0–9.5)</td>
<td>9.3 (9.0–9.5)</td>
<td>9.3 (9.0–9.5)</td>
<td>0.27</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>3.6 (3.2–3.9)</td>
<td>3.5 (3.2–3.9)</td>
<td>3.6 (3.2–3.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>92.0 (85.0–100.0)</td>
<td>95.0 (89.0–104.0)</td>
<td>89.0 (83.0–96.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>4.9 (4.0–6.0)</td>
<td>4.8 (4.0–5.9)</td>
<td>5.1 (4.2–6.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hyperuricemia</td>
<td>455 (11.3)</td>
<td>178 (8.8)</td>
<td>277 (13.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>188.0 (166.0–213.0)</td>
<td>186.0 (165.0–209.0)</td>
<td>190.0 (168.0–217.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>1,519 (37.6)</td>
<td>691 (34.2)</td>
<td>828 (41.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein (g/dL)</td>
<td>0.8 (0.7–0.9)</td>
<td>0.8 (0.7–0.9)</td>
<td>0.8 (0.7–0.9)</td>
<td>0.85</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>12.0 (10.0–15.0)</td>
<td>13.0 (10.0–15.0)</td>
<td>12.0 (10.0–14.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>2,769 (72.9)</td>
<td>1,638 (86.5)</td>
<td>1,131 (59.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>803 (21.2)</td>
<td>223 (11.8)</td>
<td>580 (30.5)</td>
<td></td>
</tr>
<tr>
<td>CKD-EPI eGFR (mL/min/1.73 m²)</td>
<td>100.0 (88.5–109.1)</td>
<td>100.1 (88.6–109.1)</td>
<td>99.9 (88.4–109.1)</td>
<td>0.76</td>
</tr>
<tr>
<td>Baseline urine laboratory finding</td>
<td></td>
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<tr>
<td>Dipstick urine albumin</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Negative</td>
<td>2,769 (72.9)</td>
<td>1,638 (86.5)</td>
<td>1,131 (59.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trace</td>
<td>803 (21.2)</td>
<td>223 (11.8)</td>
<td>580 (30.5)</td>
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<tr>
<td>1+</td>
<td>191 (5.0)</td>
<td>28 (1.5)</td>
<td>163 (8.6)</td>
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<tr>
<td>≥2+</td>
<td>33 (0.9)</td>
<td>4 (0.2)</td>
<td>29 (1.5)</td>
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</tr>
<tr>
<td>Dipstick urine RBC (/HPF)</td>
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<td>0.32</td>
</tr>
<tr>
<td>&lt; 1</td>
<td>1,628 (50.0)</td>
<td>929 (49.3)</td>
<td>699 (50.8)</td>
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<tr>
<td>1–4</td>
<td>1,442 (44.3)</td>
<td>852 (45.3)</td>
<td>590 (42.9)</td>
<td></td>
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<tr>
<td>≥5</td>
<td>188 (5.7)</td>
<td>102 (5.4)</td>
<td>86 (6.3)</td>
<td></td>
</tr>
</tbody>
</table>

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

BUN, blood urea nitrogen; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HPF, high power field; RBC, red blood cell; SBP, systolic blood pressure.
Figure 3. Proportional comparisons of each metabolic risk factor between living kidney donors and matched healthy non-donor controls from 1995 to 2016. (A) Hypertension. (B) Hyperuricemia. (C) Hypercholesterolemia. (D) Overweight and obesity. (E) Composition of three or more metabolic risk factors.
Table 3. Multiple logistic regression analysis with interaction terms between era and donor status to compare time-trend between donors and matched healthy non-donors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Matched non-donor</th>
<th>Donor</th>
<th>p-value</th>
<th>OR (95% CI)</th>
<th>Matched non-donor</th>
<th>Donor</th>
<th>p-value</th>
<th>OR (95% CI)</th>
<th>Matched non-donor</th>
<th>Donor</th>
<th>p-value</th>
<th>OR (95% CI)</th>
<th>Matched non-donor</th>
<th>Donor</th>
<th>p-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
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<td>Reference</td>
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<td></td>
</tr>
<tr>
<td>Hyperuricemia</td>
<td>Reference</td>
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<td></td>
<td>Reference</td>
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<td>Hypercholesterolemia</td>
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<td>Reference</td>
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<tr>
<td>Overweight and obesity</td>
<td>Reference</td>
<td></td>
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<td>Metabolic risk factors ≥ 3</td>
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<td></td>
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<td>Reference</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Matched non-donor</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor</td>
<td>0.66 (0.3–1.48)</td>
<td>0.32</td>
<td>0.14</td>
<td>1.49 (0.84–2.65)</td>
<td>0.17</td>
<td>0.14</td>
<td>2.25 (1.39–3.92)</td>
<td>0.01</td>
<td>0.02</td>
<td>2.76 (1.61–4.74)</td>
<td>0.00</td>
<td>0.00</td>
<td>2.32 (1.50–3.69)</td>
<td>0.00</td>
<td>0.00</td>
<td>1.64 (1.09–2.46)</td>
</tr>
<tr>
<td>Year</td>
<td>Reference</td>
<td></td>
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<td></td>
<td>Reference</td>
<td></td>
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OR, odds ratio; CI, confidence interval.

The Kidney Donor Profile Index (K DPI) Registry indicated that among 4,839 total cases from 2014 to 2018, 62.8% of kidney transplants were from living donors [3], which indicates that living kidney transplantations comprise a major portion of total kidney transplants in Korea. Living donor kidney transplant is increasing rapidly because more recent technologies have addressed several obstacles, including ABO and HLA mismatch [5,6]. This is especially relevant because the data indicate that in the past, kidneys were only donated to genetically related persons including parent to child, siblings, and relatives, whereas living kidney transplants are now conducted among couples who are not genetically related, and this approach is the second most common out of all total living kidney donations.

Predonation metabolic and lifestyle risk factors are included in KDIGO clinical practice guidelines on the Evaluation and Care of Living Kidney Donors; however, all of the recommendations are “non-graded” due to insufficient evidence from eligible studies [19]. In the United States, the number of living kidney transplants has decreased by 13% since 2004, unlike in other countries [20]. The reasons for this are likely multifactorial, but it may be that the proportion of unhealthy individuals in the United States is increasing, due to factors such as obesity [12,21]. Therefore, it is important to identify differences in metabolic risk factors between living kidney donors and healthy control groups. To our knowledge, this is the first study to include a multicenter comparison of metabolic risk factors between kidney donors matched with a healthy non-donor comparison group. Overall, with the exception of overweight/obese patients, the proportions of each metabolic risk in recipients compared to matched non-donors were low (Fig. 3).

Current guidelines indicate that predonation blood pressure and target organ damage should be evaluated carefully [19] because the associated reduction of kidney mass and function are related to a progressive increase in blood pressure [19,22,23]. Kidney donation is not contraindicated if blood pressure is well-controlled in patients that take 1 to 2 antihypertensive medications. However, donor candidates should be informed that their blood pressure may increase over time and that the kidney donation may accelerate a rise in blood pressure. In a recent study with an average of 56 months of follow-up for 190 living kidney donors, 10% of donors developed hypertension, and the predonation blood pressure, proteinuria, and fasting glucose values were higher in the group of individuals with new-onset hypertension [24].
In this study, although there was no significant difference between the two groups in the proportion of patients that were diagnosed with hypertension over time, the increase in the absolute number of individuals with hypertension in the donor population should not be taken lightly as it could indicate the risk for a future increase in new-onset hypertension cases. Additional studies are needed to determine the long-term outcomes for donors with higher blood pressure and confirm their level of risk as donors.

Serum uric acid concentrations are not specifically mentioned in the guidelines for living kidney donors. However, higher uric acid levels are associated with the progression of renal deterioration [25], and predonation serum uric acid levels could be an important indicator of the postoperative renal function for donors [26,27]. Additionally, prenephrectomy uric acid levels are a potential predictor for new-onset DM after kidney transplantation in living donors [28]. Based on this data, we hypothesize that serum uric acid levels are an important factor that should be monitored carefully in living kidney donors considering that they will age with a single kidney. The prevalence of hyperuricemia in the general population of Korea has been reported in different ways. Koo et al. [29] reported that the prevalence of hyperuricemia was 133.25 per 1,000 persons in men and 8.17 per 1,000 persons in women (2004–2013). In another study [13], the proportion of hyperuricemia was 11.4% (17.0% in men and 5.9% in women). Although there were a limited number of reports on the time-trend of hyperuricemia, studies have indicated that the prevalence of gout increased 5.17-fold from 0.39% in 2002 to 2.01% in 2015 [30]. Similar to this report, our study indicated that the prevalence of hyperuricemia increased rapidly in both living kidney donors and non-donors. Therefore, additional studies are needed to investigate the association between predonation hyperuricemia and long-term clinical outcomes for kidney donors.

Although there is currently controversial evidence on lipid profiles and long-term outcomes in donors, one study reported that abnormal preoperative elevation of total cholesterol and low-density lipoprotein (LDL) levels of living kidney donors were predictive for developing CKD after nephrectomy [31]. In the Korean population, although the definition of hypercholesterolemia was different from the definition applied in this study, the prevalence of hypercholesterolemia (total cholesterol more than 240 mg/dL or taking lipid drugs) increased from 9.0% in 2007 to 20.7% in 2018 [32,33]. Although hypercholesterolemia did not rapidly increase over time compared to other risk factors in both donors and controls in this study, half of the individuals had hypercholesterolemia based on total cholesterol.

One of the major metabolic risks is overweight or obese status because worldwide obesity has nearly tripled since 1975 [34]. Obesity and DM are highly interrelated diseases, and since DM is the main cause of ESKD, it is necessary to emphasize control of body weight, which is a modifiable factor and preventative for DM. The prevalence of obesity in patients in Korea increased from 29.7% in 2009 to 32.4% in 2015 [35]. In the present study, we investigated whether the obesity rate increased to the same extent in the kidney donors and non-donors and found that the proportion of overweight/obese patients was 32.7% and 22.9%, respectively, from 1995 to 2000, which was a higher percentage than the 2000s. This might be due to the small number of enrolled donors during the time period, as this study only included 177 individuals. Therefore, even though the absolute number of overweight/obesity patients was small, the proportion of overweight/obese patients may be underestimated because health reports have indicated that the percentage of overweight/obese patients has increased gradually since 2001.

Type 2 DM is a leading cause of CKD and ESKD worldwide [36,37]. Most of the guidelines for kidney donor evaluation recommend that individuals diagnosed with DM should be excluded from living kidney donation [38,39]. Only a few guidelines [38] have indicated that individuals with DM could be considered as candidates for kidney donation after a rigorous evaluation of the lifetime risk of cardiovascular and CKD after nephrectomy. Accordingly, we performed two analyses and excluded DM as a matching variable and found that the number of individuals with DM rapidly increased in both groups. However, the number of DM patients overall was small, therefore, the results could not determine whether kidney donation approval is gradually increasing based on individual decisions to accept poor health outcomes or associated complications after nephrectomy. Consequently, further analyses with a large-scale donor cohort will be needed to confirm this finding.

Because metabolic risk factors are not typically isolated indications, we conducted additional analyses to determine the proportion of subjects with ≥3 metabolic risks. The analysis could have benefited from clear application of metabolic syndrome criteria from the NCEP III, but there was...
a lack of data on waist circumference, triglyceride levels, and LDL cholesterol in the data sets. A direct comparison was not possible because the definitions of composite metabolic risk in this study and metabolic syndrome differed. Lee et al. [40] reported that the age-adjusted prevalence of metabolic syndrome increased from 28.8% in 2009 to 30.5% in 2013. Although the increase over time was more rapid in healthy non-donors, the proportion of individuals with ≥3 various metabolic risks increased in both groups over time (Fig. 3E). Because composite metabolic risks are increasing in living donors despite detailed evaluation before kidney donation, clinicians should consider this phenomenon and inform patients of the associated risks and challenges.

To date, only a few studies have evaluated the metabolic risks between living kidney donors and a matched population partly because it is difficult to identify participants from the general population. Therefore, the present study adds to the evidence on metabolic risk in living kidney donors. Because various metabolic risks in the general population are increasing, we assessed whether the metabolic risk factors for kidney donors are similarly increasing. Despite the advantages of the study, there were some limitations. For example, national data from Korea were not sufficient to allow comparison of each metabolic risk due to the cross-sectional study design. Thus, it is necessary to evaluate whether the long-term outcomes including death, ESKD, and incident DM are worse in donors with high metabolic risks. Furthermore, all of the medical records of donors were carefully reviewed, but a part of the data, which was entered before the EMR implementation, was subject to human error. Moreover, all of the variables were only measured once; therefore, this study did not include serial changes. Finally, all elements that corresponded with metabolic syndrome could not be evaluated because this study was retroactive and focused on previous data.

In conclusion, there are no definitive differences in metabolic risk factor trends between living kidney donors and matched healthy non-donor controls. However, absolute metabolic risks are increasing in living kidney donors despite attempts to select healthy individuals based on various preoperative evaluations. Because most metabolic risk factors are modifiable, physicians should recommend lifestyle modifications, including weight loss, to candidate donors. Additionally, further studies are needed to evaluate the long-term outcomes based on predonation metabolic risks.

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

Funding
This study was supported by the National Evidence Collaborating Agency project number NECA-A-20-005. The funder had no role in the study conduct and the study was performed independently by the authors.

Acknowledgments
The authors would like to thank Ji Su Yang (Seoul National University Hospital), Hyun Ju Lim (Seoul National University Hospital), and Seong Eun Park (Keimyung University Hospital) for their review of electrical medical records in this cohort study. We also would like to thank Pf. Jung Nam An (Hallym University Sacred Heart Hospital) and Jong Cheol Jeong (Seoul National University Bundang Hospital) who guided our efforts in this research process.

Authors’ contributions
Conceptualization: EK, YK, HL
Formal analysis: EK, JP, SP, MP
Funding acquisition: HL
Investigation: All authors
Writing–original draft: EK, JP
Writing–review & editing: HL
All authors read and approved the final manuscript.

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References


Metformin use and cardiovascular outcomes in patients with diabetes and chronic kidney disease: a nationwide cohort study

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Background: Metformin has recently been shown not to increase the risk of lactic acidosis in patients with chronic kidney disease (CKD). Thus, the criteria for metformin use in this population has expanded. However, the relationship between metformin use and clinical outcomes in CKD remains controversial.

Methods: This study considered data from 97,713 diabetes patients with an estimated glomerular filtration rate of <60 mL/min/1.73 m². The primary outcome was major adverse cardiac and cerebrovascular events (MACCE), and the secondary outcomes were all-cause mortality and incident end-stage renal disease (ESRD).

Results: Metformin users had a significantly higher risk of MACCE than non-users (hazard ratio [HR], 1.20; 95% confidence interval [CI], 1.14–1.26; p < 0.001). However, metformin users had a lower risk of all-cause mortality (HR, 0.78; 95% CI, 0.74–0.81; p < 0.001) and ESRD (HR, 0.44; 95% CI, 0.42–0.47; p < 0.001) during follow-up than non-users did. The relationships between metformin use and clinical outcomes remained consistent in propensity score matching analyses and subgroup analyses of patients with adequate adherence to anti-diabetes medication.

Conclusion: Treatment with metformin was associated with an increased risk of MACCE in patients with diabetes and CKD. However, metformin users had a lower risk of all-cause mortality and ESRD during follow-up than non-users did. Therefore, metformin needs to be carefully used in patients with CKD.

Keywords: Chronic kidney disease, Mortality, Metformin, Renal insufficiency
Introduction

Chronic kidney disease (CKD) is becoming a global health challenge [1]. Because it causes premature death and substantial healthcare costs, the increasing prevalence of CKD is a socioeconomic burden [2,3]. Diabetes mellitus (DM) is one of the most important causes of CKD in several countries [4], and the prevalence of DM in adults worldwide is expected to be 7.7% by 2030 [5]. Moreover, both DM and CKD are important risk factors for cardiovascular disease (CVD). The burden of CVD increases continuously as renal function declines [6], and individuals with both DM and CKD have an exceptionally high risk of CVD [7]. Therefore, proper DM treatment is an indispensable issue in CKD management.

Although new classes of anti-diabetes drugs have shown significant benefits in preventing CVD development [8,9], metformin is still the first-line drug for DM management [10,11]. Traditionally, metformin use has not been recommended for patients with CKD because of the risk of lactic acidosis. However, several clinical trials and observational studies have reported that the risk of fatal and nonfatal lactic acidosis did not increase with metformin use, even in patients with advanced CKD [12–14]. Accordingly, the U.S. Food and Drug Administration (FDA) of the United States expanded the criteria for metformin use. Nevertheless, prescribing metformin to advanced CKD patients, those whose estimated glomerular filtration rate (eGFR) is less than 30 mL/min/1.73 m², remains controversial. Moreover, current clinical practice guidelines suggest diverse criteria for metformin use in patients with CKD [14], reflecting a lack of consensus about the relationship between metformin use and clinical outcomes in patients with CKD. Recent observational studies have shown inconsistent relationships between metformin use and CVD [15–17], all-cause mortality [13,18], and end-stage renal disease (ESRD) [13,19,20]. Clearly, physicians have reason to be confused about prescribing metformin to CKD patients.

Therefore, we evaluated the relationship between metformin use and the incidence of major adverse cardiac and cerebrovascular events (MACCE), all-cause mortality, and ESRD in CKD patients using data from a large Korean health screening cohort.

Methods

Study population

This retrospective observational study was conducted using cohort data from the National Health Insurance Service (NHIS) database. The NHIS data contain medical service claims, pharmacy claims, and health screening data for the whole population of the Republic of Korea. Detailed information about the NHIS database has been described previously [21]. The NHIS provides a national health screening program for adults aged ≥40 years, and approximately three-quarters of all eligible Koreans participate in those health screenings every year. Among them, we selected 267,442 individuals who participated in a national health screening between 2009 and 2015, were prescribed anti-diabetes medications, and had an eGFR of <60 mL/min/1.73 m². From them, we excluded individuals who had type 1 diabetes (n = 4,655), had ESRD requiring dialysis or kidney transplantation (n = 583), had taken anti-diabetes medications for less than 90 days (n = 163,913), or had experienced the clinical outcomes before taking anti-diabetes medication for 90 days (n = 1,064). However, we did not exclude patients with a history of CVD, such as myocardial infarction (MI), congestive heart failure, peripheral vascular disease, or stroke. Consequently, we included 97,713 patients in this study (Fig. 1). If patients underwent multiple health screenings, they were included at the earliest health screening that satisfied the inclusion criteria. Those patients were then followed up until December 31, 2019. This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Soonchunhyang University Seoul Hospital (No. 2019-06-014) with a waiver for informed consent.

Data collection

All health screening procedures met the internal and external quality control standards of the Korean Association of External Quality Assessment Service, as judged by detailed procedures described previously [22]. Briefly, data on lifestyle habits, including cigarette smoking and alcohol consumption, were obtained using a standardized questionnaire. The laboratory measurements and anthropometric
parameters were obtained by trained healthcare providers. Blood pressure was measured three times, and the average of the last two measurements was used. Blood samples were obtained after 8 hours of fasting, and eGFR was calculated using the CKD Epidemiology Collaboration equation [23]. Proteinuria was assessed by the dipstick test and was defined as ≥1+. Medication history was obtained using the prescription database of the NHIS. Metformin, sulfonylureas, thiazolidinediones, α-glucosidase inhibitors, dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-1 receptor agonists, sodium-glucose cotransporter-2 inhibitors, and insulin were considered anti-diabetes medications. In addition, we collected information about the use of aspirin, statins, and renin-angiotensin system blockade (RASB). Medical histories were obtained using the diagnosis codes in the NHIS database, which are based on the Korean Classification of Disease (KCD)-7. Medical histories were confirmed when patients visited an outpatient clinic at least twice or were hospitalized at least once due to disease before their first health screening day. The Charlson comorbidity index (CCI) was calculated using medical histories [24].

Definition of metformin users

In the Republic of Korea, prescriptions for chronic diseases, including DM, are generally given for 90 days. Therefore, we defined metformin users as individuals who had metformin prescriptions for at least 90 days after the health screening day. Metformin non-users were defined as diabetes patients who had prescriptions for other anti-diabetes medications (but not metformin) for the same period. Therefore, we defined the index date of study entry as the 90th day of the anti-diabetes medication prescription after the health screening day.

Outcome assessment

The primary outcome was MACCE, a composite of nonfatal coronary heart disease (CHD) (MI and unstable angina) (KCD code I20-I23) and nonfatal stroke (ischemic and hemorrhagic stroke) (I60-I63) requiring hospitalization for more than 2 days. The secondary outcomes were incident ESRD and all-cause mortality. Incident ESRD was defined as having prescription codes for hemodialysis or peritoneal dialysis for at least 90 days or having a prescription code for renal transplantation. Mortality data were linked with data prepared by Statistics Korea that record all deaths in the Republic of Korea. As a sensitivity analysis, we used cause of death data from Statistics Korea and defined cardiovascular mortality as death caused by MACCE.

Statistical analysis

Continuous variables are expressed as means ± standard deviations, and categorical variables are presented as numbers and percentages. For comparisons between groups, Student t-test was used for continuous variables, and Pearson chi-square testing was used for categorical variables. The cumulative incidences of the outcomes were assessed using the Kaplan-Meier method. A multivariable analysis was conducted using a Cox proportional hazard regression model, and the results are expressed as hazard ratios (HRs) and 95% confidence intervals (CIs). As a sensitivity analysis, we conducted a cause-specific hazard regression analysis with mortality as a competing risk to evaluate the relationship between metformin use and MACCE or ESRD. We also used propensity score matching (PSM) analyses. The

Figure 1. Flow chart for patient enrollment.
CKD, chronic kidney disease; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease.
propensity scores were obtained using a logistic regression with the nearest neighbor technique, and metformin users were matched with metformin non-users in a ratio of 1:1. The logistic regression model was derived by adjusting for the following variables: age; sex; hypertension history; MI; congestive heart failure (CHF); peripheral vascular disease; stroke; cancer; use of aspirin, a statin, or RASB; body mass index (BMI); systolic blood pressure (SBP); fasting glucose; eGFR; serum total cholesterol; presence of proteinuria; smoking status with pack-years; and alcohol consumption. The PSM analyses were performed for the entire cohort and at each CKD stage. We also performed a sensitivity analysis using the subgroup with adequate adherence to anti-diabetes medication. Adequate adherence was defined as a proportion of days covered (PDC) with anti-diabetes medication greater than 80% of the entire follow-up period (PDC of metformin ≥ 80% for metformin users and PDC of other anti-diabetes medications (but not metformin) ≥ 80% for metformin non-users) [25]. Finally, we performed another PSM analysis in the subgroup whose PDC was ≥ 80%. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). A p-value of less than 0.05 was considered statistically significant.

Results

Baseline characteristics

Baseline characteristics according to metformin use are presented in Table 1. Among the 97,713 participants, 77,666 were classified as metformin users. The mean ages of the metformin users and non-users were 66.3 ± 9.5 and 66.0 ± 8.9 years, respectively. Compared with the non-users, the metformin user group was younger, more female, and had a lower history of hypertension, MI, CHF, stroke, and cancer. Thus, the CCI among the metformin users was significantly lower than that in the metformin non-users group (p < 0.001). The use of aspirin was similar between groups, but metformin users took fewer statins and RASB than non-users. In addition, metformin users had lower BMI, SBP, and serum creatinine, but they had higher fasting glucose, eGFR, total and high-density lipoprotein cholesterol, and triglyceride levels than non-users. The metformin user group had fewer smokers and individuals with proteinuria but more heavy drinkers than the non-user group.

Risk of major adverse cardiac and cerebrovascular events by metformin use status

During a mean follow-up of 5.3 years, a total of 11,434 MACCE (11.7%) occurred, for a corresponding incidence rate of 22.2 per 1,000 patient-years. The incidence rate of MACCE in metformin users was 22.5 per 1,000 patient-years, producing a significantly higher risk of MACCE than found in non-users (HR, 1.20; 95% CI, 1.14–1.26; p < 0.001) (Table 2). When comparing the components of MACCE, the risk of CHD increased marginally (HR, 1.07; 95% CI, 1.00–1.14; p = 0.05), but the risk of stroke increased significantly in metformin users (HR, 1.40; 95% CI, 1.30–1.51; p < 0.001) compared with non-users. In the cause-specific hazard regression analysis with mortality as a competing risk, metformin users still had an increased risk of MACCE (HR, 1.36; 95% CI, 1.28–1.44; p < 0.001). In the subgroups by CKD stage, the increased risk of MACCE among metformin users was observed in CKD stages 3a and 3b but attenuated in CKD stages 4 and 5 (Supplementary Table 1, available online).

Risk of all-cause mortality and end-stage renal disease by metformin use status

During the follow-up period, the incidence rates of all-cause mortality and ESRD were 24.1 and 7.6 per 1,000 patients-years, respectively. The risk of mortality during that period was significantly lower in metformin users (HR, 0.78; 95% CI, 0.74–0.81; p < 0.001) than in non-users (Table 3). The incidence of cardiovascular mortality was 3.9 and 2.8 per 1,000 patients-years in metformin non-users and users, respectively. Thus, risk of cardiovascular mortality was also significantly lower in metformin users (HR, 0.70; 95% CI, 0.62–0.79; p < 0.001) (Supplementary Table 2, available online). However, due to their increased risk MACCE, metformin users had a higher risk than non-users of experiencing the composite cardiovascular outcome (HR, 1.16; 95% CI, 1.10–1.22; p < 0.001), which was a composite of MACCE and cardiovascular mortality. In addition, the risk of ESRD was significantly lower in metformin users (HR, 0.44; 95% CI, 0.42–0.47; p < 0.001) than in non-users (Table 3). In the cause-specific hazard regression analysis with mortality as a competing risk, metformin users still had a significantly lower risk of ESRD (HR, 0.48; 95% CI, 0.44–0.52; p < 0.001)
than non-users. In the subgroups of CKD stages, the lower risks of all-cause mortality and ESRD during follow-up in metformin users were consistently present in all CKD stages (Supplementary Table 3, available online).

Risk of clinical outcomes by metformin use status after propensity score matching

Because the patients’ characteristics differed significantly

Table 1. Baseline characteristics of patients according to metformin use

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<td>Age (yr)</td>
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<td>66.0 ± 8.9</td>
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<td>Sex</td>
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<tr>
<td>Male</td>
<td>13,972 (69.7)</td>
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<td>Female</td>
<td>6,075 (30.3)</td>
<td>28,387 (36.6)</td>
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<td>Comorbid condition</td>
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<td>Hypertension</td>
<td>17,447 (87.0)</td>
<td>64,528 (83.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>567 (2.8)</td>
<td>1,716 (2.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart failure</td>
<td>1,072 (5.3)</td>
<td>2,844 (3.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>2,383 (11.9)</td>
<td>9,424 (12.1)</td>
<td>0.34</td>
</tr>
<tr>
<td>Stroke</td>
<td>3,127 (15.6)</td>
<td>10,767 (13.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cancer</td>
<td>1,153 (5.8)</td>
<td>3,419 (4.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Medication use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>7,787 (38.8)</td>
<td>30,020 (38.7)</td>
<td>0.62</td>
</tr>
<tr>
<td>Statin</td>
<td>9,218 (46.0)</td>
<td>33,026 (42.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RASB</td>
<td>14,852 (74.1)</td>
<td>53,834 (69.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Charlson comorbidity index</td>
<td>2.6 ± 1.6</td>
<td>2.2 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline measurement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (g/m²)</td>
<td>25.2 ± 3.2</td>
<td>25.1 ± 3.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>130.9 ± 16.6</td>
<td>129.5 ± 16.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>77.1 ± 10.3</td>
<td>77.2 ± 10.0</td>
<td>0.51</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>131.8 ± 47.0</td>
<td>136.6 ± 48.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.7 ± 2.1</td>
<td>1.5 ± 2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>46.1 ± 12.3</td>
<td>50.7 ± 9.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>178.4 ± 44.1</td>
<td>180.4 ± 43.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>48.1 ± 18.6</td>
<td>49.2 ± 19.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>98.9 ± 45.1</td>
<td>99.2 ± 73.9</td>
<td>0.46</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>163.8 ± 114.9</td>
<td>168.5 ± 118.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proteinuria*</td>
<td>4,665 (23.3)</td>
<td>10,773 (13.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>10,283 (51.3)</td>
<td>42,231 (54.4)</td>
<td></td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>5,872 (29.3)</td>
<td>20,408 (26.3)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>3,892 (19.4)</td>
<td>15,027 (19.3)</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption (unit/wk)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0</td>
<td>8,565 (42.7)</td>
<td>33,695 (43.4)</td>
<td></td>
</tr>
<tr>
<td>1–7</td>
<td>5,733 (28.6)</td>
<td>21,080 (27.1)</td>
<td></td>
</tr>
<tr>
<td>8–14</td>
<td>2,392 (11.9)</td>
<td>9,172 (11.8)</td>
<td></td>
</tr>
<tr>
<td>≥15</td>
<td>3,357 (16.8)</td>
<td>13,719 (17.7)</td>
<td></td>
</tr>
</tbody>
</table>

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

BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RASB, renin-angiotensin system blockade.

*Dipstick test, ≥1+. 
between groups, we performed PSM between metformin users and non-users in a 1:1 ratio. The baseline characteristics after PSM are presented in Supplementary Table 4 (available online). After PSM, the cumulative MACCE-free survival was significantly lower in metformin users than in non-users \( (p < 0.001) \) (Fig. 2). Meanwhile, metformin users had significantly higher cumulative survival and ESRD-free survival than non-users \( (p < 0.001, \text{all}) \). In the multivariable analysis, the HR of MACCE for metformin use was 1.15 (95% CI, 1.09–1.22) (Table 4). When we performed a PSM anal-

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**Table 2. Risk of MACCE according to metformin use**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Metformin group</th>
<th>Event, n (%)</th>
<th>Incidence (per 1,000 patient-yr)</th>
<th>Crude HR (95% CI)</th>
<th>p-value</th>
<th>Adjusted* HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MACCE</td>
<td>Non-users</td>
<td>2,082 (10.4)</td>
<td>21.1</td>
<td>(Reference)</td>
<td>-</td>
<td>1.20 (1.14–1.26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Users</td>
<td>9,352 (12.0)</td>
<td>22.5</td>
<td>1.06 (1.01–1.11)</td>
<td>0.021</td>
<td>1.20 (1.14–1.26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CHD</td>
<td>Non-users</td>
<td>1,261 (3.3)</td>
<td>12.8</td>
<td>(Reference)</td>
<td>-</td>
<td>(Reference)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Users</td>
<td>4,983 (6.4)</td>
<td>12.0</td>
<td>0.93 (0.88–0.99)</td>
<td>0.028</td>
<td>1.07 (1.00–1.14)</td>
<td>0.05</td>
</tr>
<tr>
<td>Stroke</td>
<td>Non-users</td>
<td>821 (4.1)</td>
<td>8.3</td>
<td>(Reference)</td>
<td>-</td>
<td>(Reference)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Users</td>
<td>4,369 (5.6)</td>
<td>10.5</td>
<td>1.25 (1.16–1.35)</td>
<td>&lt;0.001</td>
<td>1.40 (1.30–1.51)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CHD, coronary heart disease; CI, confidence interval; HR, hazard ratio; MACCE, major adverse cardiac and cerebrovascular events.

*Adjusted for age, sex, smoking, alcohol consumption, history of hypertension, myocardial infarction, congestive heart failure, peripheral vascular disease, stroke, cancer, body mass index, systolic blood pressure, fasting glucose, estimated glomerular filtration rate, total cholesterol, presence of proteinuria, and use of aspirin, a statin, or renin-angiotensin system blockade.

**Table 3. Risk of all-cause mortality and ESRD according to metformin use**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Metformin group</th>
<th>Event, n (%)</th>
<th>Incidence (per 1,000 patient-yr)</th>
<th>Crude HR (95% CI)</th>
<th>p-value</th>
<th>Adjusted* HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause mortality</td>
<td>Non-users</td>
<td>3,370 (16.8)</td>
<td>32.5</td>
<td>(Reference)</td>
<td>-</td>
<td>0.78 (0.74–0.81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Users</td>
<td>9,830 (12.7)</td>
<td>22.2</td>
<td>0.66 (0.63–0.68)</td>
<td>&lt;0.001</td>
<td>0.78 (0.74–0.81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESRD</td>
<td>Non-users</td>
<td>1,966 (9.8)</td>
<td>20.1</td>
<td>(Reference)</td>
<td>-</td>
<td>0.44 (0.42–0.47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Users</td>
<td>2,116 (2.7)</td>
<td>4.8</td>
<td>0.23 (0.22–0.25)</td>
<td>&lt;0.001</td>
<td>0.44 (0.42–0.47)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CI, confidence interval; ESRD, end-stage renal disease; HR, hazard ratio.

*Adjusted for age, sex, smoking, alcohol consumption, history of hypertension, myocardial infarction, congestive heart failure, peripheral vascular disease, stroke, cancer, body mass index, systolic blood pressure, fasting glucose, estimated glomerular filtration rate, total cholesterol, presence of proteinuria, and use of aspirin, a statin, or renin-angiotensin system blockade.

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**Figure 2. Cumulative event-free survival according to use of metformin in patients with chronic kidney disease and taking diabetes medication, after propensity score matching.** (A) Major adverse cardiac and cerebrovascular events (MACCE), (B) all-cause mortality, and (C) end-stage renal disease (ESRD).
ysis in each CKD stage, an increased risk of MACCE was observed only in CKD stage 3a; the risk was attenuated in advanced stages. In addition, the HRs of all-cause mortality and ESRD with metformin use were 0.76 (95% CI, 0.73–0.80) and 0.45 (95% CI, 0.42–0.48), respectively, and the benefits of metformin use for delaying death and preventing ESRD were seen in all CKD stages.

Risk of clinical outcomes by metformin use status in the subgroups with adequate adherence

A further sensitivity analysis was performed in the subgroup of patients with adequate adherence to anti-diabetes medication. The metformin users in this subgroup still had a significantly increased risk of MACCE (HR, 1.66; 95% CI, 1.57–1.76; p < 0.001), and that relationship was observed in CKD stage 3, but not in stages 4 and 5 (Table 5). Furthermore, the HR for all-cause mortality during follow-up was still significantly lower for metformin users in this subgroup (HR, 0.88; 95% CI, 0.83–0.93; p < 0.001), but the survival benefit from metformin use was attenuated in patients with eGFR of <45 mL/min/1.73 m². Meanwhile, the HR for ESRD in metformin users was 0.29 (95% CI, 0.26–0.33) and consistently lower in metformin users than in non-users in all CKD stages.

Risk of clinical outcomes by metformin use status after PSM in the subgroups with adequate adherence

Finally, we conducted PSM in the subgroup of patients with adequate adherence to anti-diabetes medication. We found that metformin use still carried a significantly increased risk of MACCE (HR, 1.65; 95% CI, 1.55–1.75; p < 0.001) (Table 6). When PSM was conducted for each CKD stage, that relationship was attenuated in the higher CKD stages, but it remained significant. Meanwhile, the survival benefit from metformin use also remained (HR, 0.89; 95% CI, 0.84–0.95; p < 0.001), but it disappeared in patients with eGFR of <45 mL/min/1.73 m². The risk of ESRD was significantly lower in metformin users (HR, 0.30; 95% CI, 0.26–0.33; p < 0.001), and that benefit was observed in all CKD stages.

Discussion

In this study, we found that metformin use was associated with a significantly increased risk of MACCE in patients

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**Table 4. Risk of clinical outcomes according to metformin use after propensity score matching**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Overall (n = 40,094)</th>
<th>CKD stage (eGFR, mL/min/1.73 m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude</td>
<td>Adjusted ( \text{a} )</td>
</tr>
<tr>
<td></td>
<td>MACCE (n = 40,094)</td>
<td>1.15 (1.09–1.22)</td>
</tr>
<tr>
<td></td>
<td>CHD (n = 40,094)</td>
<td>1.01 (0.93–1.09)</td>
</tr>
<tr>
<td></td>
<td>Stroke (n = 40,094)</td>
<td>1.37 (1.26–1.50)</td>
</tr>
<tr>
<td></td>
<td>All-cause mortality</td>
<td>0.78 (0.74–0.82)</td>
</tr>
<tr>
<td></td>
<td>ESRD (n = 40,094)</td>
<td>0.47 (0.44–0.51)</td>
</tr>
</tbody>
</table>

Data are expressed as hazard ratio (95% confidence interval). CHD, coronary heart disease; CKD, chronic kidney disease; ESRD, end-stage renal disease; MACCE, major adverse cardiac and cerebrovascular events.

\( \text{a} \) Adjusted for age, sex, smoking, alcohol consumption, history of hypertension, myocardial infarction, congestive heart failure, peripheral vascular disease, stroke, cancer, body mass index, systolic blood pressure, fasting glucose, eGFR, total cholesterol, presence of proteinuria, and use of aspirin, a statin, or renin-angiotensin system blocker.
### Table 5. Risk of clinical outcomes according to metformin use in subgroups with adequate adherence<sup>a</sup> to diabetes medication

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Overall (n = 55,201)</th>
<th>CKD stage (eGFR, mL/min/1.73 m&lt;sup&gt;2&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude</td>
<td>Adjusted&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MACCE</td>
<td>1.39 (1.32–1.47)</td>
<td>1.66 (1.57–1.76)</td>
</tr>
<tr>
<td>CHD</td>
<td>1.23 (1.15–1.32)</td>
<td>1.47 (1.36–1.58)</td>
</tr>
<tr>
<td>Stroke</td>
<td>1.65 (1.51–1.79)</td>
<td>1.96 (1.80–2.15)</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>0.70 (0.67–0.74)</td>
<td>0.88 (0.83–0.93)</td>
</tr>
<tr>
<td>ESRD</td>
<td>0.12 (0.11–0.13)</td>
<td>0.29 (0.26–0.33)</td>
</tr>
</tbody>
</table>

Data are expressed as hazard ratio (95% confidence interval).

<sup>a</sup>Adequate adherence was defined as proportion of days covered ≥80%.

<sup>b</sup>Adjusted for age, sex, smoking, alcohol consumption, history of hypertension, myocardial infarction, congestive heart failure, peripheral vascular disease, stroke, cancer, body mass index, systolic blood pressure, fasting glucose, eGFR, total cholesterol, presence of proteinuria, and use of aspirin, a statin, or renin-angiotensin system blockade.

### Table 6. Risk of clinical outcomes according to metformin use after propensity score matching in subgroups with adequate adherence<sup>a</sup> to diabetes medication

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Overall (n = 32,744)</th>
<th>CKD stage (eGFR, mL/min/1.73 m&lt;sup&gt;2&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude</td>
<td>Adjusted&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MACCE</td>
<td>1.59 (1.49–1.69)</td>
<td>1.65 (1.55–1.75)</td>
</tr>
<tr>
<td>CHD</td>
<td>1.39 (1.29–1.51)</td>
<td>1.46 (1.35–1.59)</td>
</tr>
<tr>
<td>Stroke</td>
<td>1.89 (1.72–2.08)</td>
<td>1.92 (1.75–2.12)</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>0.88 (0.82–0.93)</td>
<td>0.89 (0.84–0.95)</td>
</tr>
<tr>
<td>ESRD</td>
<td>0.22 (0.19–0.24)</td>
<td>0.30 (0.26–0.33)</td>
</tr>
</tbody>
</table>

Data are expressed as hazard ratio (95% confidence interval).

<sup>a</sup>Adequate adherence was defined as proportion of days covered ≥80%.

<sup>b</sup>Adjusted for age, sex, smoking, alcohol consumption, history of hypertension, myocardial infarction, congestive heart failure, peripheral vascular disease, stroke, cancer, body mass index, systolic blood pressure, fasting glucose, eGFR, total cholesterol, presence of proteinuria, and use of aspirin, a statin, or renin-angiotensin system blockade.
with CKD. However, metformin users had significantly lower risks of all-cause mortality and ESRD during follow-up than non-users did. These relationships were consistent in the subgroups made using a PSM analysis, adequate adherence to anti-diabetes medication, and both together. The higher risk of MACCE and lower risk of mortality during follow-up in metformin users were mitigated at advanced CKD stages; however, the benefit for ESRD was consistently shown in all CKD stages.

Although several new classes of anti-diabetes drugs have been released, metformin is still the first-line drug for DM management. In Korea, metformin is even the most commonly used anti-diabetes medication in patients with early CKD [26]. Beyond glycemic control, it has been reported that metformin has pleiotropic effects that control lipids, body weight, and blood pressure [27,28]. Nevertheless, previous studies have raised uncertainties about the effectiveness of metformin in reducing cardiovascular outcomes in the general population [29–31]. In 2016, the U.S. FDA allowed metformin use in patients with CKD stage 3, and the Korean Ministry of Food and Drug Safety followed the U.S. FDA in that ruling in 2019. However, the relationship between metformin use and cardiovascular outcomes has remained inconclusive in patients with CKD.

In a Swedish study of 51,675 patients with type 2 diabetes, treatment with metformin showed a CVD benefit when compared with insulin treatment, but not when compared with other oral hypoglycemic agents [17]. In addition, those researchers found no significant relationship between metformin use and the risk of any CVD in the subgroup of CKD stage 3 patients. Charytan et al. [15] reported that metformin users in CKD stage 3, but not those in CKD stages 4 and 5, had a lower risk of cardiovascular death than non-users. A recent study by Roumie et al. [16] reported that metformin monotherapy was associated with a lower risk of MAACE than sulfonylurea monotherapy in patients with CKD. In contrast, we found that metformin users with CKD faced a significantly increased risk of MACCE. Some patient characteristics differed between those previous studies and this study. First, the mean BMI in the studies conducted by Charytan et al. [15] and Roumie et al. [16] was greater than 30 kg/m$^2$, which is higher than that in our study population (25.1 kg/m$^2$). A previous study found that metformin is efficacious in lowering serum glucose regardless of BMI, but the weight reduction effect was observed only in obese patients [32]. Therefore, the cardiovascular protective effect of metformin found in previous studies might have been caused by weight reduction, which would not be expected to apply to our study population. Second, the patients in our study had a lower incidence of CHD and higher incidence of stroke than the patients in previous studies. In particular, the high risk of MACCE we found was largely caused by a high risk of stroke in metformin users, and the HR of stroke was higher than that of CHD in all our analyses. Activating the AMP-activated protein kinase (AMPK) pathway is one of the main molecular mechanisms of metformin [33]. However, AMPK activation in the brain is remarkably increased after an ischemic injury [34]. Moreover, some experimental studies have reported that metformin treatment and subsequent AMPK activation can aggravate acute cerebral infarction [35]. Importantly, at a concentration higher than the therapeutic dose, metformin can further activate the AMPK pathway [33,36]. Because metformin is eliminated by the kidney, its concentration can increase when renal function declines [37]. Therefore, a previous observational study found that metformin use was associated with an increased risk of stroke in ESRD patients on dialysis [38]. Accordingly, although we could not examine the specific doses of metformin used, high concentrations of it might have produced the high incidence of stroke and MACCE found in our study. The risk of CHD was also significantly increased in CKD stage 3 in our data. In fact, there is a lack of evidence to support increased CHD in metformin users. The high risk of CHD with metformin use might be caused by lactic acidosis [39]. However, as aforementioned, the risk of lactic acidosis with metformin use is very low, and the risk has been reported not to increase even in CKD stage 5 [12–14]. One previous study reported that metformin use could delay the endothelial recovery of a drug-eluting stent via an AMPK-dependent pathway, which could cause stent thrombosis [40]. Poor glycemic control might be another cause of the increased CHD in metformin users in our study because the baseline level of fasting glucose in metformin users was significantly higher than that in non-users. Because this study was conducted using data from a health screening cohort, we could not examine other glycemic control parameters, such as glycated hemoglobin. However, the low risk of mortality and ESRD become difficult to explain if metformin users had poor glycemic control during the follow-up period.
Despite the increased risk of MAACE, metformin users had a significantly lower risk of cardiovascular mortality than non-users in our study. Interestingly, this paradoxical result was also reported in a previous study. A post hoc analysis of a randomized controlled trial reported that patients with metformin exposure had significantly lower risks of all-cause and cardiovascular mortality during follow-up than those without exposure \[41\]. However, the risk of stroke did not differ between groups (HR, 0.97; 95% CI, 0.68–1.39), and the risk of MI was marginally increased in patients with metformin exposure (HR, 1.23; 95% CI, 0.92–1.65). It is difficult to find evidence to support that contradictory result. As aforementioned, although AMPK activation caused by metformin can be harmful in patients with CKD, the AMPK pathway is also a well-known underlying protective mechanism for CVD \[42\]. Moreover, the role of AMPK in angiogenesis is known to be contradictory. Metformin suppresses retinal angiogenesis and exerts a protective effect against retinopathy \[43\], and it also suppresses tumor angiogenesis \[44\]. On the other hand, metformin promotes angiogenesis and revascularization under hypoxic and ischemic injury \[45\]. Thus, AMPK activation by metformin could have different angiogenic effects in different cellular microenvironments \[46\], which could affect its role in the development of CVD. Moreover, the metformin users in our study were younger and had fewer comorbidities and higher eGFR than the non-users. Therefore, metformin users might have survived CVD better than non-users. In addition, because we defined our outcomes based on diagnostic codes, not medical records, our operational definitions of the outcomes might not adequately reflect real clinical events, though hospitalization with a diagnostic code is a widely used method to define outcomes in big data studies \[13,16\].

Metformin users had a significantly lower risk of all-cause mortality during follow-up than non-users did in our study, which is inconsistent with previous studies \[15,19\]. This result might reflect the lower comorbidity levels of metformin users. However, survival benefits remained when we matched comorbidities in the PSM analysis. It is noteworthy that the survival benefit of metformin use was attenuated in patients at an advanced CKD stage in our sensitivity analysis. In a study by Hung et al. \[13\], metformin use was associated with an increased risk of all-cause mortality during follow-up in patients with CKD stage 5. They also reported that the mortality risk increased dose-dependently with metformin use in that population. Therefore, renal function depletion and consequent metformin accumulation might not be beneficial for mortality at an advanced CKD stage. Nevertheless, that study also reported a significantly decreased risk of ESRD in CKD stage 5. In our study, the decreased risk of ESRD conferred by metformin use was observed across all CKD stages. Recent experimental studies demonstrated that metformin prevented renal fibrosis and retarded CKD progression in murine models \[47,48\]. Nevertheless, because metformin use also conferred an increased risk of MACCE in our study, further experimental studies are needed to reveal the effects of metformin on other organs, beyond its protective effects on the kidney.

This study has several limitations. First, because we defined metformin use as 90 days of use before the index date, we could not consider metformin use before the health screening day. Thus, the actual duration of metformin use could differ from that used in the analysis. Second, we could not consider newly added, switched, or discontinued anti-diabetes medications (other than metformin) during the follow-up period. Therefore, the effects of other anti-diabetes medications might confound our findings. Third, we could not include the dose of metformin in the analysis. Thus, dose-dependent relationships between metformin and clinical outcomes were not presented. Fourth, although we conducted PSM analyses, hidden confounding factors might still have affected the relationship between metformin use and clinical outcomes. Fifth, some health screening centers might not have used isotope dilution mass spectrometry–traceable creatinine measurements. Thus, the possibility of CKD stage misclassification exists in this study. Finally, the study population in this work was Korean, and our results might not be generalizable to other ethnic groups. Despite those limitations, our study also has several strengths. Because this was a nationwide cohort study, a large number of patients were included. In addition, the NHIS database includes claims for all medical facilities in Korea, and data from Statistics Korea include all cases of mortality. Therefore, few outcomes in the data were missing. Moreover, we conducted sensitivity analyses for subgroups with PDC of ≥80% and found consistent relationships between metformin use and clinical outcomes. Thus, our results were not confounded by medication discontinuation.
In conclusion, despite the current trend to expand metformin use in patients with CKD, we found that metformin use could be associated with an increased risk of MACCE in this population. On the other hand, metformin users had a lower risk of all-cause mortality and ESRD during follow-up than non-users did. Therefore, metformin needs to be used carefully under strict surveillance for CVD occurrence in patients with CKD.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

This study was supported by a Young Investigator Research Grant from the Korean Society of Nephrology (2019), a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (2019R1G1A1100498), and the Soonchunhyang University Research Fund.

Authors’ contributions

Conceptualization: HN, DRR
Data curation: MHK
Formal analysis: MHK
Funding acquisition: HK
Writing–original draft: HJO, SHK, JSJ, HN, DCH, HK, DRR
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Characteristics of pediatric rhabdomyolysis and the associated risk factors for acute kidney injury: a retrospective multicenter study in Korea

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Background: The clinical features of pediatric rhabdomyolysis differ from those of the adults with rhabdomyolysis; however, multicenter studies are lacking. This study aimed to investigate the characteristics of pediatric rhabdomyolysis and reveal the risk factors for acute kidney injury (AKI) in such cases.

Methods: This retrospective study analyzed the medical records of children and adolescents diagnosed with rhabdomyolysis at 23 hospitals in South Korea between January 2007 and December 2016.

Results: Among 880 patients, those aged 3 to 5 years old composed the largest subgroup (19.4%), and all age subgroups were predominantly male. The incidence of AKI was 11.3%. Neurological disorders (53.6%) and infection (39.0%) were the most common underlying disorder and cause of rhabdomyolysis, respectively. The median age at diagnosis in the AKI subgroup was older than that in the non-AKI subgroup (12.2 years vs. 8.0 years). There were no significant differences in body mass index, myalgia, dark-colored urine, or the number of causal factors between the two AKI-status subgroups. The multivariate logistic regression model indicated that the following factors were independently associated with AKI: multiorgan failure, presence of an underlying disorder, strong positive urine occult blood, increased aspartate aminotransferase and uric acid levels, and reduced calcium levels.

Conclusion: Our study revealed characteristic clinical and laboratory features of rhabdomyolysis in a Korean pediatric population and highlighted the risk factors for AKI in these cases. Our findings will contribute to a greater understanding of pediatric rhabdomyolysis and may enable early intervention against rhabdomyolysis-induced AKI.

Keywords: Creatine kinase, Etiology, Muscles, Renal insufficiency
**Introduction**

Rhabdomyolysis is a syndrome involving skeletal muscle damage caused by conditions such as severe trauma, physical exertion, seizure, drug use, toxins, biological agents, metabolic disorders, prolonged immobilization, and genetic defects. Subsequently, the breakdown of the damaged skeletal muscle leads to the release of its contents into plasma, thereby causing complications, such as acute kidney injury (AKI), electrolyte disturbance, compartment syndrome, and disseminated intravascular coagulation [1,2]. AKI is the leading cause of death in patients with rhabdomyolysis. Myoglobin-induced renal toxicity leads to rhabdomyolysis-associated AKI because of increased oxidative stress, inflammation, endothelial dysfunction, vasoconstriction, tubular obstruction, and apoptosis [1,3].

Rhabdomyolysis appears more frequently among male individuals, African-Americans, those aged younger than 10 or older than 60 years, and those with body mass index (BMI) values of greater than 40 kg/m² [4]. However, the etiology of rhabdomyolysis and the incidence of AKI may vary depending on age [4–7]. Furthermore, large-scale multicenter studies on rhabdomyolysis and the associated risk factors for AKI in the pediatric population are scarce. The primary aim of this study was to investigate the characteristics of rhabdomyolysis in a Korean pediatric population. The secondary aim was to identify the risk factors for AKI among patients with rhabdomyolysis.

**Methods**

**Study design**

This retrospective study analyzed the medical records of children and adolescents diagnosed with and treated for rhabdomyolysis at 23 hospitals in South Korea from January 2007 to December 2016. This study was approved by the Institutional Review Board of Pusan National University School of Medicine and informed consent was waived due to the nature of the retrospective study (No. 05-2018-021).

**Study population**

Rhabdomyolysis was defined by a creatine kinase (CK) level of at least 1,000 U/L or myoglobin level of at least 100 ng/mL. Patients eligible for inclusion were those aged between one month and 18 years who met the definition of having rhabdomyolysis (n = 1,046). Individuals were excluded from this study if they had myocarditis or myocardial infarction, had received cardiopulmonary resuscitation (n = 118) or had insufficient medical records (n = 48). Patients with muscular dystrophy were included only if they had not yet been diagnosed with such (n = 18). All subjects (n = 880) were divided into two AKI-status subgroups (with or without AKI) for comparison (Fig. 1).

**Data collection**

Medical records were reviewed to observe the following: age at diagnosis, sex, anthropometric data, symptoms, underlying illnesses, causal factors and complications of rhabdomyolysis, laboratory results, methods of treatment, and outcomes. All individuals were divided into five age subgroups because the incidence of rhabdomyolysis and the rate of AKI are not linearly associated with age [6]. Demographic data such as height, weight, and BMI were transformed into z-scores using the 2017 Korean National Growth Charts for Children and Adolescents (https://knhanes.cdc.go.kr/knhanes/sub08/sub08_02.do) and lambda–mu–sigma method [8]. AKI was defined as an increase in serum creatinine of at least 0.3 mg/dL within 48 hours or at least 1.5 times the minimum creatinine value during the hospitalized stay, regardless of the time period [9], which was established according to the creatinine criteria of the Kidney Disease:

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**Figure 1. CONSORT flow diagram.**

AKI, acute kidney injury.
Improving Global Outcomes (KDIGO) guidelines [10]. Oliguria was defined as a urine output of less than 0.5 mL/kg/hr for 12 hours. Urine output was determined based on details from the medical history, such as the time of last urination and medical records of urine output in the hospital. Multiorgan failure was defined as the failure of at least two of the major organ systems. Short-term and long-term outcomes were stratified based on a cutoff of 90 days after the date of AKI occurrence. The recovery duration of CK and myoglobin levels was defined as the period from the onset of rhabdomyolysis to the date of restoring the value in question to within the normal range. Volume overload was defined as the presence of pulmonary edema or peripheral edema. Chronic kidney disease was defined as a decrease in the glomerular filtration rate to less than 90 mL/min/1.73 m², which was calculated using the updated Schwartz formula as follows: 0.413 × [height (cm)/serum creatinine (mg/dL)]. Laboratory parameters were analyzed by the following or equivalent methods: serum CK was analyzed by a CK N-acetyl-cystein–activated procedure; serum myoglobin was analyzed by chemiluminescence immunoassay; qualitative and quantitative urine myoglobin levels were analyzed by ammonia sulfate precipitation testing with urine test strips and electrochemiluminescence immunoassay, respectively; and serum creatinine was analyzed using the modified Jaffe’s kinetic method.

Statistical analysis

For the comparison of AKI and non-AKI subgroups, the Student t test or Mann-Whitney U test was performed for continuous variables, and the chi-squared test or Fisher exact test was performed for categorical variables. Multiple responses were allowed when surveying underlying diseases, causes, and complications of rhabdomyolysis. Statistical significance was set at p < 0.05.

We investigated whether AKI can be predicted by risk factors and initially accessible clinical information of patients with rhabdomyolysis. Multiple logistic regression analysis was performed to determine the independent risk factors associated with AKI. Candidate variables with p-values of less than 0.1 in the univariate analysis were entered into the model through variable selection by a stepwise method. Variance inflation factors were checked to eliminate multiple coherences. The results of the generated model were confirmed by the receiver-operating characteristic (ROC) curve and area under the ROC curve. All analyses were performed using R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Demographic data and clinical symptoms

According to the study inclusion and exclusion criteria, 880 patients were included. The overall age distribution of these patients showed a bimodal peak (3-5 and 15-18 years, respectively). Among the five age subgroups, the largest one was those aged three to 5 years old in the total group and non-AKI subgroup, whereas it was 15 to 18 years old in the AKI subgroup. The median age at diagnosis was older in the AKI subgroup than in the non-AKI subgroup (12.2 years vs. 8.0 years; p < 0.001). There were 2.5 times more boys (ratio of boys:girls, 634:246), and all age subgroups were predominantly male (range of sex proportion, 2.0–3.6). Boys totaled 68.0% to 69.6% of patients younger than 12 years and 76.8% to 78.0% of those older than 12 years (Table 1).

Height, weight, and BMI z-scores did not significantly deviate from zero in the total rhabdomyolysis group. For those in the AKI subgroup, the actual height, weight, and BMI were higher, but their z-scores were not significantly different between the two subgroups. Body surface area was also higher in the AKI subgroup, but the z-scores could not be compared between the AKI-status subgroups because of a lack of data. Among the 880 patients, myalgia or muscle weakness was observed in 38.9% and dark-colored urine was present for 14.0%. Although oliguria occurred in only 4.1% of all rhabdomyolysis cases, it affected one-third of the AKI subgroup. Only 3.4% of all rhabdomyolysis cases had two or more causes of rhabdomyolysis. There were no significant differences in myalgia or muscle weakness, dark-colored urine, or the number of causal factors between the two AKI-status subgroups (Table 1).

Underlying disease

Of the 880 patients, 28.6% had underlying diseases. A detailed list of underlying disorders is provided in the supplementary data (Supplementary Table 1, available online). Neurological disorders were the most common underlying
disorder (53.6%), followed by nephrological disorders, endocrinological and metabolic disorders, genetic and syndrome disorders, and cardiological disorders (Fig. 2A). The frequency of neurological disorders had a bimodal distribution and peaked in patients aged zero to 2 years and those aged 15 to 18 years, respectively. Most cardiological disorders were observed in patients younger than 9 years of age, whereas most psychiatric disorders were observed in those older than 9 years of age. No characteristic age distribution was identified for the other disorders (Fig. 2B). The underlying disorder that showed a significantly higher proportion in the AKI group included neurological disorders (26.3% vs. 14.0%; p = 0.002) and oncologic disorders (4.0% vs. 0.6%; p = 0.008). The proportion of total underlying disorders was higher in the AKI subgroup (53.5% vs. 25.5%; p < 0.001).

### Etiology

The most common cause of rhabdomyolysis was infection (39.0%), followed by trauma or surgery (21.1%), prolonged convulsions (21.0%), and unknown causes (9.6%). Other causes were diverse and heterogeneous, with a proportion of less than 3.0% (Fig. 3A). Influenza virus was the most common infectious cause. A detailed list of etiologies is shown in the supplementary data (Supplementary Table 2, available online).

Some causes of rhabdomyolysis were characterized by age; the greatest frequency of seizures and infections occurred at 0 to 2 years of age and 3 to 5 years of age, respectively. The frequency of trauma increased with age, with most cases occurring in patients aged 15 to 18 years (Fig. 3B-I). In the AKI group, although trauma was still prominent in older patients, the age pattern of infection and prolonged seizures in the total group became indistinctive. However, AKI was more frequently caused by relatively rare causes, such as drugs, multiorgan failure, cardiac arrhythmias, and metabolic disorders (Fig. 3B-II, III). Similarly, the differences in the frequency of infection (ratio of AKI:non-AKI subgroups, 36.1%:40.8%; p = 0.12) and prolonged seizure (18.1%:22.5%; p = 0.18) were not prominent, but those for multiorgan failure (12.4%:1.2%; p < 0.001) and drugs (6.7%:1.2%; p < 0.004) were significantly higher in the AKI subgroup.

### Table 1. Characteristics of patients with acute rhabdomyolysis with or without AKI

<table>
<thead>
<tr>
<th>Variable</th>
<th>AKI (n = 99)</th>
<th>Non-AKI (n = 781)</th>
<th>Total (n = 880)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>65 (65.7)</td>
<td>569 (72.9)</td>
<td>634 (72.0)</td>
<td>0.17</td>
</tr>
<tr>
<td>Age at diagnosis (yr)</td>
<td>12.2 (6.0–15.1)</td>
<td>8.0 (4.0–13.4)</td>
<td>8.0 (4.0–14.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0–2</td>
<td>12 (12.1)</td>
<td>140 (17.9)</td>
<td>152 (17.3)</td>
<td></td>
</tr>
<tr>
<td>3–5</td>
<td>11 (11.1)</td>
<td>160 (20.5)</td>
<td>171 (19.4)</td>
<td></td>
</tr>
<tr>
<td>6–8</td>
<td>9 (9.1)</td>
<td>119 (15.2)</td>
<td>128 (14.5)</td>
<td></td>
</tr>
<tr>
<td>9–11</td>
<td>11 (11.1)</td>
<td>86 (11.0)</td>
<td>97 (11.0)</td>
<td></td>
</tr>
<tr>
<td>12–14</td>
<td>22 (22.2)</td>
<td>142 (18.2)</td>
<td>164 (18.6)</td>
<td></td>
</tr>
<tr>
<td>15–18</td>
<td>34 (34.3)</td>
<td>134 (17.2)</td>
<td>168 (19.1)</td>
<td></td>
</tr>
<tr>
<td>Height z-score</td>
<td>0.17 (−1.12 to 1.16)</td>
<td>0.29 (−0.70 to 1.35)</td>
<td>0.29 (−0.76 to 1.30)</td>
<td>0.57</td>
</tr>
<tr>
<td>Weight z-score</td>
<td>0.01 (−1.36 to 0.84)</td>
<td>−0.12 (−1.05 to 0.78)</td>
<td>−0.11 (−1.09 to 0.78)</td>
<td>0.95</td>
</tr>
<tr>
<td>BMI z-score(^a) (n = 780)</td>
<td>−0.03 (−1.29 to 0.86)</td>
<td>−0.26 (−1.09 to 0.71)</td>
<td>−0.23 (−1.10 to 0.72)</td>
<td>0.54</td>
</tr>
<tr>
<td>BSA(^b)</td>
<td>1.28 (0.83–1.60)</td>
<td>0.96 (0.70–1.39)</td>
<td>0.97 (0.72–1.44)</td>
<td>0.00</td>
</tr>
<tr>
<td>Myalgia or muscle weakness</td>
<td>41 (41.4)</td>
<td>301 (38.5)</td>
<td>342 (38.9)</td>
<td>0.66</td>
</tr>
<tr>
<td>Dark-colored-urine</td>
<td>20 (20.2)</td>
<td>103 (13.2)</td>
<td>123 (14.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Oliguria, yes</td>
<td>36 (36.4)</td>
<td>0 (0)</td>
<td>36 (4.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. of causal factors(^c)</td>
<td>92 (92.9)</td>
<td>758 (97.1)</td>
<td>850 (96.6)</td>
<td>0.07</td>
</tr>
<tr>
<td>Single</td>
<td>7 (7.1)</td>
<td>23 (2.9)</td>
<td>30 (3.4)</td>
<td></td>
</tr>
</tbody>
</table>

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

AKI, acute kidney injury; BMI, body mass index; BSA, body surface area.

\(^a\)National database of BMI only includes individuals older than 2 years. \(^b\)BSA z-score was not available in our study. \(^c\)Multiple responses allowed the collection of causal factor data.
Laboratory data

When the AKI and non-AKI subgroups were compared, most laboratory results showed significant differences between these two groups. A significantly greater proportion of AKI cases was associated with more positive urine occult blood (OB) (p < 0.001). The proportions of hematuria and urine myoglobin were higher in the AKI subgroup (p < 0.001 for both). Initial and peak CK and peak myoglobin levels were also significantly higher in the AKI subgroup, while the initial calcium levels were lower in the AKI subgroup. The initial and peak blood levels of parameters of interest, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine, uric acid, and phosphorus, were significantly higher in the AKI subgroup (Table 2).

Among the results of univariate analysis (Table 1, 2), clinical information, which was initially assessable and whose p-value was less than 0.1, underwent stepwise variable selection, and the following variables were selected: age subgroup, the presence of underlying disease, multorgan failure, urine OB, CK, AST, uric acid, calcium, and phosphorus. Based on the multiple logistic regression model for predicting AKI in rhabdomyolysis, variables that were
independently associated with AKI development includ-
ed the presence of underlying disease, multiorgan failure, 
urine OB, AST, uric acid, and calcium. Patients aged 15 to 18 
years had an odds ratio of 2.3 relative to that of the reference 
group (3–5 years), but the p-value was insignificant. Urine 
OB 4+ and multiorgan failure-induced rhabdomyolysis had 
the highest odds ratios (Table 3). The discrimination value 
of the predictive model was established by means of an 
ROC curve, with an area under the ROC curve of 0.946 (p < 
0.001) (Fig. 4).

Outcomes

CK and myoglobin were recovered within 5 to 6 days after

Figure 3. Cause of rhabdomyolysis. (A) Proportion of each cause. Infection, including both influenza and other infections, was the most prevalent cause (39.0%). (B) Age distribution of the causes. AKI, acute kidney injury.
On the first day of rhabdomyolysis, and there was no difference between the AKI and non-AKI subgroups. Short-term outcomes in the AKI subgroup were characterized by a higher proportion of volume overload (33.8% vs. 0.7%; p < 0.001) and longer hospitalization (18 days vs. 6 days; p < 0.001). The median oliguria recovery time was 2.5 days in the AKI subgroup. The long-term outcomes of 522 patients, excluding those with missing data, revealed a greater proportion of chronic kidney disease and recurrent rhabdomyolysis in the AKI subgroup (p < 0.001) (Table 4).

### Complications

Only 15.4% of patients with rhabdomyolysis experienced complications. The total incidence of AKI was 11.3%; the incidence rates for the 0 to 9 years, 9 to 11 years, 12 to 14 years, and 15 to 18 years age subgroups were 6.4% to 7.9%, 11.3%, 13.4%, and 20.2%, respectively. The AKI subgroup present-

### Table 2. Laboratory results of acute rhabdomyolysis in those with and without AKI

<table>
<thead>
<tr>
<th>Variable</th>
<th>AKI (n = 99)</th>
<th>Non-AKI (n = 781)</th>
<th>Total (n = 880)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine occult blood</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Negative</td>
<td>27 (27.3)</td>
<td>544 (69.7)</td>
<td>571 (64.9)</td>
<td></td>
</tr>
<tr>
<td>Trace</td>
<td>0 (0)</td>
<td>12 (1.5)</td>
<td>12 (1.4)</td>
<td></td>
</tr>
<tr>
<td>1+</td>
<td>9 (9.1)</td>
<td>54 (6.9)</td>
<td>63 (7.2)</td>
<td></td>
</tr>
<tr>
<td>2+</td>
<td>17 (17.2)</td>
<td>52 (6.7)</td>
<td>69 (7.8)</td>
<td></td>
</tr>
<tr>
<td>3+</td>
<td>38 (38.4)</td>
<td>106 (13.6)</td>
<td>144 (16.4)</td>
<td></td>
</tr>
<tr>
<td>4+</td>
<td>8 (8.1)</td>
<td>13 (1.7)</td>
<td>21 (2.4)</td>
<td></td>
</tr>
<tr>
<td>Urine RBC count (/HPF)</td>
<td>34 ± 67</td>
<td>12 ± 43</td>
<td>15 ± 46</td>
<td>0.002</td>
</tr>
<tr>
<td>True hematuria, RBC ≥ 5/HPF</td>
<td>45 (45.5)</td>
<td>105 (13.4)</td>
<td>150 (17.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urine myoglobin, positive (n = 267)</td>
<td>28 (82.4)</td>
<td>96 (41.2)</td>
<td>124 (46.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood test results at initial consult</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>3,161 (1,050−12,772)</td>
<td>1,986 (1,078−5,698)</td>
<td>2,144 (1,077−6,262)</td>
<td>0.05</td>
</tr>
<tr>
<td>Myoglobin (mg/mL)</td>
<td>2,072 (512−6,085)</td>
<td>2,266 (243−12,449)</td>
<td>2,159 (278−12,449)</td>
<td>0.93</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>179 (78−973)</td>
<td>86 (49−213)</td>
<td>90 (50−263)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>88 (24−382)</td>
<td>34 (17−120)</td>
<td>36 (17−138)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>1,264 (756−3,766)</td>
<td>677 (531−1,152)</td>
<td>725 (542−1,354)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>25.4 (15.5−53.5)</td>
<td>10.8 (8.3−13.5)</td>
<td>11.2 (8.4−14.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.5 (0.9−3.0)</td>
<td>0.5 (0.4−0.7)</td>
<td>0.5 (0.4−0.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>8.0 (5.6−12.5)</td>
<td>4.2 (3.2−5.9)</td>
<td>4.5 (3.2−6.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.6 (7.5−9.2)</td>
<td>9.2 (8.8−9.6)</td>
<td>9.1 (8.7−9.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>5.1 (4.1−6.5)</td>
<td>4.4 (3.9−5.1)</td>
<td>4.5 (3.9−5.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The median oliguria recovery time was 2.5 days in the AKI subgroup. The long-term outcomes of 522 patients, excluding those with missing data, revealed a greater proportion of chronic kidney disease and recurrent rhabdomyolysis in the AKI subgroup (p < 0.001) (Table 4).

### Complications

Only 15.4% of patients with rhabdomyolysis experienced complications. The total incidence of AKI was 11.3%; the incidence rates for the 0 to 9 years, 9 to 11 years, 12 to 14 years, and 15 to 18 years age subgroups were 6.4% to 7.9%, 11.3%, 13.4%, and 20.2%, respectively. The AKI subgroup present-

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

AKI, acute kidney injury; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CK, creatine kinase; HPF, high-power field; LDH, lactate dehydrogenase; RBC, red blood cell.
Table 3. Multiple logistic regression model for predicting AKI in patients with acute rhabdomyolysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group (yr)</td>
<td>Reference = 3–5 yr&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0–2</td>
<td>0.96 (0.32–2.87)</td>
<td>0.95</td>
</tr>
<tr>
<td>6–8</td>
<td>0.84 (0.24–2.89)</td>
<td>0.78</td>
</tr>
<tr>
<td>9–12</td>
<td>0.72 (0.21–2.44)</td>
<td>0.59</td>
</tr>
<tr>
<td>12–14</td>
<td>1.15 (0.43–3.1)</td>
<td>0.79</td>
</tr>
<tr>
<td>15–18</td>
<td>2.30 (0.89–5.93)</td>
<td>0.09</td>
</tr>
<tr>
<td>Presence of underlying disorder</td>
<td>3.35 (1.91–5.88)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Multiorgan failure</td>
<td>6.28 (1.92–20.57)</td>
<td>0.002</td>
</tr>
<tr>
<td>Urine occult blood&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Reference = negative</td>
<td></td>
</tr>
<tr>
<td>Trace</td>
<td>0 (0–infinity)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>1+</td>
<td>2.05 (0.78–5.38)</td>
<td>0.15</td>
</tr>
<tr>
<td>2+</td>
<td>2.69 (1.07–6.74)</td>
<td>0.03</td>
</tr>
<tr>
<td>3+</td>
<td>4.46 (2.22–8.97)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4+</td>
<td>5.21 (1.53–17.81)</td>
<td>0.008</td>
</tr>
<tr>
<td>CK/100&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1 (1.00–1.00)</td>
<td>0.43</td>
</tr>
<tr>
<td>AST/100&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.05 (1.01–1.09)</td>
<td>0.01</td>
</tr>
<tr>
<td>Uric acid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.23 (1.14–1.34)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78 (0.65–0.94)</td>
<td>0.008</td>
</tr>
<tr>
<td>Phosphorus&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03 (0.89–1.19)</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Initial creatinine was included in the multivariable logistic regression analysis to reveal uric acid, phosphorus, and calcium as risk factors for AKI, independent of creatinine.

AKI, acute kidney injury; AST, aspartate aminotransferase; CI, confidence interval; CK, creatine kinase; OR, odds ratio.

<sup>a</sup>Laboratory findings at initial consult. <sup>b</sup>OR of CK/100 and AST/100 can be interpreted as 100-unit increases in the values of CK and AST. <sup>c</sup>Age range of 3 to 5 years was selected as the reference because this age group had the lowest incidence of AKI.

![Figure 4. Receiver-operating characteristic curve of the multiple logistic regression model for predicting acute kidney injury.](image)

Treatment

In this study, 24.2% of patients did not require treatment, with a significantly higher proportion of these patients existing in the non-AKI group (p = 0.002). Volume replacement was the most commonly used treatment (72.1%). Alkalization and mannitol administration were performed significantly more often in the AKI subgroup (p < 0.001). Dialysis was performed in 31.3% of the 99 patients with AKI. Peritoneal dialysis was performed in only one patient, while the others required continuous renal replacement therapy (CRRT) (Table 5). The average dialysis period was 10.6 days.

Discussion

Rhabdomyolysis has been defined in various ways in previous studies. A CK level of at least 1,000 U/L or exceeding 5 times the upper limit of the normal range has been commonly used as the cutoff value for diagnosis [4], but those of at least 5,000 U/L and at least 10,000 U/L have also been
Table 4. Outcomes and complications of acute rhabdomyolysis in patients with or without AKI

<table>
<thead>
<tr>
<th>Variable</th>
<th>AKI (n = 99)</th>
<th>Non-AKI (n = 781)</th>
<th>Total (n = 880)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short-term outcomes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK recovery time (day)</td>
<td>7.0 (3.5–12.5)</td>
<td>6.0 (4.0–8.0)</td>
<td>6.0 (4.0–9.0)</td>
<td>0.11</td>
</tr>
<tr>
<td>Myoglobin recovery time (day)</td>
<td>5.0 (3.0–11.0)</td>
<td>5.0 (3.0–9.0)</td>
<td>5.0 (3.0–9.0)</td>
<td>0.44</td>
</tr>
<tr>
<td>Oliguria recovery time (day)</td>
<td>2.5 (2.0–8.0)</td>
<td>NA</td>
<td>2.5 (2.0–8.0)</td>
<td>0.005</td>
</tr>
<tr>
<td>Volume overload, yes (n = 529)a</td>
<td>24 (33.8)</td>
<td>3 (0.7)</td>
<td>27 (5.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of hospitalization (day)</td>
<td>18.0 (7.0–38.0)</td>
<td>6.0 (4.0–10.0)</td>
<td>7.0 (4.0–12.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Long-term outcome (n = 522)b</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>None</td>
<td>39 (82.9)</td>
<td>460 (96.8)</td>
<td>499 (95.6)</td>
<td></td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>3 (6.4)</td>
<td>0 (0)</td>
<td>3 (0.6)</td>
<td></td>
</tr>
<tr>
<td>Recurrent rhabdomyolysis</td>
<td>5 (10.5)</td>
<td>15 (3.2)</td>
<td>20 (3.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Complications</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>10 (10.1)</td>
<td>734 (94.0)</td>
<td>744 (84.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AKI</td>
<td>99 (100)</td>
<td>0 (0)</td>
<td>99 (11.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Compartment syndrome</td>
<td>0 (0)</td>
<td>2 (0.3)</td>
<td>2 (0.2)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>DIC</td>
<td>12 (12.1)</td>
<td>3 (0.4)</td>
<td>15 (1.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Electrolyte disturbance</td>
<td>25 (25.3)</td>
<td>29 (3.7)</td>
<td>54 (6.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Death</td>
<td>14 (14.1)</td>
<td>14 (1.8)</td>
<td>28 (3.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as median (interquartile range) or number (%).
AKI, acute kidney injury; CK, creatine kinase; DIC, disseminated intravascular coagulopathy; NA, not applicable.

aThe number of patients except missing value.
bMultiple responses allowed the collection of data on complications.

Figure 5. Distribution of complications by age in rhabdomyolysis patients.
AKI, acute kidney injury; DIC, disseminated intravascular coagulopathy.

adopted [8–10]. Another criterion that is less frequently used is myoglobin, whose reference level in previous studies ranged from at least 80 ng/mL to at least 150 ng/mL [7,11,12]. We used either CK or myoglobin level as the inclusion criteria for our study to maximize the number of participants.

Because of the diversity of definitions and subjects, the incidence of rhabdomyolysis varies from 4% to 50% in adult studies [3,11]. The exact incidence of pediatric rhabdomyolysis is unclear, but it has been reported to range from 5% to 37.5% [5–7]. Approximately 4% to 50% of cases of rhabdomyolysis lead to AKI, while up to 15% of AKI cases can be attributed to rhabdomyolysis [1–3]. The incidence of AKI in our study was 11.3%. We believe that the use of a single,
universal definition of rhabdomyolysis is necessary because it will aid in comparing incidence rates between studies and lead to advancements in this field.

A pediatric study performed in Korea investigated rhabdomyolysis in 39 children and reported the findings of a higher proportion of boys (2.5-fold more) among the affected children, epilepsy as the most common underlying disorder, and infection as the most common cause, similar to our results [7]. Another pediatric study that involved 172 children in Taiwan reported the existence of a higher proportion of boys (3.7-fold more) among the total cases and found viral myositis to be the most common cause. However, these authors also reported an absence of sex differences in AKI incidence [6]. Similarly, our study also revealed a male sex dominance. The higher proportion of adolescent males in rhabdomyolysis cases suggests that sex hormones or greater physical activity, which leads to more muscle mass, may contribute to the development of rhabdomyolysis. There were no sex differences in the proportion of causes, underlying disease, or complications of rhabdomyolysis in our study. Further studies are needed to determine whether sex contributes to rhabdomyolysis. We also did not observe any differences in the incidence of AKI according to sex. Therefore, male sex is not a risk factor for rhabdomyolysis-induced AKI.

The influence of anthropometric data on rhabdomyolysis and AKI has not been fully elucidated, and reports on the association between rhabdomyolysis and obesity are limited. Increased BMI could reflect a large muscle mass but usually implies excess adipose tissue that could induce pathological changes in the kidney and increase baseline circulating levels of nephrotoxic inflammatory molecules [13]. Several adult studies have indicated that obesity and high BMI are linked to rhabdomyolysis and AKI [14–17]. In a single-center cohort study on obesity and AKI unrelated to rhabdomyolysis, obese patients were more likely to develop AKI, and each 5-kg/m² increase in BMI was associated with a 10% greater risk of severe AKI [14]. However, BMI was not associated with AKI in a previous pediatric study [6], and the z-scores of height, weight, and BMI did not differ between the AKI and non-AKI subgroups in our study. At this time, the reason why there is a contradiction regarding the influence of obesity on rhabdomyolysis and AKI between adults and children remains unclear. With respect to the different results between pediatric and adult studies, limitations of BMI, body composition, and duration of obesity might affect the risk of AKI.

Although myalgia, weakness, and dark-colored urine have been known as the classic triad of symptoms of rhabdomyolysis, these findings are observed in less than 10% of affected patients. In a previous report, more than 50% of patients did not have myalgia or weakness, while dark-colored urine was observed in only 3.6% of the cases [2]. Our cases showed a relatively greater proportion of the classic triad than found in previous studies, but these symptoms were not distinguishing features of the AKI subgroup.

It is well known that infants and toddlers are vulnerable to febrile illness and seizures and that outside activities and accidents tend to increase in adolescents. The etiologic distribution of bimodal distribution in our study reflects these characteristics and may influence the frequency of rhabdomyolysis in each age group. Therefore, these etiologic results vary depending upon the subpopulation. Previous adult studies suggested that trauma and illicit drug abuse are primary causes of rhabdomyolysis [18–20]. In our study, trauma was the second most common cause of rhabdomyolysis and was more prevalent in adolescent patients. However, drug-induced rhabdomyolysis accounted for only 1.7% of cases, and there were no cases of illicit drug use. Relatively low frequencies of trauma-induced
and drug-induced rhabdomyolysis are believed to reflect the characteristics of the pediatric population and low prevalence of drug abuse in Korea [21,22]. Therefore, we suggest that the causes of rhabdomyolysis should be investigated in specific subpopulations.

Patients who experience seizures are considered vulnerable to rhabdomyolysis for two reasons. First, seizures lead to postictal alterations in several blood parameters, such as CK [23]. It is well known that skeletal muscle injury can be caused by convulsive seizures or status epilepticus [24]. Second, the majority of antiepileptic drugs, such as valproic acid and levetiracetam, are relevant to rhabdomyolysis [25]. Some neuromuscular disorders, such as muscular dystrophy, are accompanied by increased CK levels, which are susceptible to triggering factors such as fever, exercise, bisphosphonate use, and anesthesia [26–28]. A retrospective study reported 13 patients with rhabdomyolysis featuring muscular dystrophy; the median duration between the first episodes of rhabdomyolysis and genetic diagnosis was 2 years, and the authors suggested that persistently increased CK levels with recurrent rhabdomyolysis warrant a workup for underlying muscular dystrophy [28].

Muscle-derived components are effective biomarkers for renal damage, resulting in hyperphosphatemia; hypercalcemia; hyperuricemia; increased plasma LDH and AST levels; and increased urinary excretion of creatinine, uric acid, and glucose [29]. Although CK is a cornerstone of rhabdomyolysis diagnosis, it remains controversial whether CK itself is related to the risk of AKI and mortality. Some studies have shown that the initial or peak CK value is not a reliable marker of AKI or mortality outcome [30–32]. However, other studies have suggested that the initial and peak values of CK and myoglobin are risk factors for AKI [6,20]. Our study showed that the initial CK level was significantly increased, but that of myoglobin was not. These contradictory results regarding CK and myoglobin may be affected by differences in the time interval from symptom onset to arrival at the hospital. CK levels rise in rhabdomyolysis within 12 hours of the onset of muscle injury, peak within 24 to 72 hours, and normalize approximately 5 days after the cessation of muscle injury. The half-life of CK is approximately 36 hours [33]. Meanwhile, myoglobin has a short half-life (2–4 hours) and may return to normal within 6 to 8 hours [2]. In future studies, it is worth considering adjusting the initial CK level according to the timing of muscle injury.

Our study revealed significant differences in most traditional biomarkers, including AST, ALT, LDH, uric acid, calcium, and phosphorus. Although they were not included in our study, constant albuminuria and hypoalbuminemia have been suggested as independent risk factors for AKI in previous research [7,20]. In this respect, timely measurements of traditional biomarkers are useful and practical for predicting AKI. The McMahon score predicts the risk of renal failure requiring renal replacement therapy (RRT) or resulting in mortality in patients with rhabdomyolysis. The variables include age; female sex; origin of rhabdomyolysis; and initial values of creatinine, calcium, CK, and phosphate. A McMahon score of 6 points or higher has greater sensitivity and specificity than a CK level of greater than 5,000 U/L in predicting the risk of RRT [34]. Although the McMahon score was developed for adult patients, modification of variables based on our and other pediatric studies may give us a scoring system applicable to children in future studies.

Dipstick urine OB can yield false-positive results caused by dehydration, exercise, hemoglobinuria, and myoglobinuria, which are commonly seen in rhabdomyolysis [1,35]. However, it is an inexpensive and rapid test that is useful for detecting the risk-estimation markers of AKI. The correlation between AKI and urine OB shown in our study supports the usefulness of the urine dipstick test. In our study, dark-colored urine did not show any difference between the AKI-status subgroups, while true hematuria was significantly higher in the AKI subgroup. Thus, the influence of hematuria on the degree of urine OB is greater than that of myoglobin in this group, and these results suggest a role of hematuria in AKI. Hematuria has a pathophysiological mechanism that is involved in aspects of renal damage, such as direct tubular damage, oxidative stress, and secretion of inflammatory cytokines, which occurs during rhabdomyolysis [36]. Hematuria is presumed to aggravate kidney function in patients with rhabdomyolysis. Therefore, the risk of AKI could increase in cases of true hematuria and patients with higher OB scores following urinalysis.

Fluid resuscitation has been widely used to treat rhabdomyolysis, and it was also the most commonly used method in our study. Although fluid therapy is emphasized by consensus [5,37,38], other treatment methods are still controversial. Dawley [37] reported that management consists of rapidly aggressive intravenous resuscitation to maintain urine output and limited use of bicarbonate for acidosis.
and mannitol for oliguria, respectively. Michelsen et al. [38] reported guidelines that recommend early fluid resuscitation using crystalloids but not the routine use of diuretics, mannitol, alkalization, or RRT. CRRT effectively removes myoglobin and manages AKI [38]. A Cochrane systematic review concluded that CRRT provides some benefits, but the evidence is insufficient [39]. However, significantly decreased myoglobin levels; improved BUN, creatinine, and potassium levels; and reduced oliguria and hospitalizations were reported relative to when conventional therapy was used. Mortality rates exhibited significant differences, but data on long-term outcomes are lacking [39].

To our knowledge, this is the first large-scale pediatric multicenter study. First, our study clarified the age distribution, proportions of boys and girls, and anthropometric data in rhabdomyolysis, which have been reported in previous studies. Second, our detailing of underlying diseases and causes of rhabdomyolysis can help in understanding their influence in the pediatric population. Third, we found that the independent risk factors for AKI in rhabdomyolysis were multiorgan failure; the presence of underlying disorders; increased levels of urine OB, AST, and uric acid; and decreased levels of calcium. Unlike in previous adult studies, sex, BMI, and CK were not included as risk factors. Fourth, the role of urinalysis was newly highlighted for predicting AKI.

However, there are some limitations to account for as this was a retrospective multicenter study. First, some medical record data regarding etiology, underlying disorders, and laboratory data were missing, which led to the exclusion of some cases. Second, AKI and oliguria could be underestimated or overestimated because of the methodological issues with baseline creatinine level and urine output. Third, some laboratory data, such as CK and myoglobin levels and urine red blood cell count exceeding the upper reference limit, were reported only as values over the upper limit instead of the exact value, which might have led to underestimation of their influence. In addition, the analysis methods and equipment used likely varied among the involved hospitals. Fourth, underlying disorders were classified based on the involved organ or pathology of the disease for the purpose of detailed descriptions, but the criteria for classification were vague for disorders with multisystemic involvement, and severity was not considered. Thus, the interpretation of the influence of underlying disorders on rhabdomyolysis and AKI is limited.

Rhabdomyolysis is a well-known disease, but its epidemiology, risk factors, incidence rates of AKI, and mortality have not been elucidated because of the heterogeneity caused by varying definitions and etiologies. Our study revealed characteristic clinical and laboratory features of rhabdomyolysis in a multicenter Korean pediatric population as well as the predictive factors for AKI. These findings will contribute to a broader understanding of pediatric rhabdomyolysis and enable early intervention against rhabdomyolysis-induced AKI.

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Conflicts of interest

All authors have no conflicts of interest to declare.

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References


Clinical outcomes and prognostic factors of mortality in liver cirrhosis patients on continuous renal replacement therapy in two tertiary hospitals in Korea

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Background: Data on liver cirrhosis (LC) patients undergoing continuous renal replacement therapy (CRRT) are lacking despite of the dismal prognosis. We therefore evaluated clinical characteristics and predictive factors related to mortality in LC patients undergoing CRRT.

Methods: We performed a retrospective observational study at two tertiary hospitals in Korea. A total of 229 LC patients who underwent CRRT were analyzed. Patients were classified into survivor and non-survivor groups. We used multivariable Cox regression analyses to identify predictive factors of in-hospital mortality.

Results: During a median follow-up of 5 days (interquartile range, 1–19 days), in-hospital mortality rate was 66.4%. In multivariable analysis, the Acute Physiology and Chronic Health Evaluation II (APACHE II) score (hazard ratio [HR], 1.03; 95% confidence interval [CI], 1.01–1.06; p = 0.02), Model for End-Stage Liver Disease (MELD) score (HR, 1.08; 95% CI, 1.04–1.11; p < 0.001), and delivered CRRT dose (HR, 0.95; 95% CI, 0.92–0.98; p = 0.002) were significant risk factors for in-hospital mortality. Patients with a CRRT delivered dose < 25 mL/kg/hr had a higher mortality rate than those with a delivered dose > 35 mL/kg/hr (HR, 3.13; 95% CI, 1.62–6.05; p = 0.001). Subgroup analysis revealed that a CRRT delivered dose < 25 mL/kg/hr was a significant risk factor for in-hospital mortality among LC patients with a MELD score ≥ 30.

Conclusion: High APACHE II score, high MELD score, and low delivered CRRT dose were significant risk factors for in-hospital mortality. CRRT delivered dose impacted mortality significantly, especially in patients with a MELD score ≥ 30.

Keywords: Acute kidney injury, Continuous renal replacement therapy, Liver cirrhosis, Mortality, Prognosis
Introduction

Acute kidney injury (AKI) is a well-known complication of liver cirrhosis (LC) that occurs in approximately 50% of patients and is strongly associated with increased mortality [1]. Continuous renal replacement therapy (CRRT) has become an important kidney replacement therapy (KRT) modality in the intensive care unit (ICU) setting worldwide [2]. Cirrhotic patients with acute illness frequently experience intravascular volume depletion and hepatic encephalopathy resulting from splanchnic arterial vasodilatation and impaired detoxification of ammonia [3,4]. Under these circumstances, CRRT could be the best KRT option for LC patients because it can maintain hemodynamic stability, prevent elevated intracranial pressure, and reduce serum ammonia levels [5].

Patient prognosis has improved as the proportion of patients undergoing CRRT in the ICU has increased [2]. A recent national epidemiological study in Korea showed that use of CRRT as an AKI treatment has increased over time (2005 to 2007, 4,667 patients [62%]; 2014 to 2016, 13,414 patients [80%]), whereas the in-hospital mortality rate has simultaneously decreased (2005 to 2007, 63.4%; 2014 to 2016, 53.7%) [6]. Similarly, in a previous report from our hospital, the short-term mortality rate of patients for whom CRRT was initiated in the ICU was 57.3% [7]. Despite these changes, the mortality rate of cirrhotic patients who require dialysis due to liver failure but who are noncandidates for liver transplantation remains extremely high at above 80% [8,9].

Traditionally, KRT has been considered to be a bridging therapy in patients with hepatorenal syndrome (HRS) who are possible candidates for liver transplantation [10]. Providing KRT to acute tubular necrosis (ATN) patients who suffer multiple organ failure has been considered inappropriate. Due to the lack of outcome data for noncandidates for liver transplantation with AKI undergoing KRT, it is difficult to determine whether KRT should be offered to these non-listed patients [11]. However, the widespread use of CRRT has resulted in cirrhotic patients undergoing CRRT in the form of acute KRT. Efforts have recently been made to explore the clinical outcomes of LC patients with AKI. Since the International Club of Ascites Criteria for AKI (ICA-AKI) staging system was adopted, several studies have reported that mortality rate increases with AKI staging [12,13]. Although some studies have analyzed mortality rates according to AKI type, the results have been inconsistent [14,15]. Furthermore, use of different inclusion criteria for AKI staging complicates comparison of study findings. Literature on LC patients with AKI is difficult to generalize because of the variability in regional practice patterns and available medical treatment options such as terlipressin [3]. Clinical outcomes for this high-risk population in Korea are lacking. Therefore, our goal in this study was to evaluate the clinical characteristics and risk factors for in-hospital mortality in non-listed LC patients receiving CRRT.

Methods

Study subjects

This was a retrospective study based on clinical data obtained from LC patients admitted to Pusan National University Hospital from January 2013 to December 2018 and to Pusan National University Yangsan Hospital from January 2017 to February 2020. Initially, we investigated all LC patients who started CRRT for the first time during their hospitalization. Exclusion criteria were as follows: (1) subjects under the age of 18 years; (2) subjects who underwent a liver transplantation; and (3) subjects who had undergone maintenance hemodialysis. Patients were classified into survivor and non-survivor groups.

We received appropriate approval from the Institutional Review Board (IRB) of Pusan National University Hospital (No. 1909-011-083) and the IRB of Pusan National University Yangsan Hospital (No. 05-2020-097). The IRBs waived the requirement for informed consent because of the retrospective design of the study.

Data collection and definitions

We collected demographic data and information on comorbidities, including diabetes, hypertension, and chronic kidney disease (CKD), from electronic medical records. We also obtained the last serum creatinine value within 3 months before admission. CKD was defined as a baseline estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m². Clinical data, laboratory results, and radiologic findings were reviewed to identify complications related to LC and to ascertain causes of AKI. To estimate the severity of acute illness, Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Health Evaluation II (APACHE II)
scores were calculated on the day of ICU admission [16,17]. The severity of LC was assessed using the Model for End-Stage Liver Disease (MELD) score [18], which was obtained at the time of CRRT initiation. The application of CRRT was based on clinical judgment by a treating physician. Study investigators reviewed medical records to confirm the diagnosis and need for KRT. The delivered dose of CRRT, expressed as mL/kg/hr, was calculated by effluent flow.

The diagnosis of HRS was based on the 2015 ICA-AKI and was as follows [19]: (1) diagnosis of cirrhosis and ascites; (2) diagnosis of AKI according to ICA-AKI criteria; (3) no response after two consecutive days of diuretic withdrawal and plasma volume expansion with albumin at 1 g/kg body weight; (4) absence of shock; (5) no current or recent use of nephrotoxic drugs; and (6) no macroscopic signs of structural kidney injury. Shock was defined as a systolic blood pressure of less than 80 mmHg or use of vasopressors. Gastrointestinal bleeding complications were also evaluated. Other LC complications were ascites, lactic acidosis, spontaneous bacterial peritonitis, hepatic encephalopathy, hepatocellular carcinoma, and bleeding episodes except gastrointestinal bleeding due to coagulopathy.

Continuous renal replacement therapy prescription

CRRT was applied to critically ill LC patients with AKI who had acidemia, hyperkalemia, pulmonary edema, hepatic encephalopathy, or needed solute removal according to the judgment of their physicians. CRRT was performed using the Prismaflex set with the AN 69 ST 100 membrane (Baxter International Inc., Deerfield, IL, USA) in continuous venovenous hemodiafiltration mode. The initial prescribed dose of CRRT ranged from 30 to 40 mL/kg/hr in most cases. Additional modifications were made according to the catabolic state or the presence of metabolic acidosis or hyperkalemia. Heparin-free, heparin, or nafamostat mesylate anticoagulants were selected depending on patient bleeding tendency.

Outcomes

Study subjects were followed up until discharge, transfer to another hospital, or death in the study hospital. Primary outcome was in-hospital, all-cause mortality during the study period.

Statistical analysis

Continuous variables are presented as means ± standard deviations or medians (interquartile range). Categorical variables are presented as numbers (percentages). The Kolmogorov-Smirnov test was performed to assess if variables followed a normal distribution. We performed the t test or Mann-Whitney U test to assess the significance of differences in continuous variables between survivors and non-survivors, and the chi-square test to assess the significance of differences in categorical variables between these two groups. We used natural log transformation to normalize urine output (UO) and CRRT duration values. Mortality according to the reason for requiring CRRT and delivered CRRT dose was analyzed using the Kaplan-Meier method with a log-rank test. CRRT delivered dose was analyzed as both a continuous and categorical variable and patients were divided into three groups according to CRRT delivered dose: <25, 25–35, and >35 mL/kg/hr groups. We used univariable and multivariable Cox regression analyses to identify risk factors for in-hospital mortality. Multivariable analysis was performed by selecting variables that showed significance in the univariable analysis, excluding the component variables of the APACHE II or MELD scores except for sex and age. Additionally, we performed subgroup analysis according to LC severity based on the MELD score. We also used the maximum Youden’s index in the receiver operating characteristic (ROC) curve to assess the optimal cutoff value of the MELD score. Associations are presented as hazard ratios (HRs) with corresponding 95% confidence intervals (CIs). All collected data were analyzed using IBM SPSS for Windows version 23.0 (IBM Corp., Armonk, NY, USA). The p-values less than 0.05 were considered statistically significant.

Results

Clinical characteristics of patients

A total of 229 LC patients who did not undergo liver transplantation but who underwent CRRT at two tertiary hospitals were analyzed. Patients were grouped into survivors (n = 77) and non-survivors (n = 152). The baseline characteristics of the study patients are shown in Table 1. Mean age was 58.1 ± 10.5 years, and 179 patients (78.2%) were male. The proportion of patients with CKD was evaluated based on 172
Table 1. Baseline clinical characteristics of liver cirrhosis patients undergoing CRRT

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Survivor</th>
<th>Non-survivor</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>229</td>
<td>77</td>
<td>152</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>58.1 ± 10.5</td>
<td>57.8 ± 10.2</td>
<td>58.2 ± 10.6</td>
<td>0.78</td>
</tr>
<tr>
<td>Male sex</td>
<td>179 (78.2)</td>
<td>62 (80.5)</td>
<td>117 (77.0)</td>
<td>0.54</td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>74 (32.3)</td>
<td>21 (27.3)</td>
<td>53 (34.9)</td>
<td>0.25</td>
</tr>
<tr>
<td>Hypertension</td>
<td>70 (30.6)</td>
<td>24 (31.2)</td>
<td>46 (30.3)</td>
<td>0.89</td>
</tr>
<tr>
<td>CKD*</td>
<td>24 (14.0)</td>
<td>6 (3.5)</td>
<td>18 (10.5)</td>
<td>0.27</td>
</tr>
<tr>
<td>ICU risk factor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventilator use</td>
<td>123 (53.7)</td>
<td>36 (46.8)</td>
<td>87 (57.2)</td>
<td>0.13</td>
</tr>
<tr>
<td>Vasopressor use</td>
<td>158 (69.0)</td>
<td>38 (49.4)</td>
<td>120 (78.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>107 ± 22</td>
<td>117 ± 21</td>
<td>103 ± 21</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SOFA score</td>
<td>13.0 ± 4.8</td>
<td>11.2 ± 4.2</td>
<td>14.0 ± 4.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>26.0 ± 7.2</td>
<td>23.9 ± 6.3</td>
<td>27.1 ± 7.4</td>
<td>0.0002</td>
</tr>
<tr>
<td>MELD score</td>
<td>31.9 ± 5.6</td>
<td>29.4 ± 5.4</td>
<td>33.2 ± 5.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>UO for 6 hr before CRRT (mL)</td>
<td>80 (20–183)</td>
<td>125 (30–365)</td>
<td>60 (20–144)</td>
<td>0.002</td>
</tr>
<tr>
<td>CRRT duration (hr)</td>
<td>35.0 (16.0–91.0)</td>
<td>36.0 (15.0–101.0)</td>
<td>32.0 (16.3–83.5)</td>
<td>0.44</td>
</tr>
<tr>
<td>CRRT prescription</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRRT downtime (hr)</td>
<td>1.0 (0.0–4.5)</td>
<td>1.0 (0.0–4.5)</td>
<td>1.0 (0.0–4.8)</td>
<td>0.76</td>
</tr>
<tr>
<td>Prescribed dose (mL/kg/hr)</td>
<td>38.9 ± 4.8</td>
<td>39.7 ± 5.0</td>
<td>38.5 ± 4.7</td>
<td>0.08</td>
</tr>
<tr>
<td>Delivered dose (mL/kg/hr)</td>
<td>33.4 ± 6.0</td>
<td>35.1 ± 5.5</td>
<td>32.4 ± 6.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Laboratory finding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White blood cell (10^3/µL)</td>
<td>13.8 ± 9.8</td>
<td>12.4 ± 7.0</td>
<td>14.5 ± 11.0</td>
<td>0.09</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>9.4 ± 2.4</td>
<td>9.4 ± 2.1</td>
<td>9.4 ± 2.5</td>
<td>0.97</td>
</tr>
<tr>
<td>Platelet (10^3/µL)</td>
<td>86.1 ± 61.2</td>
<td>93.0 ± 59.5</td>
<td>82.6 ± 62.0</td>
<td>0.23</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>5.7 ± 7.0</td>
<td>4.9 ± 5.2</td>
<td>6.1 ± 7.8</td>
<td>0.21</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>5.4 ± 1.1</td>
<td>5.6 ± 1.2</td>
<td>5.2 ± 1.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.8 ± 1.7</td>
<td>2.8 ± 0.8</td>
<td>2.8 ± 2.0</td>
<td>0.79</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>3.6 ± 6.0</td>
<td>3.6 ± 2.3</td>
<td>3.5 ± 7.1</td>
<td>0.99</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>49.5 ± 30.3</td>
<td>47.0 ± 29.7</td>
<td>50.7 ± 30.6</td>
<td>0.39</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>134.0 ± 8.1</td>
<td>132.1 ± 6.0</td>
<td>135.0 ± 8.9</td>
<td>0.004</td>
</tr>
<tr>
<td>PT-INR</td>
<td>2.4 ± 1.5</td>
<td>2.1 ± 0.9</td>
<td>2.6 ± 1.7</td>
<td>0.004</td>
</tr>
<tr>
<td>Total CO₂ (mmol/L)</td>
<td>13.4 ± 6.6</td>
<td>13.6 ± 7.1</td>
<td>13.3 ± 6.3</td>
<td>0.77</td>
</tr>
<tr>
<td>pH</td>
<td>7.3 ± 0.1</td>
<td>7.3 ± 0.1</td>
<td>7.2 ± 0.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Causes of CRRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRS</td>
<td>18 (7.9)</td>
<td>3 (3.9)</td>
<td>15 (9.9)</td>
<td>0.11</td>
</tr>
<tr>
<td>Acute kidney injury</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With shock</td>
<td>172 (75.1)</td>
<td>57 (74.0)</td>
<td>115 (75.7)</td>
<td>0.79</td>
</tr>
<tr>
<td>Without shock</td>
<td>39 (17.0)</td>
<td>17 (22.1)</td>
<td>22 (14.4)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

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

APACHE II, Acute Physiology and Chronic Health Evaluation II; BUN, blood urea nitrogen; CRRT, continuous renal replacement therapy; HRS, hepatorenal syndrome; ICU, intensive care unit; MELD, Model for End-Stage Liver Disease; PT-INR, prothrombin time-international normalized ratio; SBP, systolic blood pressure; SOFA, Sequential Organ Failure Assessment; UO, urine output.

*The proportion of patients with CKD was evaluated based on 172 patients with an available baseline estimated glomerular filtration rate value.

patients with an available baseline eGFR value. Twenty-four patients (14.0%) had underlying CKD, of which 23 patients had advanced CKD (eGFR category of G3b or higher). We presented baseline, admission, and peak serum creatinine values in Supplementary Table 1 (available online). There were no significant differences in age, sex, or comorbidities.
between survivor and non-survivor groups. The most common cause of admission was infection (25.8%) followed by other LC complications (24.5%), gastrointestinal (GI) bleeding (17.0%), HRS or AKI (9.6%), cardiovascular events (5.7%), and other factors such as trauma or transcatheter arterial chemoembolization (17.5%).

On initiation of CRRT, 123 (53.7%) and 158 patients (69.0%) required mechanical ventilation and vasopressors, respectively. Not only was the use of vasopressors significantly higher (49.4% vs. 78.9%, respectively; p < 0.001) but systolic blood pressure was significantly lower (117 ± 21 mmHg vs. 103 ± 21 mmHg, respectively; p < 0.001) in the non-survivor group than the survivor group. The survivor group had lower SOFA and APACHE II scores on ICU admission and a lower MELD score on initiation of CRRT than the non-survivor group (11.2 ± 4.2 vs. 14.0 ± 4.8, respectively, p < 0.001; 23.9 ± 6.3 vs. 27.1 ± 7.4, respectively, p = 0.002; 29.4 ± 5.4 vs. 33.2 ± 5.2, respectively, p < 0.001). The survivor group had a higher UO for the 6 hours before initiation of CRRT compared to the non-survivor group (125 mL [30–365 mL] vs. 60 mL [20–144 mL], respectively; p = 0.002). The prescribed dose of CRRT was similar between the survivor and non-survivor groups (39.7 ± 5.0 mL/kg/hr vs. 38.5 ± 4.7 mL/kg/hr, respectively; p = 0.08); however, the actual delivered dose was higher in the survivor group than in the non-survivor group (35.1 ± 5.5 mL/kg/hr vs. 32.4 ± 6.0 mL/kg/hr, respectively; p = 0.001). Sodium and prothrombin time-international normalized ratio (PT-INR) levels were significantly lower (132.1 ± 6.0 mmol/L vs. 135.0 ± 8.9 mmol/L, respectively, p = 0.004; 21.0 ± 0.9 vs. 22.6 ± 1.7, respectively, p = 0.004) in survivors, whereas total protein was significantly higher in survivors compared with non-survivors (5.6 ± 1.2 g/dL vs. 5.2 ± 1.1 g/dL, respectively; p = 0.020). The leading indication for CRRT was AKI with shock (75.1%). The proportion of HRS tended to be higher in non-survivors than survivors (9.9% vs. 3.9%, respectively). However, no statistically significant differences in reason for requiring CRRT were observed between the two groups.

Risk factors for in-hospital mortality

Causes of death are presented in Table 2. During a median follow-up period of 5 days (interquartile range, 1–19 days), the in-hospital mortality rate was 66.4%. Twenty-one patients (9.2%) died within 24 hours of being admitted to the ICU. The most common causes of death were LC complications (36.2%) and infection (22.4%), except for spontaneous bacterial peritonitis. The Kaplan-Meier curve showed that the cumulative survival rate was not significantly different according to the CRRT causes of HRS, AKI with shock, and AKI without shock (Fig. 1). Cumulative survival rate was also similar between the HRS and AKI groups (p = 0.26).

Table 2 shows the Cox regression analysis results for in-hospital mortality. In univariable analysis, indicators of acute disease severity such as SOFA score (HR, 1.06; 95% CI, 1.03–1.10; p < 0.001), APACHE II score (HR, 1.04; 95% CI, 1.02–1.06; p = 0.001), and MELD score (HR, 1.08; 95% CI, 1.05–1.11; p < 0.001) were significantly correlated with increased in-hospital mortality. Low log UO, log CRRT duration, and CRRT delivered dose were also associated with increased in-hospital mortality (HR, 0.90; 95% CI, 0.83–0.98; p = 0.01/ HR, 0.77; 95% CI, 0.68–0.87; p < 0.001/ and HR, 0.95; 95% CI, 0.92–0.98; p = 0.001, respectively). In multivariable Cox regression analysis, APACHE II score (HR, 1.03; 95% CI, 1.01–1.06; p = 0.02), MELD score (HR, 1.08; 95% CI, 1.04–1.11; p < 0.001), and delivered CRRT dose (HR, 0.95; 95% CI, 0.92–0.98; p = 0.002) were significant risk factors for in-hospital mortality. We further investigated the association with in-hospital mortality by using the CRRT delivered dose as a categorical variable. Kaplan-Meier curves for cumulative survival rates stratified by CRRT delivered dose are presented in Fig. 2. When comparing the three CRRT delivered dose groups, the survival rate was lowest in the group with a CRRT delivered dose < 25 mL/kg/hr (log-rank p < 0.001). The mortality rate was significantly higher in patients with a CRRT delivered dose < 25 mL/kg/hr compared with a dose > 35 mL/kg/hr after adjustment (HR, 3.13; 95% CI, 2.6–3.8).

Table 2. Causes of death for non-survivors

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Non-survivor (n = 152)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRS or AKI</td>
<td>17 (11.2)</td>
</tr>
<tr>
<td>Gastrointestinal bleeding</td>
<td>29 (19.1)</td>
</tr>
<tr>
<td>Other LC complications*</td>
<td>55 (36.2)</td>
</tr>
<tr>
<td>Infection</td>
<td>34 (22.4)</td>
</tr>
<tr>
<td>Cardiovascular event</td>
<td>6 (3.9)</td>
</tr>
<tr>
<td>Others*</td>
<td>11 (7.2)</td>
</tr>
</tbody>
</table>

Data are expressed as number (%).

AKI, acute kidney injury; HRS, hepatorenal syndrome; LC, liver cirrhosis.
*A includes lactic acidosis, spontaneous bacterial peritonitis, hepatic encephalopathy, hepatocellular carcinoma, and a bleeding episode due to coagulopathy except gastrointestinal bleeding. *Includes trauma, anaphylactic shock, and unknown causes.
Effects of continuous renal replacement therapy delivered dose on mortality according to liver cirrhosis severity

We divided study patients into two groups based on a MELD score of 30, which was previously reported to have a mortality rate of above 50% [20,21]. ROC analysis showed that the area under the curve was 0.707 (p < 0.001) and the maximum Youden’s index was 28.5, which is similar to the conventional cutoff value for predicting mortality. A CRRT delivered dose < 25 mL/kg/hr was a significant risk factor for in-hospital mortality in the severe LC group after adjustment (MELD score ≥ 30; HR, 2.80; 95% CI, 1.32–5.96; p = 0.007) (Table 4). However, there was no significant correlation between CRRT delivered dose and in-hospital mortality in patients with a MELD score < 30.

Discussion

AKI in LC patients, especially those who are not candidates for liver transplantation, is extremely challenging to treat. Several previous studies demonstrated that development of AKI was an independent prognostic factor for mortality in LC patients [12,22–25]. In the current study, we investigated the clinical characteristics of LC patients initiating CRRT and explored factors associated with in-hospital mortality. We observed that non-listed LC patients needing CRRT had an in-hospital mortality rate of 66.4%, which is higher than that reported in a previous study of middle-aged (55–64 years) patients from our hospital for whom CRRT was initiated [7]. A high APACHE II score, high MELD score, and low CRRT delivered dose were significant risk factors for in-hospital mortality. The mortality rate was significantly higher in patients with a CRRT delivered dose < 25 mL/kg/hr than those with a delivered dose > 35 mL/kg/hr after adjustment. This association was significant only in patients with severe LC (MELD score ≥ 30).

Previous multicenter clinical trials have investigated the relationship between CRRT dose and mortality [26,27]. These trials reported a lower mortality rate when the CRRT delivered dose was >19–22 mL/kg/hr, although the higher
CRRT delivered dose group did not have decreased mortality compared to the lower CRRT delivered dose group [28]. In other words, maintaining the CRRT delivered dose in an appropriate range reduced mortality. Our results support the hypothesis that there is a minimum required CRRT delivered dose for improvement of mortality in susceptible patients.

We found that those patients with a higher MELD score group were more affected by CRRT delivered dose than those with a lower MELD score. CRRT is widely used as an extracorporeal technique to support both kidney and liver function in liver failure patients. We would expect to observe an improvement in mortality rate due to a reduction in complications from accumulated toxins caused by liver dysfunction. Consistent with this, several clinical studies have reported that CRRT can reduce serum bilirubin and ammonia levels [29,30]. However, literature regarding the relationship between bilirubin, ammonia excretion, and mortality is sparse. Cardoso et al. [5] reported a 38% reduction in serum ammonia from day 1 to 3 as well as a reduction in 21-day all-cause mortality with CRRT. In our study, because serum ammonia levels were not collected, we were unable to analyze the association between serum ammonia levels and mortality.

Table 3. Cox regression analysis results for in-hospital mortality

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>1.00 (0.98–1.01)</td>
<td>0.79</td>
</tr>
<tr>
<td>Male sex (vs. female)</td>
<td>1.00 (0.65–1.39)</td>
<td>0.79</td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.14 (0.81–1.59)</td>
<td>0.46</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.95 (0.67–1.34)</td>
<td>0.76</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>1.17 (0.71–1.94)</td>
<td>0.55</td>
</tr>
<tr>
<td>ICU risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventilator use</td>
<td>1.42 (1.03–1.96)</td>
<td>0.04</td>
</tr>
<tr>
<td>Vasopressor use</td>
<td>2.47 (1.67–3.67)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SOFA score</td>
<td>1.06 (1.03–1.10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>1.04 (1.02–1.06)</td>
<td>0.001</td>
</tr>
<tr>
<td>MELD score</td>
<td>1.08 (1.05–1.11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log UO</td>
<td>0.90 (0.83–0.98)</td>
<td>0.011</td>
</tr>
<tr>
<td>Log CRRT duration</td>
<td>0.77 (0.68–0.87)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prescribed dose (per mL/kg/hr)</td>
<td>1.00 (0.94–1.01)</td>
<td>0.23</td>
</tr>
<tr>
<td>Delivered dose (per mL/kg/hr)</td>
<td>0.95 (0.92–0.98)</td>
<td>0.001</td>
</tr>
<tr>
<td>Laboratory finding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White blood cell (per 10^3/µL)</td>
<td>1.01 (0.99–1.03)</td>
<td>0.22</td>
</tr>
<tr>
<td>Hemoglobin (per g/dL)</td>
<td>0.98 (0.91–1.05)</td>
<td>0.55</td>
</tr>
<tr>
<td>Platelet (per 10^3/µL)</td>
<td>1.00 (1.00–1.00)</td>
<td>0.2</td>
</tr>
<tr>
<td>Total bilirubin (per mg/dL)</td>
<td>1.01 (0.99–1.04)</td>
<td>0.21</td>
</tr>
<tr>
<td>Total protein (per g/dL)</td>
<td>1.00 (0.90–1.00)</td>
<td>0.002</td>
</tr>
<tr>
<td>Albumin (per g/dL)</td>
<td>0.96 (0.84–1.10)</td>
<td>0.56</td>
</tr>
<tr>
<td>Creatinine (per mg/dL)</td>
<td>1.00 (0.96–1.03)</td>
<td>0.84</td>
</tr>
<tr>
<td>BUN (per mg/dL)</td>
<td>1.00 (0.99–1.00)</td>
<td>0.57</td>
</tr>
<tr>
<td>Sodium (per mmol/L)</td>
<td>1.04 (1.01–1.06)</td>
<td>0.001</td>
</tr>
<tr>
<td>PT-INR</td>
<td>1.21 (1.10–1.33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total CO2 (per mmol/L)</td>
<td>0.99 (0.97–1.02)</td>
<td>0.46</td>
</tr>
<tr>
<td>pH</td>
<td>0.21 (0.07–0.65)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Adjusted factors are age, sex, APACHE II score, MELD score, log UO, log CRRT duration, delivered dose, and total protein.

APACHE II, Acute Physiology and Chronic Health Evaluation II; BUN, blood urea nitrogen; Cl, confidence interval; CRRT, continuous renal replacement therapy; HR, hazard ratio; ICU, intensive care unit; MELD, Model for End-Stage Liver Disease; PT-INR, prothrombin time-international normalized ratio; SOFA, Sequential Organ Failure Assessment; UO, urine output.
mortality rates. Further investigation is needed to provide a rationale for advocating for CRRT for liver support in populations with high morbidity.

In CRRT treatment, an optimal prescription is important, including the appropriate CRRT dose [28]. The CRRT delivered dose is usually lower than the prescribed dose [31]. Furthermore, CRRT is often discontinued due to clotting filters or hypotension, which frequently occur in LC patients with cardiovascular instability and coagulopathy [32]. Non-survivors had lower blood pressures and required more vasopressor use than survivors in our study. This may have contributed to delivery of a lower CRRT dose in non-survivors than survivors. However, there was no significant difference in CRRT downtime between the two groups, so other factors need to be considered. Prior studies have demonstrated that the discrepancy between the prescribed and delivered CRRT dose is due to multiple factors [33–35]. Malfunction of the catheter and filter may contribute to reduced blood flow and

Table 4. Effects of CRRT delivered dose on mortality according to MELD group

<table>
<thead>
<tr>
<th>CRRT delivered dose</th>
<th>Total (n = 212)</th>
<th>MELD &lt;30 (n = 75)</th>
<th>MELD ≥30 (n = 121)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Continuous variable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivered dose (per mL/kg/hr)</td>
<td>0.95 (0.92–0.98)</td>
<td>0.002</td>
<td>0.95 (0.88–1.02)</td>
</tr>
<tr>
<td>Categorical variable (mL/kg/hr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;35</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>25–35</td>
<td>1.62 (1.08–2.44)</td>
<td>0.02</td>
<td>1.23 (0.55–2.73)</td>
</tr>
<tr>
<td>&lt;25</td>
<td>3.13 (1.62–6.05)</td>
<td>0.001</td>
<td>2.09 (0.41–10.54)</td>
</tr>
</tbody>
</table>

Adjusted factors are age, sex, APACHE II score, MELD score, log urine outcome, log CRRT duration, delivered dose, and total protein. CI, confidence interval; CRRT, continuous renal replacement therapy; HR, hazard ratio; MELD, Model for End-Stage Liver Disease.
lead to machine disconnection from the patient. In addition, a decrease in membrane permeability, an increase in urea production, and a larger distribution volume of uremic solutes can also increase the gap between the prescribed and delivered amount of CRRT.

The non-survivor group had lower serum albumin levels and a more prolonged PT-INR than the survivor group, implying worsened LC severity in the former. Furthermore, a higher APACHE II score and higher MELD score were independent risk factors for in-hospital mortality. Our results suggest that multiorgan failure is correlated with mortality, consistent with previous studies [36,37]. In a meta-analysis, the ICU score, which refers to the degree of organ damage, was a predictive factor for short-term mortality in LC patients. In particular, liver and kidney dysfunction affect long-term morality [38].

The survivor group had lower serum sodium levels despite a lower MELD score than the non-survivor group. There was no significant difference in serum sodium levels at hospitalization between the survivor and non-survivor groups (131.4 ± 6.0 vs. 132.9 ± 6.6, respectively; \(p = 0.08\)). This indicates that the non-survivor group had greater serum sodium fluctuations than the survivor group. In addition, a previous study showed that serum sodium variation (≥6 mEq/L) during hospital stay was a predictor of the mortality rate of hospitalized patients [39]. Thus, we further analyzed the relationship between mortality and serum sodium variation (ΔNa, defined as the absolute difference between the serum sodium value at hospitalization and before CRRT initiation). Compared to the survivor group, the non-survivor group had a higher ΔNa (2.6 mEq/L [0.0–7.1 mEq/L] vs. 0.9 mEq/L [0.0–2.6 mEq/L], \(p = 0.002\)) and a higher proportion of patients with ΔNa ≥ 6 mEq/L (28.3% vs. 11.7%). In univariable analysis, log ΔNa (HR, 1.29; 95% CI, 1.10–1.52; \(p = 0.002\)) and ΔNa ≥ 6 mEq/L (HR, 1.73; 95% CI, 1.21–2.47; \(p = 0.002\)) were significantly correlated with increased in-hospital mortality. However, multivariable analysis showed that log ΔNa (HR, 1.13; 95% CI, 0.83–1.52; \(p = 0.44\)) and ΔNa ≥ 6 mEq/L (HR, 1.43; 95% CI, 0.72–2.84; \(p = 0.30\)) were not significant predictors of in-hospital mortality in the present study.

Although the importance of AKI is increasingly being recognized, there are no gold-standard diagnostic criteria for AKI in LC patients. The International Club of Ascites announced new criteria for staging AKI in this population in 2015 [19]. UO has been deleted from the 2015 ICA-AKI diagnostic criteria because cirrhotic patients are frequently oliguric with sodium retention, even though kidney function is normal [19]. Although UO for 6 hours before CRRT was lower in non-survivors than in survivors, this was not correlated with mortality rate. However, measurement of UO for only six hours immediately before CRRT may be too short a time window. In addition, UO does not fully reflect deterioration of kidney function in LC patients, so CRRT should be initiated based on consideration of the dysfunction of various organs. Consequently, more meticulous management of LC patients with severe acute illness is required. Clinicians should modify the precipitating factors of AKI, restore intravascular volume with albumin infusion with continuous assessment of organ dysfunction, and assess the need for CRRT.

Reasons for initiating CRRT were not significantly correlated with mortality, consistent with a previous study. Allegretti et al. [14] reported that the 6-month mortality rate in KRT was 85%, and that the cause of AKI (ATN vs. HRS) was not associated with mortality in non-listed LC patients requiring KRT. However, this finding should be interpreted with caution. Most existing studies have been retrospective in nature, involving patients with different baseline characteristics, CRRT initiation criteria, and prescriptions. In addition, there may have been some difficulties in clearly distinguishing categories of HRS and AKI, although there are HRS diagnostic criteria for LC patients [19].

In a previous meta-analysis of LC patients admitted to the ICU, patients admitted for variceal bleeding had a lower mortality rate than patients admitted for other reasons [38]. In our study, there were no significant associations between the cause of admission (i.e., gastrointestinal bleeding, HRS or AKI, other LC complications, infection, cardiovascular events) and in-hospital mortality (\(p = 0.22\)). Our study findings may be different from those of previous studies because we focused on critically ill patients who needed CRRT.

We conducted this study because previous studies on CRRT outcomes of non-listed LC patients have reported inconsistent findings, and Korean data are insufficient. However, there are several limitations to our study. First, as a retrospective observational study, potential biases that we did not account for may have been present. Second, we collected data from two tertiary hospitals with liver transplantation centers, but not within the same time period. Finally, there might have been variability in clinical diagnoses, timing of CRRT,
and prescription for CRRT among the treating physicians.

In conclusion, the outcomes of LC patients needing CRRT remain poor. High APACHE II score, high MELD score, and low CRRT delivered dose were significant risk factors for increased in-hospital mortality in LC patients requiring CRRT. A low CRRT delivered dose had a greater impact on in-hospital mortality in patients with high LC severity than those with low LC severity. Therefore, achieving an effective CRRT dose is crucial, especially in patients with severe LC.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

This work was supported by clinical research grant from Pusan National University Hospital in 2020.

Authors’ contributions

Conceptualization: YHJ, IYK, SHS, HJK
Data curation, Formal analysis: YHJ, IYK, GSJ, SHS, HJK
Funding acquisition: HJK
Investigation: All authors
Project administration: HJK
Writing–original draft: YHJ, IYK, HJK
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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Jeon, et al. Outcomes in liver cirrhosis patients on CRRT
Background: Endothelial cell (EC) dysfunction is a frequent feature in patients with end-stage renal disease (ESRD). The aim of this study was to generate human induced pluripotent stem cells, differentiate ECs (hiPSC-ECs) from patients with ESRD, and appraise the usefulness of hiPSC-ECs as a model to investigate EC dysfunction.

Methods: We generated hiPSCs using peripheral blood mononuclear cells (PBMCs) isolated from three patients with ESRD and three healthy controls (HCs). Next, we differentiated hiPSC-ECs using the generated hiPSCs and assessed the expression of endothelial markers by immunofluorescence. The differentiation efficacy, EC dysfunction, and molecular signatures of EC-related genes based on microarray analysis were compared between the ESRD and HC groups.

Results: In both groups, hiPSCs and hiPSC-ECs were successfully obtained based on induced pluripotent stem cell or EC marker expression in immunofluorescence and flow cytometry. However, the efficiency of differentiation of ECs from hiPSCs was lower in the ESRD-hiPSCs than in the HC-hiPSCs. In addition, unlike HC-hiPSC-ECs, ESRD-hiPSC-ECs failed to form interconnecting branching point networks in an in vitro tube formation assay. During microarray analysis, transcripts associated with oxidative stress and inflammation were upregulated and transcripts associated with vascular development and basement membrane extracellular matrix components were downregulated in ESRD-hiPSC-ECs relative to in HC-hiPSC-ECs.

Conclusion: ESRD-hiPSC-ECs showed a greater level of EC dysfunction than HC-hiPSC-ECs did based on functional assay results and molecular profiles. hiPSC-ECs may be used as a disease model to investigate the pathophysiology of EC dysfunction in ESRD.

Keywords: CD31, Endothelial cell dysfunction, End-stage renal disease, Microarray, Pluripotent stem cell
Introduction

The rapid increase in patients with end-stage renal disease (ESRD) is a global health problem. In Korea, for example, the number of patients with ESRD was 28,046 in 2002 but had increased rapidly to 93,884 in 2016 [1]. Despite improvements in survival due to advances in dialysis therapy, the mortality rate is still much higher among those with ESRD than in the general population; cardiovascular disease is a leading cause of mortality, accounting for 30% to 50% of all deaths in ESRD patients [2]. In addition, a significant proportion of patients receiving long-term dialysis experience cardiovascular complications, which can reduce their quality of life and represents a major economic burden [3]. Therefore, methods to limit cardiovascular complications in ESRD are critical.

Many traditional risk factors, such as diabetes mellitus, hypertension, chronic kidney disease, and mineral bone disease, are thought to increase cardiovascular complications in patients with ESRD [3]. Of note, endothelial dysfunction is a common underlying mechanism connecting these risk factors to cardiovascular complications [4]. Indeed, endothelial dysfunction and atherosclerosis are observed in nearly all patients with ESRD and are invariably associated with thrombosis and vascular hypertension [5, 6]. Therefore, it is necessary to investigate the pathogenesis of endothelial cell (EC) dysfunction in ESRD.

The entire range of cell types found in the human body can be evaluated using human induced pluripotent stem cells (hiPSCs). Therefore, hiPSC technology and the increasingly refined ability to differentiate hiPSCs into disease-relevant target cells have far-reaching implications for understanding disease pathophysiology, identifying disease-causing genes, and developing more precise therapeutics [7, 8]. For example, hiPSC-derived neurons and neural progenitor cells [9, 10], hiPSC-derived hepatocytes to model inherited metabolic disorders of the liver [11], and hiPSC-derived cardiomyocytes to model hypertrophic cardiomyopathies and diabetes mellitus-induced cardiomyopathies [12, 13] represent encouraging routes of investigation. Collected findings suggest that ECs differentiated from patient-derived hiPSCs can be used to develop a platform for studies of the mechanisms underlying endothelial dysfunction in ESRD.

We characterized ECs differentiated from hiPSCs (hiPSC-ECs) taken from patients with ESRD and from hiPSC-ECs taken from healthy controls (HCs), respectively. In particular, we generated hiPSCs using peripheral blood mononuclear cells (PBMCs) from patients with ESRD and HCs. Using these cell lines, we compared the efficacy of EC differentiation and cell functions. We further used a microarray approach to identify transcripts associated with EC dysfunction in hiPSC-ECs from patients with ESRD.

Methods

Reprogramming of PBMCs

A peripheral blood sample was obtained from three patients with ESRD and three HCs. PBMCs were isolated by centrifugation using Ficoll-Paque PLUS (GE Healthcare, Chicago, IL, USA). Baseline clinical characteristics are presented in Table 1. Isolated PBMCs were cultured in StemSpan Animal Component-free media (Stem Cell Technologies, Vancouver, BC, Canada) supplemented with StemSpan CC110 (Stem Cell Technologies) for 4 days. Then, mononuclear cells were transferred to 24-well plates manually coated with recombinant human vitronectin (BD BioCoat; Corning, Corning, NY, USA), and Sendai virus (CytoTune hiPSC 2.0 Reprogramming Kit; Thermo Fisher Scientific, Waltham, MA, USA) was added at a multiplicity of infection of three. Medium was changed daily until hiPSC colonies formed. After manual picking, hiPSC lines were maintained on vitronectin (Invitrogen, Carlsbad, CA, USA)-coated plates in TeSR-E8 medium (Stem Cell Technologies). On day 12 after transduction, emerging hiPSC colonies were picked individually and expanded for characterization. From day 3 to day 21 after transduction, cells were cultured in a 37°C incubator with 5% CO₂.

All subjects gave their informed consent for inclusion before they participated in this study. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board of Seoul St. Mary’s Hospital (No. KC16TISI0774).

EC differentiation from PBMC-hiPSCs

We used the induced pluripotent stem cell (iPSC) culture method without karyotypic abnormalities or the loss of pluripotency and the iPSCs used were less than 20 passages
To initiate differentiation, confluent cultures of hiPSCs were incubated with 1 mg/mL type IV collagenase for 10 minutes and transferred to ultra-low attachment dishes containing differentiation medium for four days to form embryoid bodies (EBs). The differentiation medium consisted of α-minimum Eagle’s medium, 20% fetal bovine serum, L-glutamine, β-mercaptoethanol (0.05 mmol/L), and 1% nonessential amino acids supplemented with 50 ng/mL of bone morphogenetic protein-4 (BMP-4) (PeproTech, Rocky Hill, NJ, USA) and 50 ng/mL of vascular endothelial growth factor (VEGF) A (PeproTech). For the generation of heterogeneous hiPSC-ECs, the 4-day EBs were reattached to gelatin-coated dishes in the presence of VEGF-A for another 10 days before purification. On day 14 of differentiation, the ECs were purified by magnetic cell sorting. Differentiated cells were dissociated into single cells with Accutase (Life Technologies) for 20 minutes at 37°C, washed with 1× phosphate-buffered saline (PBS) containing 5% bovine serum albumin, and passed through a 70-μm cell strainer. They were next incubated with the CD31 MicroBead Kit (#130-091-935; Miltenyi Biotech, Bergisch Gladbach, Germany) for 30 minutes and a MidiMACS separator with an LS column (Miltenyi Biotech). The purified hiPSC-ECs were expanded in Endothelial Growth Medium-2MV (EGM-2MV) media (Lonza, Basel, Switzerland). Differentiation medium was replaced every 2 days for EC differentiation.

**Table 1. Baseline characteristics of patients with ESRD and healthy controls**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ESRD Patient 1</th>
<th>ESRD Patient 2</th>
<th>ESRD Patient 3</th>
<th>Healthy control Patient 1</th>
<th>Healthy control Patient 2</th>
<th>Healthy control Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>40</td>
<td>56</td>
<td>42</td>
<td>42</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Primary renal disease</td>
<td>IgAN</td>
<td>DM</td>
<td>IgAN</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Dialysis duration (mo)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Smoking history</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>20.4</td>
<td>24.2</td>
<td>24.7</td>
<td>20.9</td>
<td>21.5</td>
<td>20.7</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>5.41</td>
<td>5.84</td>
<td>7.10</td>
<td>0.64</td>
<td>0.98</td>
<td>0.80</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>45.4</td>
<td>76.1</td>
<td>56.4</td>
<td>15.6</td>
<td>14.1</td>
<td>17.3</td>
</tr>
<tr>
<td>WBC (10⁹/L)</td>
<td>5.65</td>
<td>4.53</td>
<td>4.06</td>
<td>4.20</td>
<td>5.35</td>
<td>5.21</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.6</td>
<td>10.9</td>
<td>10.5</td>
<td>13.0</td>
<td>15.2</td>
<td>13.8</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>31.7</td>
<td>33.7</td>
<td>30.8</td>
<td>38.8</td>
<td>45.4</td>
<td>40.1</td>
</tr>
<tr>
<td>Platelet (10⁹/L)</td>
<td>174</td>
<td>198</td>
<td>154</td>
<td>266</td>
<td>432</td>
<td>498</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>162</td>
<td>143</td>
<td>241</td>
<td>213</td>
<td>144</td>
<td>55</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>118</td>
<td>140</td>
<td>190</td>
<td>205</td>
<td>142</td>
<td>202</td>
</tr>
<tr>
<td>HDL</td>
<td>34</td>
<td>48</td>
<td>40</td>
<td>65</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>LDL</td>
<td>53*</td>
<td>82*</td>
<td>89</td>
<td>90</td>
<td>67</td>
<td>137</td>
</tr>
</tbody>
</table>

BUN, blood urea nitrogen; CRP, C-reactive protein; DM, diabetes mellitus; ESRD, end-stage renal disease; HDL, high-density lipoprotein; IgAN, immunoglobulin A nephropathy; LDL, low-density lipoprotein; NA, not applicable; WBC, white blood cell.

*Any type of 3-hydroxy-3-methyl-glutaryl-coenzyme reductase inhibitor (statin) therapy.

Flow cytometry

hiPSC colonies were dissociated using TrypLE Express (Life Technologies) and washed with PBS, and the cell suspension was stained with stage-specific embryonic antigen-4 (SSEA-4) (813-70, 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and TRA-1-81 (TRA-1-80, 1:100; Santa Cruz Biotechnology) surface antibodies for 30 minutes. Intracellular staining for NANOG (1E6C4, 1:100; Santa Cruz Biotechnology) was performed by sequential incubations with fixation and permeabilization solutions (A and B Fix & Perm Solutions, Thermo Fisher Scientific). Cells were incubated with NANOG, followed by with a fluorescein isothio-
cyanate-conjugated secondary antibody (BD BioSciences, San Jose, CA, USA).

Confocal microscopic analysis

hiPSCs were grown on plastic cover slide chambers and fixed with 4% paraformaldehyde. The following antibodies were used: NANOG (1E6C4, 1:100), SSEA-4 (813-70, 1:100), and TRA-1-81 (TRA-1-80, 1:100). ECs from ESRD-PBMC-hiPSCs were grown on plastic cover slide chambers and fixed with 4% paraformaldehyde. The following antibodies were used: CD31 (WM-59; eBioscience, San Diego, CA, USA), CD34 (4H11; eBioscience), CD133 (TMP4; eBioscience), von Willebrand factor (VWF) (sc-53466, 1:100; Santa Cruz Biotechnology), fetal liver kinase 1 (Flk-1) (sc-6251, 1:100; Santa Cruz Biotechnology), and VEGF receptor (Flt) (sc-271789, 1:100; Santa Cruz Biotechnology). Alexa Fluor 488-conjugated streptavidin (Molecular Probes, Eugene, OR, USA) was used according to the manufacturer’s instructions. The stained sections were visualized under a Zeiss microscope (LSM 510 Meta; Carl Zeiss, Oberkochen, Germany) at magnifications of 200× and 400×. The profiles of EC markers were evaluated in each stained tissue section at ×200 magnification using a color image analyzer (TDI Scope Eye, version 3.0, for Windows; Olympus, Tokyo, Japan). All data are presented as mean ± standard error values; unpaired t tests were used for comparisons among groups. Differences with p-values of less than 0.05 were considered statistically significant. All statistical analyses were conducted using GraphPad Prism version 5 (GraphPad Software Inc., San Diego, CA, USA).

Alkaline phosphatase

After 5 days in culture, colonies were assayed for phosphatase alkaline enzymatic activity using the Alkaline Phosphatase Detection Kit (Merck-Millipore, Burlington, MA, USA) according to the manufacturer’s instructions.

In vitro EC tube formation assay

Endothelial tube formation assays were performed in 96-well plates coated with Matrigel (354230; BD, Franklin Lakes, NJ, USA), a reconstituted basement membrane matrix. Approximately 2 × 10⁴ ECs were seeded into each well with 200 µL of EGM-2MV + 20% human serum media. Capillary-like networks were monitored and images were obtained after 4 hours [16]. The area of endothelial tube formation was measured in samples using the ImageJ software (original magnification, 50×; National Institutes of Health, Bethesda, MD, USA).

Cell viability assay

Differentiated ECs were seeded in 96-well plates at a density of 2 × 10⁵/well for 24 or 48 hours, respectively. Before the end of the specified periods, Cell Counting Kit-8 solution (Dojindo Molecular Technologies, Kumamoto, Japan) was added to each well for 2 hours. Absorbance was measured at 450 nm using a VersaMax enzyme-linked immunosorbent assay reader (Molecular Devices, Sunnyvale, CA, USA).

Microarray analysis

RNA purity and integrity were evaluated using the ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA). The Affymetrix Whole Transcript Expression Array (Thermo Fisher Scientific) was used to estimate transcript levels according to the manufacturer’s protocol (GeneChip WT Pico Reagent Kit; Thermo Fisher Scientific). Complementary DNA was synthesized using the GeneChip WT Pico Amplification Kit, as described by the manufacturer (Thermo Fisher Scientific). The sense complementary DNA was fragmented and biotin-labeled with terminal deoxynucleotidyl transferase using the GeneChip WT Terminal Labeling Kit (Thermo Fisher Scientific). Approximately 5.5 µg of labeled DNA target was hybridized to the Affymetrix GeneChip Human 2.0 ST Array (Thermo Fisher Scientific) at 45°C for 16 hours. Hybridized arrays were washed and stained on a GeneChip Fluidics Station 450 system (Thermo Fisher Scientific) and scanned on a GCS3000 scanner (Thermo Fisher Scientific). Signal values were computed using the GeneChip Command Console software (Thermo Fisher Scientific).

Raw data preparation and statistical analyses

Raw data were extracted automatically, following the Affymetrix data-extraction protocol, using the GeneChip Command Console software. After importing CEL files, data were summarized and normalized by the robust multi-aver-
age (RMA) method implemented in the Affymetrix Expression Console software. A gene-level RMA analysis and differentially expressed gene (DEG) analysis were performed. Significant differences in expression level were determined based on fold-change values and the local pooled errors test, in which the null hypothesis was not different among groups. The false discovery rate (FDR) was controlled by adjusting the p-value using the Benjamini-Hochberg algorithm. For a DEG set, a hierarchical cluster analysis was performed using complete linkage and Euclidean distances as a measure of similarity. Gene enrichment and functional annotation analyses for the significant probe list were performed using the Gene Ontology (GO; www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg/) databases. All data analyses and the visualization of DEGs were conducted using R version 3.1.2 (www.r-project.org; R Foundation for Statistical Computing, Vienna, Austria).

Protein-protein interaction network analysis

It is known that the Search Tool for the Retrieval of Interacting Genes (STRING) (https://string-db.org) database, which integrates both known and predicted protein-protein interactions (PPIs), can be applied to predict functional interactions of proteins [17]. In this study, to seek potential interactions between DEGs according to different tissues, the STRING tool was employed. Active interaction sources, including text mining, experiments, databases, and co-expression, were applied, together with limiting the species to "Homo sapiens" and the interaction score to greater than 0.4, to construct the PPI networks. The Cytoscape software version 3.8.2 (an open-source software platform for visualizing networks) was used to visualize the PPI network. In this kind of network, the nodes correspond to the proteins and the edges represent the interactions, respectively.

Results

Characterization of hiPSC in patients with ESRD and HCs

The pluripotency-associated markers NANOG, SSEA4, and TRA-1-81 on hiPSCs from patients with ESRD or HCs were detected by confocal microscopy (Fig. 1A) and flow cytometry (Fig. 1B). Differentiation assays demonstrated that hiPSCs are pluripotent. Pluripotency was assessed by alkaline phosphatase staining (Fig. 1C).

EC differentiation using ESRD-hiPSCs and HC-hiPSCs

We examined the efficiency of hiPSC differentiation into ECs. hiPSCs were differentiated via the EB formation method in the presence of BMP-4 (50 ng/mL) and VEGF (50 ng/mL), as depicted in Fig. 2A. In addition, we evaluated the properties for characteristics of mature ECs using endothelial markers such as CD31, CD34, CD133, VWF, Flk (kinase insert domain receptor), and Flt-1 (VEGF receptor 1) (Fig. 2B). Before and after purification with CD31, ESRD-hiPSC-ECs demonstrated reduced immunoreactivity for endothelial markers relative to HC-hiPSC-ECs. Furthermore, fewer hiPSC-ECs were generated from ESRD-hiPSC-ECs than from HC-hiPSC-ECs using our standard differentiation procedure (average yields: 33% and 4% of total differentiated cells, respectively; p < 0.01) (Fig. 2C). These results show that cell line-specific differences in the efficiency of endothelial differentiation were detected.

Clear dysfunction of ESRD-hiPSC-ECs in comparison with HC-hiPSC-ECs

To compare the angiogenic potential of ESRD-hiPSC-ECs and HC-hiPSC-ECs, we first seeded the ECs onto Matrigel in vitro and evaluated the formation of branching point structures (Fig. 3A). Morphogenic differentiation into vascular plexus-like networks or branching point structures was induced. The plating of HC-hiPSC-ECs on Matrigel resulted in the formation of branching point networks. However, ESRD-hiPSC-ECs failed to form interconnecting branching point networks (average areas: 35% and 10% tube formation; p < 0.01) (Fig. 3A, B). We also evaluated the viability of differentiated EC from hiPSCs; as shown in Fig. 3C, ESRD-hiPSC-ECs exhibited worsened cell viability relative to HC-hiPSC-ECs.

Phenotypic and transcriptional profiles of ESRD-hiPSC-ECs and HC-hiPSC-ECs

We performed a microarray analysis of ESRD-hiPSC-ECs (n = 3) and HC-hiPSC-ECs (n = 3) (Fig. 4A) and identified a total of 226 DEGs. We further identified the leading-edge
**Figure 1.** Characterization of hiPSCs from patients with ESRD and healthy controls. (A) Patient-derived PBMCs were reprogrammed to hiPSCs. The hiPSCs expressed the pluripotency markers NANOG (red), SSEA4 (green), and TRA-1-81 (green), as evaluated by immunofluorescence staining. The scale bar represents 50 µM. Nuclei were counterstained with DAPI. (B) hiPSCs expressed the pluripotency markers NANOG, SSEA4, and TRA-1-81 based on flow cytometry analysis. (C) hiPSCs were stained with alkaline phosphatase substrate to assess pluripotency (100×).

ESRD, end-stage renal disease; hiPSCs, human induced pluripotent stem cells; PBMC, peripheral blood mononuclear cell; DAPI, 4',6-diamidino-2-phenylindole.
Figure 2. EC differentiation of hiPSCs from patients with ESRD and HCs. (A) Schematic overview of EC differentiation. (B) Expression levels of the EC markers CD31, CD34, CD133, VWF, Flk-1, and Flt in purified hiPSC-ECs before and after CD31+ isolation, as determined by immunofluorescence staining with quantitative analysis. Scale bar represents 50 µM. **p < 0.05 relative to HC-iPSC-ECs. (C) CD31+ expression on hiPSC-ECs differentiated from patients with ESRD and HCs, as determined by flow cytometry analysis. **p < 0.01 relative to HC-iPSC-ECs.

EC, endothelial cell; ESRD, end-stage renal disease; Flk-1, fetal liver kinase 1; Flt-1, vascular endothelial growth factor receptor 1; HC, healthy control; hiPSC, human induced pluripotent stem cell; SSC, side scatter; vWF, von Willebrand factor.
gene set (i.e., the core set of genes accounting for this enrichment) from each hiPSC-EC group and distinguished a core of 144 upregulated (Supplementary Table 1, available online) and 75 downregulated (Supplementary Table 2, available online) genes. Genes with expression level differences exceeding the established threshold (i.e., upregulated or downregulated by at least 1.5-fold) were further evaluated (see scatterplot in Fig. 4B). The expression levels of 37 genes were higher (upregulated by at least five-fold) and seven genes were lower (downregulated by less than five-fold) in ESRD-hiPSC-ECs than in HC-hiPSC-ECs. Among genes with increased expression, the levels of the following 10 genes were increased by more than 10-fold: MT1H, NLRP7, MIR302C, DPPA3, MT1G, ESRG, L1TD1, SLC7A3, ZNF729, and PRDM14. Meanwhile, among genes with reduced expression, the levels of the following seven were decreased by more than five-fold: CEMIP, COL12A1, TYRP1, TXNIP, IL18, PCDH10, and GDF6. Fourteen upregulated genes in ESRD-hiPSC-ECs were associated with oxidative stress [18] and inflammation, i.e., MT1H, MT1G, NLRP7, NLRP2, SOX2, IFIT1, IFIT2, IFIT3, CXCL1, IFI6, IFIH1, IFI44, FOXI3, and IFI44L, and four upregulated genes in ESRD-hiPSC-ECs were associated with the inhibition of EC migration and proliferation (i.e., MIR302C, MIR302B, MIR302D, and
Figure 4. RNA microarray analysis of hiPSC-derived ECs from patients with ESRD and HCs. (A) Hierarchical clustering analysis of gene expression in hiPSC-derived ECs from patients with ESRD and HCs, respectively. Heatmap showing 219 significantly (p < 0.05) differentially expressed transcripts between HC-iPSC-ECs (n = 3) and ESRD-hiPSC-ECs (n = 3). The 219 genes were selected based on the criteria described in the Methods section. Expression levels are normalized for each gene; yellow represents high expression and blue represents low expression. (B) Scatterplot of expression levels in HC-iPSC-ECs and ESRD-hiPSC-ECs. (C) Enriched KEGG pathways in ESRD-hiPSC-ECs in comparison with HC-hiPSC-ECs. EC, endothelial cell; ESRD, end-stage renal disease; HC, healthy control; hiPSC, human induced pluripotent stem cell, KEGG, Kyoto Encyclopedia of Genes and Genomes. *indicates pathways associated with EC dysfunction.

Eight upregulated genes in ESRD-hiPSC-derived ECs were associated with basement membrane extracellular matrix (ECM) components (i.e., EPCAM, CDH1, claudin 10, CLDN7, CLDN6, GPC4, ADGRV1, and SFRP2). Eleven downregulated genes were involved in vascular development and basement membrane ECM components, including COL12A1, PCDH10, VTN, CALB2, ITGA11, EFEMP1, PCDHB14, FBN1, CDH5, FBLN5, and FBN2. Therefore, microarray analysis showed that transcripts associated with the ECM cell adhesion pathway were downregulated in ESRD-hiPSC-ECs (Supplementary Table 2).

KEGG pathway enrichment analyses of DEGs

Based on a KEGG pathway enrichment analysis, DEGs that were more upregulated in ESRD-hiPSC-ECs as compared with in HC-hiPSC-ECs were mainly enriched in cell adhesion molecules, tight junctions, the toll-like receptor signaling pathway, and leukocyte transendothelial migration (e.g., CD34, CLDN10, CDH5, CDH1, CLDN6, CLDN7, PDCD1LG2,
MARVELD2, CCL5, SPP1, and CXCL10). Downregulated DEGs were significantly enriched with regard to functions in ECM-receptor interactions and focal adhesion (i.e., ITGA11, VTN, LAMA1, SPP1, and TNC) (Fig. 4C, Table 2).

**PPI network between genes more expressed in ESRD-hiPSC-ECs relative to in HC-hiPSC-ECs**

The PPI network among genes was expressed more strongly in ESRD-hiPSC-ECs than in HC-hiPSC-ECs. A total of 144 upregulated and 75 downregulated unique gene identities were analyzed using the STRING database and the Cytoscope software. The required confidence score was set to 0.400. In the networks, the nodes corresponded to the proteins and the edges represented the interactions. In Fig. 5A and B, red ovals depict cellular metabolism, while blue ovals depict ECM remodeling and green ovals indicate additional GO terms (i.e., cytokine and immune response). In ESRD-hiPSC-EC, the major hubs are those that are involved in the remodeling of the ECM, cellular metabolism, cytokines, and immune responses. Detailed GO terms are described in Supplementary Tables 3, 4 (available online).

**Discussion**

It is well known that significant EC dysfunction is detectable in ESRD patients, which can induce major cardiovascular complications, resulting in higher mortality and morbidity rates in this patient population [5,6]. Therefore, it is necessary to establish an appropriate platform that can

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**Table 2. KEGG pathway enrichment analyses of DEGs**

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</tr>
</tbody>
</table>

DEG, differentially expressed gene; ECM, extracellular matrix; FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes.

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**Figure 5. Protein-protein interaction network analysis between genes expressed in ESRD-hiPSC-ECs as compared with HC-hiPSC-ECs.** (A) Results of upregulated genes. (B) Results of downregulated genes.
be used for disease pathology research as well as new drug development to combat EC dysfunction. In the past decade, advances in iPSC reprogramming technology have enabled scientists to create specific organ tissues or cells with characteristics of specific diseases [12,19,20]. In this study, we successfully generated hiPSCs and hiPSC-ECs using PBMCs from ESRD patients and showed that hiPSC-ECs may represent EC dysfunction in terms of functional and molecular assays. These findings suggest that an *in vitro* model of hiPSC-ECs from patients with ESRD may improve our understanding of EC dysfunction and may be an effective platform for screening drug candidates.

Our first aim was to reprogram hiPSCs, which can be a source for ESRD-hiPSC-ECs, using PBMCs from ESRD patients. Several reprogramming techniques have been developed for generating hiPSCs via transferring genes corresponding to the four Yamanaka transcriptional factors to various types of somatic cells, such as dermal fibroblasts, PBMCs, or urine cells [21,22]. We decided to use PBMCs as a platform for hiPSC reprogramming because collecting blood is less invasive than performing a skin biopsy to gather dermal fibroblasts and ESRD patients are also mostly familiar with the former process. For reprogramming, we employed the Sendai virus-transfection method. Subsequently, we did not observe any differences between cells from patients with ESRD and HCs with regard to reprogramming efficacy or the expression of markers of pluripotency, such as NANOG, SSEA4, and TRA-1-81; hence, hiPSCs from patients with ESRD were expected to have a similar pluripotent potential to that of cells from normal subjects.

The next consideration was whether reprogrammed hiPSCs could retain the specific characteristics of a disease. Until now, disease modeling employing hiPSCs has been mainly focused on hereditary or familial disorders because reprogrammed hiPSCs essentially retain the germline genetic defects found in somatic cells. Therefore, target cell types differentiated from hiPSCs with genetic defects are expected to show the phenotype or molecular signature of the genetic disease associated with the mutations. Indeed, the cause of ESRD in three patients was either diabetes mellitus or immunoglobulin A nephropathy, which basically do not have significant germline mutations at baseline [23–25]. Recently, it was suggested that hiPSCs generated using somatic cells from patients with aging-related diseases, including chronic kidney disease, may retain the somatic memory associated with cell identity [26]. Indeed, in contrast with the complete loss of germ cell memory in embryonic stem cells, there is some evidence that hiPSCs partially retain the characteristics of their origin somatic cells, such as the age of the donor [27,28]. Therefore, some recent studies have focused on generating hiPSCs from diseased somatic cells to use as a source for regeneration or for research into the mechanism of disease or the senescence of target cells or tissues.

So, we presumed that ESRD-hiPSCs may retain the characteristics of ESRD and performed a comparison between ESRD-hiPSC-ECs and HC-hiPSC-ECs in the following three aspects: efficacy of EC differentiation, results of functional assay using a tube formation assay, and results of molecular signature analysis by microarray. During differentiation into hiPSC-ECs, we observed differences between ESRD-hiPSCs and HC-hiPSCs in terms of differentiation efficacy. ESRD-hiPSC-ECs consistently generated fewer hiPSC-ECs (estimated as the proportion of CD31+ cells) in comparison with HC-hiPSC-ECs. Indeed, patient-derived hiPSCs can be defective in disease-related cell differentiation; for example, hiPSCs derived from a patient with Prader-Willi syndrome exhibit neuronal differentiation defects [29].

Next, we evaluated the functional differences between ESRD-hiPSC-ECs and HC-hiPSC-ECs. For the evaluation of EC dysfunction in an *in vitro* model, tube formation assay, a test designed to evaluate angiogenesis, has been widely employed [16,30]. We observed that, unlike HC-hiPSC-ECs, ESRD-hiPSC-ECs failed to form interconnecting branching point networks. Our findings suggest that ESRD-hiPSC-ECs lose their vasculature formation ability. It is speculated that somatic cells exposed to a uremic state for a long time undergo an epigenetic change, which is memorized and partially retained in the reprogrammed hiPSCs. [27] This epigenetic defect in ESRD-hiPSC-ECs may result in their functional disability in terms of angiogenesis, but further investigation is necessary to clarify this issue.

Lastly, we conducted a microarray analysis to investigate the underlying mechanism associated with the vasculature functional disability observed in ESRD-hiPSC-ECs in comparison with HC-hiPSC-ECs. As a result, it was determined that genes associated with the regulation of the basement membrane, ECM degradation, and EC migration were differentially expressed between ESRD-hiPSC-ECs and HC-hiPSC-ECs. Endothelin 1 (*EDN1*), mainly produced by
vascular ECs, induces vasoconstriction in physiological conditions [31]. Angiopoietin 2 (ANGPT2) is involved in angiogenesis and modulates EC differentiation, survival, and stability [32]. Matrix-remodeling associated 5 (MXRA5) is involved in adhesion and remodeling [33]. Membrane metallo-endopeptidase (MME) regulates the inflammatory response and insulin signaling in white preadipocytes [34]. Finally, cell migration-inducing hyaluronan binding protein (CEMIP) promotes cell migration [35]. The observed defects in the transcription of essential molecules in hiPSC-ECs from patients with ESRD may contribute to the defects in angiogenesis revealed by the tube formation assay [36].

This study had some limitations. First, we did not clearly demonstrate the epigenetic changes in ESRD-hiPSC or ESRD-hiPSC-ECs—for example, the methylation of specific genes. Robust gene-sequencing analysis may help to clarify this issue. Second, the number of patients who participated was limited and their underlying renal diseases varied. Indeed, it is possible to consider that the characteristics of endothelial dysfunction in diabetic patients are fundamentally different from those of nondiabetic patients. However, a considerable effort is required to reprogram hiPSCs and differentiate hiPSC-ECs in order to conduct research using a large patient group. The development of more efficient and faster iPSCs reprogramming techniques will help to mitigate this issue. Lastly, one of the inherent limitations of the predictive power of in vitro iPSC-based model systems is that they may not demonstrate sufficient complexity to approximate in vivo physiology. To overcome this, it would be preferable to use self-assembled vascular organoids and vasculature-on-a-chip platforms for studying the effects of disease processes and drugs on iPSC-derivated vasculature.

Lastly, two of the three ESRD patients or HCs were female. There are sex differences in the remodeling of DNA methylation marks; hence, these sex differences can be connected to differences in developmental potential between female and male iPSCs [37,38]. Therefore, it may be necessary to conduct further research among subjects of the same sex.

In conclusion, our results suggest that hiPSC-ECs derived from patients with ESRD may demonstrate functional and molecular characteristics of EC dysfunction. Accordingly, our in vitro model of hiPSC-ECs in patients with ESRD may be helpful to elucidate EC dysfunction and establish a platform for screening drug candidates.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding sources

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science and Technology, Republic of Korea (NRF-2020R1C1C1008346), and by financial support from the Catholic Medical Center Research Foundation provided in the program year of 2020.

Acknowledgments

We thank the blood donors who gave their time to participate in this study.

Authors’ contributions

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References


Neutrophil extracellular traps and heparin-induced antibodies contribute to vascular access thrombosis in hemodialysis patients

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\textbf{Background:} Anti-heparin/platelet factor 4 (PF4) antibodies may trigger severe thrombotic complications in hemodialysis (HD) patients. Tetrameric PF4 has a high affinity for extracellular DNA, which is a key component of neutrophil extracellular traps (NETs); therefore, the interactions between anti-heparin/PF4 antibodies and NETs can contribute to prothrombotic events.

\textbf{Methods:} Anti-heparin/PF4 antibody levels were measured by enzyme-linked immunosorbent assay and an optical density > 1.8 was regarded as clinically significant. We additionally measured serum nucleosome levels as representative markers of NETs, and the contributions of anti-heparin/PF4 and increased serum nucleosome levels to the primary functional patency loss of vascular access was assessed.

\textbf{Results:} The frequency of anti-heparin/PF4 antibodies was significantly higher in incident HD patients compared to prevalent HD patients (23.6\% vs. 7.7\%). Serum nucleosome levels, as well as the white blood cell counts, neutrophil counts, and high-sensitivity C-reactive protein levels, were significantly higher in anti-heparin/PF4 antibody-positive patients compared to the control. Platelet counts tended to be lower in the patients with anti-heparin/PF4 of >1.8 than in the controls. Relative risk calculations showed that the presence of anti-heparin/PF4 antibodies increased the risk of primary functional patency failure by 4.28-fold, and this risk increased further with higher nucleosome levels. Furthermore, in the anti-heparin/PF4 antibody-positive group, the time to first vascular intervention was much shorter, and the risk of repeated intervention was higher, compared to the controls.

\textbf{Conclusion:} In incident HD patients, the presence of anti-heparin/PF4 antibodies was associated with increased NET formation; this could be a strong predictor of vascular access complications.

\textbf{Keywords:} Extracellular traps, Platelet factor 4, Renal dialysis, Thrombosis, Vascular access

Received: April 12, 2021; Revised: May 10, 2021; Accepted: June 3, 2021
Editor: Soon Hyo Kwon, Soonchunhyang University, Seoul, Republic of Korea
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Introduction

Heparin-induced thrombocytopenia (HIT) is a serious immune reaction to heparin often characterized by severe thrombosis; the morbidity and mortality rates of HIT patients are high [1,2]. HIT is caused by the formation of a complex of abnormal autoantibodies that bind to platelet factor 4 (PF4) and heparin, together known as HIT antibodies [3]. HIT antibodies activate platelets, which can cause catastrophic arterial and venous thrombosis. HIT occurs in approximately 5% of patients exposed to heparin, regardless of the dose, schedule, or route of administration [4–6].

Patients undergoing continuous renal replacement therapy or intermittent hemodialysis (HD) typically require anticoagulation treatment to prevent the clotting of extracorporeal circuits. Heparin is the drug most commonly used for this purpose [7]. Although long-term heparin exposure is associated with a high incidence of anti-heparin antibodies, the prevalence and clinical significance of HIT antibody formation are currently unknown [8–10]. A previous study examining 700 incident HD and peritoneal dialysis patients reported a relatively high incidence of anti-heparin/PF4 antibodies [8]. However, they found no correlation between the presence of anti-heparin/PF4 and thrombocytopenia or thrombosis, and their study did not predict clinical outcomes. As a result, routine testing for the presence of HIT antibodies is not currently recommended for HD patients [8,11,12]. However, the mortality rate of untreated HIT has been reported to be as high as 20% in HD patients [13–15].

There are several phases of HIT, including an acute phase and various recovery phases [16]. Subclinical HIT, or subacute HIT, refers to the state in which a patient has recovered from an acute episode of HIT, but has persistent HIT antibodies; in this case, the presence of HIT antibodies may not be associated with clinical outcomes [17]. Furthermore, some patients develop clinically silent or insignificant anti-PF4 antibodies, which do not cause thrombocytopenia. Therefore, the timing of the detection and evaluation of anti-heparin/PF4 antibodies may determine their prognostic significance [6].

Unlike most other forms of drug-induced immune thrombocytopenia, HIT is associated with vascular thrombosis, with the primary mechanism of platelet activation and resultant injury to endothelial cells [18]. Recently, neutrophil extracellular traps (NETs), or NETosis, has been implicated in the development of thrombosis in individuals with HIT [6,19–21]. HIT immune complexes can activate neutrophils either directly, via the Fc-gamma receptor IIA (FcrRIIA), or indirectly, via P-selectin and its ligand P-selectin glycoprotein ligand-1. Furthermore, PF4 adheres readily to NET DNA, and the resultant PF4-NET complex contributes to the prothrombotic nature of HIT [19,20,22]. A mouse model study of HIT revealed that both the depletion of neutrophils and the blockage of neutrophil nuclear decondensation caused by a NETosis inhibitor prevented thrombosis, even though thrombocytopenia was unaffected, thus demonstrating the critical role of NETosis in HIT thrombosis [19].

Given that vascular access complications are largely due to the thrombotic occlusion of a stenotic fistula or graft [23,24], we assessed whether anti-heparin/PF4 antibodies played a role in adverse HD vascular access outcomes. We compared the prevalence of heparin-induced anti-heparin/PF4 antibodies between incident HD patients, who had recently been exposed to heparin for the first time, and maintenance HD (MHD) patients, who had been undergoing regular dialysis for more than 3 months with long-term heparin use. As a negative control, we also measured anti-heparin/PF4 antibodies in HD patients who did not use heparin. Furthermore, we measured serum nucleosome levels as a representative marker of NET in vivo. In this manner, we evaluated the utility of the association between anti-heparin/PF4 antibodies and nucleosome levels for predicting adverse HD vascular access outcomes in incident HD patients.

Methods

Study population

This observational, single-center study included incident HD patients, who had recently started dialysis treatment, and MHD patients, who underwent regular HD (three times per week), between January 2016 and June 2017. Of the 105 incident HD patients, 31 were excluded for the following reasons; presence of an acute infection at the time of starting HD (n = 3), decompensated liver cirrhosis (n = 2), decompenated heart failure with ejection fraction of <30% (n = 6), hematologic diseases (n = 1), nonachievement of long-term vascular access (n = 10), death or recovery of renal function within 3 months (n = 3), or loss to follow up or transfer to another clinic (n = 6). Of the 169 MHD patients, 26 were...
excluded due to the presence of hematologic diseases (n = 3), decompensated liver cirrhosis (n = 2), decompensated heart failure with ejection fraction of <30% (n = 4), nonuse of heparin due to recent gastrointestinal bleeding (n = 5), or some other reason (n = 10). The study protocol was approved by the Institutional Review Board of Hallym University Sacred Heart Hospital (No. 2016-I067), and informed consent was obtained from all patients.

Data collection and blood sampling

Baseline demographic data were obtained, including age, sex, and comorbidities, along with clinical data regarding the underlying cause of renal disease. Venous blood samples were collected into K2-ethylenediaminetetraacetic acid (EDTA)-coated tubes and 0.109 M trisodium citrate-coated tubes (Becton Dickinson, Franklin Lakes, NJ, USA). For the isolation of plasma, the blood was separated by centrifugation at 1,500 × g for 20 minutes at 4°C. Isolated plasma samples were aliquoted and stored at –80°C until analysis.

For MHD patients, sampling was performed immediately before the HD session in the middle of the week. Biochemical analyses of white blood cells (WBCs), neutrophils, lymphocytes, platelets, hemoglobin, serum albumin, cholesterol, blood urea nitrogen, and creatinine were performed. The neutrophil to lymphocyte ratio was also calculated. Levels of the high-sensitivity C-reactive protein (hs-CRP) inflammatory cytokine were also measured.

Anti-heparin/platelet factor 4 antibody and neutrophil extracellular trap quantification assays

The anti-heparin/PF4 antibodies were detected using a commercial heparin/PF4 enzyme-linked immunosorbent assay (ELISA) kit (PF4 IgG, HAT 45G; Immucor GTI Diagnostics, Waukesha, WI, USA) [17]. Diluted, citrated plasma (1:50) was added to microwells, which were coated with immobilized PF4 complexed to polyvinyl sulfonate. After incubation for 35 minutes in a 37°C water bath, the microwells were washed and incubated with alkaline phosphatase-conjugated anti-human immunoglobulin (IgG) antibody. Following a second incubation for 35 minutes in a 37°C water bath, the microwells were washed and incubated with p-nitrophenyl phosphate substrate for 30 minutes. Optical density (OD) was measured by subtracting the absorbance reading at 490 nm (a reference filter) from the absorbance reading at 405 nm. According to a previous study, OD values of <0.4 indicated an absence of antibodies. OD values equal to or greater than 1.0 indicated the presence of antibodies; values higher than 1.8 were regarded as clinically significant (strongly positive). The differences between the duplicate wells were <20% [11,25].

To evaluate NET formation, histone-DNA complex (nucleosome) NET biomarkers were measured using a Cell Death Detection ELISA Plus Kit (Roche, Mannheim, Germany). According to the manufacturer’s instructions, a mixture containing anti-histone-biotin antibody, anti-DNA-peroxidase antibody, and EDTA-plasma was placed in streptavidin-coated wells. After incubation for 2 hours, the wells were washed and reacted with a substrate for 20 minutes. The reaction was stopped using a stop solution. OD was calculated in the same way as in the heparin-PF4-IgG antibody assay. As we reported previously, the highest quartile of serum nucleosome (Q4) was used as the cutoff value for significant increase of NETs [26].

Study endpoints

The primary outcomes of this study were primary functional patency loss, defined as thrombotic occlusion of vascular access (including abandonment of the access site) requiring surgical or percutaneous endovascular intervention (percutaneous transluminal angioplasty [PTA] or thrombectomy) following initial cannulation, and achievement of adequate dialysis within 6 months of the first dialysis session [1,27].

The secondary outcomes were as follows; the time taken for successful cannulation in three consecutive HD sessions, the time to the first intervention and frequency of first interventions within 3 months, and the time between the first intervention and reintervention. We also analyzed the abandonment rate of initial vascular access during follow-up.
Statistical analysis

As an exploratory study, the sample size of the study was planned based on the expected mean differences between the incident HD, MHD, and control populations (non-heparin users) of 20% of the anti-PF4 antibody level. Using nonparametric sample size estimation, the sample size calculation for the incident HD patients resulted in a sample size of 50 to 60 patients and control groups who were eligible for the study in the same study period to achieve a power of at least 80%. Variables with normal distributions are expressed as the means ± standard deviations, and the Kolmogorov-Smirnov test was used to analyze the normality of the distribution of each parameter. Categorical variables are expressed as percentages and were compared using the chi-square test. Cumulative survival curves were derived using the Kaplan-Meier method, and differences between survival curves were compared using the log-rank test. Pearson’s correlation analysis was used to clarify the relationship between the number of immune cells and anti-PF4 antibody levels. A Cox proportional hazards model was used to identify factors independently associated with vascular access abandonment, based on hazard ratios (HRs) with 95% confidence intervals (CIs). A p-value less than 0.05 was regarded as significant. All statistical analyses were performed using IBM SPSS version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Baseline characteristics

During the 18-month period from January 2016 to June 2017, a total of 105 incident HD and 169 MHD patients were enrolled in this study, and a total of 74 and 143 patients were evaluated. The median dialysis duration for MHD patients was 39.7 months. Table 1 presents the baseline characteristics of the patients. Incident HD patients were significantly older (p = 0.001) than MHD patients and used arteriovenous grafts (AVGs), as opposed to arteriovenous fistulas (AVFs), more frequently than the MHD patients (p = 0.01). Preoperative Doppler ultrasound was typically performed prior to long-term vascular access to identify suitable vessels for AVF.

Table 1. Baseline characteristics of all patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Heparin-free</th>
<th>Heparin use</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>19</td>
<td>55</td>
<td>143</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>70.6 ± 11.2</td>
<td>70.9 ± 11.3</td>
<td>64.9 ± 12.5</td>
</tr>
<tr>
<td>Male sex</td>
<td>17 (63.2)</td>
<td>28 (50.9)</td>
<td>75 (52.4)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.6 ± 4.6</td>
<td>24.1 ± 4.3</td>
<td>23.6 ± 3.5</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>19 (68.5)</td>
<td>23 (41.8)</td>
<td>86 (60.1)</td>
</tr>
<tr>
<td>Previous CVD</td>
<td>3 (15.7)</td>
<td>5 (9.1)</td>
<td>28 (19.6)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>137 ± 25</td>
<td>142 ± 17</td>
<td>140 ± 20</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>77 ± 20</td>
<td>76 ± 12</td>
<td>74 ± 12</td>
</tr>
<tr>
<td>HD duration (mo)</td>
<td>1.1 (0.2–1.7)</td>
<td>1.0 (0.3–1.8)</td>
<td>39.7 (16–80)</td>
</tr>
<tr>
<td>Anti-platelet agent use</td>
<td>3 (15.7)</td>
<td>23 (41.8)</td>
<td>81 (56.6)</td>
</tr>
<tr>
<td>Vascular access type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVF</td>
<td>6 (31.6)</td>
<td>36 (68.5)</td>
<td>118 (82.5)</td>
</tr>
<tr>
<td>AVG</td>
<td>5 (26.3)</td>
<td>17 (31.5)</td>
<td>25 (17.5)</td>
</tr>
<tr>
<td>Anti-PF4 antibody, OD</td>
<td>0.39 ± 0.19</td>
<td>1.34 ± 0.65</td>
<td>0.93 ± 0.47</td>
</tr>
<tr>
<td>&gt;1.8, strong positive</td>
<td>NA</td>
<td>13 (23.6)</td>
<td>11 (7.7)</td>
</tr>
<tr>
<td>1.0–1.8, weak positive</td>
<td>NA</td>
<td>19 (34.5)</td>
<td>32 (22.3)</td>
</tr>
<tr>
<td>0.4–1.0, intermediate</td>
<td>NA</td>
<td>23 (41.8)</td>
<td>93 (65.0)</td>
</tr>
<tr>
<td>&lt;0.4, negative</td>
<td>NA</td>
<td>0</td>
<td>7 (4.9)</td>
</tr>
</tbody>
</table>

Number (%) only, mean ± standard deviation, or median (interquartile range). AVF, arteriovenous fistula; AVG, arteriovenous graft; BP, blood pressure; CVD, cardiovascular disease; HD, hemodialysis; NA, not applicable; OD, optical density; PF4, platelet factor 4.

*p-value for comparison between incident HD and maintenance HD.
placement; if no suitable vessels were present, an AVG was placed. Other baseline comorbidities were comparable between the two dialysis groups.

We divided the patients into three groups; incident HD patients with heparin free, incident HD patients with heparin use, and MHD patients. We then compared the anti-heparin/PF4 antibody levels among the three groups. As expected, anti-heparin/PF4 antibodies were rarely detected in non-heparinized HD patients (mean OD, 0.39 ± 0.11). Notably, the mean anti-heparin/PF4 antibody OD value was significantly higher in incident HD patients compared to MHD patients, even though both HD patient groups were regularly exposed to heparin (1.34 ± 0.65 vs. 0.93 ± 0.47, p < 0.001) (Fig. 1). Applying the cutoff OD value of 1.8 (i.e., strongly positive), the prevalence of clinically significant levels of anti-heparin/PF4 antibodies was 23.6% in incident HD patients (13 of 55) and 7.7% in MHD patients (11 of 143). The prevalence of heparin-induced antibodies in the MHD group was comparable to previously reported data.

Focusing on the incident HD group, we examined the relationships between clinical and biochemical parameters and clinically significant anti-heparin/PF4 antibody levels (Table 2). Patients with antibody OD values of >1.8 showed an increased tendency toward higher systolic and diastolic blood pressures than the control group. Notably, patients with strongly positive anti-heparin/PF4 antibody levels had significantly higher WBC and neutrophil counts compared to the controls, even though all cell counts were within normal ranges (Fig. 2A, Table 3). However, the number of platelets was slightly lower in patients with clinically significant antibody OD values. Additionally, the levels of hs-CRP, a serum inflammatory marker, were significantly higher in patients with strongly positive antibody levels (B).

**Figure 1. Differences in anti-heparin/PF4 antibody levels among groups.** In patients without heparin use (heparin-free), the antibody level was very low (negative control). In patients with heparin use, the level was significantly higher in incident HD patients compared to maintenance HD patients.

**Figure 2. The relationship between anti-heparin/PF4 Ab levels and immune cell counts.** (A, B) The Ab level had a positive correlation with WBC and neutrophil counts but had a negative relationship with platelet count. Also, the levels of hs-CRP, a serum inflammatory marker, were significantly higher in patients with strongly positive Ab levels (B). Ab, antibody; hs-CRP, high-sensitivity C-reactive protein; OD, optical density; PF4, platelet factor 4; WBC, white blood cell.
Table 2. Comparison of biochemical data and vascular outcomes according to PF4 antibody level

<table>
<thead>
<tr>
<th>Variable</th>
<th>Incident HD with heparin use (n = 55)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD ≤ 1.8 (n = 42)</td>
<td>OD &gt; 1.8 (n = 13)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>70.8 ± 11.2</td>
<td>69.1 ± 11.7</td>
</tr>
<tr>
<td>Male sex</td>
<td>21 (50.0)</td>
<td>5 (38.5)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>24 (57.1)</td>
<td>6 (46.1)</td>
</tr>
<tr>
<td>Previous CVD</td>
<td>3 (7.1)</td>
<td>2 (15.3)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>139.6 ± 19.0</td>
<td>148.5 ± 16.4</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>74.0 ± 13.0</td>
<td>80.1 ± 9.9</td>
</tr>
<tr>
<td>Vascular access type</td>
<td></td>
<td>0.27</td>
</tr>
<tr>
<td>AVF</td>
<td>29 (69.0)</td>
<td>8 (61.5)</td>
</tr>
<tr>
<td>AVG</td>
<td>13 (31.0)</td>
<td>5 (38.5)</td>
</tr>
<tr>
<td>Biochemical parameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC count</td>
<td>6,427 ± 1,960</td>
<td>9,205 ± 3,580</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.5 ± 1.3</td>
<td>10.9 ± 1.3</td>
</tr>
<tr>
<td>Neutrophil count</td>
<td>4,350 ± 1,509</td>
<td>6,435 ± 3,116</td>
</tr>
<tr>
<td>Neutrophil/lymphocyte ratio</td>
<td>4.0 ± 2.9</td>
<td>4.3 ± 2.6</td>
</tr>
<tr>
<td>Platelet (10^9/mL)</td>
<td>200 ± 40</td>
<td>168 ± 70</td>
</tr>
<tr>
<td>&lt;100 × 10^6/mL</td>
<td>2 (4.7)</td>
<td>2 (15.4)</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>57.6 ± 17.6</td>
<td>54.4 ± 22.6</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>6.8 ± 1.9</td>
<td>6.3 ± 1.9</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.6 ± 0.6</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>154.1 ± 29.3</td>
<td>148.6 ± 36.9</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>1.10 ± 1.04</td>
<td>3.50 ± 2.70</td>
</tr>
<tr>
<td>Vascular outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of primary patency in 6 mo</td>
<td>9 (21.4)</td>
<td>7 (53.8)</td>
</tr>
<tr>
<td>Abandonment in 6 mo</td>
<td>0 (0)</td>
<td>4 (30.7)</td>
</tr>
<tr>
<td>First intervention in 6 mo</td>
<td>9 (21.4)</td>
<td>6 (46.2)</td>
</tr>
<tr>
<td>Secondary outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to first needling (day)</td>
<td>55.5 ± 16.7</td>
<td>72.7 ± 34.3</td>
</tr>
<tr>
<td>Time to first intervention (mo)</td>
<td>5.2 ± 4.0</td>
<td>13.4 ± 6.9</td>
</tr>
<tr>
<td>First intervention in 3 mo</td>
<td>6 (14.3)</td>
<td>6 (46.2)</td>
</tr>
<tr>
<td>Time to reintervention</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Re-PTA in 3 mo</td>
<td>4 (9.5)</td>
<td>5 (38.4)</td>
</tr>
<tr>
<td>Abandonment during follow-up</td>
<td>3 (7.1)</td>
<td>6 (46.1)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or number (%).
AVG, arteriovenous graft; BP, blood pressure; BUN, blood urea nitrogen; CVD, cardiovascular disease; HD, hemodialysis; hs-CRP, high-sensitivity C-reactive protein; OD, optical density; PF4, platelet factor 4; PTA, percutaneous transluminal angioplasty; WBC, white blood cell.

Table 3. Correlation analysis of Anti-PF4 antibody with nucleosome level and blood cell variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>WBC</th>
<th>Neutrophil</th>
<th>Platelet</th>
<th>Nucleosome level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Anti-PF4 antibody</td>
<td>0.397</td>
<td>0.003</td>
<td>0.37</td>
<td>0.006</td>
</tr>
<tr>
<td>WBC</td>
<td>-</td>
<td>-</td>
<td>0.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Neutrophil count</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

PF4, platelet factor 4; WBC, white blood cell.
significant anti-heparin/PF4 antibody levels. The frequency of thrombocytopenia (defined as a platelet count < 1 × 10^8/mL) was higher in the patients with high anti-heparin/PF4 antibody levels; however, because of the low number of cases, this finding was not statistically significant. Levels of the inflammatory marker hs-CRP were also statistically higher in patients with high anti-heparin/PF4 antibody levels (Fig. 2B). The other metabolic parameters were similar between patients with and without high anti-heparin/PF4 antibody levels. No relationship was found between the type of vascular access (AVF or AVG) and the anti-heparin/PF4 antibody positivity rate.

Vascular outcomes of patients positive for anti-heparin/platelet factor 4 antibodies

Anti-heparin/PF4 antibodies are associated with platelet activation and endothelial cell damage; therefore, we conducted thorough follow-up examinations to evaluate the HD vascular outcomes of all patients. Vascular complications are largely due to thrombosis and stenosis following endothelial damage and inflammation. Our data showed that patients with strong positive anti-heparin/PF4 antibodies had significantly higher rates of primary functional patency loss within 6 months of the first HD compared to the control group (53.8% vs. 21.4%, p = 0.03) (Fig. 3A). Relative risk calculations revealed that patients with an anti-heparin/PF4 antibody OD of >1.8 were 4.28 times more likely to experience primary functional patency failure compared to those with an anti-heparin/PF4 antibody OD of ≤1.8. One-third of the patients exhibiting strong positive antibodies experienced vascular access abandonment within 6 months, whereas no control group patients lost vascular access within the same period. In addition, around twice as many patients in the strongly positive anti-heparin/PF4 antibody group underwent vascular interventions within 6 months of vascular access creation (46.2% vs. 21.4%).

We then examined the relationships between the presence of clinically significant anti-heparin/PF4 antibody levels and the times of first cannulation and first intervention. There were no cases of primary maturation failure up to 3 months after the creation. However, the time taken for successful cannulation in three consecutive HD sessions was significantly longer in the strongly positive anti-heparin/PF4 antibody group compared to the control group; this was likely due to the higher vascular intervention rate in the strong positive group compared to the controls (72.7 ± 34.3 days vs. 55.5 ± 16.7 days, p = 0.03). Supporting this, patients who had clinically significant anti-heparin/PF4 antibody levels experienced earlier vascular intervention compared to control patients (Fig. 3A). The mean times until the first intervention were 5.2 and 13.4 months in the strong positive and control

Figure 3. Vascular outcomes according to strong positivity for anti-heparin/PF4 Abs. (A) Patients with anti-heparin/PF4 Abs underwent more frequent and earlier vascular interventions compared to the control group. Therefore, they had a much higher incidence of vascular access failure. (B, C) The abandonment risk was also significantly higher in patients with Abs. Ab, antibody; PF4, platelet factor 4.
groups, respectively. Furthermore, about half of the patients in the strong positive group underwent repeated interventions within 3 months of the initial intervention. Clinically, patients requiring frequent vascular intervention due to recurrent access obstruction could be easily identified. Taken together, our data suggested that platelet activation triggered by anti-heparin/PF4 antibody formation may be associated with early and repeated intravascular thrombotic occlusion of HD vascular access.

Additionally, we compared the rates of access abandonment between the strongly positive anti-heparin/PF4 antibody group and the control group (Fig. 3B, C). During follow-up examinations conducted 28.8 ± 14.1 months after this study, there were nine cases of abandonment (six and three in the strong positive and control groups, respectively). According to the Cox proportional HR, patients who were strongly positive for anti-heparin/PF4 antibodies had a 7.29-fold higher risk of vascular access abandonment (95% CI, 1.79–25.65 in univariate analysis). Even after adjusting for age, sex, diabetes, vascular type, and hs-CRP, the presence of clinically significant anti-heparin/PF4 antibodies increased the abandonment risk 7.32-fold (95% CI, 1.31–40.88) (Table 4).

### Relationship of serum nucleosome level with anti-heparin/platelet factor 4 positivity

Due to the close association between strong anti-heparin/PF4 antibody positivity and high WBC and neutrophil counts in peripheral blood, we evaluated the relationship between anti-heparin/PF4 antibody and serum nucleosome levels. Consistent with our previous findings, the serum nucleosome levels varied significantly among the HD patients.

| Table 4. Cox proportional HRs for predicting abandonment of vascular access |
|-----------------------------|-----------------------|----------------|
| Variable                    | HR (95% CI)           | p-value       |
| **Univariate analysis**     |                       |               |
| Anti-PF4/heparin antibody, >1.8 | 7.29 (1.79–25.65)    | 0.004         |
| **Multivariate analysis**   |                       |               |
| Age, per 1 yr               | 1.14 (0.96–1.34)      | 0.13          |
| Diabetes mellitus, presence | 1.06 (0.90–2.27)      | 0.34          |
| Vascular access type (AVG)  | 3.10 (0.66–14.46)     | 0.15          |
| hs-CRP (mg/L), >1.0         | 3.07 (0.32–15.11)     | 0.19          |
| Anti-PF4/heparin antibody, >1.8 | 7.32 (1.31–40.88)    | 0.02          |

AVG, arteriovenous graft; CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; HR, hazard ratio; PF4, platelet factor 4.

**Figure 4. The relationship between anti-heparin/PF4 strong positivity and serum nucleosome level.** (A) Patients with strongly positive anti-heparin/PF4 Abs had significantly higher levels of nucleosomes. (B, C) When divided into four groups according to the nucleosome highest quartile and an OD level of 1.8, the highest rates of primary patency loss were observed in patients with an Ab OD > 1.8 and nucleosome OD > 0.52 (Q4). Ab, antibody; OD, optical density; PF4, platelet factor 4.
The median serum nucleosome OD value was 0.16, and the cutoff value for the highest quartile (Q4) was 0.52. Serum nucleosome levels exhibited a close relationship with anti-heparin/PF4 antibody levels ($r = 0.309; p = 0.03$) (Table 3). As shown in Fig. 4A, the median nucleosome OD value was significantly higher in the strongly antibody-positive group compared to the control group (0.72 vs. 0.32, $p = 0.04$). We then divided the total population into four groups according to anti-heparin/PF4 antibody OD values (> or ≤1.8) and nucleosome OD values (> or ≤the Q4 cutoff value of 0.52) and compared the risk of primary patency loss and vascular access abandonment among the four groups (Fig. 4B, C). The highest rates of primary patency loss were observed in patients with an antibody OD of >1.8 and nucleosome OD of >0.52. Even in patients with clinically significant anti-heparin/PF4 antibody levels, the risk significantly increased with higher nucleosome levels.

**Discussion**

In this study, we investigated the potential impact of anti-heparin/PF4 antibodies on HD vascular access outcomes and their relationship with NET levels. There were several strengths to our study, the first being that we measured anti-heparin/PF4 antibody levels in both incident HD and MHD patients, which enabled direct comparison of the two groups. Given our results, we suggest that timing is an important factor when testing for anti-heparin/PF4 antibodies. Another strength of our study was that we only focused on vascular access. The causes of cardiovascular and cerebrovascular events in HD patients are complex and multifactorial; although the causes are similarly complex for vascular outcomes, the main pathogenesis of vascular complications is thrombotic occlusion with stenosis. Furthermore, we measured serum nucleosome levels alongside performance of the anti-heparin/PF4 antibody test since neutrophil dysregulation and increased NET formation have been reported to contribute to thrombosis in uremic conditions. Platelet-neutrophil interactions could be key in the formation of vascular access clots.

According to previous HIT studies, anti-heparin/PF4 antibody positivity rates were within the range of 2.3% to 10.3% in HD patients. The presence of HIT antibodies was not found to be associated with adverse clinical outcomes; however, previous studies only examined MHD patients, without considering incident HD patients [3,8,9,11,12,28]. There are multiple HIT phases, including the acute phase, subacute phase, and several recovery phases. MHD patients may fall within the subclinical or subacute HIT phase. Moreover, MHD patients may harbor clinically silent or insignificant anti-heparin/PF4 antibodies that do not cause thrombocytopenia or thrombosis. Therefore, we hypothesized that the prognostic significance of HIT antibodies should be determined in incident HD patients who have recently been exposed to heparin. In this study, we compared the prevalence of anti-heparin/PF4 antibodies between incident HD patients and MHD patients and found that the frequency of HIT antibodies was much lower in MHD patients compared to incident patients (7.7% vs. 21.7%). Supporting this, a previous study also reported that the incidence of heparin-induced antibodies was highest during the first 90 days of HD (20%). However, by 3 months, approximately 9% of patients had antibodies [8]. One potential explanation could be that patients who had developed anti-heparin/PF4 antibodies experienced vascular complications earlier during the treatment course and had therefore already died or been hospitalized/transferred to nursing care facilities prior to this study, and thus were not included in our MHD group. Consistent with this explanation, the average age of the MHD patients was significantly lower, and AVF was the main vascular access route rather than AVG. Another explanation for this finding is that anti-heparin/PF4 antibodies may be degraded following repeated exposures to heparin; however, this issue is beyond the scope of this investigation.

In this report, we focused on the incident HD group and further evaluated the prognostic role of anti-heparin/PF4 antibodies in vascular outcomes. Notably, patients with clinically significant antibody levels had higher WBC and neutrophil counts than those without. Consistent with this finding, serum nucleosome levels were much higher in the antibody-positive group. Taken together, this suggests that the presence of anti-heparin/PF4 antibodies was accompanied by neutrophil activation and increased NET formation in vivo. The platelet counts appeared to be lower in patients with anti-heparin/PF4 antibodies; however, the difference was not statistically significant. Therefore, an increased neutrophil count with or without thrombocytopenia may be a characteristic of anti-heparin/PF4 antibody-positive patients. Furthermore, the expression level of the inflammatory marker hs-CRP was higher in the positive antibody group...
compared to the control group. The increased NET levels, positive anti-heparin/PF4 antibodies, and hyperinflammatory condition may simultaneously contribute to vascular thrombosis in vivo.

We evaluated the value of anti-heparin/PF4 antibodies for predicting functional vascular access (defined as successfully matured and used for dialysis) and the subsequent loss of primary functional patency within 6 months of its first use. Because the main objective of the study was to investigate the thrombotic occlusion associated with anti-heparin/PF4 antibodies, patients with decompensated heart failure or decompensated liver cirrhosis were specifically excluded to rule out potential inflow problems with respect to vascular access. An interval of <3 months between vascular access creation and first use likely indicates that any subsequent loss of primary functional patency is not due to anatomical problems or low blood flow. Our results showed that the presence of anti-heparin/PF4 antibodies was a powerful predictor of vascular access failure (odds ratio, 7.3; p = 0.02), even after adjustment for diabetes and inflammatory conditions. In addition, the risks of vascular intervention within 3 and 6 months were 3.1 and 6.3 times higher, respectively, in the positive anti-heparin/PF4 antibody group than in the control group. Positive HIT antibodies were linked to a higher incidence of repeated vascular intervention within 3 months of the first vascular intervention and to the abandonment of vascular access. Consistent with our data, O’shea et al. [10] reported that hypercoagulable states are common in HD patients with recurrent vascular access site thrombosis.

Another notable finding in this study was that patients with positive anti-heparin/PF4 antibodies had higher serum nucleosome levels than the control group, and the vascular outcomes were worse in patients with both higher nucleosome levels and strongly positive antibodies. Given the strong association of the anti-heparin/PF4 antibodies with serum neutrophil and nucleosome levels, it is reasonable to suggest that an interaction between the anti-heparin/PF4 antibodies and excessive NET could be a risk factor for vascular thrombosis. Supporting our data, Perdomo et al. [21] recently reported that the HIT immune complex induces NETosis by interacting with FcRIIa on neutrophils and through neutrophil-platelet associations. They found neutrophil-platelet aggregates in the blood of patients with HIT and then demonstrated the involvement of NETosis in thrombi generated ex vivo. Furthermore, they showed that neutrophil depletion abolished thrombus formation in vivo.

Similarly, another recent report showed that anti-heparin antibodies could activate neutrophils and cause them to selectively bind to injured endothelia, where they released NETs; these NETs generated immunogenic complexes, which could then induce prothrombotic conditions. Moreover, the complexes are resistant to endonuclease digestion, which leads to repeated and prolonged clot formation [19,29].

A limitation of this study was that a relatively small number of patients were analyzed, all of whom were from a single center. We tried to include more patients by extending the study period, but the availability of anti-heparin/PF4 antibody commercial kits was limited due to their infrequent use in clinical practice. Therefore, the results are not representative. However, our data are nonetheless valuable; by comparing the antibody levels between MHD and incident HD patients, we discovered a relationship between anti-heparin/PF4 antibody levels and the time of heparin exposure. Relatively few studies including incident HD patients have been published. Another limitation of this study was that although we measured serum nucleosome levels, we were unable to directly visualize NETs in vivo. Furthermore, we arbitrarily determined the cutoff OD value for positive anti-heparin/PF4 antibodies (i.e., 1.8). The prevalence of anti-heparin/PF4 antibodies differs largely among existing reports, because it is influenced by the kit and the detection method used. Even for the same kit, the cutoff value differs among studies. In this study, we applied the same cutoff value used in a previous one, and also used the same ELISA kit; the positive control yielded OD values > 1.8 [25]. Warkentin et al. [25] reported that the probability of a strongly positive result in a serotonin ELISA was about 90% when a cutoff OD value of 1.8 to 2.0 was applied.

In conclusion, the frequency of anti-heparin/PF4 antibodies was significantly higher in incident HD patients compared to MHD patients. This likely explains why previous studies involving MHD patients failed to show any significant prognostic role of anti-heparin/PF4 antibodies. In incident HD patients, we found that the presence of anti-heparin/PF4 antibodies was associated with increased NET formation and that this could be a strong predictor for subsequent vascular access complications. This provides evidence for a pathological interaction between platelet activation, increased NET formation, and endothelial damage in vivo.
Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

This research was supported by a National Research Foundation grant funded by the Korean government (2020R1A2C110138611) and the Hallym University Research Fund.

Authors’ contributions

Conceptualization, Funding acquisition: JKK, SGK
Data curation: JNA, HSL
Formal analysis: JKK, SGK, JNA, HSL
Investigation: HWL, JKK, YRS, HJK
Methodology: YRS, HJK
Writing–original draft: HWL, JKK
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Effects of the route of erythropoietin administration on hemoglobin variability and cardiovascular events in hemodialysis patients

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Introduction: Despite of the routine use of erythropoietin in hemodialysis patients to correct anemia, its administration route’s effects on hemoglobin variability and cardiovascular events remain elusive. Herein, we determined different erythropoietin administration routes’ effects on hemoglobin variability in hemodialysis patients and the associated factors of hemoglobin variability and cardiovascular events.

Methods: This is a post hoc analysis of a prospective, controlled, randomized, unblinded study with 78 Korean hemodialysis patients receiving intravenous (n = 40) or subcutaneous (n = 38) erythropoietin therapy. We evaluated hemoglobin variability by calculating the frequency of hemoglobin measurements outside the target range during all visits. The high-frequency group was defined by those with hemoglobin variability over the median value (25%) while the low-frequency group was defined by those with hemoglobin variability of <25%.

Results: In this analysis, 37 patients (51.1%) were male, and the mean age was 50.6 ± 12.5 years. The frequency of the value being outside the target hemoglobin range was higher in the subcutaneous group compared to the intravenous group (p = 0.03). The low-frequency group required significantly lower erythropoietin doses compared to the high-frequency group. In the adjusted Cox analysis, the parameter high group was a significant independent risk factor for cardiovascular events (p = 0.03).

Conclusion: The risk out of the target hemoglobin range increased with subcutaneous administration compared with intravenous erythropoietin administration in hemodialysis patients. An increased frequency of the value being outside the target hemoglobin range was also associated with an increased risk of cardiovascular events.

Keywords: Anemia, Cardiovascular diseases, Erythropoietin, Renal dialysis
Introduction

Anemia is a common complication in end-stage renal disease (ESRD) patients undergoing hemodialysis (HD) and leads to a poor prognosis due to cardiovascular complications and increased mortality [1]. After erythropoiesis-stimulating agents had been introduced as treatment options for anemia, the frequency of blood transfusions decreased and the quality of life improved in patients with ESRD [2,3]. The Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines from the National Kidney Foundation recommend maintaining an appropriate hemoglobin (Hb) target of 11 to 12 g/dL in HD patients, while Hb values above 13 g/dL should be avoided [4]. However, in patients undergoing HD, it is difficult to maintain Hb levels in the adequate range for various reasons such as blood loss during dialysis, iron deficiency, malnutrition, chronic inflammation, secondary hyperparathyroidism, and insufficient dialysis doses. Therefore, only 30% of patients are within the target range while the others over- or undershoot the recommended target range [5]. Although the degree of Hb variability has decreased over the past 10 years [6], it remains a very common issue among HD patients, increasing the risk of overall mortality in these patients [7–9].

Erythropoietin (EPO) administration is one of the main treatment options for anemia in patients with ESRD. However, the effects of the EPO administration route on Hb variability and cardiovascular outcomes in HD patients remain controversial. Recent studies have reported an increase in all-cause mortality in patients with high Hb variability [6,7,10]. Another study involving 5,037 HD patients found no association between cardiovascular mortality and Hb variability [11]. We previously reported that the risk of vascular access failure may be greater with subcutaneous (SC) EPO administration compared to intravenous (IV) EPO administration [12]. Since proliferative and other biological actions of EPO on vascular cells may be maximized by sustained EPO receptor activation and SC administration may yield a more sustained activation of EPO receptors than the IV route, SC administration may result in poorer outcomes regarding vascular access. However, cardiovascular outcomes did not significantly differ between the two groups in this study.

In the present study, we investigated the effects of different routes of EPO administration on Hb variability and the association between Hb variability and cardiovascular events in maintenance HD patients.

Methods

Study population

This is a post hoc analysis of a prospective, controlled, randomized, unblinded study comparing IV administration with the SC administration of EPO [12]. A previous randomized trial was conducted between October 1, 2000 and February 28, 2007 at the Hallym University Kangnam Sacred Heart Hospital and the Hallym University Chuncheon Sacred Heart Hospital in Korea. The enrollment criteria for patients were (1) patients aged 18 years or older receiving HD for more than 6 months, (2) patients receiving regular erythropoiesis-stimulating agents for anemia, and (3) patients with adequate iron storage (transferrin saturation ≥ 20% and serum ferritin ≥ 100 ng/mL). The exclusion criteria were patients with (1) severe hyperparathyroidism (serum intact parathyroid hormone ≥ 800 pg/mL), (2) acute infection or systemic underlying inflammatory diseases, malignancy, or epilepsy, (3) severe congestive heart failure (New York Heart Association Classes III and IV), (4) gastrointestinal bleeding in the previous 3 months, (5) platelet count exceeding 500 × 10^3/µL, (6) pregnancy or lactation, (7) the use of androgens or immunosuppressive drugs during the past 3 months, (8) blood transfusion within 2 months before enrollment in the study, or (9) a history of hypersensitivity to EPO.

This study was approved by the Institutional Review Board of Hallym University Kangnam Sacred Heart Hospital (No. 2021-06-005). Due to retrospective nature of the study, the informed consent was waived. All clinical investigations were conducted in accordance with the guidelines of the 2008 Declaration of Helsinki.

Study design

Patients were randomly assigned to receive EPO beta (Recormon; F. Hoffmann-La Roche, Ltd., Basel, Switzerland) by either the IV or SC route. Randomization was performed centrally using a random permuted block with stratification according to the clinical center, age (≥60 or <60 years), and EPO dose (50 to 99, 100 to 149, or 150 to...
200 U/kg/week). Following the enrollment of patients in this study, EPO was administered through IV or SC routes at the same doses that had been administered through the SC route prior to enrollment.

During the study, the EPO dose was increased or reduced by 25% compared to the previous dose when the Hb value decreased or increased by at least 1 g/dL, respectively. When Hb dropped below 9 g/dL, the EPO dose was increased by 25%, and when it was increased to at least 12 g/dL, the dose was reduced by 50%. At the time of enrolling patients in the study, the target Hb in the KDOQI guidelines was 11 to 12 g/dL [4]. However, in the present study, Hb was titrated within the range of 9 to 12 g/dL using the above algorithm to prevent excessive Hb corrections.

All the patients enrolled in the study received EPO two or three times/week. During the study period, patients were given oral or IV iron as needed (transferrin saturation < 20% or serum ferritin < 100 ng/mL). Blood specimens for laboratory analyses were drawn from the dialysis tubing before HD monthly. Single-pool Kt/V was determined using two-point urea modeling based on the intradialytic decrease in blood urea levels and intradialytic weight loss. It was computed with the use of the following modified rate equation: single-pool Kt/V = –ln [(1 – urea reduction ratio) – 0.008 × session length] + [4 – 3.5 × (1 – urea reduction ratio)] × ultrafiltration / postdialysis weight [13].

Evaluation of hemoglobin variability

Hb variability was assessed by the frequency exceeding the titrated target Hb range of 9 to 12 g/dL. During the entire study period, we measured Hb at 1-month intervals to calculate Hb variability. To evaluate the effects of Hb variability, we divided the study population into two groups based on whether the frequency of missing the target Hb exceeded 25% in an enrolled patient. Furthermore, Hb cycling was quantified by measuring Hb excursions defined as a series of decreasing or increasing monthly Hb values differing by at least 1.5 g/dL [14]. In addition, the degree of Hb variability was calculated by comparing the conventional standard deviation (SD), residual SD, and coefficient of Hb variation for each group [15]. For the assessment of EPO responsiveness according to the administration route, we used the erythropoiesis-stimulating agent responsiveness index (ERI), calculated as the average weekly EPO dose per kg body weight divided by the average Hb level (ERI = [EPO / body weight] / Hb) [16]. The laboratory values used in the analysis were baseline values. However, the EPO dose, ferric sucrose dose, ERI, SD, residual SD, and coefficient of Hb variation were time-averaged values.

The primary outcome was to investigate the degree of Hb variability according to the EPO administration route, while the secondary outcome was the occurrence of cardiovascular events according to the Hb variation. Cardiovascular events were defined as the occurrence of myocardial infarction, heart failure, and stroke.

**Statistical analysis**

All normally distributed numerical variables were expressed as the mean ± SD, whereas variables with skewed distributions were expressed as the median and interquartile range. Analyses of the differences in baseline characteristics according to the route of EPO administration were performed using the t test for continuous variables and the chi-square test for categorical variables. The Kaplan-Meier method was used to compare cardiovascular event-free survival curves, and differences were assessed using the log-rank test. Patients were censored when cardiovascular events occurred. Multivariate Cox regression analysis of cardiovascular event-free survival was performed with adjustments for age, the high-frequency group, diabetes, previous cardiovascular disease, and vintage dialysis (>18 months). Statistical analyses were performed using IBM SPSS version 26.0 (IBM Corp., Armonk, NY, USA). For all analyses, results were considered statistically significant if p < 0.05.

**Results**

**Baseline characteristics**

A total of 78 patients were enrolled and randomly assigned to the IV (40 patients) and SC (38 patients) groups. Among those, seven patients dropped out for the following reasons: five protocol violations; one withdrawal of consent; one history of blood transfusion. Accordingly, 71 patients (38 in the IV group and 33 in the SC group) completed the study. The mean follow-up period was 50.7 months (4–77 months). Thirty-seven patients (51.1%) were men, and the mean age was 50.6 ± 12.5 years. The rate of patients who had diabetes
mellitus was 35.2%, and the average number of Hb measurements was 46.8 ± 27.5 times.

The baseline characteristics of the two groups were similar except for the intact parathyroid hormone level, which was significantly higher in the IV group compared to the SC group (95.1 ± 87.3 pg/mL vs. 32.7 ± 33.1 pg/mL, p < 0.001) (Table 1). In the SC group, 54.5% of patients received iron treatment, which was significantly higher than the IV group (p < 0.001). There were no differences in administered EPO doses, transferrin saturation, and ferritin levels between the two groups. In addition, there was no significant difference between the two groups in iron saturation or ferritin during the follow-up period.

Hemoglobin variability by the route of erythropoietin administration

When comparing the Hb variability between the two groups, the frequency of the value being outside the target Hb range was significantly lower in the IV group than in the SC group (0.27 ± 0.12 vs. 0.36 ± 0.19 per visit, p = 0.03). However, the conventional SD, residual SD, and coefficient values of the other parameters did not differ between the two study groups (0.97 ± 0.23 vs. 1.02 ± 0.28, p = 0.43; 0.90 ± 0.23 vs. 0.97 ± 0.36, p = 0.31; and 0.10 ± 0.02 vs. 0.11 ± 0.03, p = 0.15, respectively). When the ERI was evaluated, there was no statistically significant difference between the IV group and the SC group (10.9 ± 3.0 vs. 11.7 ± 4.8 U/kg/week/g/dL, p = 0.61) (Table 2).

Based on the frequency of the value being outside the target Hb range, the study population was divided into groups of patients with frequencies above and below 25%, and the differences between these two groups were compared (32 patients in the low-frequency group and 39 patients in the high-frequency group) (Table 3). There was no statistically significant difference in the proportion of diabetes mellitus between the two groups. However, the proportion of diabetic patients in the high-frequency group was 46.2%, which was higher than that of the low-frequency group (21.9%) (p = 0.06). In the low-frequency group, 19 patients (59.4%) received EPO through IV administration and 13 patients (40.6%) received EPO through SC administration. In the high-frequency group, 19 patients (48.7%) received EPO through IV administration and 20 patients (51.3%) received EPO through SC administration. There was no difference between the two groups according to the route of administration (p = 0.51). The coefficient of Hb variation was significantly lower in the low-frequency group compared

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics at baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
</tr>
<tr>
<td>No. of patients</td>
</tr>
<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>Male sex</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Previous cardiovascular disease</td>
</tr>
<tr>
<td>Dialysis vintage (mo)</td>
</tr>
<tr>
<td>Predialytic SBP (mmHg)</td>
</tr>
<tr>
<td>Predialytic DBP (mmHg)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
</tr>
<tr>
<td>Intact-PTH (pg/mL)</td>
</tr>
<tr>
<td>Single-pool Kt/V</td>
</tr>
<tr>
<td>Iron treatment</td>
</tr>
<tr>
<td>Erythropoietin dose (U/kg/wk)</td>
</tr>
</tbody>
</table>

Data expressed as number only, mean ± standard deviation, number (%), or median (interquartile range). DBP, diastolic blood pressure; PTH, parathyroid hormone; SBP, systolic blood pressure.
to the high-frequency group (0.10 ± 0.02 vs. 0.11 ± 0.03, p = 0.009). In the low-frequency group, the frequency of the lower limit level (<9 g/dL) for Hb was 0.15 ± 0.07 (per visit), and in the high-frequency group, it was 0.43 ± 0.16 (per visit) with Hb being significantly more frequent than the lower limit level of Hb (<9 g/dL) in the high-frequency group (p < 0.001). However, there was no difference in the initial laboratory test, EPO dose, ferric sucrose dose, ERI, conventional SD, and residual SD between these two groups. During the follow-up period, absolute Hb values did not differ between the two groups. However, the EPO requirement was significantly lower in the low-frequency group compared to the high-frequency group at 12, 24, and 48 months (81.8 ± 31.9 vs. 114.5 ± 38.6 U/kg/week, p < 0.001; 83.4 ± 19.2 vs. 112.3 ± 51.4 U/kg/week, p = 0.01; and 85.9 ± 34.7 vs. 126.7 ± 36.7 U/kg/week, respectively; p = 0.001) (Fig. 1A). Similarly, the ERI was lower in the low-frequency group than in the high-frequency group at 12, 24, 36, and 48 months (8.8 ± 3.9 vs. 12.1 ± 5.7 U/kg/week, p = 0.001; 8.8 ± 2.2 vs. 12.1 ± 5.7 U/kg/week, p = 0.007; 10.5 ± 3.9 vs. 13.8 ± 4.9 U/week, p = 0.001).

### Table 2. Hemoglobin (Hb) variability by route of erythropoietin administration

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intravenous group (n = 38)</th>
<th>Subcutaneous group (n = 33)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb of &gt;12, &lt;9 g/dL (n/visit)</td>
<td>0.27 ± 0.12</td>
<td>0.36 ± 0.19</td>
<td>0.03</td>
</tr>
<tr>
<td>Hb excursion (≥1.5) (n/visit)</td>
<td>0.20 ± 0.08</td>
<td>0.17 ± 0.12</td>
<td>0.27</td>
</tr>
<tr>
<td>Standard deviation of Hb</td>
<td>0.97 ± 0.23</td>
<td>1.02 ± 0.28</td>
<td>0.43</td>
</tr>
<tr>
<td>Residual standard deviation of Hb</td>
<td>0.90 ± 0.23</td>
<td>0.97 ± 0.36</td>
<td>0.31</td>
</tr>
<tr>
<td>Coefficient of Hb variation</td>
<td>0.10 ± 0.02</td>
<td>0.11 ± 0.03</td>
<td>0.15</td>
</tr>
<tr>
<td>ESA responsiveness index (U/kg/week/g/dL)</td>
<td>10.9 ± 3.0</td>
<td>11.7 ± 4.8</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation
ESA, erythropoiesis-stimulating agent.

### Table 3. Comparisons of variables by frequency out of the target hemoglobin (Hb) range

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low-frequency group, &lt;25% (n = 32)</th>
<th>High-frequency group, ≥25% (n = 39)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>51.8 ± 12.6</td>
<td>49.6 ± 11.6</td>
<td>0.44</td>
</tr>
<tr>
<td>Male sex</td>
<td>18 (56.3)</td>
<td>19 (48.7)</td>
<td>0.69</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7 (21.9)</td>
<td>18 (46.2)</td>
<td>0.06</td>
</tr>
<tr>
<td>Previous cardiovascular disease</td>
<td>4 (14.7)</td>
<td>5 (10.8)</td>
<td>0.73</td>
</tr>
<tr>
<td>Dialysis vintage (mo)</td>
<td>45.7 ± 34.7</td>
<td>38.6 ± 32.2</td>
<td>0.38</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>9.5 ± 0.8</td>
<td>9.1 ± 0.9</td>
<td>0.11</td>
</tr>
<tr>
<td>Albumin (g/mL)</td>
<td>3.7 ± 0.3</td>
<td>3.7 ± 0.3</td>
<td>0.67</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.6 ± 1.0</td>
<td>8.7 ± 1.0</td>
<td>0.56</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4.7 ± 1.8</td>
<td>4.4 ± 1.4</td>
<td>0.55</td>
</tr>
<tr>
<td>Hb A1c (%)</td>
<td>5.9 ± 1.2</td>
<td>6.3 ± 1.6</td>
<td>0.40</td>
</tr>
<tr>
<td>Intact parathyroid hormone (pg/mL)</td>
<td>80.3 ± 91.3</td>
<td>57.8 ± 59.4</td>
<td>0.23</td>
</tr>
<tr>
<td>Single-pool Kt/V</td>
<td>1.40 ± 0.30</td>
<td>1.37 ± 0.24</td>
<td>0.62</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>201.2 ± 46.7</td>
<td>205.4 ± 40.0</td>
<td>0.68</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>50.5 ± 27.6</td>
<td>50.9 ± 25.2</td>
<td>0.95</td>
</tr>
<tr>
<td>EPO administration route, IV</td>
<td>19 (59.4)</td>
<td>19 (48.7)</td>
<td>0.51</td>
</tr>
<tr>
<td>Erythropoietin dose (U/kg/wk)</td>
<td>89.9 ± 48.5</td>
<td>106.1 ± 54.6</td>
<td>0.20</td>
</tr>
<tr>
<td>Ferric sucrose dose (mg/yr)</td>
<td>81.9 ± 217.0</td>
<td>95.3 ± 249.3</td>
<td>0.82</td>
</tr>
<tr>
<td>ESA responsiveness index (U/kg/wk/g/dL)</td>
<td>9.8 ± 5.9</td>
<td>11.9 ± 6.4</td>
<td>0.17</td>
</tr>
<tr>
<td>Standard deviation of Hb</td>
<td>0.94 ± 0.17</td>
<td>1.03 ± 0.30</td>
<td>0.12</td>
</tr>
<tr>
<td>Residual standard deviation of Hb</td>
<td>0.86 ± 0.16</td>
<td>1.00 ± 0.37</td>
<td>0.06</td>
</tr>
<tr>
<td>Coefficient of Hb variation</td>
<td>0.10 ± 0.02</td>
<td>0.11 ± 0.03</td>
<td>0.009</td>
</tr>
<tr>
<td>Hb of &lt;9 g/dL (n/visit)</td>
<td>0.15 ± 0.07</td>
<td>0.43 ± 0.16</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation, or number (%).
EPO, erythropoietin; ESA, erythropoiesis-stimulating agent; IV, intravenous.
kg/week/g/dL, p = 0.02; 8.3 ± 3.5 vs. 13.2 ± 4.4 U/kg/week/g/dL, respectively; p = 0.001) (Fig. 1B). The results of the unadjusted and adjusted logistic regression analyses for the high-frequency group are presented in Table 4. In the multivariate logistic regression analysis, the presence of diabetes mellitus (odds ratio [OR], 4.49; 95% confidence interval [CI], 1.38–14.61; p = 0.01) and EPO dose (OR, 1.03; 95% CI, 1.01–1.05; p = 0.002) significantly affected the high-frequency group. However, the route of EPO administration was not related to the high-frequency group.

### Hemoglobin variability and cardiovascular outcomes

During the study period, 19 out of 71 patients (26.8%) died including nine cases (12.7%) of cardiovascular events and five cases (7.0%) of infection. Cardiovascular events oc-
occurred in 22 patients (31.0%) during the study period including six patients in the low-frequency group and 16 patients in the high-frequency group. In the survival analysis, the occurrence of cardiovascular events was significantly higher in the high-frequency group than in the low-frequency group ($p = 0.02$) (Fig. 2). However, seven patients (21.9%) died in the low-frequency group and 12 patients (30.8%) died in the high-frequency group with no significant difference between the two groups in all-cause mortality (log-rank $p = 0.29$). In cardiovascular mortality, there were three (9.4%) and six patients (15.4%), respectively, who did not have any significance between the two groups (log-rank $p = 0.36$).

The results of the unadjusted and adjusted Cox proportional hazard regression analyses for cardiovascular events are presented in Tables 5. In the adjusted Cox analysis, the high-frequency group (hazard ratio [HR], 2.96; 95% CI, 1.03–8.79; $p = 0.04$), presence of diabetes mellitus (HR, 3.34; 95% CI, 1.31–8.76; $p = 0.01$), vintage dialysis > 18 months (HR, 3.07; 95% CI, 1.02–9.21; $p = 0.045$), and older age > 60 years (HR, 2.80; 95% CI, 1.12–7.02; $p = 0.028$) were independent risk factors for the development of cardiovascular events.

**Discussion**

This *post hoc* analysis of a clinical study comparing IV administration with SC administration of EPO showed that the frequency of the value being outside the target Hb range was associated with the route of EPO administration, and that the high-frequency group was independently associated with cardiovascular events in HD patients.

In this study, there was no difference in the SD, residual SD of Hb, and coefficient of Hb variation according to the EPO administration route. Since it is difficult to maintain Hb values within the narrow target range, we categorized Hb variability according to the frequency of the value being outside this range (i.e., low-frequency vs. high-frequency groups). Our study showed that patients of the IV group were less frequently outside the target Hb range compared to those of the SC group. Furthermore, the coefficient of Hb variation was lower and Hb was less frequent than the lower limit level of Hb (<9g/dL) in the low-frequency group.

Hb variability in HD patients was first described by Lacson et al. [17] and Berns et al. [18] in 2003. Kalantar-Zadeh and Aronoff [15] reported that factors influencing Hb vari-
Table 5. Univariate and multivariate analysis of predictors associated with cardiovascular events according to the out of target range

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age, &gt;60 yr</td>
<td>2.44 (1.02–5.86)</td>
<td>0.045</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.03 (0.44–2.38)</td>
<td>0.95</td>
</tr>
<tr>
<td>High-frequency group, ≥25%</td>
<td>2.93 (1.14–7.51)</td>
<td>0.03</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4.84 (1.96–11.96)</td>
<td>0.001</td>
</tr>
<tr>
<td>Previous cardiovascular disease</td>
<td>2.92 (1.14–7.49)</td>
<td>0.03</td>
</tr>
<tr>
<td>Dialysis vintage, &gt;18 mo</td>
<td>3.20 (1.08–9.54)</td>
<td>0.04</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>1.18 (0.69–2.02)</td>
<td>0.55</td>
</tr>
<tr>
<td>Albumin (g/mL)</td>
<td>0.80 (0.18–3.52)</td>
<td>0.77</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>0.98 (0.76–1.28)</td>
<td>0.90</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>1.13 (0.73–1.74)</td>
<td>0.58</td>
</tr>
<tr>
<td>Intact parathyroid hormone (pg/mL)</td>
<td>1.00 (0.99–1.01)</td>
<td>0.88</td>
</tr>
<tr>
<td>Erythropoietin dose (U/kg/wk)</td>
<td>1.00 (0.99–1.01)</td>
<td>0.62</td>
</tr>
<tr>
<td>Ferric sucrose dose (mg/yr)</td>
<td>1.00 (0.99–1.00)</td>
<td>0.68</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>1.00 (0.99–1.01)</td>
<td>0.63</td>
</tr>
<tr>
<td>Single-pool Kt/V</td>
<td>0.32 (0.07–1.50)</td>
<td>0.15</td>
</tr>
<tr>
<td>EPO administration route, IV</td>
<td>0.49 (0.21–1.14)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

CI, confidence interval; EPO, erythropoietin; HR, hazard ratio; IV, intravenous.

ability included drug-related factors, patient characteristics, iron storage, infection, and inflammation. Of these factors, the EPO dose and administration intervals are modifiable factors in the management of HD-associated anemia [19]. In a previous study, Wright et al. [20] reported that the administration of EPO through the SC route reduced the EPO dose, increased its effectiveness, and improved patient outcomes compared to IV EPO administration. By contrast, Bommer et al. [21] reported no differences in Hb levels and EPO doses between IV and SC administration in HD patients within 48 weeks. Previous studies involved the analysis of the relationship between the route of EPO administration and the degree of Hb increase, and few studies have directly compared the relationship with Hb variability.

This study observed the outcome over 51 months using a single formulation of EPO. Therefore, our study exclusively evaluated the impact of the EPO administration route on Hb variability. Our result is similar to that of the post hoc analysis in a previous study [22]. Patel et al. [22] reported that Hb variability was slightly higher in SC administration compared to the IV administration of EPO. They suggested that the wide range of bioavailability (18%–80%) was large because of differences in absorption from the SC tissue and the specific epoetin-dosing algorithm. In their study, EPO doses were adjusted by an absolute amount rather than as a percentage of the previously prescribed dose. Therefore, EPO administration through the SC route had a longer half-life, and the change in Hb may have been greater for a given change in EPO dose compared to the IV route. In the present study, the administered EPO dose was determined according to the degree of change in Hb, rather than administering an absolute amount of EPO. Therefore, the relationship between the EPO administration route and Hb variability was more accurately evaluated in our study even though similar results were obtained in both studies.

Recent studies have reported an increase in all-cause mortality in patients with high Hb variability [6,7,10], but no prospective study has analyzed Hb variability and cardiovascular outcomes to date. In the present prospective study, the frequency of the value being outside the target Hb range was independently related to cardiovascular events, and the high-frequency group was associated with higher incidences of cardiovascular events.

In our study, Hb variability did not exhibit a significant association with cardiovascular mortality and all-cause mortality. In a recent meta-analysis, Zhao et al. [7] demonstrated a 9% increase in the adjusted rate of death for each 1 g/dL increase in Hb variability. However, this relationship between Hb variability and cardiovascular mortality is inconsistent across studies. Eckardt et al. [11] reported the...
lack of an association between cardiovascular mortality and Hb variability in 5,037 HD patients. In their study, Hb variability was assessed using the parameters within-patient SD, residual SD, and method of fluctuation across the target range (11.0–12.5 g/dL). They also reported that Hb variability was not related to all-cause mortality in HD patients [11]. By contrast, Lin et al. [23] demonstrated in a retrospective study that high Hb variability is an independent risk factor for cardiovascular mortality in HD patients. Since the criteria for Hb variability defined in each study differed and because of the nature of retrospective studies, it is difficult to accurately evaluate the relationship between Hb variability and cardiovascular mortality.

Unlike previous studies, the current study was conducted as a prospective trial. The high-frequency group was associated with more cardiovascular events and higher Hb variation compared to the low-frequency group. Although the proportion of diabetic patients did not differ between the two groups, the proportion tended to be high in the high-frequency group. In previous studies, it is known that chronic inflammation, autonomic neuropathy, and microvascular damage caused by diabetes mellitus blunt the response of EPO in chronic kidney disease [24,25]. The proportion of diabetes affects Hb variability, possibly leading to increased cardiovascular events in the high-frequency group. However, in multivariate Cox analysis, the high-frequency group was related to the occurrence of cardiovascular events independent of the presence of diabetes. This finding suggests that the occurrence of cardiovascular events in HD patients may be related to Hb variability. The high-frequency group tended to exhibit a poor response to EPO and thus, a relatively large amount of EPO was required to reach the target Hb level, significantly increasing the number of cardiovascular events. Moreover, a high EPO dose has been described as an independent predictor of adverse cardiovascular outcomes [26,27]. Additionally, exogenous EPO can stimulate cellular proliferation and matrix accumulation in blood vessels, increase platelet production and calcium signaling, and contribute to prothrombotic effects. In agreement with this, the incidence of cardiovascular events was high in patients of the high-frequency group even though no correlation between Hb variability and cardiovascular mortality was observed.

The results of our study should be interpreted with caution given the following limitations. First, this study was a post hoc analysis of a previous randomized controlled trial and was not designed specifically for this aim. Therefore, the sample size may be too small to confirm statistical significance for some observations. Second, at the time the study was performed, laboratory tests to assess inflammatory status, such as C-reactive protein, were not conducted. Third, we studied the response to one EPO formulation. The effects may differ for other EPO formulations. Finally, there was a difference between the target Hb at the time of the study and the current anemia treatment guidelines. Therefore, further randomized studies on the administration route of EPO according to the current guidelines are necessary.

The strengths of the study are that it is a prospective study and that it has been followed up for a long period of time.

In conclusion, IV EPO administration in HD patients can better maintain Hb levels within the target range, and a decreased frequency of missing these target values prevents cardiovascular events from occurring.

Conflicts of interest

All authors have no conflicts of interest to declare.

Authors’ contributions

Conceptualization: YKL, JRK, JWN, JWY
Data curation: DHK, JK, KSY, JRK
Formal analysis: DHK, AJC, HCP
Methodology: DHK, AJC, HCP, YKL, JWN
Supervision: YKL, JWY, JRK, JWN
Writing—original draft: DHK, HCP
Writing—review & editing: All authors
All authors read and approved the final manuscript.

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Kim, et al. Hemoglobin variability and cardiovascular events

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ACKNOWLEDGEMENTS

The following is a list of reviewers who contributed to KRCP by evaluating manuscripts in 2021. All editors, authors, and readers have been benefited by the reviewers' generosity and expertise. we all owe these peer reviewers a debt and express our gratitude.

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

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

1. A cover letter. It must include your name, address, telephone and fax numbers, e-mail address, and state that all authors have contributed to the paper and have never submitted the manuscript, in whole or in part, to other journals.
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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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These are expected to present major advances and important
new research results. Section headings should include Abstract, Introduction, Methods, Results, Discussion, Conflicts of interest, Acknowledgments (if applicable), and References. The text should be limited to 4,000 words (excluding tables, figures and references) and 40 references.

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

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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.

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

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

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

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

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

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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.

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

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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 (\textsuperscript{a}, \textsuperscript{b}, \textsuperscript{c}, ...) should be used for special remarks.

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

DOSEAGE AND ADMINISTRATION
- Semiautomatic patient use

Initial dose
The usual dose of NESPI in adult patients is 20 µg, to be administered as a single intravenous injection once weekly. Initial dose at the switching from erythropoietin preparations: See Precautions related to Dosage and Administration

Maintenance dose
When correction of anemia is achieved, the usual dose of NESPI in adult patients is 15-40 µg as darbepoetin alfa (parental reconstitution), to be administered as a single intravenous injection once weekly. If correction of anemia is maintained by once every two weeks injection, the frequency of administration can be changed to once every four weeks. In this case, the initial dose in adult patients is 40-120 µg administered as a single intravenous injection once every two weeks. In all cases, the dose should be adjusted in terms of the degree of anemic symptoms and the patient's age, and should not exceed 180 µg as a single injection. The target of anemia correction is around 11 g/dL of hemoglobin level.

Precautions related to Dosage and Administration
1. Initial dose at the switching from an erythropoietin preparation.
   a. When NESPI is started in substitution for an erythropoietin preparation, the dose and the frequency of administration should be determined on the basis of the data of the erythropoietin preparation that has been used. See the table (package insert).
   b. Patients who have been treated with an erythropoietin preparation twice weekly or three times weekly calculate the total dose of the erythropoietin preparation administered during the week before the switching, and then determine the initial dose of NESPI according to the table below. The treatment should be started on once weekly basis.
   c. Patients who have been treated with an erythropoietin preparation once weekly or once every two weeks calculate the total dose of the erythropoietin preparation administered during the two weeks before the switching, and then determine the initial dose of NESPI according to the table below. The treatment should be started on once every two weeks basis. See the insert text.

2. Dose adjustment
   a. Dose adjustment is required, for example, when the increase in the hemoglobin concentration of the hematopoiesis level cannot be achieved in correction phase, or when the hemoglobin concentration of the hematopoiesis level deviates from the target range for successive two weeks in maintenance phase, the dose should be increased or decreased according to the table below. Any dose increase should be performed by stage in principle.

PRECAUTIONS
- See the package insert.

STORAGE
Store in a lightproof container at 2-8 °C and avoid freezing

PACKAGING
1 vial, 10 vials for NESPI 20 µg, 50 µg, 60 µg, 120 µg, respectively

MANUFACTURED BY:
Taiyo Pharmaceutical Co., Ltd.
1042-22 Matsunoki Takaotain-cho Oita, Japan
Kyowa Hakko Kirin Co., Ltd.
100-1 Higashiaika-cho, Tachikawa-shi, Tokyo, Japan

IMPORTED BY:
Kyorin Pharmaceutical Co., Ltd.
119 Aso Tower 800, Nishinari-ku, Osaka, 545-0001, Japan
TEL: 02-2847-4820 FAX: 02-2847-4822
http://www.kyowa-kirin-kome.com
요독소를 흡착하여
투석 시작을 지연시키는
"만성신부전 진행억제제"
1-3

크레메진 세랍
KREMEZIN

spherical adsorptive carbon 2g

MIRCERA exists because
life is long

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

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

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

MIRCERA exists because we believe in the power of longer stability

A long-lasting changes caused by long-acting effects Including Non-dialysis CKD, PD, and HD

Purple Effect MIRCERA

© (R), (C) 2019/2020 Kidney Disease) (D) (Patent/Registration) (B) (Reference/Design)


© (R), (C) 2019/2020 Kidney Disease) (D) (Patent/Registration) (B) (Reference/Design)

© (R), (C) 2019/2020 Kidney Disease) (D) (Patent/Registration) (B) (Reference/Design)

© (R), (C) 2019/2020 Kidney Disease) (D) (Patent/Registration) (B) (Reference/Design)
4. Undesirable Effects

Common (≥1/100, <1/10): Headache, dizziness, vertigo, paraesthesia, visual disturbances, tinnitus, hypotension and effects on the cardiovascular system. (2) Uncommon (≥1/1,000, <1/100): ARBs (e.g. valsartan), ACE inhibitors (e.g. captopril), NEP inhibitors (e.g. sacubitril), extracorporeal treatments. (3) Anaphylactoid reactions during desensitization:

<table>
<thead>
<tr>
<th>Common Effects</th>
<th>Uncommon Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypersensitivity/Angioedema: Angioedema of the face, extremities, lips, mucous membranes, tongue, glottis and/or larynx can occur. This may be an indication of increased risk of angioedema associated with ACE inhibitors or ARBs.</td>
<td>Risk factors for the development of hyperkalemia include those with renal insufficiency, worsening of renal function, age (&gt; 70 years), diabetes mellitus, concomitant use of thiazide diuretics and potassium-sparing diuretics.</td>
</tr>
</tbody>
</table>

5. Precautions for use

- Pregnancy: When pregnancy is diagnosed, treatment with ACE inhibitors should be stopped immediately. Alternative treatments with better established safety profiles during breast-feeding are preferable.
- Hypersensitivity: Angioedema of the face, extremities, lips, mucous membranes, tongue, glottis and/or larynx can occur. This may be an indication of increased risk of angioedema associated with ACE inhibitors or ARBs. Risk factors for the development of hyperkalemia include those with renal insufficiency, worsening of renal function, age (> 70 years), diabetes mellitus, concomitant use of thiazide diuretics and potassium-sparing diuretics. (2) Uncommon (≥1/1,000, <1/100): ARBs (e.g. valsartan), ACE inhibitors (e.g. captopril), NEP inhibitors (e.g. sacubitril), extracorporeal treatments. (3) Anaphylactoid reactions during desensitization:

6. Contraindications
- Known hypersensitivity to ACE inhibitors, ARBs, NEP inhibitors, extracorporeal treatment, or any component of this product.
- History of angioedema associated with previous ACE inhibitors.
- Breastfeeding: Alternative treatments with better established safety profiles during breast-feeding are preferable.

For your patients of Hypertension CAD Heart Failure

Initiate ACERTIL® early!

<table>
<thead>
<tr>
<th>24h BP control</th>
<th>CVD risks reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACERTIL® 4mg, 8mg</td>
<td>ACERTIL® 5mg, 10mg</td>
</tr>
</tbody>
</table>


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

- Due to its short half life (5~8 min), its anticoagulant activity is almost limited to extracorporeal circuit.² ³ ⁴
- Increase of bleeding risk was not noted in HD patients with bleeding risk.⁵ ⁶ ⁷
- The filter-life is significantly prolonged during CRRT⁸ ⁹ ¹⁰

Summary of Prescribing Information¹

**PRODUCT NAME IN KOREA**
- Futhan for Inj. (nafamostat mesilate)
- Futhan50 for Inj. (nafamostat mesilate)

**INGREDIENT**
- Futhan for Inj. : 1 vial contains 10mg of nafamostat mesilate
- Futhan50 for Inj. : 1 vial contains 50mg of nafamostat mesilate

**INDICATION AND USAGE**
1. For improvement of acute symptoms of pancreatitis (acute pancreatitis, acute exacerbation of chronic pancreatitis, acute postoperative pancreatitis, ERCP-induced acute pancreatitis, traumatic pancreatitis) - Futhan for Inj. only
2. Disseminated intravascular coagulation (DIC)
3. To prevent coagulation of blood during extracorporeal blood circulation (ex. hemodialysis, plasmapheresis) in patients with bleeding complications or bleeding tendency.

**DOSAGE AND ADMINISTRATION**
- For priming, wash and fill the blood route with 20mg of nafamostat mesilate dissolved in 500mL of saline after dissolving in the small amount of 5% glucose solution or water for injection. After beginning of extracorporeal circulation, inject continuously at a rate of 20~50mg/hr as nafamostat mesilate dissolved in 5% glucose solution into anticoagulant injection line. The dosage should be appropriately adjusted according to the patient’s symptoms. The average dosage from clinical study is 35mg/hr as nafamostat mesilate.

Manufactured by Yuhan corporation. Distributed by SK chemicals

Revised: May 28, 2018.

For the details, you are recommended to check on prescribing information. The latest approved label is available on the website following. http://nedrug.mfds.go.kr

References

**FTN-HA06-201903-01**

HD: hemodialysis, CRRT: continuous renal replacement therapy
We provide one-stop service by building an integrated pipeline.

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

We devote for continuous product development and service improvement.

We work with therapists to find the optimal solution.
 Patients with aHUS can be at continuous risk of the life-threatening consequences of unpredictable complement-mediated TMA\textsuperscript{1,2}.

Chronic, uncontrolled complement activity in aHUS leads to ongoing endothelial injury, organ damage, and sudden death\textsuperscript{3}.

Improving lives together

Fresenius Medical Care is the world’s leading provider of dialysis products and services, offering life-sustaining care for people living with chronic kidney failure.

In Asia Pacific, we draw on our decades of experience and expertise to deliver our vision – **Creating a future worth living. For patients. Worldwide. Every day.**
Simplicity reinforced.
The 1st launched medicine of Calcium polystyrene sulfonate in Korea

The most prescribed treatment agent of Hyperkalemia in Korea

Various formulations for medication convenience (Powder/Granule/Suspension)

Treatment agent of Hyperkalemia

KALIMATE
Powder / Granule / Suspension

REFERENCES
1. 식품의약품안전처, 위장내위약도서관, 약학정보센터, 카리메트
2. 2019 IQVIA DATA 기준(국내 고혈압증 치료제 판매량)

카리메트 산/포함

Monitoring the dialysis dose continuously and in real-time

Only those who are aware of the nature of the path are able to reach their destination safely and quickly.

Adimea® stands for Accurate Dialysis Measurement (precise measurement of the dialysis conditions). This real-time measurement system is able to determine the Kt/V precisely in any given dialysis treatment scenario.

The measuring principle of this innovative system from B. Braun is simple: a UV light sensor installed in the dialysate drain of the Dialog+ machine measures the absorption of light and thus changes in the concentration of uremic substances as they drain off. This means that insufficient dosages are identified immediately.

The advantages are obvious: the user is able to adjust relevant parameters during treatment so as to model the Kt/V, meaning efficient and optimized dialysis treatment is guaranteed for the patient at all times and without any detours. That's for sure.

Dialog+ and Adimea®
Monitoring the dialysis dose continuously and in real-time

Only those who are aware of the nature of the path are able to reach their destination safely and quickly.

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OPTIMIZE TROUGH LEVEL
START LIFE-LONG JOURNEY\textsuperscript{1,2}

\textsuperscript{1} Program* 제출(2020.05.14).

* 프로그램* 제출(2020.05.14).
REXEED™ Series
Hemodialyzer / REXEED™

Our Perfect Solution for HD
Combining the Best with a Large Line-UP
WET TYPE Polysulfone

REXEED-A
The high flux dialyzer with best removal performance among our wet-type product range for all patient groups.

REXEED-M
Dialyzer with best removal performance among our wet-type high flux product range.

REXEED-L
Dialyzer with best removal performance among our wet-type low flux product range.

Improved design for ideal dialysate flow
Effective removal of small toxins and low molecule weight proteins
Superior biocompatibility

AY Trading Co., Ltd.
TEL: 02-585-7661 / FAX: 02-585-7664
Slow ADPKD. Preserve Hope.

Introducing Samsca – The first and only treatment proven to slow cyst progression

Samsca® Tablet ADPKD product information summary [INDICATION] To slow the progression of cyst development and renal insufficiency of autosomal dominant polycystic kidney disease (ADPKD) in adults with CKD stage 1 ~ 4 at initiation of treatment with evidence of rapidly progressing disease. [DOSAGE & ADMINISTRATION] Tolvaptan must only be prescribed by physicians who got registered in Risk Management Program to the patients who have agreed and signed on conditions specified in Risk Management Program. Patient should follow this program. And, to mitigate the risk of significant and/or irreversible liver injury, blood testing for hepatic transaminases and bilirubin is required prior to initiation of SAMSCA, continuing monthly for 18 months and at regular 3 monthly intervals thereafter. The initial dose is 60 mg tolvaptan per day as a split-dose regimen of 45 mg + 15 mg (45 mg taken upon waking and prior the morning meal and 15 mg taken 8 hours later). The initial dose is to be titrated upward to a split-dose regimen of 90 mg tolvaptan (60 mg + 30 mg) per day and then to a target split-dose regimen of 120 mg tolvaptan (90 mg + 30 mg) per day, if tolerated, with at least weekly intervals between titrations. Dose titration has to be performed cautiously to ensure that high doses are not poorly tolerated through overly rapid up-titration. Patients may down-titrate to lower doses based on tolerability. Patients have to be maintained on the highest tolerable tolvaptan dose. x Samsca® Tablet has an indication for hyponatremia as well. For further information, please refer to the latest prescribing information at www.otsuka.co.kr.
COUNT ON FABRAZYME

Treat your Fabry disease patients with Fabrazyme

1 mg/kg

once every 2 weeks

References: